COOPERATIVE RESEARCH REPORT

NO. 166

METHODOLOGY OF FISH DISEASE SURVEYS

Report of an ICES Sea-Going Workshop held on

U/F "Argos" 16-23 April 1988

https://doi.org/10.17895/ices.pub.7959
ISBN 978-87-7482-633-0
ISSN 2707-7144

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July 1989

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A Abstract

Through a combination of discussions, formal presentations and practical trials, the ICES workshop held on board U/F *Argos* from 16-23 April 1988 considered the methodology of fish disease* surveys. Particular emphasis was placed on assessing and updating the proposals of the 1984 workshop in the light of four years of practical application. Substantial differences still exist regarding the pathological conditions that are monitored and regarding diagnostic interpretations, particularly when diseases occur at low levels. The major source of sampling variability still existing was identified as the difficulty and reliability in detecting light cases of disease (particularly lymphocystis). For ICES reporting purposes it is recommended that only a restricted number of disease conditions be included and that, to improve reliability of data for international comparison, results below clearly defined levels of disease severity should be excluded.

Fish disease survey data currently being made available to ICES indicates the range of diseases present, their broad distribution and the variability in their prevalence, but does not permit adequate trend analysis of prevalence levels. The recommendation is made that in-depth studies should be carried out at a series of sampling positions throughout the area, each ICES country bordering the North Sea and Baltic taking responsibility for sampling and reporting on at least two, on an annual or more frequent basis. Positions should be selected to represent differences in contamination levels. Standardised methods for sampling, diagnosis and reporting of fish disease should be followed and the studies closely integrated with other biotic and abiotic investigations of the fish and sampling stations in order to characterise the area and to search for factors potentially influencing fish health.

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* The term disease refers throughout the paper to pathological conditions, including those of unknown aetiology.

Major problems still existing in surveying marine fish diseases include difficulties in obtaining suitable reference data for comparison with results from polluted areas, uncertainty about the dynamics and aetiology of many fish diseases and the scarcity of studies that are closely integrated with other biotic and environmental factors.

B Introduction

At its 1986 meeting the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) recommended the holding of a second sea-going workshop on the Methodology of Fish Disease Surveys, a proposal which was adopted by ICES at its 73rd Statutory Meeting as Council Resolution 1986/2:30. At the 1987 meeting of the WGPDMO the Swedish representative offered the use of the research ship U/F *Argos* for the purpose in April-May 1988. This offer was accepted and the second ICES Sea-going Workshop on Methodology of Fish Disease Surveys took place on board U/F *Argos* from 16-23 April 1988. Twelve scientists from seven countries, most of whom have worked on national fish disease surveys on a regular basis, participated in the workshop (Appendix I).

The aim of the workshop was to revise the 1984 workshop report (ICES Cooperative Research Report 140) in the light of field experience over the last four years and to update the recommendations for such surveys. It is hoped that the report of this second workshop will encourage and facilitate new investigators to initiate regular work in this field. A final goal of the workshop was also to construct a simple diagnostic and reporting system so that future survey data can be computerised by ICES.

C Continuing Problems

The 1984 workshop on methodology of fish disease surveys identified a series of factors contributing to variability in results. Although considerable improvements have resulted from the wide-ranging recommendations that were made then, subsequent practical experience has shown that significant problems still exist. The participants of this workshop considered it valuable, in order to stimulate further work, to list these problem areas again with an indication of where further progress is still required.

Problems identified by the 1984 workshop:

1. Fish parasites in relation to pollution. Literature reviews have been published concerning effects of pollution on parasites of marine fish (see Moller (1987), International Journal of Parasitology, 17, 353-362), but little recent progress has been reported in this field.

2. Abnormal gill conditions in wild fish. X-cell gill lesions of dab have been investigated since 1984 by McVicar, Bucke, Watermann and Dethlefsen (1987) (Diseases of Aquatic Organisms, 2, 197-204) and Diamant and McVicar (1987) (Aquaculture, 67, 127-133). There is still a lack of established cause/effect relationship between pollution and general gill pathology (Overstreet 1988) (Aquatic Toxicology, 11, 213-239).

3. There is still insufficient knowledge on the behaviour of diseased fish and their catchability in relation to different fishing gears.

4. Reference maps of fishing intensity have not yet been provided to establish the possible impact of injuries due to fishing gear on the health status of fish.

5. From the field data available, there is still a lack of information on the dynamics of most diseases in marine fish particularly with respect to the relationship between prevalence and incidence. Experimental work is required which might enlighten aetiological questions as well as the significance of certain diseases in relation to host survival.

6. The influence of diet composition, nutritional value of the food and the condition of marine fish on their health is still poorly understood. This item is covered by the recommendations of the present workshop concerning requirements for accompanying data in disease surveys (see page 16).

7. Present marine fish disease programmes still concentrate largely on the monitoring of external diseases. Internal organ surveys with emphasis on the liver using a standardised method of recording should be included in these programmes.

The participants of this workshop recognised several additional problem areas which should be considered.

Although collection of data from wide-ranging extensive studies (eg in cooperation with stock assessment cruises) has considerable value in indicating the range of diseases present, because of their broad distribution and the variability occurring it is recognised that such data have limited value in the analysis of trends. Even in disease dedicated cruises comparison of fish disease levels in different areas has proved difficult because of the marked spatial variability of all fish diseases being studied. Consequently there have been problems in finding true reference areas to compare with test (eg polluted) areas of interest. In general, there have been insufficient long-term studies in limited areas on an international level to allow adequate trend analysis of fish disease levels in the North Sea. The restricted number of fish disease studies integrated with studies on the chemical, physical and biotic components of the environment also makes interpretation of data in relation to pollution difficult. Similarly, there is a lack of knowledge of fish population delineation, migration, physiology and behaviour in relation to fish disease. A particular problem is that in all of these there is a lack of information on what data is required. Requirements and constraints of studies carried out by individual nations present significant problems in developing integrated sampling programmes on an international scale. This is compounded by the absence of a reliable method of providing information to relevant specialists on proposed national disease sampling programmes. The reporting of such proposals through the Chairman of WGPDMO should be encouraged. Finally, the continuing variability in the sampling, diagnosis and recording of fish disease and in the presentation of data, is the major factor preventing international comparison and pooling of data. Standardised methods appropriate for international use are required and suitable instruction (including training aids suitable for use on board ships) need to be provided in order to improve the quality and quantity of compatible data.

D Main Objectives of the Second Workshop

Recognising that the solutions to some of the major problems which have been identified still lay outside the possibility of immediate practical feasibility, the workshop set itself some broadly-based objectives.

1. Establishment of guidelines for an integrated international programme to determine long-term trends in fish disease prevalence levels in the North Sea. The realisation of this objective would require agreement on minimum sampling requirements, using standardised methods of diagnosis and reporting.

2. Establishment of internationally agreed cut-off points for the principal disease conditions included in pollution monitoring studies, below which data would be excluded from international reporting.

E Proceedings

Twelve scientists from Denmark, Finland, FRG, the Netherlands, Norway, Sweden and the UK participated in the second Sea-going Workshop on Methodology of Fish Disease Surveys. There were gaps in the regional coverage due to the absence of representatives from other ICES member countries.

The format of the workshop allowed the group to participate in practical exercises, discussions and presentations working around catches from 11 hauls in the Kattegat (Appendix II). As a basis for programme activities the first workshop's results were presented in summary and a critical assessment made of shortcomings identified by four years of practical experience (Section C).

A summary and detailed report of the practical aspects of the workshop are presented (Section F, Appendix III). The first practical exercises were used to investigate whether differences in the expertise of workers could significantly affect results and subsequent exercises allowed the participants to practise and discuss standardisation procedures. When the recording of fish disease was investigated experimentally it became clear that, for international reporting purposes to ICES, "cut-off" points were necessary. This meant eliminating some conditions including certain parasites, which present a more subjective interpretation at sea and would require further detailed study for accurate diagnosis.

Whereas the first workshop report had shown that suitable fish species for disease monitoring in the North Sea were the dab, *Limanda limanda*, and possibly the cod, *Gadus morhua*, recent experiences, while confirming the suitability of dab showed that for inshore and estuarine waters the flounder, *Platichthys flesus*, was also suitable. Because of its mobility, cod was not considered to be suitable for long-term monitoring of fish diseases in the North Sea in relation to pollution, but was suggested as a suitable species for the Baltic Sea in conjunction with the flounder.

For this workshop, effort was concentrated on dab diseases, with only a cursory interest in flounder because they were uncommon in catches. Only for lymphocystis was it possible to define and test the use of a "cut-off" point: other externally recognised diseases were scarce, and the proposed "cut-off" points are based on experience. Proposed diseases to be included in ICES international monitoring of dab were lymphocystis, skin hyperplasia/papilloma, skin ulcers and x-cell gill lesions. For internal diseases the target organ of choice was the liver. A variety of changes, both normal and pathological have been revealed in this organ and agreement was reached on a suitable "cut-off" point based on nodule size to ensure compatibility of results for international reporting. Parasites and other cysts occurring in the liver and other internal organs would not be included, but individual workers should be encouraged to make their own in-depth studies of all internal and external pathologies encountered.

Exercises to establish suitable sample numbers of fish per station indirectly indicated that minimum numbers would depend on the statistical evaluation required (associated with disease prevalence levels) and practical constraints. However, these practical exercises also indicated that, due to the relative abundance of length-groups, optimum length for external diseases of dab was 20-25 cm. However, as liver changes did not appear in significant numbers until the fish were larger than 25 cm emphasis should be placed on these larger fish during investigations of livers.

Once these criteria were established and tested, the participants designed new disease recording forms for dab and flounder. Subsequently, recording forms suitable for reporting national disease data to ICES were produced.

In order to facilitate the assessment of the significance of fish diseases in relation to pollution monitoring, the workshop participants discussed and suggested a coordinated long-term programme of fish disease monitoring drawing up guidelines which would allow the detection of any trends that may exist (Section G).

Presentations on long-term disease monitoring reflecting the participants own experiences gave insight into the various countries broader ongoing practical studies. Dethlefsen reviewed his 10 years of investigations in the North Sea. He concluded that prevalences of external diseases in dab identified on the Dogger Bank were higher compared with other areas, and this data could be correlated with residues of heavy metals and organochlorine in the fish. Vethaak whose studies commenced in 1983 has found consistently higher prevalences of certain epidermal diseases and liver-nodules in flounders and dabs sampled from contaminated areas than in this species from reference areas. However, he found higher prevalences of lymphocystis in dab in his reference area off the Dutch coast. He concluded that these variations could reflect that different diseases have multiple aetiologies. McVicar found disease prevalences in dab to be significantly higher on reference stations adjacent to sewage dump-sites than on the actual dump-sites off the Firth of Forth. His message was a warning that both "negative" and "positive" correlations between pollution and fish diseases should be treated with a degree of caution before making conclusions on causal relationships and that it was more important to monitor trends on sites rather than prevalence. Ap Rheinallt outlined preliminary data from a study of external and internal abnormalities of dab and flounder in the Thames estuary. He emphasised that, due to the small number of surveys, no definite conclusions could be drawn, but there was good correlation between macroscopial lesions and histological diagnoses of the liver changes.

Lindesjöö reported that by monitoring fin erosion of perch near to a pulp mill effluent discharge in the Baltic Sea, he was able to show a clear gradient in the prevalence of the condition along a line of stations leading from the discharge to cleaner water. In addition, improvement in the quality of the effluent was associated with a change from fin erosion to healed fin erosion. Similarly Mellergaard described an association between stress caused by oxygen deficiency in a sea area north west of Helgoland and west of Denmark and the prevalence of diseases in dab. In 1982 in those areas dead benthic animals were also associated with the oxygen deficiency. Diseases of dab showed increased prevalences from 1983 to 1985, followed by a progressive decrease in disease prevalence in the total population back to previous levels. However, each affected year classes persisted with higher levels of lymphocystis throughout their life. Bylund related his experiences with fish diseases in the Baltic Sea. He suggested that because fresh water fish inhabit these waters they may be at their limit of their endurance because of the low salinities. Any small environmental change may cause further stress thus resulting in disease.

Bucke presented his experiences on the effectiveness of disease monitoring on different types of vessels, stating that research cruises dedicated solely to fish disease work were to be preferred, although a major disadvantage was the cost. To reduce costs it may be better to participate on cruises with multiple aims, eg benthic sampling, hydrographics etc. He considered that there were advantages in using routine fish stock assessment cruises which covered a wide sea area because they gave an overall impression of background levels of disease which could be compared annually. Furthermore, a great deal of other biological and physical data became available. However, conflict could arise due to the different priorities of the different research programmes. Charter vessels were particularly useful for inshore work and *ad hoc* observations, and costs were usually low. The main disadvantages were that inadequate facilities reduced the intensity and diversity of the work.

Presentations on liver changes were given by Bucke, Kranz, Vethaak and Ap Rheinallt. Agreement was reached on the importance of assessing liver nodules histologically because if they were diagnosed as stages of hepatoma, they may be related to environmental changes. The recording of other macroscopical lesions and changes in livers was uncoordinated and not useful for ICES international reporting. All agreed that more work was needed to investigate the possibility of a cause-effect relationship between contaminants and liver anomalies.

During the workshop the participants identified the need for fish disease studies to be combined with other biotic, chemical and physiological studies. They also identified that illustrated guides would aid training in fish disease monitoring and discussed the needs of workers involved in disease monitoring in the Baltic Sea.

The workshop concluded its meeting on Saturday 23 April by drawing up a number of conclusions and recommendations to be presented through ICES.

F Practical Work

As the workshop was held on board ship, material was readily available to test proposals and recommendations under practical conditions. A total of 11 catches from the Kattegat were taken (Appendix III) and by agreement, emphasis was placed on disease conditions of common dab, *Limanda limanda*. With an overall aim of standardising the diagnosis and recording of internal and external diseases, specific objectives of different exercises included: a) familiarisation with the diseases present in the Kattegat area, b) reduction of variability and standardisation of the diagnosis and recording of external diseases, c) standardisation of observations on internal conditions and d) the development and testing of diagnostic and recording procedures which could be used for international reporting. Details of observations and results are presented in Appendix III.

From this practical work it was concluded that although the wide range of diseases present were recognised by most observers, not all were routinely recorded and that agreement was necessary on which diseases should be recorded. The significant variability found between different subsamples from the same catch was largely attributed to fish with minimal signs of disease being missed (by both rapid and intensive examination) and to differences in interpretation of clinical signs particularly at the lower ranges of disease classification scales. On the basis of these observations, it was suggested that clearly defined "cut-off" points should be used for the purposes of international comparisons, recognising that some fish with some (light) signs of disease would be excluded. In the case of lymphocystis, only dab with at least two or more surface nodules (together or separate) would be reported and only ulcers in which the skin surface was incomplete should be included, ie "acute". Problems were experienced in the diagnosis and recording of liver abnormalities and a satisfactory solution was to include only nodules larger than 2 mm diameter and to exclude liver colour and discolourations. It was also agreed that internal parasitic cysts, unless specifically identified, should be excluded from international reporting. Practical use of the proposed standardised diagnostic and recording system indicated its value in reducing variability.

G Long-term Coordinated Fish Disease Studies

a) Objectives

A requirement was identified by the workshop for the analysis of trends in disease prevalence in relation to pollution in selected fish species and areas of investigation. Use of standardised sampling and diagnostic methods would allow the international comparisons of long-term trends in spatial and temporal distribution patterns. Integration of the study with "biological effects" monitoring programmes on biotic and abiotic environmental aspects could lead to the identification of factors affecting fish health.

b) Recommendations

1. Introductory remarks

The following guidelines are recommended by the workshop as minimum requirements for long-term monitoring of fish disease levels and the analysis of trends in relation to pollution. Investigators should endeavour to meet these requirements and if possible to exceed them in terms of numbers of fish and of sites examined. Data submitted from studies where all these requirements have not been met should be accompanied by an appropriate note.

The recommendations are made on the basis of the practical experiences of the workshop and the background knowledge of the workshop participants; consequently they are most appropriate for the North Sea and Kattegat. In different areas other fish species and diseases may be selected depending on their suitability, but the same general principles governing their use should still apply.

In studies designed to identify and monitor changes in fish disease in relation to pollution it is clearly essential for a range of environmental, chemical and biotic factors to be measured in order to characterise the sampling areas. The lack of relevant expertise in these topics by many of the workshop participants was recognised and the assistance in identifying appropriate parameters for measurement requested of specialist ICES Working Groups (note recommendations).

2. Species

On the basis of their distribution, abundance, availability and disease prevalences the following fish species have been selected as suitable for examination in the North Sea.

Common dab	(*Limanda limanda*)
Flounder	(*Platichthys flesus*)
Cod	(*Gadus morhua*)

3. Diseases

For each host species the following disease signs were selected as appropriate for study on the basis of:

i) their possible responsiveness, expressed as changes in prevalence in response to surrounding environmental conditions as indicated by current published and unpublished data.

ii) ease and reliability of diagnosis.

For some cases clear "cut-off" points above the lower limits of visual detection are recommended for international reporting to increase reliability of diagnosis and allow international comparison of results, but it should be noted that data outside these limits may be useful for other purposes.

Host	Disease	Minimum requirement for international reporting
Common dab	Lymphocystis Epidermal papilloma Skin ulcers (acute and healing) x-cell gill lesion Liver nodule/tumours	More than one surface nodule Lesions larger than 2 mm diameter Open lesion One or more filaments affected Larger than 2 mm diameter
Flounder	Lymphocystis Skin ulcers (acute and healing) Liver nodule/tumours	One or more nodules Open lesion Larger than 2 mm diameter
Cod	Skin ulcers (acute and healing) Skeletal deformities Pseudobranchial swelling *Cryptocotyle*	Open lesion Grossly or by filleting Grossly observable One or more cysts

4. Sampling stations and areas

Each ICES member country should select and report on at least two stations which are known for their differences in contamination levels, with international cooperation if required to ensure a sufficiently broad spread of locations covering the areas of interest. As an example, study positions indicated by members of the workshop for which they could possibly take regular responsibility are indicated in Figure 1. The final selection of the fixed positions to be used for long-term monitoring should be made carefully with particular attention being paid to the choice of areas of national and international interest (eg dump sites) and of areas removed from pollution input as reference areas. Fish species availability, disease occurrence and the presence of existing knowledge on pollution, fish stock movements and disease should be taken into account.

Figure 1. Proposed disease sampling positions.

5. Sampling

For the purpose of this international study:

a) Sampling should be accurately positioned on a nominated latitude and longitude with all repeat hauls being within clearly defined limits eg a radius of two nautical miles.

b) Sampling should be conducted on a long-term basis, once a year within the same narrow time window (two weeks to one month) or if possible at two periods to provide separate data from the summer and winter periods.

c) Where resources permit, the minimum recommended sampling requirement should be exceeded in terms of areas, frequency and numbers of fish sampled.

d) Catches should be stratified into groups by length and a minimum number of fish per length-group examined for disease. Sample sizes are based on statistical requirements (a total sample of 250 fish allows the detection of a disease prevalence of at least 1.5% with 95% confidence limits) and also the fact that prevalence increases with fish length. For example, the minimum sampling requirements considered appropriate for dab, flounder and cod are as follows:

Dab	Disease examination	
Size group (cm)	External	Internal
15-19	100	
20-24	100	(50)
≥25	50	50

Flounder Size group (cm)	Disease examination External	Internal
20-24	100	
25-29	100	(50)
≥30	50	50

Cod Size group (cm)	Disease examination External
29	100
30-44	100
≥45	50

If 50 fish of the largest size group of dab and flounder are not available for internal examination on the sampling station additional specimens to make up the total should be taken from the upper range of the middle size group.

d) The average length and sex ratio of all fish within each length-group should be recorded and in addition the sex ratio of diseased fish within each length group noted.

6. Other data from sampling stations

To facilitate interpretation of long-term disease data and to characterise sampling stations additional investigations of biotic and abiotic characteristics are required. The complete list of appropriate complementary investigations needs to be compiled through consultation with the relevant ICES working groups (eg WG BEC) but some members of this workshop suggested the following as a possible initial framework:

a) Fish characteristics

Age/length relationship

Condition factor

Gonadosomatic Index

Stomach food composition

Contamination of liver

Enzyme induction

Immune competence

Catch per unit effort as an index of population density

b) Biotic

Characteristics of the benthic community

Fish fauna - catch composition of other species

c) Abiotic

Hydrography

Sediment composition

Sediment contamination characteristics

7. Data recording sheets

The method of recording data in the field will be a matter of individual preference and programmes. However, in order to indicate the minimum suggested requirements from individual fish used in the long-term disease investigations, examples of recording sheets for dab, flounder and cod diseases are included (Appendix IV):

8. ICES reporting format

It is essential that summarised data from the long-term fish disease studies carried out by individual Laboratories are presented to ICES in an internationally standardised manner and in a format which could be easily computerised. The workshop recommends that the format in Appendix V be used with dab, flounder and cod diseases.

H Conclusions

The workshop came to the following conclusions:

1. Most fish disease investigations have involved sampling from both pollution and reference areas. As it is established that disease levels may differ spatially for natural reasons, selection of reference areas should ensure as much conformity as possible in biological, chemical and physical parameters. Ideally, the only difference should be the anthropogenic agent(s) under consideration.

2. For the North Sea, the common dab was selected as the fish of choice for disease monitoring offshore and the flounder for inshore and estuarine waters. It was recognised that other species may be more appropriate in other areas eg cod and flounder in the Baltic were acceptable species for disease monitoring.

3. A limited number of diseases should be reported to ICES. For dab these are lymphocystis, skin hyperplasia/papilloma, skin ulcers, gill x-cell lesions and liver nodules/tumours. For flounders lymphocystis, skin ulcers and liver nodules/tumours are included. For cod ulcers, pseudobranchial "tumours", skeletal deformities and *Cryptocotyle* are included.

Because new or better parameters for investigating the relationship between fish disease and pollution should be continually sought, individual workers should be encouraged to investigate fish diseases using broadly-based programmes whenever possible

4. For international comparison by ICES, long-term monitoring of fish diseases using standardised methods of sampling, diagnosis and reporting is essential.

5. There are difficulties in determining cause-effect relationships between pollution and fish diseases from the epidemiological methods used in field studies. It is therefore important to note that with most data currently available (a) the absence of proven cause-effect relationships in such studies does not imply such associations do not exist and (b) the coincidence of high fish disease prevalence levels with areas of pollution does not necessarily indicate pollution induced effects. In order to elucidate environmental effects on fish disease, studies should be supported where possible by biological, physical and chemical investigations of the fish and environment and the use of tank and translocation studies under controlled conditions is encouraged.

6. Although there has been considerable progress toward solving some of the problems that were identified by the 1984 workshop, especially in the standardisation of techniques, major gaps in knowledge about fish diseases can still be identified (Section I).

7. Liver nodules (tumours) in dab and flounder have an unknown aetiology, possibly non-infectious, and their occurrence in older fish may be useful in indicating a pollution-related effect.

8. Because of difficulties in detection of minimal cases of disease and of problems in their diagnosis even by experienced observers a requirement was identified by the workshop for clearly defined "cut-off" points for the main diseases used in investigations, below which data would not be used for ICES reporting. For lymphocystis in dab this was determined by the presence of two clearly defined "pearl-like" nodules, whereas in flounder experience has shown that this disease is easily recognisable in all stages of infection. The "cut-off" point for skin hyperplasia/papilloma was determined as 2 mm diameter lesions and only skin ulcers with the skin broken would be included.

9. ICES grid squares may be inappropriate for reporting many fish disease prevalences, as some diseases are extremely localised. Therefore, additional information including station code and accurate sampling position in latitude and longitude should be included in ICES reports.

I Additional requirements identified by the second workshop

1. There is a need for age-length relationships of fish used in disease surveys when comparison of data from different sampling sites in different areas is desired.

2. Data is required on the short- and long-term movement patterns of fish used in disease surveys.

3. More information is required on the variability in prevalence of fish diseases in repeated hauls at the same sampling site and between sites separated by short distances.

4. More information is required on fish diseases in the Baltic using standardised sampling and diagnostic methods.

J Recommendations

The members of the ICES Workshop on Methodology of Fish Disease Surveys held on board U/F *Argos* from 16-23 April 1988 recommend for consideration by appointed members of the ICES Working Group on Pathology and Diseases of Marine Organisms that:

1. The ICES member countries, particularly those bordering the North Sea and the Baltic, should annual gather fish disease data using recommended sampling, diagnostic and reporting methods, from at least two sampling stations selected for their known differences in contamination levels (eg polluted and reference) and should annually submit the results to WGPDMO for evaluation of long term trends in disease levels.

2. In disease monitoring programmes ICES member countries should include examination for liver nodules in dab and flounder and should carry out detailed research investigations into their aetiology and pathogenicity.

3. ICES should prepare, through the WGPDMO, training aids for non-specialists in the field of fish diseases. Nominated members of the second Sea Going Workshop would prepare illustrated examples of the more common diseases with brief descriptions, to be used as guides for disease identification on field investigations.

4. After three years the WGPDMO should review progress and consider whether it would be useful to recommend a workshop to update aspects of sampling procedures including details of the international fish disease trend monitoring programme.

5. This workshop report should be published as soon as possible in the ICES Cooperative Research Report Series.

K Acknowledgements

The participants of the second ICES Sea-going Workshop on Methodology of Fish Disease Surveys wish to thank Dr B I Dybern of the Institute of Marine Research, Lysekil, Sweden for having provided excellent facilities for the workshop, and the Captain and crew of the U/F *Argos* for their hospitality and cooperation. We also wish to thank Dr J Thulin of the Swedish Environmental Protection Board, Oregrund and Fk E Lindesjöö, Uppsala University for their preparatory work in organising the workshop. Further we wish to thank Ann-Christin Rudolphi of the Institute of Marine Research, Lysekil for her invaluable work both with fish hauls and patiently typing minutes and drafts through long evenings.

The members of the workshop are deeply saddened by the death, during the editing of this report, of one of their colleagues from *Argos*, Professor Emmy Egidius. She has played a leading role in the concept of ICES Sea-going workshops on fish disease and will be greatly missed as a friend and also as an astute and innovative scientist. She contributed so much to the spirit of lively debate and cooperation which have been a feature of the Anton Dohrn and *Argos* workshops and her enthusiasm, wise guidance and encouragement during our studies will be irreplaceable.

L Appendix

Appendix 1

Participants in the Workshop on Methodology of Fish Disease Surveys held on U/F *Argos* 16-23 April 1988

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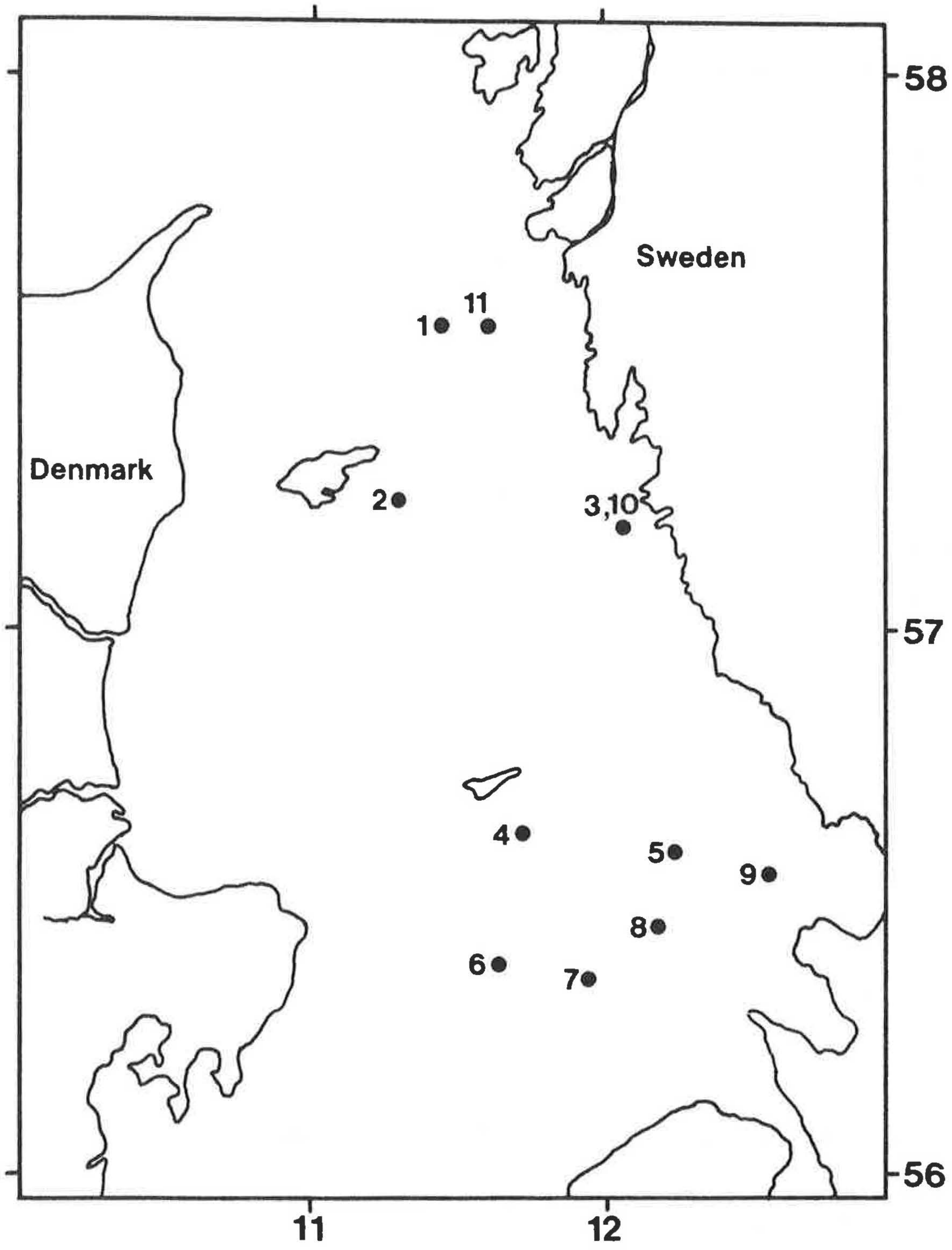
Appendix II Chart of the positions sampled during the workshop cruise.



Appendix III

Practical Work

The practical exercises concentrated on dab and their general purpose was to develop and test a standardised method of sampling, diagnosing and recording internal and external diseases of fish on which consensus could be reached by the international participants. The use of trawl catches of U/F *Argos* can be subdivided according to their objectives:

i) Initial exercises aimed at familiarising all participants with the full range of diseases present in dab in the Kattegat area.

ii) Exercises aimed at identifying the source of and of reducing inter-observer variability in the recording of skin diseases.

iii) Exercises designed to assist in the development of a standardised system for recording macroscopically visible internal abnormalities.

iv) A final exercise to perfect the suggested procedure for examining and recording data for the coordinated study.

Objective I. Familiarisation

Dab and flounder from the first two hauls were examined for internal and external diseases: in general, each observer used the methods with which he or she was familiar.

Results: Table A

There were differences among observers in the conditions that they normally recorded, eg *Cryptocotyle.* It was decided that agreement on the list of conditions recorded was necessary during later exercises. It was found that large nodules and tumours in livers could be recognised quite easily, even by workers who had no prior experience.

Objective II. Variability in external disease diagnoses

a) Variability among separate subsamples of fish examined by different observers

Subsamples of dab from three hauls, covering a similar range of sizes, were examined by several teams.

Results: Tables B, C and D

Initially there appeared to be differences among observers in their ability to detect certain diseases, eg lymphocystis (Table C). However, it was not clear to what extent differences arose from different intensities of examination as opposed to differences in diagnosis of very light signs of the disease. Variability was therefore examined in more detail.

b) Variability in the diagnosis of disease in individual fish by different observers

Ten fish, chosen to have very light signs, or to show conditions which could be open to more than one interpretation, were given to a number of observers for independent diagnosis.

Results: Table E

Despite individual differences in the grading system used for lymphocystis, and the fact that some observers did not include healed net injuries in their diagnoses, agreement on disease diagnosis was generally good. However, for these particular individual fish where the signs were very light, agreement was not universal, even when an indefinite time was available for examining them. It was therefore proposed that data for international comparison should only include signs of lymphocystis above a certain intensity. First, however, it was decided to examine the interaction between the time taken to examine fish and the number of diseased fish that were identified.

c) Variability in the diagnosis of disease due to differing intensity of examination

Two subsamples of dab from a single haul were examined rapidly and diseases noted; these fish were subsequently re-examined in greater detail. The exercise was repeated with the teams doing the rapid and detailed examination reversed, so that possible biases due to individual observers were controlled. In addition, during the latter exercise the fish found to be diseased during the detailed examination were given back to the original observers for re-examination.

Results: Tables F and G

Some fish with very light signs of disease were missed during rapid examination. This occurred irrespectively of the identity of the observers who performed the examination. Some but not all of these fish bore signs so light that no agreement could be reached as to whether or not they should be classified as diseased (Table G). In order to eliminate variability arising from this lack of agreement, and to reduce variability arising from differing intensities of examination, it was decided that for the purposes of international comparisons only dab with at least two surface nodules (together or separate) should be recorded as having lymphocystis. It was recognised that in this way some fish definitely having lymphocystis would be omitted from the data, but it was felt that this objection did not negate the prime necessity of achieving standardisation.

Objective III. Development of a standardised system for recording internal abnormalities

As a result of the initial exercises and ensuing discussions, a system for the recording of liver diseases was devised. This included the recording of liver colour and discolourations as well as spots and nodules of all sizes. The system was later simplified and applied on a number of subsequent occasions, culminating in a second visit to an area where the prevalence of liver nodules in dab appeared to be high.

Results: Tables H and I

The initial recording system for liver abnormalities appeared to be unsuitable for presenting data in a standardised form. For example, 23 different terms had been used to describe liver colour. In addition, there appeared to be great variability among observers in the recording of spots and nodules smaller than 2 mm in diameter, but the variability was less when only those larger than 2 mm were included (Table H). In this and subsequent exercises, the system was revised to include only those nodules larger than 2 mm, and to exclude liver colour and discolourations. The participants appeared to be satisfied with the new system, but numbers of diseased fish were very low. The final exercise in this section (Table I) showed clearly that, even when spots and nodules smaller than 2 mm were excluded, certain sites could still be recognised as having a high prevalence of liver abnormalities.

Objective IV. Final standardised system for recording external and internal disease

Dab from the last haul were examined for external and internal (liver, gut, spleen) abnormalities and the data recorded on the proposed recording forms.

Results: Table J

As a result of this exercise it was agreed that the list of diseases to be recorded, and the method of stratifying fish by length, were both appropriate. Some final modifications were made to the recording forms.

TABLE A

Fish species	*L. limanda*						*P. flesus*	
Haul	1		2				1	
Team:	-		I		II		-	
n fish examined:	45		66		76		44	
Length (cm)	13-28		9-24		20-33		21-42	
Diseased fish	n	%	n	%	n	%	n	%
Ly	3	6.7	5	7.6	10	13.2	11	25.0
Ulc	0	0.0	2	3.0	1	1.3	1	2.3
Pap	1	2.2	0	0.0	0	0.0		
X-Cl	0	0.0	4	6.1	2	2.6		
Pgm	-	-	3	4.5	4	5.3	5	11.4
Net	-	-	-	-	-	-	-	-
Cr	-	-	-	-	22	28.9		
St	-	-	24	36.4	20	26.3		
Ac	-	-	0	0.0	1	1.3		

Ly: Lymphocystis
Ulc: Ulceration (acute, healing, healed)
Pap: Epidermal papilloma/hyperplasia
X-Cl: X-cell gills
Pgm: Pigment anomaly
Net: Net damage (healing, healed)
Cr: Cryptocotyle sp.
St: Stephanostomum sp.
Ac: Acanthochondria sp.
0: Not detected
-: Not examined

TABLE B

Fish species:	*L. limanda*					
Team:	I		II		III	
n fish examined:	57		50		46	
Length (cm)	15-26		12-24		15-26	
Diseased fish	n	%	n	%	n	%
Ly	8	14.1	8	16.0	12	26.1
Ulc	1	1.8	1	2.0	0	0.0
Pap	3	5.3	1	2.0	4	8.7
X-Cl	0	0.0	0	0.0	0	0.0
Pgm	-	-	4	8.0	-	-
Net	4	8.8	4	8.0	4	8.7
Cr	38	66.7	29	58.0	21	45.7
St	32	56.1	30	60.0	26	56.2
Ac	0	0	4	8.0	3	6.5

TABLE C

Fish species:	*L. limanda*					
Team:	I		II		III	
n fish examined:	53		50		113	
Length (cm)	21-29		16-28		13-30	
Diseased fish	n	%	n	%	n	%
Ly	14	26.4	0	0.0	19	16.8
Ulc	9	17.0	1	2.0	0	0.0
Pap	3	5.7	0	0.0	2	1.8
X-Cl	-	-	-	-	-	-
Pgm	1	1.9	0	0.0	4	3.5
Net	-	-	-	-	-	-
Cr	14	26.4	4	10.0	13	11.5
St	23	43.4	15	30.0	38	33.6
Ac	3	5.7	0	0.0	6	5.3

TABLE D

Fish species:	*L. limanda*							
Team: n fish examined: Length (cm)	I 50 16-29		II 76 20-26		III 17 22-27		IV 50 18-32	
Diseased fish	n	%	n	%	n	%	n	%
Ly	1	2.0	2	2.6	1	5.9	2	4.0
Ulc	1	2.0	1	1.3	1	5.9	0	0.0
Pap	11	22.0	10	13.2	3	17.6	8	16.0
X-Cl	-	-	-	-	-	-	-	-
Pgm	-	-	4	5.3	-	-	-	-
Net	-	-	-	-	-	-	-	-
Cr	5	10.0	22	28.9	6	35.3	19	38.0
St	17	34.0	20	26.3	5	29.4	26	52.0
Ac	0	0.0	1	1.3	1	5.9	3	6.0

TABLE E

Fish species:	*L. limanda*					
n of observers	n non-diseased	n Ly 1	n Ly 2	n Ulc	n Pap	n Net
5	0	1	4	4	0	1
8	0	4	4	0	0	0
8	0	4	4	0	0	0
8	1	0	0	0	7	0
7	7	0	0	0	0	0
7	7	0	0	0	0	0
7	3	0	0	0	0	4
6	5	0	0	0	0	1
6	1	5	0	0	0	1
6	5	1	0	0	0	0

TABLE F

Fish species:	*L. limanda*			
Team: n fish examined	I 240		II 82	
Diseased fish	n	%	n	%
Original prevalence after rapid examination:	28	11.7	12	14.6
New prevalence after detailed examination:	41	17.1	21	25.6
Prevalence missed during the original examination:	13	5.4	9	11.0

TABLE G

Fish species:	*L. limanda*	
Team: n fish examined: Length (cm) Time/fish (second) (original examination):	I 100 13-30 26	II 100 11-32 39
Diseased fish	%	%
Original prevalence after rapid examination: Ly Ulc acute Pap	 12.0 2.0 0.0	 15.0 1.0 6.0
Total prevalence:	14.0	22.0
Total prevalence after detailed examination: (1) agreed by original examiners: (2) not agreed by original examiners:	 20.0 23.0	 25.0 28.0
Number of fish missed during original examination:	6-9	5-8

TABLE H

Fish species:			*L. limanda*			
Team:		I	II	III	IV	V
Diseased fish		n	n	n	n	n
(1) L nod <2 mm						
Length:	20-24 cm (n=10)	2	0	2	2	4
	25-30 cm (n=10)	2	7	0	6	4
(2) L nod >2 mm						
Length:	20-24 cm (n=10)	1	1	0	0	0
	25-30 cm (n-10)	1	1	3	1	0

TABLE I

Fish species:	*L. limanda*						
Team:	I		II		III		I + II + III
Length (cm)	n exam	n L nod	n exam	n L nod	n exam	n L nod	% overall
20-25	47	2	30	2	2	0	5.1
>25	3	0	1	0	21	7	28.0

n exam: number of fish examined
n L nod: number of fish with liver nodules >2 mm

TABLE J

Fish species:	*L. limanda*			
Team: Length (cm):	I 15-29		II 15-29	
Diseased fish	n	%	n	%
(1) External				
n exam	79	(100.0)	56	(100.0)
Ly	8	10.1	11	19.6
Pap	1	1.3	0	0.0
(2) Internal				
n exam	13	(100.0)	23	(100.0)
L nod	3	23.1	1	4.3

Appendix IVa. Dab disease recording form.

COUNTRY: FISH SPECIES: DAB *(LIMANDA LIMANDA)*
STATION CODE OBSERVER: INSPECTION TIME/FISH (MIN.)
(LONG./LAT.): DATE: QUALITY OF FISH:
HAUL NO: (DAY/MONTH/YEAR) DAMAGED/UNDAMAGED

LGTH	SEX	LY	PAP	ULC	X-CL	LIVN	REMARKS	LGTH	SEX	LY	PAP	ULC	X-CL	LIVN	REMARKS

LGTH=TOTAL LENGTH IN CM BELOW
SEX 1=MALE
2=FEMALE
LY= LYMPHOCYSTIS > 1 surface nodule
PAP= EPIDERMAL HYPERPLASIA/ PAPILLOMA
ULC= SKIN ULCERS
X-CL= X-CELL GILL LESION
LIVN= LIVER NODULE/TUMOUR > 2 mm (No; Diame

Appendix IVb. Flounder disease recording form.

COUNTRY: FISH SPECIES: FLOUNDER *(PLATICHTHYS FLESUS)*
STATION CODE (LONG./LAT.): OBSERVER: INSPECTION TIME/FISH (MIN.)
DATE: (DAY/MONTH/YEAR) QUALITY OF FISH: DAMAGED/UNDAMAGED
HAUL NO:

LGTH	SEX	LY	ULC			LIVN	REMARKS	LGTH	SEX	LY	ULC			LIVN	REMARKS

LGTH=TOTAL LENGTH IN CM BELOW
SEX 1=MALE
2=FEMALE
LY=LYMPHOCYSTIS
ULC= SKIN ULCERS
LIVN= LIVER NODULE/TUMOUR > 2 mm (No; Diameter)

Appendix IVc. Cod disease recording form.

COUNTRY:

STATION CODE (LONG./LAT.):

HAUL NO:

FISH SPECIES: COD *(GADUS MORHUA)*

OBSERVER:

DATE: (DAY/MONTH/YEAR)

INSPECTION TIME/FISH (MIN.)

QUALITY OF FISH: DAMAGED/UNDAMAGED

LGTH	SEX	ULC	SKD	PBT	CR		REMARKS	LGTH	SEX	ULC	SKD	PBT	CR		REMARKS

LGTH= TOTAL LENGTH IN CM BELOW

SEX 1=MALE

2=FEMALE

ULC= SKIN ULCERS

PBT= PSEUDOBRANCHIAL TUMOURS

SKD=SKELETAL DEFORMITIES

CR=CRYPTOCOTYLE

Appendix Va. Dab disease reporting form

ICES REPORTING FORMAT

COUNTRY:
STATION CODE:
LONG/LAT:
ICES SQUARE NO:
NO OF HAULS:

OBSERVER:
INSPECTION TIME/FISH (MIN):
DATE (DAY/MONTH/YEAR):

FISH SPECIES: **DAB** *(LIMANDA LIMANDA)*

SIZE GROUP: 15 - 19 CM

MEAN LENGTH OF SIZE GROUP ± SD

	TOT. NO EXAMINED		TOT. NO AFFECTED		PREVAL (%)
	MALE	FEMALE	MALE	FEMALE	
LYMPHOCYSTIS					
EPIDERMAL PAPILLOMA					
SKIN ULCER					
X-CELL GILL LESION					

SIZE GROUP: 20 - 24 CM

MEAN LENGTH OF SIZE GROUP ± SD

	TOT. NO EXAMINED		TOT. NO AFFECTED		PREVAL (%)
	MALE	FEMALE	MALE	FEMALE	
LYMPHOCYSTIS					
EPIDERMAL PAPILLOMA					
SKIN ULCER					
X-CELL GILL LESION					

SIZE GROUP: ≥25 + CM

MEAN LENGTH OF SIZE GROUP ± SD

	TOT. NO EXAMINED		TOT. NO AFFECTED		PREVAL (%)
	MALE	FEMALE	MALE	FEMALE	
LYMPHOCYSTIS					
EPIDERMAL PAPILLOMA					
SKIN ULCER					
X-CELL GILL LESION					
LIVER NODULE/TUMOUR					

Appendix Vb. Flounder disease reporting form

ICES REPORTING FORMAT

COUNTRY:
STATION CODE:
LONG/LAT:
ICES SQUARE NO:
NO OF HAULS:

OBSERVER:
INSPECTION TIME/FISH (MIN):
DATE (DAY/MONTH/YEAR):

FISH SPECIES: **FLOUNDER** *(PLATICHTHYS FLESUS)*

SIZE GROUP: 20 - 24 CM

MEAN LENGTH OF SIZE GROUP ± SD

	TOT. NO EXAMINED		TOT. NO AFFECTED		PREVAL.
	MALE	FEMALE	MALE	FEMALE	(%)
LYMPHOCYSTIS					
SKIN ULCER					

SIZE GROUP: 25 - 29 CM

MEAN LENGTH OF SIZE GROUP ± SD

	TOT. NO EXAMINED		TOT. NO AFFECTED		PREVAL.
	MALE	FEMALE	MALE	FEMALE	(%)
LYMPHOCYSTIS					
SKIN ULCER					

SIZE GROUP: ≥30 + CM

MEAN LENGTH OF SIZE GROUP ± SD

	TOT. NO EXAMINED		TOT. NO AFFECTED		PREVAL.
	MALE	FEMALE	MALE	FEMALE	(%)
LYMPHOCYSTIS					
SKIN ULCER					
LIVER NODULE/TUMOUR					

Appendix Vc. Cod disease reporting form

ICES REPORTING FORMAT

COUNTRY:
STATION CODE:
LONG/LAT:
ICES SQUARE NO:
NO OF HAULS:

OBSERVER:
INSPECTION TIME/FISH (MIN):
DATE (DAY/MONTH/YEAR):

FISH SPECIES: **COD** *(GADUS MORHUA)*

SIZE GROUP: -29 CM

MEAN LENGTH OF SIZE GROUP ± SD

	TOT. NO EXAMINED		TOT. NO AFFECTED		PREVAL.
	MALE	FEMALE	MALE	FEMALE	(%)
SKIN ULCER					
PSEUDOBR. TUMOUR					
SKELETAL DEFORMITY					
CRYPTOCOTYLE					

SIZE GROUP: 30 - 44 CM

MEAN LENGTH OF SIZE GROUP ± SD

	TOT. NO EXAMINED		TOT. NO AFFECTED		PREVAL.
	MALE	FEMALE	MALE	FEMALE	(%)
SKIN ULCER					
PSEUDOBR. TUMOUR					
SKELETAL DEFORMITY					
CRYPTOCOTYLE					

SIZE GROUP: ≥45 + CM

MEAN LENGTH OF SIZE GROUP ± SD

	TOT. NO EXAMINED		TOT. NO AFFECTED		PREVAL.
	MALE	FEMALE	MALE	FEMALE	(%)
SKIN ULCER					
PSEUDOBR. TUMOUR					
SKELETAL DEFORMITY					
CRYPTOCOTYLE					

Indication of spine colours

Reports of the Advisory Committee on Fishery Management	Red
Reports of the Advisory Committee on Marine Pollution	Yellow
Fish Assessment Reports	Grey
Pollution Studies	Green
Others	Black

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