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REPORT OF THE ICES ADVISORY COMMITTEE ON THE MARINE ENVIRONMENT 1997

Copenhagen, Denmark
9–14 June 1997

International Council for the Exploration of the Sea
Conseil International pour l'Exploration de la Mer

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Dr R.C.A. Bannister[2]		Chairman, Consultative Committee
Mr M. Nicholson[1]		Chairman, WGSAEM

[1]Invited participant. [2]Participated part-time.

All countries were represented by *Members* except Denmark, Estonia, Portugal, Sweden, the UK (represented by *Alternate Members*), and France (represented by Dr M. Chaussepied, ICES *Delegate* for France).

*The Mariculture Committee Chairman was unable to attend the meeting.

Secretariat members participating in the meeting or portions thereof:

Ms M. Karlson	ICES Departmental Secretary
Mr J.R. Larsen	ICES Environment Data Scientist
Ms M. Sørensen	ICES Environment Assistant

EXECUTIVE SUMMARY

The ICES Advisory Committee on the Marine Environment (ACME) met from 9–14 June 1997 at ICES Headquarters in Copenhagen. As part of its work during this period, the ACME prepared responses to the requests made to ICES by the Oslo and Paris Commissions and the Helsinki Commission. This report contains these responses. In addition to responses to direct requests, some sections of this report summarize the deliberations of ACME on topics for which advice was not directly requested but for which the ACME felt that there was information that would be of potential interest to the Commissions, ICES Member Countries, and other readers of this report.

Information in direct response to requests from, or which is relevant to, the work of both the Oslo and Paris Commissions and the Helsinki Commission

Monitoring

In 1997, the ACME continued work on the development of biological effects monitoring programmes. In particular, the ACME reviewed and revised the lists of recommended and promising biological effects monitoring techniques, which had been developed in 1995 as part of an integrated marine environmental monitoring strategy (Section 4.3.2). In addition, based on the results of the ICES Special Meeting on the Use of Liver Pathology in Flatfish for Monitoring Biological Effects of Contaminants, guidelines for the use of fish pathology in biological effects monitoring programmes have been developed (Section 4.3.3). Finally, some information has been given relevant to the statistical design of biological effects monitoring programmes (Section 4.3.1).

In response to the OSPAR request for ICES to prepare monitoring guidelines for PAHs in sediments and biota, the ACME has accepted detailed guidelines for monitoring PAHs in sediments (Annex 1). Information and recommendations on the choice of organisms for monitoring concentrations of PAHs and their trends, as well as for PAH-related chemical and biological monitoring have also been provided (Section 4.4.2). The remainder of these guidelines will be completed in 1998.

This report also contains information on statistical considerations relative to monitoring programmes. In particular, consideration is given to the development of harmonized statistical methods for the analysis of temporal trends in atmospheric and riverine inputs of nutrients and contaminants (Section 4.7.1). This is based on the outcome of the ICES/OSPAR Workshop on the Identification of Statistical Methods for Trend Detection. In Section 4.7.2, the ACME reports on a review of the U.S. Mussel Watch Program, primarily in terms of the power of the programme to detect trends in contaminant concentrations on a regional and national basis. Finally, preliminary information is given on new approaches to assessing temporal trends in contaminant concentrations (Section 15.2).

In the context of identifying which contaminants can be monitored on a routine basis with adequate interlaboratory comparability, Section 4.2 lists trace elements and their compounds that can be monitored in biota, sediments and sea water, and also provides information on the availability of relevant certified reference materials.

Annex 2 reviews the use of organic carbon determinations in chemical oceanography and estuarine studies; it concludes that, in general, there is no need to include measurements of organic carbon in monitoring programmes.

Finally, consideration is given to the recent development of monitoring guidelines, particularly for the OSPAR Joint Assessment and Monitoring Programme (JAMP), and it is noted that requirements to ensure a full integration of biological and chemical measurements need to be addressed in greater detail.

Quality Assurance and Intercomparison Exercises

The ACME reviewed the results of quality assurance-related activities conducted during the past year and has provided summaries of this work in Section 5. Attention has particularly been given to quality assurance criteria in relation to measurements of mesozooplankton and primary production in the Baltic Sea (Section 5.1.1), and new recommended procedures are summarized. For the OSPAR area, a strategy for the implementation of quality assurance programmes for biological measurements in relation to eutrophication effects is outlined in Section 5.2. In addition, an infrastructure for a quality assurance programme for biological effects techniques is proposed in Section 5.3.

In terms of chemical measurements, Section 5.4 provides a brief summary of 'Guidelines on quality assurance of chemical measurements in the Baltic Sea', that have been prepared for the monitoring programmes carried out under the Helsinki Commission. In subsequent sections, information is provided on various intercomparison activities involving chemical measurements, with details of quality assurance procedures in relation to measurements of dissolved oxygen in sea water provided in Annex 3.

Overviews of Contaminants in the Marine Environment

The ACME has considered information on the occurrence of the antifouling agent Irgarol 1051 in the marine environment (Section 8.1); a detailed review is contained in Annex 4. A discussion paper on toxaphene in the marine environment, particularly describing relevant analytical techniques to determine total toxaphene and individual congeners, was also reviewed; it is contained in Annex 5. Information on the origin, analysis, and distribution of polychlorinated diphenylethers (PCDEs) was also considered and an overview on this group of contaminants is attached as Annex 6.

Report sections responding to requests specific to the Oslo and Paris Commissions

Environmental Interactions of Mariculture

In response to a request, the ACME has provided some information on the escape of fish from mariculture operations in relation to disease transfer to wild stocks and genetic interactions between escaped farmed fish and wild populations. This information is contained in Section 14.1. Unfortunately, there is a lack of comprehensive quantitative data on the escape of fish from mariculture operations, so a full assessment of its consequences is not yet possible.

Some information is also provided on chemicals used in mariculture for the control of external and internal parasites, in relation to the potential environmental effects of these chemicals (Section 14.2).

Data Handling

The annual review of data handling activities relevant to OSPAR requirements by the ICES Environmental Data Bank is contained in Section 17.1 of this report; this includes an overview of the amounts of data on contaminants that have been submitted each year since 1977. Section 17.2 summarizes the work of the ICES Oceanographic Data Centre in handling nutrients data relevant to the OSPAR programmes.

Report sections responding to requests specific to the Helsinki Commission

Baseline Study of Contaminants in Baltic Sea Sediments

A review of a draft of the report, 'Contaminants in Baltic Sea Sediments', is provided in Section 6.1. However, as this report was not yet complete, the ACME decided that the preparation of recommendations for monitoring sediments in the Baltic Sea should be postponed until the report on the Baseline Study has been completed and reviewed by relevant Working Groups. The final report on the Baseline Study will be available in 1998.

The M-74 Syndrome in Baltic Salmon

A brief report on the progress in understanding the role of environmental factors in the aetiology of the M-74 syndrome that affects the Baltic stock of Atlantic salmon is contained in Section 6.2. There is a need for studies to investigate the occurrence of the M-74 syndrome and similar conditions in wild Atlantic salmon stocks and other fish species in areas other than the Baltic Sea.

Effects of Extraction of Marine Sand and Gravel on the Baltic Ecosystem

In Section 6.3, the ACME has provided information available on the quantities of marine sand and gravel extracted from the Baltic Sea and evaluations of the effects of these extractions in certain areas of the Baltic Sea, primarily in the Polish marine area. General information on potential effects, based mainly on studies in marine areas outside the Baltic Sea, has been provided in terms of impacts on benthos, macrophytes, fish and fisheries, seabirds, and marine mammals. The importance of following the ICES Guidelines for Environmental Impact Assessment and the ICES Code of Practice on Commercial Extraction of Marine Sediments is stressed.

Transfer of Halogenated Organic Compounds through the Pelagic Food Chain

In response to the HELCOM request on this topic, the ACME has provided information on chemical and biological factors governing the transfer of organic compounds through food chains and their bioaccumulation in marine organisms (Section 8.2). A detailed treatment of this topic is presented in Annex 7.

Ecosystem Effects of Fishing Activities

Available information on the impacts of different fishing practices in the Baltic Sea on target and non-target (including benthos, seabirds, and marine mammals) species is provided in Section 12. As much more work on this issue has been conducted in the North Sea, extensive use has been made of the results of studies in that area.

Marine Mammals

A status report on the populations of the three seal species in the Baltic Sea and the population of harbour porpoises in the Baltic Sea is contained in Section 11.1 of this report. Available data on the by-catch of marine mammals in fisheries in the Baltic Sea are provided in Section 11.2; further data are required before the implications of these by-catches on marine mammal populations can be fully assessed. In Section 11.3, some information is provided on seal and porpoise behaviour in relation to fishing gear, and on methods to protect gear and reduce the by-catch of marine mammals. Information on contaminant concentrations in marine mammals and biological effects of contaminants is presented in Section 11.4.

Information on topics of general interest

Fish Diseases

The ACME has reviewed the progress in the analysis of the fish disease prevalence data that have been submitted by ICES Member Countries (Section 7.1). The development of methods for the analysis of spatial distribution and temporal trends in estimated disease prevalence is now complete and future analyses will be able to be conducted on a regular basis. The ACME also reviewed the results of a sea-going workshop to standardize and intercalibrate methodologies for fish disease studies in the Baltic Sea (Section 7.2).

Effects of Contaminants on Marine Organisms

In view of the concern that certain contaminants may have an endocrine-disrupting effect on marine organisms, the ACME reviewed and accepted a list of biomarkers and bioassays that are currently used to investigate reproductive or endocrine-disrupting effects of contaminants on aquatic organisms (Section 8.4).

Seabird Ecology Issues

ICES has held several meetings and an International Symposium concerned with seabird ecology and seabird/fish interactions in recent years. In Section 10, the ACME provides an overview of the outcome of this work and indicates priorities in future work.

Introductions and Transfers of Marine Organisms

The ACME reviewed the status of on-going introductions of species for mariculture purposes, in particular concerning culture of the alga *Porphyra yezoensis* in the State of Maine, the alga *Undaria pinnatifida* in France, and the Red King crab *Paralithodes camtschatika* in the Barents Sea (Section 13.1).

Issues relevant to the transfer of pathogens and potentially harmful aquatic organisms via ships' ballast water and sediments are reviewed in Section 13.2. While work is being conducted on both risk assessment and management and control, further research is required to understand the types of species that can be transferred in ships' ballast. In Section 13.3, consideration is given to potential means to control alien species that have invaded a marine area and are causing problems. Several examples of such invasions are noted.

Global Ocean Observing System

The ACME considered the potential contribution of ICES to the Global Ocean Observing System (GOOS) and has made some recommendations on this in Section 16.

Sources of Information Considered by the ACME at its 1997 Meeting

At its 1997 meeting, the ACME considered, *inter alia*, information included in the most recent reports of the following ICES groups:

BEWG	Benthos Ecology Working Group
MCWG*	Marine Chemistry Working Group
SGBWS*	ICES/IOC/IMO Study Group on Ballast Water and Sediments
SGFDDS	Study Group on the Statistical Analysis of Fish Disease Data in Marine Fish Stocks
SGMBIS*	Study Group on Marine Biocontrol of Invasive Species
SGQAB	ICES/HELCOM Steering Group on Quality Assurance of Biological Measurements in the Baltic Sea
SGQAC	ICES/HELCOM Steering Group on Quality Assurance of Chemical Measurements in the Baltic Sea
SGQAE*	ICES/OSPAR Steering Group on Quality Assurance of Biological Measurements related to Eutrophication Effects
WGAGFM	Working Group on the Application of Genetics in Fisheries and Mariculture
WGBAST	Baltic Salmon and Trout Assessment Working Group
WGBEC*	Working Group on Biological Effects of Contaminants
WGBFAS	Baltic Fisheries Assessment Working Group
WGEAMS*	Working Group on Environmental Assessment and Monitoring Strategies
WGEIM	Working Group on Environmental Interactions of Mariculture (1996 report)
WGHABD	ICES/IOC Working Group on Harmful Algal Bloom Dynamics
WGITMO*	Working Group on Introductions and Transfers of Marine Organisms
WGMS*	Working Group on Marine Sediments in Relation to Pollution
WGPDMO	Working Group on Pathology and Diseases of Marine Organisms
WGSAEM*	Working Group on Statistical Aspects of Environmental Monitoring
WGSE	Working Group on Seabird Ecology
WGSEAL	Working Group on Seals and Small Cetaceans in European Seas

Reports of the following other activities were also considered:

ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants (1996)

ICES/HELCOM Workshop on Quality Assurance of Pelagic Biological Measurements in the Baltic Sea (1996)

ICES/OSPAR Workshop on the Identification of Statistical Methods for Trend Detection (1997)

*These groups report directly to ACME.

1 INTRODUCTION

The Advisory Committee on the Marine Environment (ACME) is the Council's official body for the provision of scientific advice and information on the marine environment, including marine pollution, as may be requested by ICES Member Countries, other bodies within ICES, and relevant regulatory Commissions. In handling these requests, the ACME draws on the expertise of its own members and on the work of various expert ICES Working Groups and Study Groups. The ACME considers the reports of these groups and requests them to carry out specific activities or to provide information on specific topics.

The ACME report is structured in terms of the topics covered at the ACME meeting on which it has prepared scientific information and advice; the topics include both those for which information has been requested by the Commissions or other bodies and those identified by the ACME to enhance the understanding of the marine environment. Information relevant to the Commissions' requests and specific issues highlighted by the ACME for their attention are summarized in Sections 2 and 3, where the individual work items from each Commission are listed and related to relevant sections of the main text.

2 PROGRESS ON TASKS FOR THE OSLO AND PARIS COMMISSIONS, INCLUDING DATA HANDLING

A summary of the progress on the 1997 programme of work requested by the Oslo and Paris Commissions is given below, along with reference to the relevant sections and annexes of this report where more detailed information can be found. This summary is provided according to the format of the Work Programme, with the questions on the Work Programme shown in italics and a summary of the ACME advice below in normal print.

SCIENTIFIC ADVICE

1 Monitoring Activities

1.1 to develop monitoring guidelines for polyaromatic hydrocarbons (PAHs), including attention to:

a) detection limits in sediments and biota which are in line with relevant assessment criteria;

b) the number of replicate samples required per sampling area in order to characterize the sampling area, for both sediments and biota; taking into account general guidelines if established;

c) normalization techniques (e.g., grain size, total organic carbon).

Section 4.4.1 of this report describes the status of the work on the development of guidelines for monitoring PAHs in biota and sediments. Detailed guidelines have been prepared for monitoring PAHs in marine sediments; they are contained in Annex 1 to this report. These guidelines do not, however, contain information on normalization techniques as the appropriate normalization techniques for data on PAHs in sediments have not yet been agreed by the experts (see Section 4.5.2). Section 4.4.2 contains information and recommendations on the choice of organisms for monitoring concentrations of PAHs and their trends, as well as for PAH-related integrated chemical and biological monitoring. Work will continue with the aim of providing the remaining material in 1998. Some of this further work is described in Section 4.5.1.

2 QUALITY ASSURANCE

2.1 To establish a joint ICES/OSPAR Steering Group on Quality Assurance of Biological Measurements related to eutrophication parameters (chlorophyll-a, phytoplankton, macrozoobenthos and macrophytobenthos) in order to coordinate:

a) the development of quality assurance procedures;

b) the implementation of quality assurance activities, e.g., the conduct of workshops and intercomparison exercises;

c) the preparation of appropriate taxonomic lists of species.

At the 1996 Annual Science Conference, ICES established the ICES/OSPAR Steering Group on Quality Assurance of Biological Measurements related to Eutrophication Effects. This Steering Group held its first meeting in February 1997 and a brief summary of the outcome is reported in Section 5.2. The initial work of this Steering Group was hampered by poor attendance at this first meeting. It is hoped that representatives of all relevant countries will participate in the further work of this Steering Group.

2.2 To coordinate relevant QA activities in accordance with the biological effects monitoring component of the Joint Assessment and Monitoring Programme. This will cover such measurements as DNA-adducts, metallothionein, ALA-D activity, lysosomal stability, lipid peroxidation, liver pathology, and fish disease.

In Section 6.2 and Annexes 5 and 6 of the 1996 ACME report, the ACME provided details of quality assurance (QA) procedures relevant to the above-mentioned techniques. In order to conduct intercomparison exercises and other QA activities among the laboratories that will carry out these biological effects measurements, it is necessary to establish an infrastructure to coordinate this work. Funding is also required for the preparation of materials and the conduct of the exercises. Section 5.3.1 of this report outlines an infrastructure for the coordination of QA activities for biological effects monitoring techniques.

In addition, Section 5.3.2 provides further information on biological effects techniques for the measurement of flatfish liver pathology for monitoring purposes, and associated quality assurance procedures for these measurements. This is based on the outcome of the ICES Special Meeting on the Use of Liver Pathology in Flatfish for Monitoring Biological Effects of Contaminants, which provided further development and standardization of methods for the measurement of fish liver histopathology and identification of QA requirements.

3 METHODOLOGIES

3.1 To organize in cooperation with the OSPAR Secretariat a meeting of a Joint ICES/OSPAR Workshop on the Identification of Statistical Methods for Trend Detection. The workshop should:

a) refine the criteria for harmonized statistical methods for trends analyses as at Appendix 1 in order to make these criteria applicable for trend assessments of riverine and atmospheric input data;

b) develop harmonized procedure(s) for the detection of trends in existing OSPAR datasets for riverine and atmospheric inputs to the marine environment;

c) consider whether such a procedure could also be developed for the detection of trends in existing OSPAR datasets for other types of input, e.g., direct inputs, inputs resulting from offshore activities and dumping of dredged materials;

d) apply these procedure(s) to a representative selection of available datasets;

e) assess on the basis of results obtained under point (d) the power to detect trends in inputs and recommend, if appropriate, changes in the existing monitoring programmes so as to meet the aims of OSPAR, taking into account financial and logistical constraints.

ICES organized the ICES/OSPAR Workshop on the Identification of Statistical Methods for Trend Detection in Copenhagen on 25–26 February 1997, in association with the annual meeting of the ICES Working Group on Statistical Aspects of Environmental Monitoring (WGSAEM). The attendance at the Workshop was good and the discussion covered a broad range of the topics required. However, the ACME noted that there is a need for further clarification and specification of requirements for assessments of inputs data. The choice of statistical method would also be clearer if an appropriate statistical objective were determined. The full report of the Workshop has been provided to OSPAR as document ICES CM 1997/Env:11. The ACME consideration, based on this report and a review of the outcome of the Workshop by WGSAEM, is contained in Section 4.7.1, below.

4 OTHER TOPICS

4.1 to provide information on the escape of fish from mariculture operations and to advise IMPACT 1997 on the means by which this impact could be controlled.

Section 14.1 of this report provides information on the escape of fish, primarily salmon, from mariculture operations and potential impacts on wild populations of fish. Although quantitative data on fish escapes is lacking, studies of the fish caught in areas near mariculture facilities show that in recent years a significant proportion of the catch has been farmed fish. This has also been shown to be the case for one offshore area. Owing to the lack of comprehensive quantitative data on fish escapes, the ACME considered that an overall assessment of the consequences of escapees on wild stocks is not yet possible. However, given the possible adverse effects on wild stocks, the ACME emphasized the need to take precautionary measures to minimize escapes from mariculture operations.

5 FISHERIES

5.1 to provide information on the impact of fishing activity on the growth and spatial distribution of the target fish population for commercially exploited stocks of fish and shellfish in the five OSPAR regions that are subject to regular assessment.

In October 1996, IMPACT decided that the information required in this request should be provided for five species: cod, herring, sole, mackerel, and hake. The relevant ICES fisheries assessment working groups will assemble this information for review by the ICES Advisory Committee on Fishery Management at its meeting in May 1998 and subsequent transmission to OSPAR.

5.2 to provide information on incidental mortality of marine mammals owing to fishing activities on a species (and gear type) basis for each of the five OSPAR regions.

Some initial work has been done on this topic by the Working Group on Seals and Small Cetaceans in European Seas (WGSEAL). The final compilation of information will be carried out at the WGSEAL meeting in spring 1998.

5.3 to provide information on quantities of discards by gear type for commercially exploited stocks of fish and shellfish in the maritime area subject to regular assessment.

The following ICES fisheries assessment working groups have been asked to compile data on quantities of discards by gear type for the stocks of finfish or shellfish under their remit: Arctic Fisheries Working Group, Northern Pelagic and Blue Whiting Fisheries Working Group, Herring Assessment Working Group for the Area South of 62°N, Northwestern Working Group, Working Group on the Assessment of Demersal Stocks in the North Sea and Skagerrak, Working Group on the Assessment of Mackerel, Horse Mackerel, Sardine and Anchovy,

Working Group on the Assessment of Northern Shelf Demersal Stocks, Working Group on the Assessment of Southern Shelf Demersal Stocks, Working Group on North Atlantic Salmon, *Pandalus* Working Group, Working Group on *Nephrops* Stocks, Study Group on the Biology and Assessment of Deep-Sea Fisheries Resources, and Study Group on the Assessment of Other Fish and Shellfish Species. The information from all these Working Groups will be reviewed and compiled on the basis of OSPAR regions in autumn 1997 by the Working Group on Ecosystem Effects of Fishing Activities, after which there will be review by the Advisory Committees.

5.4 to provide information on changes in abundance of individual species of non-target fish in the maritime area owing to fishing activities.

The Working Group on Ecosystem Effects of Fishing Activities will compile information on this issue for review by the Advisory Committee on Fishery Management in May 1998.

DATA HANDLING IN 1997

1 To carry out data handling activities relating to:

1.1 contaminant concentrations in biota and sediments;

1.2 contaminant concentrations in sea water;

1.3 measurements of biological effects;

1.4 the Nutrient Monitoring Programme.

2 To establish a databank for phytobenthos, zoobenthos and phytoplankton species.

The ICES Secretariat Environmental Data Centre has handled all data submitted in 1996, covering monitoring activities in 1995. As in the previous year, however, the volume of data was small, possibly owing to a lack of assessment activities.

The ICES Oceanographic Data Centre continues to maintain as complete as possible a data set on nutrients and other oceanographic parameters in the ICES area. Various products have been prepared for the OSPAR Ad Hoc Working Group on Eutrophication (EUT) which describe distributions of nutrients in eutrophied waters.

Further information on data handling activities is contained in Section 17 of this report.

3 PROGRESS ON TASKS FOR THE HELSINKI COMMISSION

The present status of work on 1997 requests by the Baltic Marine Environment Protection Commission (Helsinki Commission) is given below, along with reference to the relevant sections and annexes of this report where more detailed information can be found. The requests are shown in italics and a summary of the ACME advice is then given in normal print.

CONTINUING RESPONSIBILITIES

1. *To evaluate every third year the populations of seals and harbour porpoise in the Baltic Sea, including the size of the populations, distribution, migration, reproductive capacity, effects of contaminants and health status, and additional mortality owing to interactions with commercial fisheries (by-catch, intentional killing).*

Section 11.1 of this report contains information on the status of populations of seals and harbour porpoises in the Baltic Sea. The ACME noted with concern that there is no estimate available of the total population of harbour porpoises in the Baltic Sea; it appears, however, that the Baltic porpoises are a separate population from those in the Kattegat/Skagerrak and the North Sea. For grey seals and ringed seals, populations in the Gulf of Bothnia are constant or increasing, but their development in the southern parts of the Baltic Sea is weak.

Some information on by-catches of marine mammals in Baltic fisheries is provided in Section 11.2; however, this information is insufficient to evaluate the impact of by-catches on seal populations in the Baltic Sea. Section 11.3 provides some information on seal behaviour in relation to fishing gear and on methods to protect gear and reduce by-catches of seals. Contaminant levels and biological effects in marine mammals are covered in Section 11.4.

2. *To coordinate quality assurance activities on biological and chemical measurements in the Baltic Sea and report routinely on planned and ongoing ICES intercomparison exercises, and to provide a full report on the results.*

Information on progress in the overall development of quality assurance (QA) procedures for biological measurements is summarized in Section 5.1, while specific information on the outcome of the ICES/HELCOM Workshop on Quality Assurance of Pelagic Biological Measurements in the Baltic Sea is contained in Section 5.1.1. This Workshop examined methodology related to zooplankton monitoring, including comparison of sampling and sub-sampling techniques, and developed recommendations concerning counting procedures and analysis. Primary production measurements were considered, and demonstrations were given of the various steps in the ^{14}C incubator technique.

With regard to quality assurance of chemical measurements, the ACME reviewed and approved final general guidelines for quality assurance procedures for chemical measurements under the HELCOM monitoring programmes, as well as a series of annexes covering specific technical aspects. Additional annexes are under development and will be reviewed as they become available. A summary of this work is contained in Section 5.4.

SPECIAL STUDIES

3. *To coordinate the Baseline Study of Contaminants in Sediments 1993 and the compilation and review of results.*

Section 6.1 reviews the progress in the preparation of an overall report on the results of this Baseline Study. The ACME reviewed a draft of this report, but noted that the information contained in this draft report is not complete enough to support the development of recommendations for future monitoring of sediments in the Baltic Sea. It is anticipated that this report will be completed by the end of 1997 and recommendations prepared during early 1998.

4. *To evaluate the impact of different fishing practices (e.g., drift nets, gill nets, bottom trawling) in the Baltic Sea on target and non-target species, including, in addition to fish, invertebrates, marine mammals, and birds.*

Few studies have been conducted in the Baltic Sea concerning the impacts of different fishing practices on target and non-target species, so extensive use has been made of the work on this topic in the North Sea. The available information is provided in Section 12 of this report. ICES ACME encourages Baltic Sea countries to conduct relevant studies using the experience gained from the North Sea.

5. *To compile available evidence on causes of the M-74 syndrome in Baltic salmon and to provide a summary of the progress in understanding the relevant environmental factors influencing the occurrence of M-74, along with an account of the geographical extent in the distribution of this syndrome.*

No significant progress has been made during the past year in understanding the role of environmental factors in the aetiology of the M-74 syndrome in Baltic salmon. A progress report is provided in Section 6.2 of this report. It has been confirmed that the M-74 syndrome also occurs in Baltic sea trout populations in Swedish and Finnish waters, although with less severe consequences. Further research is needed, including research on the

potential occurrence of conditions similar to M-74 in Atlantic salmon and other species outside the Baltic Sea area.

6. *To provide information concerning the transfer of halogenated organic compounds (DDT family, HCB, CBs and dioxins) through the pelagic food chains.*

Section 8.2 contains a summary of the factors governing the transfer of halogenated organic compounds in food chains. In addition, Annex 7 provides a comprehensive introduction to the subject of biomagnification of halogenated organic contaminants in food chains.

7. *To provide information on seal behaviour in relation to fishing gear, means of protecting fishing gear from seals and/or repelling seals from such gear, and new fishing gear and/or fishing techniques to reduce by-catch of marine mammals.*

Information covering these topics is provided in Section 11.3 of this report. Further information will become available at the 1997 ICES Annual Science Conference, where a Theme Session on By-Catch of Marine Mammals: Gear Technology, Behaviour, and Kill Rates will be held.

8. *To provide information on the effects of extraction of marine sand and gravel on the Baltic ecosystem, including the extent and volume of such extractions, and known impacts on, e.g., benthos, diving seabirds, and bottom-spawning fish and invertebrates.*

Section 6.3 of this report provides available information on the effects of extraction of marine sand and gravel in the Baltic Sea. ICES ACME encourages compliance with the ICES Code of Practice for the Commercial Extraction of Marine Sediments by countries extracting sand and gravel in the Baltic Sea to avoid deleterious impacts associated with these activities.

9. *To provide advice on sampling strategies for trend monitoring of zooplankton and phytoplankton variables for the assessment of eutrophication and its effects.*

As this request was not formally transmitted to ICES until after the HELCOM Environment Committee meeting in October 1996, it was too late to have it included in the formal terms of reference of the relevant Working Groups meeting in 1997.

4 MONITORING GUIDELINES AND TECHNIQUES

4.1 Consideration of Current Guidelines on Chemical Monitoring of Fish and Shellfish in Relation to ICES Advice on Monitoring Strategies

Request

There is no specific request, but there is an on-going interest on the part of ICES Member Countries as well as OSPAR and HELCOM for advice concerning monitoring strategies, techniques, and guidelines.

Source of the information presented

The 1997 report of the Working Group on Environmental Assessment and Monitoring Strategies (WGEAMS) and ACME deliberations.

Status/background information

The report of the 1996 meeting of the OSPAR Ad Hoc Working Group on Monitoring (MON 1996) was reviewed in relation to such factors as structure and strategy, but technical matters were not addressed in detail. The main purpose of MON 1996 had been to draft monitoring guidelines and technical annexes to be used under the new OSPAR Joint Assessment and Monitoring Programme (JAMP) (and be available for adoption or advice elsewhere as other potential users felt appropriate). The documentation on chemical monitoring of fish and shellfish was structured to include a Guidelines section, which was designed to be of general applicability and to be relatively long-lived, and Technical Annexes which would be subject to more frequent revision as analytical techniques, etc., develop and become available for use in monitoring programmes. The ACME agreed that, although this system led to considerable repetition of text, it ensured that the documents were simple to read and that the complete advice on a subject could be obtained by reference to a small number of documents that were not cluttered by alternative courses of action applicable to different matrices. It was noted that the sediment Guidelines also follow the same structure.

The ACME noted, that to use the Guidelines in the design of coordinated monitoring activities, it would be necessary for a Steering Group to meet to agree on detailed objectives of the programme, and then to select those sections and topics from the overall Guidelines and Technical Annexes that were directly applicable to any particular task. The Guidelines were therefore quite comprehensive and the detailed description of particular monitoring exercises would be expected to include appropriate extracts from the Guidelines.

Some concern was expressed that the Guidelines on fish sampling might, in practice, prove difficult to follow exactly. It is a common experience that the required fish species may not be present in the necessary numbers, or covering the defined size/age range and at the time and location chosen for the sampling. Such problems can be reduced by appropriate interaction between programme designers and fisheries scientists beforehand. However, deviations from the sampling guidelines have given rise to difficulties in data assessment, where the exclusion of all data which did not meet all the Guidelines can greatly reduce the amount of data available to the assessors, while inclusion of the data could lead to uncertainties in the degree to which comparisons might be valid. It was recognized that the Sampling Guidelines had been drawn up with the intention of, for example, allowing existing programmes to continue, or minimizing the sampling variance, or investigating the spatial distribution of contaminants, and that in several cases indications had been given of the flexibility in the requirements. In some cases, the requirements seemed rather demanding and in others, rather lax. It was felt that data assessors could need some guidance on the policy to be adopted towards data on samples that did not fully meet the Sampling Guidelines. In this regard, the ACME noted that, for a successful monitoring programme, there clearly needs to be an appropriate target population (e.g., a species sampled from a given length range and a specified space-time sampling frame) which remains stable throughout the monitoring period. If this is not the case (referred to as having an unstable population), then it may be useful to distinguish between situations in which:

1) there originally was such a population, but its characteristics have changed in response to contamination;

2) there is a stable population, but the programme was set up using an unstable population; and

3) there is no stable target population which will be present throughout the monitoring period.

In the first case, this change is a major environmental response, which is of interest in its own right. In the second case, it may be sensible to acknowledge the mistake and simply start again. Alternatively, it may be possible to modify the programme by loosening the specification of the target population, e.g., by widening the space-time sampling frame sufficiently to permit the sample quota to be fulfilled. This may increase the level of sampling variability, but should prevent the results of the programme from being distorted by uncontrolled systematic variation. It will be necessary to ensure that the wider sampling frame is appropriately sampled, for example, by random sampling within the wider sampling frame. Widening the sampling frame may also be

possible in the third case. Alternatively, a completely different programme may be considered.

There still remains the problem of how to assess data when *ad hoc* modifications to the sampled population have been made in order to maintain a specified level of sampling. For example, with temporal monitoring data, samples may have periodically been collected outside the prescribed space-time sampling frame. This is a difficult problem. The bottom line is that if the sampled population is unstable, the results could be meaningless. However, the ACME recommends that in the short term, such data still be reported, together with explanatory details that can be included in the database. This information may subsequently provide suitable commentary for, e.g., unlikely trends or abnormal fluctuations. In the longer term, the ACME recommends that this problem be addressed by making appropriate corrections to the programme, as described above. If possible, when this problem can be anticipated, sensible instructions should be included in the sampling guidelines. Clearly, the dialogue with fisheries scientists and statisticians is crucial at that stage.

Concerning the Purposes of the Biota Monitoring Guidelines, contained in Annex 5 of the MON 1996 report, it was noted that the Purposes appear to have been derived partially from the Purposes of the former Joint Monitoring Programme (JMP), but have been significantly improved and clarified. Reference to monitoring to protect public health (food assurance, food quality) has been removed, and this subject is not included in the JAMP. The ACME endorsed the statements of Purpose a. (temporal trend monitoring)[1] and Purpose b. (spatial distribution monitoring)[2], and welcomed the clear statements of these Purposes. Purpose c.[3] appears more complicated, as it relates to harm to living resources and marine life and therefore covers the effects of chemicals on organisms. Purpose c.i. (to identify sites where contaminant-specific biological effects programmes should be applied) is very similar to Purpose b. (to assess the existing level of marine contamination), and is a slightly different application of rather similar data.

Purpose c.ii. ('to investigate the chemical cause of observed biological effects') represents a clear link to the monitoring objectives based around biological effects measurements. Current ICES advice is that biological effects and chemical monitoring should proceed in an integrated manner. The ACME proposed that detailed Guidelines and Technical Annexes should be completed for Biological Effects Monitoring, and that they should include such matters as sampling, sample preservation, sample pooling strategies, etc. It is likely that they will differ from those given for chemical monitoring in the Biota Monitoring Guidelines. It is therefore necessary that integrated biological and chemical programmes are planned in detail, and that the requirements in such areas as sample mass, sample preservation, pooling procedures, and data interpretation be fully considered during the design of the programme to allow full advantage to be taken of the integrated approach.

It was noted that there might be scope to reduce the cost of monitoring data through multi-purpose cruises on research vessels, for example, taking advantage of sampling opportunities on surveys such as the quarterly International Bottom Trawl Survey cruises, and that this could serve to encourage the integration of chemical and biological measurements.

The ACME agreed that the new JAMP Guidelines for chemical monitoring of contaminants in fish and shellfish were in accordance with current ICES advice on monitoring strategies, but that procedures need to be incorporated elsewhere in the JAMP Guidelines that would ensure that, in programmes addressing Purpose c., the needs of both chemical and biological measurements would be met and the programme fully integrated.

Recommendations

ICES ACME recommends that JAMP procedures for chemical monitoring of contaminants in fish and shellfish be incorporated in the JAMP Guidelines in a manner that will ensure that the needs of both chemical and biological measurements will be met and the overall programme fully integrated, particularly in programmes addressing monitoring Purpose c. as defined in the JAMP Biota Monitoring Guidelines, i.e., to assess harm to living resources and marine life.

4.2 Organic Contaminants and Trace Elements that can be Monitored in Biota, Sediment, and Sea Water on a Routine Basis

Request

There is no specific request; this updates information presented in the 1995 and 1996 ACME reports for organizations coordinating international or regional monitoring programmes on contaminants in marine media.

[1] The full text of Purpose a. is:
'to assess the effectiveness of measures taken for the reduction of marine contamination (temporal trend monitoring). Changes in contaminant inputs are reflected in the concentrations of contaminants in biota over time.'

[2] The full text of Purpose b. is:
'to assess the existing level of marine contamination (spatial distribution monitoring). Monitoring contaminant concentrations in fish, shellfish and seabird eggs can be used to indicate large-scale regional differences in contamination.'

[3] The full text of Purpose c. is:
'to assess harm to living resources and marine life. The role of chemical measurements in integrated chemical and biological effects monitoring programmes is:
i. to identify sites where contaminant-specific biological effects programmes should be applied; and
ii. to investigate the chemical cause of observed biological effects.'

Source of the information presented

The 1997 report of the Marine Chemistry Working Group (MCWG) and ACME deliberations.

Status/background information

In Table 5.4.4 of the 1996 ACME report (ICES, 1996), the ACME provided a list of organic contaminants which can be monitored on a routine basis. This list was provided by MCWG, and it was noted that MCWG has agreed that the table should be revised and has decided to implement a new procedure for routinely updating the list. Under this procedure, a small team of MCWG members will prepare a revised table, that will include organic compounds, trace metals, and nutrients, prior to the 1998 MCWG meeting. This new table will be reviewed and made available for consideration by ACME in 1998.

The following guidelines were agreed for updating the table:

1) The information produced should cater to the needs of all interested parties (e.g., HELCOM, OSPAR, AMAP, and ICES).

2) The team should provide an annual update based on current information on quality assurance/quality control (QA/QC) for chemical determinands in the marine environment, especially those of interest for marine monitoring programmes.

3) The list of determinands should include (groups of) compounds of interest to international monitoring programmes as well as new contaminants.

4) Subgroups of compounds (e.g., PCB congeners, unsubstituted or alkylated PAHs, etc.) should be specified where necessary.

5) Data from international interlaboratory studies should be presented with the appropriate references.

6) A list of currently available certified reference materials (CRMs) should be included.

7) Information should be given on the capability of laboratories in the analysis of the determinands concerned. This should include overall information on the state-of-the-art, a range of coefficient of variation (CV) % values, and the basis of the assessment parameters.

Until the new procedure is established and the table updated, the information on organic contaminants in Table 5.4.4 of the 1996 ACME report (ICES, 1996) is valid. With respect to trace metals, a list of trace elements that can be monitored on a routine basis in biota, sediments, and sea water was prepared. This list is based on information from recent intercomparison exercises as well as on the availability of certified reference materials (CRMs) and is contained in Table 4.2.

Table 4.2.a. Trace elements and their compounds that can be monitored in biota.

Element	Recent I/C data†	Supplier	QC material
Zn	QUASIMEME/NOAA	NRC	DORM-2 (dogfish muscle)
Cd	QUASIMEME/NOAA	NRC	DOLT-2 (dogfish liver)
Pb	QUASIMEME/NOAA	NRC	TORT-2 (lobster tissue)
Cu	QUASIMEME/NOAA	NRC	LUTS-1 (lobster tissue)
Cr	QUASIMEME/NOAA	IAEA	IAEA 350 (tuna fish)
Ni	QUASIMEME/NOAA	BCR	CRM 278 (mussels), CRM 414 (plankton)
As	QUASIMEME/NOAA	BCR	CRM 422 (cod muscle)
Hg	QUASIMEME/NOAA	NIST	SRM 1566a (oyster tissue)
Ag	NOAA	NIST	SRM 1566a (oyster tissue)
Se	NOAA	NIST	SRM 1566a (oyster tissue)
Sb	NOAA		
Sn	NOAA		
Fe	NOAA	NIST	SRM 1566a (oyster tissue)
MMHg	none	BCR NRC	CRM 463, CRM 464 (tuna fish) DOLT-2, TORT-2, LUTS-1, DORM-2
*As compounds	none	NRC	DORM-2
*Organotin compounds	none	NIHS (Japan)	

†This column refers to intercomparison exercises (I/C) carried out with regard to marine environmental samples.
*Not yet suitable for monitoring.

Table 4.2.b. Trace elements and their compounds that can be monitored in sediments.

Element	Recent I/C data†	Supplier	QC material
Zn	QUASIMEME/NOAA	NRC	BCSS-1, MESS-1; NBS 1646
Cd	QUASIMEME/NOAA	BCR	CRM 277, CRM 320
Pb	QUASIMEME/NOAA	NIST	SRM 1645, SRM 1646, SRM 2704
Cu	QUASIMEME/NOAA	NIST	SRM 1645, SRM 1646, SRM 2704
Cr	QUASIMEME/NOAA	NIST	SRM 1645, SRM 1646, SRM 2704
Ni	QUASIMEME/NOAA	NIST	SRM 1645, SRM 1646, SRM 2704
As	QUASIMEME/NOAA	NIST	SRM 1646
Al	QUASIMEME/NOAA	NIST	SRM 1645, SRM 1646, SRM 2704
Hg	QUASIMEME/NOAA	NRC	BEST-1
Si	NOAA	NIST	SRM 2704
Be	NOAA	NRC	BCSS-1, MESS-1
Tl	NOAA	NIST	SRM 1645, SRM 2704
Sn	NOAA	NRC	BCSS-1, MESS-1
Sb	NOAA	NIST	SRM 2704
Fe	NOAA	NIST	SRM 1645, SRM 1646, SRM 2704
Mn	NOAA	NIST	SRM 1645, SRM 1646, SRM 2704
Ag	NOAA		
Se	NOAA	NIST	SRM 2704
MMHg	none	IAEA BCR NRC	IAEA 356 CRM 580[2)] PACS-2[1)]
*Organotin compounds	none	BCR	CRM 463 (DBT, TBT)

†This column refers to intercomparison exercises (I/C) carried out with regard to marine environmental samples.
*Not yet suitable for monitoring.
[1)]In preparation.
[2)]In process of certification.

There are sufficient results from intercomparison exercises available (both QUASIMEME and the U.S. National Oceanic and Atmospheric Administration (NOAA)) to demonstrate the practicability of routine analysis of the following trace elements:

- Zn, Cu, Pb, Cd, Hg, Cr, Ni, and As in biota;
- Zn, Cu, Pb, Cd, Hg, Cr, Ni, As, and Al in sediments;
- Zn, Cu, Pb, Cd, Cr, Ni, As, Fe, and Mn in sea water.

NOAA has also successfully measured the following elements: Ag, Se, Sb, Fe, and Sn in biota and Si, Be, Tl, Ag, Se, Sn, Sb, Fe, and Mn in sediments. Additional elements and species of interest are Ag, Se, organotin compounds, arsenic speciation (As(III), As(V), monomethylarsenic (MMA), dimethylarsenic (DMA), trimethylarsenic (TMA), arsenocholine, arsenobetaine), and monomethylmercury (MMHg) in biota and sediments; Hg and MMHg, selenium speciation (Se(II), Se(IV), Se(VI)), and chromium speciation (Cr(III), Cr(VI)) in sea water. For these additional elements and species, no monitoring should be carried out until successful intercalibrations have been performed. Plans for the determination of arsenic speciation, and the analysis of methylmercury and organotin compounds in biota and sediments are currently in a preparatory phase in the QUASIMEME proficiency testing schemes.

Need for further research

Using the guidelines adopted, MCWG will prepare for 1998 a revised table on organic contaminants, trace elements, and nutrients that can be monitored on a routine basis. This information will be updated annually.

Table 4.2.c. Trace elements and trace element speciation that can be monitored in sea water.

Element/Compound	Recent I/C data†	Supplier	QC material
Zn	ICES	NRC	NASS-4 (sea water)
Cd	ICES	NRC	SLEW-2 (estuarine water)
Pb	ICES	NRC	NRC: SLRS-3 (river water)
Cu	ICES	NRC	SLRS-3 (river water)
Cr	ICES	NRC	SLRS-3 (river water)
Ni	ICES	NRC	SLRS-3 (river water)
As	ICES	NRC	SLRS-3 (river water)
Fe	ICES	NRC	SLRS-3 (river water)
Mn	ICES	NRC	SLRS-3 (river water)
Hg	QUASIMEME[1)]	none	
*MMHg	none	none	
*Ag	none	none	
*Se	none	none	
*Se(II), Se(IV), Se(VI)	none	none	
*Cr(III), Cr(VI)	none	none	

†This column refers to intercomparison exercises (I/C) carried out with regard to marine environmental samples.
*Not yet suitable for monitoring.
[1)]Exercise currently being performed.

Key to abbreviations in Table 4.2:

BCR: European Commission Bureau of Community References (now: Standards, Measurement and Testing Programme)
IAEA: International Atomic Energy Agency
NIST: U.S. National Institute of Standards and Testing
NOAA: U.S. National Oceanic and Atmospheric Administration
NRC: National Research Council of Canada
DBT: dibutyltin
MMHg: monomethylmercury
TBT: tributyltin

Recommendations

ICES ACME recommends that, for information on trace elements and organic contaminants that can be monitored on a routine basis, reference should be made to Table 4.2 in this report and Table 5.4.4 in the 1996 ACME report, respectively.

Reference

ICES. 1996. Report of the ICES Advisory Committee on the Marine Environment, 1996. ICES Cooperative Research Report, No. 217: 34, 36.

4.3 Biological Effects Monitoring

4.3.1 Guidelines on the statistical design of biological effects monitoring programmes

Request

There is no specific request, but this issue is related to current and previous requests of the Oslo and Paris Commissions.

Source of the information presented

The 1997 report of the Working Group on Biological Effects of Contaminants (WGBEC) and ACME deliberations.

Status/background information

In 1996, OSPAR made several requests to ICES for advice on the statistical design and evaluation of monitoring programmes, particularly in relation to the monitoring of polycyclic aromatic hydrocarbons (PAHs) and the detection of temporal trends. In this connection, the ACME noted that WGBEC addressed some statistical issues regarding sampling design and data analysis in biological effects monitoring programmes; the ACME endorsed the WGBEC views described here.

In connection with the sampling design of biological effects monitoring programmes, the ACME emphasized the importance of (a) having clear objectives (e.g., the detection of spatial distributions or temporal trends), (b) specifying the relevant variables for measurement (e.g., cytochrome P4501A induction), (c) identifying important covariables (e.g., sex, season), and (d) identifying the sample size and number of samples required to attain the requisite statistical power for testing the hypothesis in question. Statistical advice is essential at the stage when particular variables are being selected for monitoring programmes because it allows one to choose the optimal sampling design by taking into account known variability in order to evaluate the hypothesis in the most cost-effective manner.

The ACME considered that, following the integration of biological and chemical monitoring techniques which it has been advocating for several years, the use of multivariate analysis techniques would greatly assist in the evaluation of the resulting data. Such methods are desirable because:

- they increase the ability to indicate differences between sites ('holistic' evaluation using the available information);
- they can identify (and quantify) links between different variables determined for each individual organism, e.g., linking biological endpoints with measurements of accumulated contaminant residues;
- they can identify (and quantify) links between different variables within an ecosystem, e.g., linking biomarker change, bioassay responses, benthic community diversity, and contaminant levels.

It was emphasized that whole-ecosystem evaluations using multivariate techniques must involve measurements in several species that are sensitive to the contaminant of interest, and that any weighting of variables must be relevant to the ecosystem in question and to the natural variability within it. Furthermore, different endpoints (e.g., biomarkers and benthic community changes) respond on very different time scales, and care must therefore be taken when incorporating them into a multivariate assessment.

Need for further research

There is a need to develop multivariate statistical techniques to handle the data emerging from integrated biological and chemical monitoring programmes, in order that maximum use can be made of this information to achieve a holistic picture of contaminant effects.

4.3.2 Recommended and promising biological effects techniques

Request

There is no specific request; this updates information presented in the 1995 ACME report, and is of interest to ICES Member Countries and organizations conducting biological effects monitoring.

Source of the information presented

The 1997 report of the Working Group on Biological Effects of Contaminants (WGBEC) and ACME deliberations.

Status/background information

The ACME noted that WGBEC has reviewed and revised the lists of recommended and promising biological effects monitoring techniques which were developed in 1995 as part of an integrated marine environmental monitoring strategy (ICES, 1995). Four techniques (reproductive success in fish, induction of antioxidant enzymes, D-aminolevulinic acid dehydratase (ALA-D) inhibition by lead, and the production of fluorescent bile metabolites of PAHs) which were originally considered 'promising' were moved to the 'recommended' list. Furthermore, the measurement of intersex in *Littorina littorea* as a means of monitoring the effects of tributyltin (TBT) was placed directly on the 'recommended' list. The ACME noted that WGBEC has also agreed to consider five new techniques for possible inclusion on the 'promising' list in 1998:

1) the COMET assay for DNA strand breaks, which measures the degree of damage to the genetic material;
2) methods for measuring low levels of chemical residues by raising antibodies to them and quantifying using immunological techniques;
3) thyroid hormone analysis as a technique for studying potential interference with growth and reproductive processes;
4) measuring small DNA adducts in order to quantify chemical binding to the genetic material;
5) cellular energy allocation (see below).

WGBEC has given some preliminary consideration to cellular energy allocation, a technique which has been developed with freshwater *Daphnia*. It uses simple calorimetric methods to measure an organism's energy consumption (E_c) and energy reserves available for metabolism (E_a), and the difference between the two ($E_a - E_c$) representing the energy available for growth and reproduction. Much like the scope-for-growth (SFG) response, this available energy decreases if an animal is stressed. The technique is simpler to employ than SFG, and can be applied most appropriately to small planktonic organisms. However, there are some doubts about the sensitivity of the method, and there is a need to conduct comparisons with SFG (perhaps by using mussel tissues in collaboration with the SFG intercomparison exercise in June 1997).

The revised list of recommended methods, including also the measurement of externally visible fish diseases, is presented in Table 4.3.2.1 and the revised list of promising methods is contained in Table 4.3.2.2.

Recommendations

ICES ACME recommends the use of the methods listed in Table 4.3.2.1 in national and international monitoring programmes.

Reference

ICES. 1995. Report of the ICES Advisory Committee on the Marine Environment, 1995. ICES Cooperative Research Report, No. 212: 84–96.

Table 4.3.2.1. Recommended techniques for biological monitoring programmes at the national or international level (revised version of Table A1.1 in ICES, 1995).

Method	Organism	Refs.	Issues addressed	Biological significance
Bulky DNA adduct formation	Fish[1] Bivalve molluscs	1–6	PAHs Other synthetic organics, e.g., nitro organics, amino triazine pesticides (triazines)	Measures genotoxic effects.
AChE inhibition	Fish[1], crustacea, bivalve molluscs	12–16	Organophosphates and carbamates or similar molecules Possibly algal toxins	Measures exposure.
Metallothionein induction	Fish[1]	17–22	Measures induction of metallothionein protein by certain metals (e.g., Zn, Cu, Cd, Hg)	Measures exposure and disturbance of copper and zinc metabolism.
EROD or P4501A induction*	Fish[1]	46–51, 99	Measures induction of enzymes which detoxify planar organic contaminants (e.g., PAHs, planar PCBs, dioxins)	Possible predictor of pathology through mechanistic links. Sensitive indicator of exposure.
ALA-D inhibition	Fish[1]	74–75	Lead	Index of exposure.
Antioxidant enzymes	Fish[1]	76–78	Not contaminant specific, will respond to a wide range of environmental contaminants	Measures the presence of free radicals.
Fluorescent bile metabolites	Fish	79–80	PAHs	Measures exposure to and metabolism of PAHs.
Lysosomal stability	Fish[1] *Mytilus* spp.	23–25	Not contaminant specific but responds to a wide variety of xenobiotic contaminants and metals	Measures cellular damage and is a good predictor of pathology. Provides a link between exposure and pathological endpoints. Possibly, a tool for immuno-suppression studies in white blood cells.
Neoplastic and pre-neoplastic liver histopathology	Fish[1]	7–11	PAHs Other synthetic organics, e.g., nitro organics, amino triazine pesticides (triazines)	Measures pathological changes associated with exposure to genotoxic and non-genotoxic carcinogens.

*Intercomparisons or quality control procedures complete for some methods (e.g., Refs. 31, 40, 99, 100).
[1] May also be applicable to mammals and birds.

Table 4.3.2.1. Recommended techniques for biological monitoring programmes at the national or international level (continued).

Method	Organism	Refs.	Issues addressed	Biological significance
Whole sediment bioassays*	• *Corophium* • *Echinocardium* • *Arenicola* • *Leptocheirus* • *Grandidierella* • *Rhepoxynius* • *Ampelisca*	31–35	Not contaminant specific, will respond to a wide range of environmental contaminants in sediments	Acute/lethal and acute/sublethal toxicity only at present. May enable retrospective interpretation of community changes.
Sediment pore water bioassays*	Any water column organism including: • *Dinophilus* • sea urchin fertilization, etc. • bivalve embryo • Microtox	36–41	Will respond to a wide range of environmental contaminants	Acute and chronic toxicity, including genotoxicity, etc. Toxicity of hydrophobic contaminants might be underestimated in pore water assays.
Sediment sea water elutriates*	Any water column organism, as above	36–41	Will respond to a wide range of environmental contaminants in • dredge spoils • sediments liable to resuspension	Acute/lethal and acute/sublethal toxicity, including genotoxicity, etc.
Water bioassays*	As for pore water and elutriates (see above)	36–41	Not contaminant specific, will respond to a wide range of environmental contaminants in inshore and estuarine waters	Acute/lethal and acute/sublethal toxicity, including genotoxicity, etc.
Scope for growth	Bivalve molluscs, e.g., *Mytilus*	55–58	Responds to a wide variety of contaminants	Integrative response which is a sensitive and sublethal measure of energy available for growth.
Shell thickening	*Crassostrea gigas*	103	Specific to organotins	Disruption to pattern of shell growth.
Vitellogenin induction	Male and juvenile fish	26–30	Oestrogenic substances	Measures feminization of male fish and reproductive impairment.
Imposex	Neogastropod molluscs, e.g., dogwhelk (*Nucella lapillus*)	52–54	Specific to organotins	Reproductive interference. Estuarine and coastal littoral waters (*Nucella*) and offshore waters (*Buccinum*).
Intersex	Littorinids	101, 102	Specific to reproductive effects of organotins	Reproductive interference in coastal (littoral) waters.
Reproductive success in fish	*Zoarces viviparus*	72	Not contaminant specific, will respond to a wide range of environmental contaminants	Measures reproductive output and survival of eggs and fry in relation to contaminants in a viviparous fish species. Restricted to period when young are carried by female.
Externally visible fish disease	Fish	104–108	Measures the effects of non-specific stress by quantifying the presence of externally visible diseases, especially in dab (*Limanda limanda*)	These diseases are natural, but may be exacerbated by various stressors, including contaminants.
Benthic community analysis*	Macro-, meio-, and epibenthos	42–45, 100, 109	Responds to a wide variety of contaminants, particularly those resulting in organic enrichment	Ecosystem level. Retrospective. Particularly useful for point sources. Most appropriate for deployment when other monitoring methods indicate a problem may exist.

*Intercomparisons or quality control procedures complete for some methods (e.g., Refs. 31, 40, 99, 100).
[1] May also be applicable to mammals and birds.

Table 4.3.2.2. Promising biological effects monitoring methods which require further research before they can be recommended (revised version of Table A1.2 in ICES, 1995).

Method	Organism	Refs.	Issue addressed	Biological significance
DNA strand breaks	Fish and mussels	1–6	Not contaminant specific, will respond to a wide range of environmental contaminants	Measures genotoxic effects, but is also extremely sensitive to other environmental parameters.
Oncogenes	Fish	93–95	PAHs Other synthetic organics, e.g., nitro organics, amino triazine pesticides (triazines)	Activation of oncogenes (*ras*) or damage to tumour suppressor genes (p53). Measures genotoxic effects leading to carcinogenesis.
P4501A induction	Invertebrates	96	Induced enzyme response to PAHs, planar PCBs, dioxins and/or furans	Measures exposure to organic contaminants.
Glutathion-*S*-transferase(s) (GST)	Fish	97	Predominantly organic xenobiotics	Measures exposure and the capacity of the major group of Phase II enzymes.
Multidrug/xenobiotic resistance (MDR/MXR)	Fish, invertebrates	85–92	Organic xenobiotics	Measure of exposure.
Protein or enzyme altered foci	Fish	92	PAHs Other synthetic organics, e.g., nitro organics, amino triazine pesticides (triazines)	Indicates exposure to carcinogen(s).
Various methods of measuring immunocompetence	Fish and invertebrates	73	Not contaminant specific, will respond to a wide range of environmental contaminants	Measures factors which influence susceptibility to disease.
On-line monitoring	Mussels and crabs	98	Responds to metals and xenobiotics	Measures the effects of chemicals on heart rate using a simple and inexpensive remote biosensor. Gives an integrated response.
Degenerative liver, gill and kidney histopathology	Fish (especially flatfish such as dab (*Limanda limanda*))	59–66	General toxicological response which will respond to a wide variety of contaminants	Measures degenerative change in tissues.
Abnormalities in wild fish embryos and larvae	Many fish, including demersal and pelagic species	70–71	Not yet linked unequivocally to contaminants	Measures frequency of probably lethal abnormalities in fish larvae. Mutagenic, teratogenic.
Chronic whole sediment bioassays	Invertebrates	32	Responds to a wide range of contaminants	Measurements such as growth and reproduction, coupled to biomarker responses, which will give a measure of the bioavailability and chronic toxicity in whole sediments.
Pollution-induced community tolerance (PICT) water bioassay	Microalgae	67–69	Specific contaminants can be tested	Measure of degree of adaptation to specific pollutants. Not yet widely tested.
Allometric response in the benthic community	Macro-, meio-, and epibenthos	81–84	Not contaminant specific, will respond to a wide range of environmental contaminants	Ecosystem level. Retrospective.

References for Tables 4.3.2.1 and 4.3.2.2

1. Dunn, B.P., Black, J.J., and Maccubbin, A. 1987. ^{32}P-postlabelling analysis of aromatic DNA adducts in fish from polluted areas. Cancer Research, 47: 6543–6548.

2. Varanasi, U., Reichert, W.L., and Stein, J.E. 1989. ^{32}P-postlabelling analysis of DNA adducts in liver of wild English sole (*Parophrys vetulus*) and winter flounder (*Pseudopleuronectes americanus*). Cancer Research, 49: 1171–1177.

3. Varanasi, U., Reichert, W.L., Eberhart, B.-T., and Stein, J.E. 1989. Formation of benzo[*a*]pyrene-diolepoxide-DNA adducts in liver of English sole (*Parophrys vetulus*). Chemico-biological Interactions, 69: 203–216.

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4.3.3 Results of the ICES Special Meeting on the Use of Liver Pathology in Flatfish for Monitoring Biological Effects of Contaminants

Request

There is no specific request; the ACME considered the results of this ICES Special Meeting to be of interest to the Oslo and Paris Commissions because studies on liver histopathology and liver nodules of flatfish have been recommended as techniques for incorporation into the biological effects component of the OSPAR Joint Assessment and Monitoring Programme (JAMP). Furthermore, the issues addressed at the ICES Special Meeting covered the development of quality assurance procedures for liver pathology measurements within biological effects monitoring programmes and are, therefore, directly related to item 2.2 of the 1997 Work Programme from OSPAR.

Source of the information presented

The report of the ICES Special Meeting on the Use of Liver Pathology in Flatfish for Monitoring Biological Effects of Contaminants (SMLIPA), the 1997 reports of the Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) and the Working Group on Biological Effects of Contaminants (WGBEC), and ACME deliberations.

Status/background information

The ICES Special Meeting was held at the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) Weymouth Laboratory, Weymouth, UK, from 22–25 October 1996, under the co-convenership of Dr S.W. Feist (UK) and Dr T. Lang (Germany). The planning of the meeting was carried out by WGPDMO and the Sub-group on Statistical Analysis of Fish Disease Prevalence Data in Marine Fish Stocks (SGFDDS), with the assistance of Dr A. Köhler (Germany) as representative of WGBEC. Eighteen participants representing nine ICES Member Countries attended the meeting.

The main objective of the meeting was to bring together specialists from ICES Member Countries involved in research and monitoring programmes on contaminant-associated liver pathology of flatfish, in order to:

1) provide an overview of on-going activities in ICES Member Countries;

2) evaluate the suitability and applicability of techniques used to measure liver pathology of fish for monitoring purposes;

3) elaborate guidelines for field sampling, processing of samples, and interpretation of liver pathology for monitoring programmes, including quality assurance procedures.

The meeting was divided into two sections. The first section consisted of the presentation of keynotes and reports of national activities, as well as discussions on the formulation of recommendations on appropriate biological effects techniques and quality assurance procedures. In the second section, practical sessions were devoted to the demonstration of different techniques by selected participants and assessments on the classification and intercalibration of histopathological liver lesions using sets of prepared microscope slides submitted by the participants.

Biological effects techniques for the measurement of flatfish liver pathology for monitoring purposes, recommended by the participants in the ICES Special Meeting and accepted by ACME, are given in Table 4.3.3. They include examination for gross liver lesions and histopathology as well as cellular/subcellular and biochemical biomarker studies.

In the full report of the Special Meeting (ICES, 1997), techniques in use and the results of studies using these techniques are described in detail, taking into account their strengths and limitations for monitoring purposes. Guidelines are provided on sampling strategies and the processing of samples for histopathology and other purposes, and for the diagnosis and classification of histopathological liver lesions. Quality assurance requirements for fish liver histopathology studies are identified and necessary procedures are suggested. The ACME noted that further quality assurance procedures for the monitoring of liver histopathology were developed by WGPDMO after having reviewed the report of the Special Meeting. These are dealt with in Section 5.3.2 of this report.

Need for further research or additional data

The ACME appreciated the results of the Special Meeting and considered that they represent important progress in the establishment of the use of liver pathology for international research and monitoring programmes on biological effects of contaminants, in particular the OSPAR Joint Assessment and Monitoring Programme (JAMP). The work carried out at the Special Meeting, therefore, constituted a successful continuation of ICES activities in terms of the development and standardization of biological effects techniques.

Table 4.3.3. Biological effects techniques for the measurement of fish liver pathology recommended for monitoring purposes.

Effects Measured	Technique	Status	Contaminant Response
	macroscopic liver changes		
nodules > 2 mm in diameter	macroscopic, subsequent histological confirmation, paraffin sections, H&E staining	A	neoplasia: probably specific, carcinogens (1) non-neoplastic: probably unspecific
	liver histopathology		
general necrotic/degenerative changes	paraffin sections, H&E staining	B	probably unspecific (2)
unique degenerative changes	paraffin sections, H&E staining	B	specific, PAHs, PCBs, DDTs (1)
storage conditions	paraffin sections, H&E staining	B	probably unspecific (3)
inflammatory changes	paraffin sections, H&E staining	B	probably unspecific (3)
non-neoplastic proliferative lesions	paraffin sections, H&E staining	B	probably unspecific (3)
vascular abnormalities	paraffin sections, H&E staining	B	probably unspecific (3)
foci of cellular alteration	paraffin sections, H&E staining	B	probably specific (1)
benign tumours	paraffin sections, H&E staining	B	probably specific (1)
malignant tumours	paraffin sections, H&E staining	B	probably specific (1)
	liver histochemistry		
lysosomal membrane stability	cryo sections, neutral red	B	general toxicity
enzyme-altered foci (G6PDH)	cryo sections	B	specific
	liver immuno-histochemistry		
proliferating cell nuclear antigen (PCNA)	paraffin sections, antibodies	B	regeneration, proliferation (3)
CYP1A	paraffin sections, antibodies	B	polar contaminants, PAHs, PCBs
	liver biochemistry		
CYP1A (EROD)	fluorescence assay	B	polar contaminants, PAHs, PCBs
DNA adducts	^{32}P-postlabelling	B	genotoxicity (PAHs, others)

Status A: Quality assurance procedures are in place.
Status B: Development of quality assurance procedures and interlaboratory intercalibration are needed.
Number categories for relative importance as a biomarker for exposure/toxicity: 1 = high, 2 = medium, 3 = low.
H&E staining: haematoxylylin and eosin staining.

In the reports of the Special Meeting and the 1997 meeting of WGPDMO, the incorporation of data obtained from studies on liver histopathology and other related biomarkers into the fish disease data section of the ICES Environmental Data Centre was considered. The ACME endorsed this proposal and supported the suggestion that the co-conveners of the Special Meeting, together with the ICES Secretariat, explore ways to accomplish this (see also Section 7.1, below).

Recommendations

ICES ACME encourages ICES Member Countries to integrate studies on fish liver pathology into monitoring programmes on biological effects of contaminants. The studies should be designed according to the guidelines detailed in the report of the ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants.

Reference

ICES. 1997. Report of the Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants. ICES CM 1997/F:2. 75 pp.

4.4 Monitoring Contaminants in Marine Media

4.4.1 Monitoring guidelines for PAHs in sediments and biota

Request

Item 1.1 of the 1997 Work Programme from the Oslo and Paris Commissions.

Source of the information presented

The 1997 reports of the Working Group on Environmental Assessment and Monitoring Strategies (WGEAMS), the Working Group on Marine Sediments in Relation to Pollution (WGMS), the Marine Chemistry Working Group (MCWG), and ACME deliberations.

Status/background information

This OSPAR request was considered by three ICES Working Groups in 1997. The ACME recognized that specific guidelines on polycyclic aromatic hydrocarbons (PAHs) are needed for several aspects of monitoring within the OSPAR Joint Assessment and Monitoring Programme (JAMP) including JAMP issue 1.10 (What are the concentrations in the maritime area?) and JAMP issue 1.11 (Do PAHs affect fish and shellfish?). The draft JAMP Guidelines document produced by the 1996 meeting of the OSPAR Ad Hoc Working Group on Monitoring (MON 96/9/1-E) was reviewed to identify missing sections in the Guidelines, or sections which needed updating, in order to accommodate the monitoring of PAHs and related issues. The following list describes the work that would be required to amend the relevant sections of the JAMP Guidelines related to biota:

1) The general section of the contaminant-specific guidelines for biota (Annex 5 of the MON 1996 report, Biota Monitoring Guidelines) needs to be updated to include PAHs.

2) A technical annex addressing issues specific for analyses of PAHs in biota should be attached to the Biota Monitoring Guidelines or, alternatively, the technical annex on organic contaminants should be rewritten to include PAHs. The first alternative is preferable for the following reasons:

   a) The draft Biota Monitoring Guidelines with technical annexes, as prepared by MON 1996, have already been adopted by the OSPAR Working Group on Concentrations, Trends and Effects of Contaminants in the Marine Environment (SIME) as the official JAMP Guidelines, with their current content and format.

   b) Monitoring of PAHs in biota will only concern one of the three matrices discussed in the existing technical annexes, namely mussels.

   c) The methods of analysis for PAHs differ significantly from the methods used for the other organic contaminants included in Technical Annex 1 of the JAMP Biota Monitoring Guidelines.

Therefore, the ACME suggests that Technical Annex 1 of the JAMP Biota Monitoring Guidelines remain confined to halogenated organic contaminants and be renamed accordingly.

The ACME noted that draft general guidelines for the monitoring of PAHs in biota had been prepared by MCWG. In the review of these draft guidelines, however, it was felt that additional details were necessary to meet the requirements of guidelines for the JAMP programme. Accordingly, the ACME decided that this work should continue and that MCWG should be requested to prepare a draft technical annex addressing the analysis of PAHs in mussels, following the format of existing Technical Annexes to the JAMP Biota Monitoring Guidelines.

In terms of the guidelines for monitoring PAHs in sediments, the ACME reviewed a draft Technical Annex for the determination of PAHs in sediments, prepared by WGMS, and agreed that it met the requirements for such guidelines. Accordingly, the ACME agreed that this Technical Annex should be added to the ICES Guidelines for the Use of Sediments in Marine Monitoring (ICES, 1994, 1996) and should be forwarded to OSPAR for proposed incorporation into the JAMP Sediment Monitoring Guidelines. This document is attached as Annex 1.

The ACME also recognized that further work should be conducted on the question of the number of replicate samples required per sampling area, for both sediments and biota, as well as on the variance associated with the measurement of the spatial distributions of PAHs. This will be considered by the new Study Group on Monitoring Programmes for Contaminants in Sediments (SGMPCS), with review by WGSAEM, WGMS, MCWG, and WGEAMS. Furthermore, as WGMS had not been able to agree on the appropriate methods to normalize data on PAHs in sediments (see Section 4.5.2, below), this Working Group will be requested to continue consideration of this topic with the aim of providing a recommended method or methods in 1998.

The ACME noted that guidelines on PAH analysis are also needed for contaminant-specific biological effects monitoring (Annex 12 of the MON 1996 report). Methods to be used in PAH-specific monitoring include determinations of cytochrome P4501A, DNA adducts, PAH metabolites in fish bile, and liver histopathology. Guidelines are already in place for some of these procedures, while others require additional work. The ACME agreed that WGBEC and MCWG would need to collaborate intersessionally on the finalization of the technical annexes regarding analyses for PAH-specific biological effects monitoring.

Recommendations

ICES ACME recommends that Annex 1 to this report, on analytical methods for the determination of PAHs in sediments, be included as a Technical Annex to the ICES Guidelines for the Use of Sediments in Marine Monitoring (ICES, 1994, 1996) and also be forwarded to OSPAR for proposed incorporation into the JAMP Guidelines.

References

ICES. 1994. Report of the ICES Advisory Committee on the Marine Environment, 1993. ICES Cooperative Research Report, No. 198: 45–57.

ICES. 1996. Report of the ICES Advisory Committee on the Marine Environment, 1996. ICES Cooperative Research Report, No. 217: 100–104.

4.4.2 Matrices for determining PAHs and their metabolites in a monitoring programme

Request

This issue is relevant to item 1.1 of the 1997 Work Programme from the Oslo and Paris Commissions.

Source of the information presented

The report of the 1995 OSPAR/ICES Workshop on Biological Effects Monitoring Techniques, the 1997 report of the Working Group on Biological Effects of Contaminants (WGBEC) and ACME deliberations.

Status/background information

The OSPAR request to develop monitoring guidelines for polycyclic aromatic hydrocarbons (PAHs) was first presented to ICES in the 1996 Work Programme from the Oslo and Paris Commissions. After discussions in four ICES Working Groups and deliberations by ACME that year, the ACME pointed out that the request was phrased from a purely chemical viewpoint, omitting a biological component. The ACME recommended, based on the outcome of the OSPAR/ICES Workshop on Biological Effects Monitoring Techniques (Aberdeen, October 1995) and considering the general aims of the OSPAR Joint Assessment and Monitoring Programme (JAMP), that PAH monitoring programmes be designed to take into consideration the potential of these compounds to give rise to deleterious biological effects, i.e., that OSPAR should address the issue of PAH monitoring in the marine environment from the point of view of an integrated programme, combining chemical and biological effects measurements (ICES, 1996). The ACME noted with regret that the request had not been rephrased in the 1997 OSPAR Work Programme and encouraged a new discussion in WGBEC, based on integrated biological and chemical studies.

The ACME pointed out that an update of existing monitoring guidelines for PAHs concerning the choice of species was published in 1995 (ICES, 1995) and that one approach for evaluating the environmental effects of PAHs using fish includes measurements of cytochrome P4501A (EROD), PAH metabolites in bile, DNA adducts, and liver histopathology (OSPAR/ICES Workshop on Biological Effects Monitoring Techniques, Aberdeen, October 1995).

At its 1997 meeting, WGBEC further considered this issue through two presentations on integrated monitoring studies carried out in Sweden and Norway investigating PAH impacts from aluminium smelters and creosote discharges. The ACME took note of these studies and stressed that the choice of the target species is crucial since PAH concentrations in fish are likely to be low because of the high metabolic capacity of fish with respect to these compounds, while benthic invertebrates may accumulate varying amounts of PAHs. The highest PAH levels were observed in sea cucumber (*Stichopus tremulus*), intermediate levels in scallop (*Chlamys septemradiata*), and low levels in the sea star (*Psilaster andromeda*). Several species of fish in the gradient outside the aluminium smelter, as well as in the study in which creosote-contaminated sediment was the source of pollution, were exposed to genotoxic substances and were found to have an elevated cytochrome P4501A activity and to contain PAH metabolites in the bile.

Regarding biological effects monitoring in relation to PAHs, the ACME endorsed the view of WGBEC that species which accumulate high levels of PAHs may not necessarily be the only species to use for monitoring the biological effects of PAHs.

Need for further research

The ACME took note that biological effects techniques for a wide range of benthic invertebrates, with the exception of blue mussel, are not available at this time, and encouraged the development of such techniques for a range of other benthic invertebrates.

Recommendations

ICES ACME recommends that benthic invertebrate tissues, preferably from bivalves (e.g., mussels), should be used as matrices for monitoring the concentration levels and trends of PAHs. Fish tissues should, in general, not be used due to the high rates of metabolism of PAHs. However, measurements of PAHs in fish tissues may contribute to PAH-related integrated chemical/biological monitoring approaches in areas with expected substantial PAH concentrations.

ICES ACME recommends that the following biological effects techniques, namely measurement of cytochrome P4501A (EROD), PAH metabolites in bile, DNA adducts and liver histopathology, be used for PAH-specific biological effects monitoring in fish.

References

ICES. 1995. Report of the ICES Advisory Committee on the Marine Environment, 1995. ICES Cooperative Research Report, No. 212: 29.

ICES. 1996. Report of the ICES Advisory Committee on the Marine Environment, 1996. ICES Cooperative Research Report, No. 217: 30.

4.4.3 Use of lipids as cofactors in organic contaminant analysis

Request

There is no specific request; this is part of the continuing ICES work on the monitoring of contaminants.

Source of the information presented

The 1997 report of the Working Group on Biological Effects of Contaminants (WGBEC) and ACME deliberations.

Status/background information

The ACME noted that WGBEC reviewed data which suggest that neutral lipids have a high potential for normalizing concentrations of very persistent non-polar organic contaminants in animal tissues. It was suggested that bioaccumulation is basically caused by equilibrium partitioning between sea water and the neutral lipid pool in organisms. Intercomparability of data between tissues and species may therefore be compromised by the standard method for measuring lipids (the Bligh and Dyer method), which gives a higher yield of phospho-sphingo and glyco-lipids which are more polar than the contaminants of interest. It was agreed that it is important to consider both lipid class and partitioning processes when normalizing residue data for lipophilic persistent organic contaminants.

4.5 Techniques for Monitoring Contaminants in Sediments

4.5.1 Progress in the work of the Study Group on Monitoring Programmes for Contaminants in Sediments

Request

There is no specific request; this is part of the continuing ICES work on monitoring contaminants in sediments.

Source of the information presented

The 1997 report of the Working Group on Marine Sediments in Relation to Pollution (WGMS), information provided by the Chairman of the Study Group on Monitoring Programmes for Contaminants in Sediments (SGMPCS), and ACME deliberations.

Status/background information

The ACME was informed that its recommendation that a new Study Group on Monitoring Programmes for Contaminants in Sediments (SGMPCS) be established had been accepted by the ICES Council at the 1996 Annual Science Conference. The ACME had made this recommendation in response to suggestions by WGSAEM, WGMS, and WGEAMS, and the Study Group consists of sedimentologists, chemists and statisticians from these Working Groups.

The Study Group should meet in early 1998 to complete its development of the design, assessment and data requirements of sediment monitoring programmes related to contaminants. In particular, the ACME proposed that the Study Group should use its results to focus on the number of replicate samples per area to characterize the sampling area in monitoring for contaminants, including polycyclic aromatic hydrocarbons (PAHs), in sediments. The specific tasks to be carried out by SGMPCS should include to:

a) clarify the objectives and contrast suitable sampling schemes for 1) estimation of the average contaminant level in an area, and 2) mapping the spatial distribution of a contaminant;

b) describe appropriate methods of statistical analysis;

c) identify data requirements for estimating components of variability or other relevant parameters necessary for designing an effective programme;

d) liaise with the ICES Secretariat to identify suitable historical data sets of contaminants (including PAHs) in sediments to demonstrate the estimation of components of variability, etc.;

e) take due account of physical and chemical normalization procedures;

f) demonstrate the design of a sediment monitoring programme for contaminants (including PAHs), if possible identifying appropriate levels of sampling to achieve a specified statistical target.

The ACME endorsed the establishment of the SGMPCS because the subject of contamination in sediments has been of interest to ICES for many years. This is because sediments may successfully reflect patterns of spatial and temporal contamination. Sediment monitoring programmes are potentially valuable for detecting environmental contamination and for determining the effectiveness of control measures taken to decrease the inputs of contaminants.

A large impediment to the conduct of effective monitoring programmes has been continued disagreement about how to design and analyse sediment surveys. The ACME agreed that a resolution of these disagreements is urgently needed.

4.5.2 Normalization of organic contaminants in sediments

Request

This topic is part of continuing ICES work and is also of relevance to item 1.1 of the 1997 Work Programme from the Oslo and Paris Commissions.

Source of the information presented

The 1996 and 1997 reports of the Working Group on Marine Sediments in Relation to Pollution (WGMS) and ACME deliberations.

Status/background information

In 1993, the ACME approved Guidelines for the Use of Sediments in Marine Monitoring Programmes (ICES, 1994a). Annex 2 of these Guidelines concerns the use of normalization as a procedure to compensate for the influence of natural processes on the measured variability in the concentrations of contaminants in sediments. In this context, the ACME took note of the advantages and disadvantages of the two methods of normalization, namely, the geochemical and the fine fraction approaches (ICES, 1994a). In 1994, the ACME reviewed the results of a study on the relative efficiency of various sieving techniques on sediments and noted that dry sieving a freeze-dried mud in order to measure the quantity of fines was unacceptable (ICES, 1994b). The ACME also noted that questions had been raised on both the quantitative measurement of sediment fines and the extraction of fines for chemical analysis and stated that these should be clarified. At its 1995 meeting, WGMS studied these potential problems and concluded that wet sieving, including simultaneous ultrasonic treatment and the addition of agate balls, did not affect the concentrations of organic contaminants and that it seems to be an appropriate approach for compensating for grain size effects, except possibly for lindane and PAHs (ICES, 1995).

Further consideration of this topic was given in 1996 (ICES, 1996). Two approaches, one based on the partition theory of McKay and Paterson (1981) and the second on the use of semi-permeable membrane devices (Huckins *et al.*, 1993), were applied to metals and/or organic contaminants in sediments from the Ems-Dollard and the Western Scheldt, and the Wadden Sea, respectively. The ACME noted that the criteria of the partition (fugacity) theory of McKay and Paterson (1981), which considers that, at equilibrium, the ratio between the concentration and the uptake capacity is equal in each phase and also in each compartment within a sediment sample, were met for metals, PCBs, and PAHs when the normalization was optimized using total organic carbon (TOC) and the lutum content.

At its 1997 meeting, WGMS continued its review of the normalization of contaminant concentrations in sediments based on the partition theory of McKay and Paterson (1981). With the sediments from the Ems-Dollard, the elemental organic carbon (EOC, equal to TOC) was correlated with other parameters representing organic matter. An excellent correlation ($r^2 = 0.997$) was found between EOC and the wet oxidation method (oxidizable matter (OM) determined by bichromate oxidation). From the slope, an EOC content of 48 % was calculated for OM. Loss-on-ignition (LOI) at different temperatures showed that 550 °C was too high to represent the organic matter. LOI at 330 °C (LOI_{330}), but extended to as long as 124 hours, showed an acceptable relationship with the EOC content.

EOC, OM, and LOI_{330} showed positive correlations with both CB and PAH concentrations, but also displayed an intercept deviating from zero for the latter components. In a log-log plot, the slope becomes one for the CBs when LOI_{330} is used, but deviates from one when OM or EOC is chosen as the cofactor, especially in sandy samples. For PAHs, the slope was significantly different from unity when using EOC or OM, but approached unity when using LOI_{330}. WGMS concluded that the latter operationally defined and quite simple cofactor appears to be an efficient parameter to characterize the organic matter relationships of the organic contaminants. The commonly used parameters EOC or OM are acceptable only for CBs, but it should be clearly stated which method has been used to compare the results. No conclusion can be derived at present as to whether it is better to use the linear or log-linear relationship.

Need for further research

In view of the lack of consensus at the 1997 WGMS meeting about the use of a non-conservative measurement (TOC) as a normalizer, the ACME agreed with WGMS that more experiments are necessary to prove the wider applicability of LOI_{330} before it can be recommended as a normalizer for organic contaminants in sediments in favour over the more widely accepted EOC (TOC) and OM. The ACME noted that WGMS intends to conduct collaborative research on this issue.

References

Huckins, J.N., Manuweera, G.K., Patty, J.D., McKay, D., and Lebo, J.A. 1993. Lipid-containing semi-permeable membrane devices for monitoring organic contaminants in water. Environmental Science and Technology, 27: 2489–2496.

ICES. 1994a. Report of the ICES Advisory Committee on the Marine Environment, 1993. ICES Cooperative Research Report, No. 198: 45–57.

ICES. 1994b. Report of the ICES Advisory Committee on the Marine Environment, 1994. ICES Cooperative Research Report, No. 204: 27–29.

ICES. 1995. Report of the ICES Advisory Committee on the Marine Environment, 1995. ICES Cooperative Research Report, No. 212: 33–34.

ICES. 1996. Report of the ICES Advisory Committee on the Marine Environment, 1996. ICES Cooperative Research Report, No. 217: 37–38.

McKay, D., and Paterson, S. 1981. Calculating fugacity. Environmental Science and Technology, 15: 1006–1014.

4.6 Use of Organic Carbon Measurements in Chemical Oceanography

Request

There is no specific request; this is part of continuing ICES work on monitoring chemical components in the marine environment.

Source of the information presented

The 1997 report of the Marine Chemistry Working Group (MCWG) and ACME deliberations.

Status/background information

The ACME noted that MCWG has considered the usefulness of including organic carbon determinations in monitoring programmes. It reviewed a paper by M. Krysell (Sweden) on the use of organic carbon in chemical oceanography and studies of estuaries. The conclusion of MCWG is that there is no apparent reason to include organic carbon in monitoring programmes, but such data may be very useful in targeted experiments. The review paper is attached as Annex 2 to this report.

4.7 Statistical Aspects of Monitoring Programmes

4.7.1 Results of the ICES/OSPAR Workshop on the Identification of Statistical Methods for Trend Detection

Request

Item 3.1 of the 1997 Work Programme from the Oslo and Paris Commissions.

Source of the information presented

The 1997 reports of the ICES/OSPAR Workshop on the Identification of Statistical Methods for Trend Detection (WKSMTD) and the Working Group on Statistical Aspects of Environmental Monitoring (WGSAEM), and ACME deliberations.

Status/background information

Under item 3.1 of the 1997 OSPAR Work Programme, ICES was requested to organize, in cooperation with OSPAR, a joint ICES/OSPAR Workshop on the Identification of Statistical Methods for Trend Detection (WKSMTD). This Workshop was held in Copenhagen on 25–26 February 1997. Several data sets on atmospheric and riverine inputs of nutrients and certain contaminants were made available for use by the Workshop.

Briefly stated, the step-wise objectives presented to WKSMTD were to:

a) refine given criteria for harmonized statistical methods for the analysis of temporal trends in riverine and atmospheric inputs;

b) develop corresponding procedures;

c) consider whether these procedures could also be applied to data from other input sources;

d) apply these procedures to some data;

e) assess the power of these methods; and

f) if appropriate, recommend modifications to improve the power of the programme.

The criteria for harmonized statistical methods referred to in (a), above, relate to **what** data should be analysed (whether they should be annual or monthly loads, loads or concentrations, or flow-adjusted loads), **how** they should be statistically analysed (according to a common, unambiguous analysis providing indication of both the significance and the magnitude of any trend, and which is both powerful and robust), and **when** they should be analysed (before 1998 to provide results for the OSPAR Quality Status Report (QSR 2000)).

The ACME noted that there was still extensive debate about **what** should be measured. WKSMTD had mixed views on the value of assessing trends in estimated total annual loads (TALs). Essentially, it had been argued that for riverine inputs TALs include non-anthropogenic components which would be unchanged by control measures. For example, the natural component of phosphorus would not be changed by any input controls. Hence, TALs would not give direct evidence of the effectiveness of control measures. Furthermore, for atmospheric inputs, not all components are observed, and some are estimates based on modelling. There were also

unresolved discussions of whether concentration or load should be assessed, about the possible benefits of analysing data on a monthly basis, and about the necessity and/or advantages of adjusting riverine loads for variations in flow. Although WKSMTD discussed methods for flow-correcting riverine inputs and WGSAEM developed a simple statistical framework for comparing the efficiency of annual and monthly data, no simple conclusions were drawn.

The choice of **how** to assess trends in an inputs index depends not only on which specific index/indices are of interest, but also on what pattern(s) of temporal change OSPAR wants to be able to detect. A statistical method may be good at detecting one pattern, but poor at detecting another. To illustrate, consider the three stylized scenarios depicted in Figures 4.7.1.1.a, 4.7.1.1.b, and 4.7.1.1.c. These show, for a period of T years, (1) a linear decrease, (2) a step decrease, and (3) a period of increase followed by a period of decline, respectively. The magnitude of the signal is characterized by the value of k, the difference between the largest and the smallest values. Figures 4.7.1.2.a, 4.7.1.2.b, and 4.7.1.2.c show the corresponding powers of detecting these signals for three statistical methods: (1) linear regression (L), (2) a Mann-Kendall test (MK), and (3) a fitted Smoother (S). This last method is similar to that currently used in the OSPAR assessments of temporal trends in contaminant concentrations in biota. These figures are for $T = 10$ and for k scaled by the between-year residual standard deviation.

Figure 4.7.1.2.a shows, as would be expected, that when there is a linear trend, linear regression is uniformly more powerful than either the Mann-Kendall test or the Smoother. With a step-trend, linear regression still performs surprisingly well, and is more powerful than the Smoother (Figure 4.7.1.2.b). For $T = 10$ and short time series in general, the power of the Mann-Kendall test reaches an upper limit and eventually stops increasing with increasing k. Figure 4.7.1.2.c shows that both linear regression and the Mann-Kendall test, designed to detect monotonic trends, do extremely poorly for a case in which a period of increase is followed by a period of decline.

The ACME noted that for an assessment of TALs, WKSMTD had made some progress in measuring the likely effectiveness of the current OSPAR programme. Effectiveness is quantified in terms of the power to detect some signal and depends on both the size and the pattern of the signal and the magnitude of the residual variability. Estimates of the residual variances in the total annual loads (on a log scale) were made for eleven test data sets. These were used to estimate the percentage annual linear decrease (i.e., the slopes of the linear change in the logarithm of the total annual load) that would be detected with a 90 % power after ten years, using a test based on a smoother. These are shown in Table 4.7.1.1.

Approximately, detectable trends in total annual loads for the nutrients tend to be in the range 10–20 % per year. For cadmium, the detectable trend tends to be poorer, in the range 30–40 %.

Need for further research or additional data

The ACME noted the conclusions of WKSMTD and WGSAEM that if OSPAR wants to make an assessment of TALs (or annual average concentrations), this could easily be done. However, there is clearly a need for further scientific evaluation before a consensus about **what** to assess can be reached.

The ACME considered that there was a need for further clarification and specification of the requirements for assessments of inputs data. It may be that the assessments of both total annual input loads and concentrations, of flow-adjusted loads, or of more complex measurements all provide useful information. There may also be advantages in incorporating seasonal information by analysing, e.g., monthly data. Further research is required to resolve these fundamental issues.

If, in addition to the test data sets distributed to the WKSMTD, OSPAR has access to additional data sets consisting of monthly information on concentrations, flows and any additional covariables, the ACME recommends that they be collated and made available to WGSAEM.

Recommendations

ICES ACME considers that OSPAR should distinguish between its short-term objective of providing information for the QSR 2000 and what must be a longer-term objective of establishing a fully resolved protocol for reporting inputs.

In the longer term, ICES ACME recommends that OSPAR consider whether there is a need for closer consultation between a small number of statisticians and inputs-oriented scientists who, together, should have the technical skills to formulate technical objectives, resolve scientific issues, and assess methodology.

Figure 4.7.1.1.a. Scenario 1 shows a linear decrease over a period of T years. The value k is the difference between the largest and the smallest values.

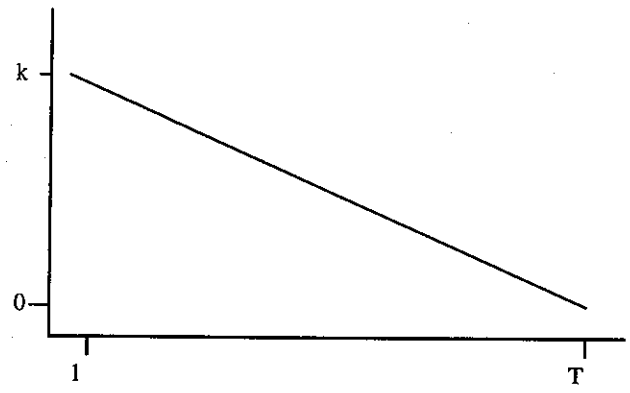


Figure 4.7.1.1.b. Scenario 2 shows a step decrease occurring within a period of T years. The value k is the difference between the largest and the smallest values.

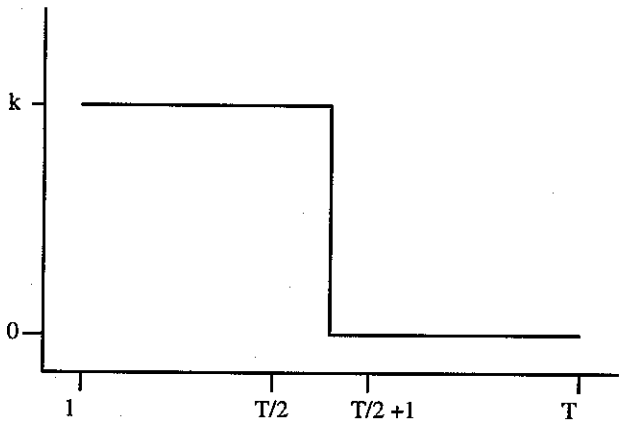


Figure 4.7.1.1.c. Scenario 3 shows a period of increase followed by a period of decrease occurring within T years. The value k is the difference between the largest and the smallest values.

Figure 4.7.1.2.a. The power of detecting the signal shown in Figure 4.7.1.1.a using linear regression (L), a Mann-Kendall test (MK), and a fitted smoother (S). $T = 10$ years.

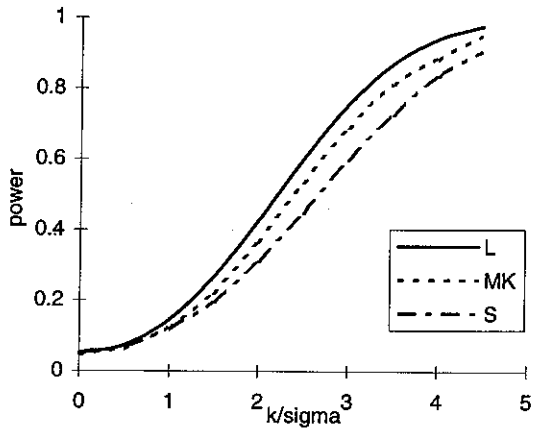


Figure 4.7.1.2.b. The power of detecting the signal shown in Figure 4.7.1.1.b using linear regression (L), a Mann-Kendall test (MK), and a fitted smoother (S). $T = 10$ years.

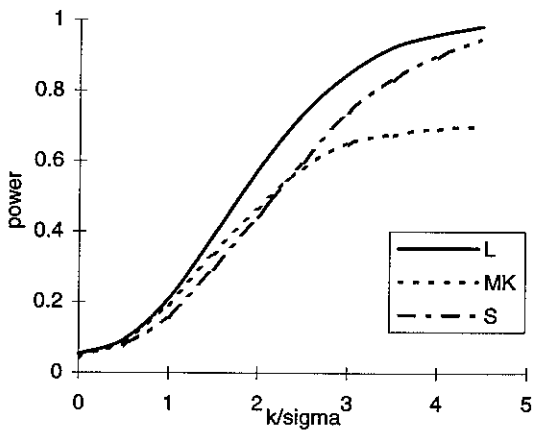


Figure 4.7.1.2.c. The power of detecting the signal shown in Figure 4.7.1.1.c using linear regression (L), a Mann-Kendall test (MK), and a fitted smoother (S). $T = 10$ years.

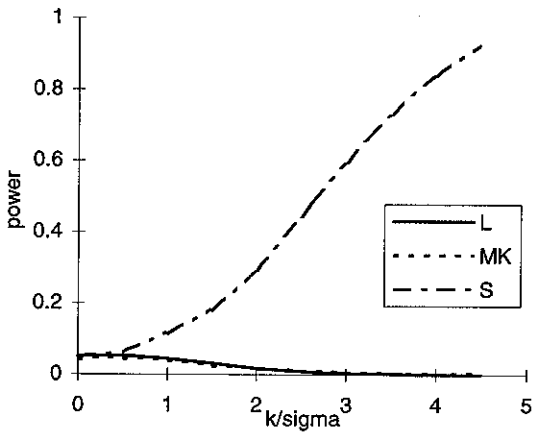


Table 4.7.1.1. Estimates of the residual variance in total annual load (on a log scale) and the percentage annual linear change that would be detected with 90% power after ten years for the data sets tested during the Workshop.

Source	Contaminant	% residual s.d.	% change per year
Atmospheric	NO_3	24	11
Atmospheric	NO_3	45	22
Atmospheric	NH_4	16	7
Riverine	NH_4	33	16
Atmospheric	Cd	77	37
Riverine	Cd	69	33
Riverine	Cd	77	37
Riverine	γ–HCH	20	10
Riverine	γ–HCH	64	31
Riverine	total N	27	13
Riverine	total P	41	20

In the short term, if OSPAR wants to make an assessment of total annual loads or concentrations, ICES ACME considers that the choice of statistical method would be clearer if an appropriate statistical objective were declared. (If there are complex or multiple objectives, an appropriate single, simple objective may serve as a convenient surrogate for the others.) For example, if OSPAR decides that its objective is

to detect any downward, monotonic trend sustained over the whole period of monitoring using a method that is not sensitive to errors of measurement and is not reliant on unrealistic statistical assumptions,

then a simple Mann-Kendall test with an appropriate significance level would probably suffice. However, if the objective is

to detect both monotonic and non-monotonic changes (e.g., a trend consisting of periods of both increase and decrease),

then it would be more appropriate to use the tests based on a smoother. The choice of method may also be tempered by the availability of suitable software and should be guided by statistical consultation.

If this objective were extended to include a target of the magnitude of trend that the method should be able to detect, then it would be possible to make a retrospective power analysis to reveal whether the programme and method of analysis are meeting this target. For example, the target could be

to detect a linear trend of approximately 7 % per year (equivalent to 50 % in ten years) with a probability of 90 % after ten years.

Indications that this target was not being achieved might suggest that a different action should be taken, such as using a less robust but more powerful method, adopting a less ambitious target, or revising the programme.

4.7.2 Review of plans for an optimized U.S. Mussel Watch Program

Request

There is no specific request; this is part of continuing ICES work to provide advice on the development of effective monitoring strategies.

Source of the information presented

The 1997 report of the Working Group on Statistical Aspects of Environmental Monitoring (WGSAEM) and ACME deliberations.

Status/background information

The ACME noted that WGSAEM had considered two aspects of the U.S. Mussel Watch Program:

1) how the precision of the programme is affected by climatic and analytical factors; and
2) the power of the programme to detect national or regional trends.

The results of these considerations are summarized below.

Effect of Environmental and Analytical Factors

The effects of factors affecting the precision of the programme were assessed using data from ten contiguous regions of the U.S. coast (Beliaeff *et al.*, 1997). Each region contained from 10 to 25 sites, selected on the basis of median air temperature and commonality of species. A linear model was applied to the log-transformed data with stepwise additions for the effects of year, temperature, precipitation, and changes in laboratory or chemical method. The analysis was performed for concentrations of eight trace elements and six groups of organic contaminants measured annually from 1986 through 1994.

Decreasing trends were found for chlorinated organic contaminants in most of the regions. Among the metals, copper and cadmium concentrations were decreasing in three regions; other metals displayed no trend, except for one region-wide trend. Although the climatic effects were not significant, there was evidence of analytical and/or laboratory effects. These latter effects imply that trends should be selectively reported, with some prior screening of data sets similar to that made in the OSPAR and HELCOM assessments.

Programme Design for Estimating a Regional Trend

The basic assessment of data from the U.S. Mussel Watch Program described above consists of a summary of results of individual Mann-Kendall tests of the trend at each site. A problem is then how to interpret results where the emphasis is upon finding evidence of national or regional changes when significant trends have been found at some sites, but not at others.

One possibility is to do a meta analysis of the collected results of the individual analyses. For example, the meta analysis may consist of a test to see whether the number of individually significant trends is greater than would have occurred by chance. The following theoretical development is presented to show how this works in practice.

Suppose there are N independent sites, and that an annual index has been measured at each site, every year, for T years. Further, suppose a trend test (e.g., a Mann-Kendall test) has been made at each site, resulting in y significant trends at the α_1 significance level.

A simple meta analysis might then pose the question: Could y significant trends have been observed by chance? If p is the probability of observing a significant trend at a site (i.e., the power of an individual test), then the meta test is equivalent to testing the hypotheses

H_0: $p \le \alpha_1$ versus H_1: $p > \alpha_1$.

If the size of this test is taken to be α_2, then an appropriate meta test based on the Binomial distribution is to reject H_0 if

$$\text{Prob}(\text{Bi}(N, \alpha_1) \ge y) \le \alpha_2.$$

In order to choose an appropriate number of sites at which to monitor, the meta power of the binomial test must be derived. Suppose there is a linear trend β, common to each site. (To be specific, β is the change in the mean index each year at each site.) Further, let σ be the residual between-year standard deviation in the annual index, again assumed common to each site.

The power of our (single-site) test, p, depends on the test used, the size of the test α_1, the signal-to-noise ratio β/σ, and the number of years, T. Now define r_N to be the smallest integer such that

$$\text{Prob}(\text{Bi}(N, \alpha_1) \ge r_N) < \alpha_2. \qquad (1)$$

The meta power of the meta test is then

$$MetaPower = \text{Prob}(\text{Bi}(N, p) \ge r_N).$$

An approximate meta power formula can be obtained by using a Normal approximation to the Binomial distribution:

$$MetaPower = 1 - \Phi[\Phi^{-1}(1-\alpha_2)\sqrt{\frac{\alpha_1(1-\alpha_1)}{p(1-p)}} - \frac{N(p-\alpha_1)}{\sqrt{Np(1-p)}}] \qquad (2)$$

where $\Phi(.)$ denotes the Normal distribution function and $\Phi^{-1}(.)$ its inverse. This formula should be adequate provided that neither of $N\alpha_1$ and Np are too close to 0 or N.

These equations can be made more explicit by incorporating specific values of p corresponding to a Mann-Kendall test carried out at each site. Values of p for other tests could, of course, be used. The values are taken from Nicholson *et al.* (1997) who present p for the Mann-Kendall test for a range of values of β/σ with $\alpha_1 = 5$ % and $T = 10$ and 20.

The number of independent sites required to achieve a specified power in the meta test is the minimum value of N such that

$$\text{Prob}(\text{Bi}(N, p) \ge r_N) \ge MetaPower \qquad (3)$$

or, using the Normal approximation,

$$N = \left[\frac{\Phi^{-1}(1-\alpha_2)\sqrt{\alpha_1(1-\alpha_1)} - \Phi^{-1}(1-MetaPower)\sqrt{p(1-p)}}{p-\alpha_1}\right]^2.$$

These results are demonstrated using estimates of the residual between-year standard deviation (on a log scale) for Cd, Hg, Pb, chlordane, DDT, and PCB, obtained from 156 time series in the U.S. Mussel Watch Program. Table 4.7.2.1 gives the 25 %, 50 %, and 75 % quantiles of the residual between-year standard deviation, σ, for each contaminant. These quantiles have been labeled as *low*, *medium*, and *high*. (To simplify the presentation and reduce the size of the tables, the values for Hg and Pb have been combined, as have the values for chlordane, DDT, and PCB. The corresponding quantiles were sufficiently similar to make this sensible.)

Table 4.7.2.1. The 25 %, 50 % and 75 % quantiles (labeled as *low*, *medium* and *high*) of the residual between-year standard deviation, σ, for specific contaminants.

	Residual between-year standard deviation σ		
	low	*medium*	*high*
Cd	0.16	0.22	0.28
Hg and Pb	0.21	0.29	0.39
Chlordane, DDT, PCB	0.34	0.45	0.61

Table 4.7.2.2 shows the number of independent sites required to detect a meta trend of $\beta = 0.01$, 0.02, 0.05, 0.10, and 0.20 over $T = 10$ and 20 years for each contaminant with 90 % power. Throughout, the sizes of both the single-site Mann-Kendall test and the meta test have been taken to be 0.05. Note that the trends are on a log scale, so, e.g., $\beta = 0.02$ corresponds to approximately a 2 % change in concentration per year. The values have been calculated using the exact binomial formulae (equations 1 and 3).

Thus, for example, to detect a regional trend of $\beta = 0.05$ in log Cd concentration over ten years with 90 % power would require somewhere between 5 and 26 independent sites. However, a similar trend for chlordane, DDT, and PCB would require somewhere between 51 and 432 independent sites. If the programme were extended to last twenty years, only two and 3–7 independent sites, respectively, would be required.

Table 4.7.2.2. Number of independent sites required to detect a meta trend β over T years with 90 % power for each contaminant or group of contaminants.

Cd						
	$T = 10$			$T = 20$		
β	$\sigma = 0.16$	$\sigma = 0.22$	$\sigma = 0.28$	$\sigma = 0.16$	$\sigma = 0.22$	$\sigma = 0.28$
0.01	-	-	-	21	50	106
0.02	91	282	765	3	6	13
0.05	5	13	26	2	2	2
0.10	3	3	4	2	2	2
0.20	3	3	3	2	2	2
Hg and Pb						
	$T = 10$			$T = 20$		
β	$\sigma = 0.21$	$\sigma = 0.29$	$\sigma = 0.39$	$\sigma = 0.21$	$\sigma = 0.29$	$\sigma = 0.39$
0.01	-	-	-	40	120	298
0.02	234	891	-	5	14	36
0.05	12	28	80	2	2	3
0.10	3	4	7	2	2	2
0.20	3	3	3	2	2	2
Chlordane, DDT, PCB						
	$T = 10$			$T = 20$		
β	$\sigma = 0.34$	$\sigma = 0.45$	$\sigma = 0.61$	$\sigma = 0.34$	$\sigma = 0.45$	$\sigma = 0.61$
0.01	-	-	-	199	415	-
0.02	2039	-	-	23	52	138
0.05	51	137	432	3	4	7
0.10	6	13	36	2	2	2
0.20	3	3	5	2	2	2

Need for further research or additional data

Designing programmes to ensure detection of regional trends provides an interesting and potentially useful example of alternative ways to specify objectives for monitoring programmes. Further development of this approach might consider the following issues.

- The theory and example above make a number of simplifying assumptions. It is important to investigate the implications of relaxing (i.e., having less stringent or fewer of) these assumptions. For example, we assumed a common trend at all sites. In practice, it might be more reasonable to assume that the trend at each site varies about a common trend with a certain distribution.
- Similarly, it may be more realistic for the residual variance at a site to also be drawn from some probability distribution. This would also obviate the need to present a range of values of N for each contaminant.
- A more powerful procedure might be to estimate the trend at each site and then use this summary statistic in a meta analysis (rather than the binary yes/no corresponding to significance/non-significance). In this case, it would not be necessary to have observed significant individual-site trends.

Recommendations

ICES ACME encourages further development of appropriate statistical objectives for monitoring programmes.

References

Beliaeff, B., O'Connor, T.P., Daskalakis, D.K., and Smith, P.J. 1997. U.S. Mussel Watch data from 1986 to 1994: temporal trend detection at large spatial scales. Annex 5. Report of the Working Group on Statistical Aspects of Environmental Monitoring. ICES CM 1997/Env:1.

Nicholson, M.D., Fryer, R.J., and Maxwell, D. 1997. A study of the power of various methods for detecting trends. Annex 9. Report of the ICES/OSPAR Workshop on the Identification of Statistical Methods for Trend Detection. ICES CM 1997/Env:11.

5 QUALITY ASSURANCE PROCEDURES AND INTERCOMPARISON EXERCISES

5.1 Quality Assurance of Biological Measurements in the Baltic Sea

Request

Item 2 of the 1997 requests from the Helsinki Commission.

Source of the information presented

The 1997 report of the ICES/HELCOM Steering Group on Quality Assurance of Biological Measurements in the Baltic Sea (SGQAB) and ACME deliberations.

Status/background information

The introduction of quality assurance (QA) procedures into the biological measurements in the HELCOM Baltic Monitoring Programme has proceeded in a systematic manner since the establishment of the ICES/HELCOM Steering Group on Quality Assurance of Biological Measurements in the Baltic Sea in late 1992. To date, the following progress has been achieved (see also Table 5.1.1):

- Almost all HELCOM countries have 'In-house Manuals', including also QA procedures for sampling and analysis, under preparation or completed.
- Laboratories in three countries (Finland, Lithuania and Sweden) have obtained national certificates or accreditation for biological variables (excluding primary production).
- Training courses on taxonomic variables have become (phytoplankton) or are becoming (zooplankton and zoobenthos) a routine annual activity.
- The annual phytoplankton training courses cover several matters related to QA of phytoplankton analyses (not only species identification).
- Standardization of methodology for monitoring phytoplankton, primary productivity, zooplankton and macrozoobenthos has progressed via the two workshops on pelagic biology organized in 1995 (chlorophyll *a*, microbiology) and 1996 (meso-zooplankton, primary production), two workshops on benthic biology (macrozoobenthos) organized in 1994 and 1996, and will continue to advance via the workshops to be organized in 1997 (microzooplankton, macrozoobenthos).

It is, however, a matter of concern that Russian scientists have not participated in any stages of the QA development.

Need for further research

A key issue for further progress in establishing quality assurance procedures for biological measurements is the need for agreement on a uniform data reporting format and a new taxonomic coding system. The present RUBIN taxonomic code is no longer being updated (see Section 17.3, below), and HELCOM has asked ICES for assistance in this matter. This need has been stressed not only for phytoplankton but also for zooplankton and macrozoobenthos, and it is of mutual concern for activities within HELCOM and OSPAR.

Another urgent matter concerns the lack of biological Reference Materials (RMs). At present, few or no Certified Reference Materials (CRMs) are available for the biological parameters covered in the HELCOM and OSPAR monitoring programmes. From the quality assurance point of view, such materials are essential for both interlaboratory and intralaboratory quality routines, and this is particularly evident in both monitoring programmes within the field of taxonomy.

Recommendations

ICES ACME recommends that the Steering Group on Quality Assurance of Biological Measurements in the Baltic Sea continues its efforts to ensure that all laboratories associated with the HELCOM monitoring programme develop their 'In-House QA Manuals' for the benefit of increased quality and comparability of data. ICES ACME also supports the efforts to continue the training courses and workshops on sampling, laboratory analysis, and taxonomy for the staff involved in the monitoring programmes. A close cooperation with the newly formed ICES/OSPAR Steering Group on Quality Assurance of Biological Measurements related to Eutrophication Effects (SGQAE) is encouraged as many issues are of joint interest. Finally, ICES ACME shares the concern of SGQAB that it is extremely important for Russian participants to join the QA activities.

5.1.1 Results of the ICES/HELCOM Workshop on Quality Assurance of Pelagic Biological Measurements in the Baltic Sea

Request

Item 2 of the 1997 requests from the Helsinki Commission.

Source of the information presented

The report of the 1996 ICES/HELCOM Workshop on Quality Assurance of Pelagic Biological Measurements in the Baltic Sea and ACME deliberations.

Table 5.1.1. Summary of the present stage of the introduction of QA procedures into biological monitoring in countries around the Baltic Sea.

Country	In-House Manual for sampling	In-House Manual for biological variables	Accreditation for sampling	Accreditation for biological variables	Remarks
Denmark	under way	under way	no	no	
Estonia		yes	no	no	financial problems in accreditation
Finland	yes	yes	yes	yes	primary production not accredited, coastal monitoring not accredited
Germany	under way	under way	no	no	accreditation is not a target
Latvia	yes	yes	no	no	
Lithuania	yes	yes	yes	yes	primary production not accredited
Poland	yes	yes	no	no	
Russia					no information available
Sweden	yes	yes	some laboratories soon	yes	accreditation is a prerequisite for BMP laboratories

Status/background information

The aim of the ICES/HELCOM Workshop on Quality Assurance of Pelagic Biological Measurements in the Baltic Sea (Warnemünde, October 1996) was to prepare well-defined recommendations for HELCOM on the revision of the Baltic Monitoring Programme and its Guidelines concerning measurements of mesozooplankton and primary productivity. As the basis for the recommendations, demonstrations and comparisons of equipment, sampling, and other techniques were conducted.

Three sampling techniques for zooplankton (WP-2 net with 55 µm, 100 µm and 200 µm mesh size, Juday-net with 160 µm mesh size, and Danish plankton pump with 100 µm mesh size) were demonstrated at sea and compared quantitatively and qualitatively during the Workshop. As could be expected, highly deviating results were produced due to the principal differences between the methods. Several items in sampling were highlighted and corresponding recommendations to HELCOM were prepared. In principle, these recommendations added some details and/or emphasized the importance of following the already accepted Guidelines.

The use of only the 100 µm WP-2 net was recommended, and therefore the pumping system used by Denmark should be replaced by this net. The Workshop drew attention to the role of the wire angle for the quality of the data and set 25 kg (in some cases, 40 kg) as the minimum weight to keep the wire vertical; it also stressed the importance of reporting the wire angle. The sampling intervals were defined as (1) sea bottom to halocline (included), (2) top of the halocline to the thermocline (included), and (3) top of the thermocline to the sea surface. If there is no thermocline, the standard haul should be 0–25 m, and if there is no halocline, the standard haul should be 75 m to the thermocline or to 25 m. Furthermore, the Workshop felt that the use of flow meters is always necessary and that attention must be paid to the rinsing of the net. For subsampling, a calibrated Stempel-pipette or a Kott splitter was recommended.

Concerning the counting procedure and analysis, the Workshop recommended that:

- the minimum microscope magnification should be 125 x;
- the minimum number of individuals to be counted should be 100, of the three most dominant groups;
- biomass factors should be used for the different taxonomic groups and developmental stages;
- in addition, direct measurements of ash-free dry mass could be conducted, on a voluntary basis.

Finally, the Workshop felt that the use of the same type of computer software would improve the data quality and facilitate and unify the data reporting.

The question of updating the taxonomic coding system was discussed and the need for a rapid solution to this question was emphasized (see also Section 17.3, below).

In this respect, the Workshop recognized coinciding needs between HELCOM and OSPAR, and proposed that this matter be considered at the next joint meeting of the ICES/HELCOM Steering Group on Quality Assurance of Biological Measurements in the Baltic Sea (SGQAB) and the ICES/OSPAR Steering Group on Quality Assurance of Biological Measurements related to Eutrophication Effects (SGQAE) in February 1998. SGQAB has recognized the urgency of this matter, but pointed out the need for external funding for such a large amount of work.

The primary production session of the Workshop consisted of demonstrations of various steps (measurements of irradiance profiles, estimation of production-irradiance (P-I) relationship) in the ^{14}C P-I incubator technique using the standard 'ICES incubator'. The Workshop reviewed the results of a questionnaire concerning details of the primary production measurements in different laboratories. It was noted that very few institutes follow the present guidelines and very different procedures are used. Therefore, the necessity for obtaining agreement on a standard method was stressed. The recommended procedure was the following:

- use of the P-I method in a standardized incubator;
- use of ^{14}C concentrations which allow statistically sufficient counts;
- incubation time of 2 hours;
- incubator temperature corresponding to *in situ* temperature;
- incubator light level preferably up to 400 $\mu E\ m^{-2}\ s^{-1}$;
- avoidance of 'outside' light;
- sampling during daytime;
- sampling from a mixed euphotic layer, with additional samples taken if the euphotic layer is stratified;
- use of a silicon hose to take integrated samples;
- termination of incubation by filtration on GF/F filters;
- only the scintillation counting technique should be used for radioactivity measurement (quench curves should be established and the efficiency of the counter should be checked using an internal standard);
- dissolved inorganic carbon (DIC) should be measured according to a standard procedure or calculated from Buch's formulas;
- light attenuation should be measured as photosynthetic available radiation (PAR, 400–700 nm), not as global irradiance.

A minimum sampling frequency of fifteen times per year in the open sea and 20–25 times per year at coastal stations was recommended. The need for updating data reporting formats was recognized.

Need for further research

The many deviations from the Guidelines in respect to the methodology used in the Baltic Monitoring Programme (BMP) clearly illustrate the need for further intercomparisons and intercalibrations. This is particularly important as the many national coastal programmes will now be linked to the open sea BMP. As the new revised version of the BMP Guidelines (to be issued by HELCOM in 1998) will contain instructions for QA procedures for each parameter, it is to be anticipated that methodological issues must be given higher priority in order to fulfill data quality requirements.

Recommendations

ICES ACME recommends that, in the light of the outcome of the workshops arranged and the new extended Baltic Monitoring Programme, the Steering Group should continue to coordinate intercomparison and intercalibration exercises. ICES ACME also recognizes the need for a uniform data reporting format and taxonomic coding system.

5.2 Quality Assurance of Biological Measurements in the OSPAR Area

Request

Item 2.1 of the 1997 Work Programme from the Oslo and Paris Commissions.

Source of the information presented

The 1997 reports of the ICES/OSPAR Steering Group on Quality Assurance of Biological Measurements related to Eutrophication Effects (SGQAE) and the Benthos Ecology Working Group (BEWG), and ACME deliberations.

Status/background information

a) SGQAE Strategy for Practical Implementation of QA Programmes

The ACME took note that, in response to a request from the Oslo and Paris Commissions, ICES established an ICES/OSPAR Steering Group on Quality Assurance of Biological Measurements related to Eutrophication Effects (SGQAE) at the 1996 Annual Science Conference. The first meeting of SGQAE was held at ICES Headquarters in February 1997. After reviewing the information available on relevant biological

monitoring programmes and any quality assurance (QA) procedures associated with them, SGQAE considered the biological measurements to be taken under the OSPAR Nutrients Monitoring Programme to determine eutrophication effects and agreed on the strategy described here for the implementation of a QA programme.

For phytoplankton/chlorophyll *a*, the priority is likely to be for international-level QA assessment, at least at the level of sampling methodology, since the same (or similar) approaches will apply throughout the OSPAR area. It is also self-evident that the habitat, i.e., the water column, is dependably present at all locations. This is in contrast to some benthos studies, where site-specific factors may determine differences in target organisms and sampling methods: not all countries will be involved in identical survey and sampling approaches. An example would be the presence or absence of a coastal rocky habitat. Also, biogeographical factors affecting phytoplankton populations and the benthos of widely distributed habitats (such as soft bottoms) may, in practice, limit the scope or necessity for intercomparisons of proficiency in species identification across all OSPAR countries. For example, biogeographical provinces across the OSPAR area range from Arctic Boreal to Lusitanean.

This suggests that a tiered approach to QA initiatives, i.e., varying from the level of the laboratory to the national or international level, would be appropriate. Such an approach would also, incidentally, highlight the priorities that would need to be given to the development of central databases for different subject areas.

It is also to be expected that there will be some examples of entrenched differences in sampling approaches between countries even for comparable habitats, e.g., where evidence for the greater efficiency of one sampling device over another is unconvincing. Here, personal preferences or historical precedents will be influential. There is no intrinsic reason why this should lead to significant problems with the quality of the resulting data, provided that acceptable documentation is available as to accuracy, precision, representativity, etc., of the data.

SGQAE emphasized the fundamental importance attached to agreement among participating countries on basic sampling issues such as mesh size, criteria for acceptance/rejection of field samples (e.g., for sediment macrofauna, based on sample volume and visual appearance), and consistency in the timing of annual or more frequent surveys. Disparities here will nullify any benefits of sound QA, when it comes to intercomparisons of the results.

It is essential that support at the national level be firmly established for the principle of sound QA of biological measurements. Strong support can also be given to the view of the ICES/HELCOM Steering Group on Quality Assurance of Biological Measurements in the Baltic Sea (SGQAB) that, in the application of this principle, emphasis should be placed upon the performance of the individual scientist or technician at the laboratory bench. Central to a successful outcome is a sound laboratory QA system, and this must be encouraged as a starting point. Also, along the path of developing effective QA procedures, the aim should be to persuade and assist, rather than simply to dismiss poor performance when measured against agreed standards of acceptability. SGQAE also endorsed the view that the aims of the monitoring programme should be carefully considered, in order to derive realistic QA targets. Such a pragmatic approach to the issue is permissible, given the early stages of development both of a coordinated monitoring programme and a supporting QA strategy.

These deliberations must be translated into a practical programme for the various subject areas. A consideration of the entire history of a sample provides a framework for identifying priority areas, i.e., from field sampling through laboratory analysis to the final analysis and then archiving the resulting data and analysed material.

The task is therefore:

a) to acknowledge the fact that the different steps in a monitoring exercise have variable influence on the accuracy and precision of the resulting data, and to develop a priority list with attention to the most important field and laboratory stages with respect to their influence on data quality. Common sense dictates that the list will be biased towards the more intractable problems, such as laboratory taxonomic issues, and quality control of key areas of field sampling activity;

b) to assess the variability in methodology of these stages as reflected in the draft OSPAR Joint Assessment and Monitoring Programme (JAMP) guidelines for the relevant monitoring components;

c) in view of Task (b), above, to identify the most critical QA elements in each step of the methodology;

d) to propose a set of priority QA areas and to identify the best means to address them (field/laboratory workshops; intercalibrations, including data analysis techniques as well as sample processing; the drafting and adoption of Standard Operating Procedures (SOPs) and the associated production of 'quality manuals'; pursuit of formal accreditation, etc.). For SOPs/quality manual production, SGQAE could offer basic guidance on 'best practice'. Examples of SOPs covering specified topics will be requested from a range of laboratories for review at the next meeting, as well as examples of actions to be taken to ensure a quality which is fit for the purpose;

e) to consider organizational aspects and realistic time scales. (Depending on the topic, tiers of activity may be identified, ranging from intralaboratory work to between-laboratory comparisons within and across

some or all countries). These will then determine the appropriate level of participation, i.e., local, national, 'regional' (groups of countries), or 'global' (all countries);

f) to consider practical implementation (including numbers of participants that are likely to be involved in different activities, and realistic workshop sizes to aim for), and also likely funding opportunities;

g) to identify 'secondary' (supplementary) variables relevant to the interpretation of biological data, and to seek guidance on which ICES or OSPAR groups are best placed to deal with them.

The ACME accepted the above strategy for the work of SGQAE and noted that several ICES Working Groups, including the Benthos Ecology Working Group (BEWG) and the Working Group on Phytoplankton Ecology (WGPE), will be requested to assist SGQAE in the development of specific QA procedures under their remits.

b) Consideration of Benthos Monitoring and Associated QA

The ACME noted that BEWG reviewed the OSPAR guidelines for benthos monitoring and made a number of suggestions to improve the text.

BEWG also considered the results of the 1997 meeting of the ICES/HELCOM Steering Group on Quality Assurance of Biological Measurements in the Baltic Sea (SGQAB). For the macrozoobenthos aspects, a relevant QA chapter will be prepared by a member of BEWG. SGQAB also decided on details of the terms of reference for three training workshops which include one workshop on benthic taxonomy in Roskilde, Denmark in November 1997.

BEWG expressed support for CD-ROM projects with taxonomic identification aids to contribute to the QA of benthos measurements in terms of taxonomy.

The ACME took note of the BEWG review of the report of the ICES/OSPAR Steering Group on Quality Assurance of Biological Measurements related to Eutrophication Effects (SGQAE). BEWG noted that the benthos habitats referred to were, in all cases, distributed across more than one country although, clearly, their relative importance varied substantially (e.g., rocky habitats). It was, therefore, likely that there would be a requirement for across-country syntheses of data covering all benthic habitats and, hence, a parallel requirement for consistent QA procedures at an international level.

Tabulations of QA issues relating to benthos studies (appearing as annexes to the SGQAE report) were considered to be acceptable. It was felt that high priority should be attached to the training element in raising the quality of the data eventually generated.

BEWG had little experience regarding the application of QA criteria for acceptance or rejection of benthos data. It was noted that commercial consultants are presently more accustomed to operating to such criteria. There would be benefits in having case studies of their application presented at future meetings of BEWG.

A compilation of an inventory of guidelines for the conduct of benthos surveys operated by different countries would be useful (in addition to recognized publications of international organizations such as ICES and HELCOM). This could include countries both within and outside the OSPAR area.

Experience with the compilation of data from the 1986 ICES North Sea Benthos Survey was instructive, as an indication of the problems that may occur in the synthesis of data from different laboratories and/or countries, especially with regard to taxonomy. The relevant information has been published in *ICES Cooperative Research Report* No. 218.

It is to be expected that the databank manager will be responsible for applying 'plausibility' controls on incoming data, in order to filter out nonsensical entries such as gross errors in station positions or sampling dates. Standard software is presently applied within ICES for data on chemical contaminants. Judgements on the scientific acceptability of the data will be the responsibility of expert assessors.

The specifications for benthos sampling at North Sea Task Force stations contained in the 1990 BEWG report were reviewed, in order to provide updated guidance on certain fundamental questions relating to the initiation of soft-bottom benthos surveys in the OSPAR context. This update highlighted key points which have been addressed in more detail in published ICES, HELCOM, and OSPAR guidelines.

The ACME endorsed BEWG guidance on basic approaches to be adopted in the conduct of surveys of soft-bottom macrofauna under OSPAR auspices, as follows:

1) Wherever possible, representative stations should be chosen to correspond with pre-existing stations for which good historical data are available.

2) Sediments should be sampled using 0.1 m^2 grabs and/or corers, and sieved to 1 mm for the macrofauna.

3) In cases where there may be advantages to additional investigation of the smaller macrofauna component (i.e., down to 0.5 mm), nested 1 mm and 0.5 mm meshes should be used and the samples should be processed separately.

4) A minimum of five replicate samples should be taken at individual stations selected for the examination of temporal trends. Depending on the monitoring objectives, stratified random sampling, with one replicate collected at a sufficient number of stations to satisfy statistical requirements, is also acceptable for this purpose.

5) Coordination in survey timing will be essential for across-country comparisons of spatial patterns and for the evaluation of temporal trends. Sampling in the period February–May is recommended, since this will tend to limit 'noise' arising from the transient presence of many newly recruited juveniles. It will also facilitate sorting of samples and will reduce taxonomic problems associated with the presence of small specimens.

6) The minimum acceptable sample size for soft sediments collected by grabs is 5 litres, with the exception of hard-packed sands, which is 2.5 litres.*

7) Identification should be to species level, wherever possible. Records should be made at the next highest taxonomic level whenever uncertainties exist.

5.3 Quality Assurance Procedures for Biological Effects Measurements

5.3.1 Proposed QA programme for biological effects techniques

Request

Item 2.2 of the 1997 Work Programme from the Oslo and Paris Commissions.

Source of the information presented

The 1997 report of the Working Group on Biological Effects of Contaminants (WGBEC) and ACME deliberations.

Status/background information

The ACME noted that WGBEC agreed that there is an urgent need to establish quality assurance (QA) procedures for the biological effects techniques in the OSPAR Joint Assessment and Monitoring Programme (JAMP) including, *inter alia,* measurements of DNA adducts, metallothionein induction, D-aminolevulinic acid dehydratase (ALA-D) activity, lysosomal stability, lipid peroxidation, and cytochrome P4501A induction. Detailed proposals for QA procedures for these techniques have already been made by ACME in 1996 (ICES, 1996). WGBEC noted that funding for such a programme is unlikely to come primarily from OSPAR Contracting Parties, but may be possible from the European Commission (EC) Standards, Measurement and Testing Programme, and that the successful QUASIMEME scheme for QA of chemical monitoring measurements might form a useful general model. It was therefore agreed that WGBEC would, intersessionally and in collaboration with other relevant ICES Working Groups and Study Groups, draft a funding bid to the EC. This would include techniques of interest to HELCOM, MEDPOL, and the Black Sea Commission, as well as OSPAR. It should be noted that the OSPAR Environmental Assessment and Monitoring Committee (ASMO) has already agreed that such a proposal is very welcome, and ASMO will do what it can to support this initiative.

It was thought essential that such a QA programme, while recommending particular techniques, would not be prescriptive about them, but would instead be results-driven, i.e., the precise methods are irrelevant if the results are correct.

The infrastructure of this QA programme should consist of three tiers:

1) participating laboratories;

2) a Central Steering Group, which would set overall policy, control the budget, appoint experts to each of four Subject/Area Subgroups, and communicate results externally;

3) expert laboratories, appointed under contract by the Subject/Area Subgroups, responsible for driving forward the development of QA for particular methods by organizing practical workshops for participating laboratories, providing training, agreeing acceptable limits of variation, providing at least three reference materials, and evaluating the reproducibility of results.

Finally, the expert laboratories would evaluate the repeatability of reference results generated by participating laboratories over a period of time, and would assess their performance against agreed criteria. They would then decide which laboratories were in compliance with the scheme at any one time.

The four Subject/Area Subgroups would initially cover the techniques listed in Table 5.3.1.1.

WGBEC identified a number of scientists who were willing, with others, to help prepare a funding bid to the EC, and who would form the nucleus of the eventual Central Steering Group. It was recognized that scientists associated with several ICES groups (WGBEC, WGPDMO, BEWG, WGPE, WGZE, SGQAB, SGQAE)

* Further details on criteria for judging the acceptability of samples at the time of collection can be found in the report of the 1994 ICES/HELCOM Workshop on Quality Assurance of Benthic Measurements in the Baltic Sea (ICES CM 1994/E:10) and in a forthcoming revision of *ICES Techniques in Marine Environmental Sciences* No. 8.

Table 5.3.1.1. Proposed infrastructure of a QA programme for biological effects monitoring techniques, with examples of techniques that could be included in each Subject/Area Subgroup.

CENTRAL STEERING GROUP			
SUBJECT/AREA SUBGROUPS			
Pathology	Community	Biochemistry	Bioassays and Physiology
Liver histopathology	Benthic communities	P4501A induction	Water bioassays
Liver nodules	Phytoplankton assemblages	Metallothionein induction	Sediment bioassays
Lysosomal stability	Zooplankton assemblages	PAH metabolites	Fish reproductive success (viviparous blenny)
Imposex/intersex		DNA adduct formation	[Scope for growth]
Oyster shell thickening		ALA-D induction	
Externally visible fish diseases		Antioxidant enzyme induction	
		[Vitellogenin induction]	
		[AChE inhibition]	

Note: Square brackets indicate that the technique is not currently part of standard international monitoring suites, but may soon become so.

should be included in this bid. For each method, WGBEC also identified a number of experts whose parent organizations (see Annex 5 of ICES, 1997) would be suitably experienced expert laboratories within the scheme, although final decisions on this subject will be left to the Central Steering Group.

The ACME endorsed these proposals.

References

ICES. 1996. Report of the ICES Advisory Committee on the Marine Environment, 1996. ICES Cooperative Research Report, No. 217: 105–119.

ICES. 1997. Report of the Working Group on Biological Effects of Contaminants. ICES CM 1997/Env:5.

5.3.2 Quality assurance plan for studies of fish liver histopathology and externally visible fish diseases

Request

Item 2.2 of the 1997 Work Programme from the Oslo and Paris Commissions.

Source of the information presented

The 1997 report of the Working Group on Pathology and Diseases of Marine Organisms (WGPDMO), the report of the ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants, and ACME deliberations.

Status/background information

The ACME took note of the 1997 report of WGPDMO, which provided information on the status of quality assurance with regard to studies on externally visible fish diseases, macroscopic liver nodules, and liver histopathology of the common dab (*Limanda limanda*), all of which are techniques to be incorporated into the OSPAR JAMP according to the draft OSPAR Guidelines for General Biological Effects Monitoring.

WGPDMO noted that some of the tables in Technical Annex 1 of the draft OSPAR Guidelines for General Biological Effects Monitoring relating to the above studies did not accurately reflect the ICES standard methodologies and, therefore, required revision. A revised version of these tables was prepared by WGPDMO. The ACME reviewed these revised tables and accepted them (see Table 5.3.2.1) for transmission to OSPAR.

The ACME endorsed the view of WGPDMO that quality assurance procedures for studies of externally visible diseases and macroscopic liver nodules of dab and flounder (*Platichthys flesus*) have been developed and assessed by ICES and are now fully established. These QA procedures concern all steps in the process from sampling to data reporting and submission to the ICES Environmental Data Centre, as well as statistical analysis. Therefore, the quality assurance plan for the incorporation of externally visible diseases and macroscopic liver nodules into the JAMP can be considered complete and operational.

Table 5.3.2.1. Proposals for revised Tables 1.2.3, 1.2.4, and 1.3.1 in Technical Annex 1 of the draft Guidelines for General Biological Effects Monitoring under the OSPAR Joint Assessment and Monitoring Programme (JAMP).

Table 1.2.3. Liver Histopathology

Target organ/organism:	Liver in dab (or other target species beyond the distribution limits of dab)
Effect measured:	Unique degenerative changes (hepatocellular and nuclear polymorphism, megalocytic hepatosis/hepatic megalocytosis, hydropic vacuolization of biliary epithelial cells and/or hepatocytes) Foci of cellular alteration Benign tumours Malignant tumours
Means of interpretation:	Contaminant-related, particularly carcinogens
Methodology:	An ICES TIMES report should be available in 1998
Quality assurance/control:	Initiated due to the ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants
Suitability for application: B. Procedures for diagnosis of histopathology still need further quality assurance	

Table 1.2.4. Macroscopic Liver Nodules

Target organ/organism:	Liver in dab (or other target species beyond the distribution limits of dab)
Effect measured:	Sampling of macroscopic liver nodules > 2 mm in diameter and subsequent quantification of histologically identified liver neoplasms
Means of interpretation:	Specifically related to liver carcinogenesis
Methodology:	ICES has published a standard protocol in TIMES No. 19 (Bucke *et al.*, 1996)
Quality assurance/control:	Procedures have been established within ICES
Suitability for application: A. Quality assurance procedures are in place.	

Table 1.3.1. Externally Visible Fish Diseases

Target organ/organism:	Dab (or other species beyond the distribution limits of dab)
Effect measured:	Lymphocystis Epidermal hyperplasia/papilloma Skin ulcerations X-cell gill disease
Means of interpretation:	Unspecific indicator of environmental stress Data are of value for general biological effects monitoring and for subsequent temporal trend monitoring.
Methodology:	ICES has published a standard protocol in ICES TIMES No. 19 (Bucke *et al.*, 1996). Data are stored in the ICES Environmental Data Centre. Standardized methodologies for data submission and statistical analysis have been developed by ICES.
Quality assurance/control:	Procedures are well-established and include sea-going workshops, interlaboratory calibration exercises, and quality control of data submitted to the ICES Environmental Data Centre.
Suitability for application: A. Quality assurance procedures are in place.	

WGPDMO, however, recognized the need for a similar quality assurance plan for the incorporation of liver histopathology. This issue was addressed during the ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants (see Section 4.3.3, above). Quality assurance guidelines for sampling and processing were produced during that ICES Special Meeting. However, guidelines for training and intercalibration for the diagnosis of histopathological liver lesions are still incomplete.

WGPDMO proposed that a training and intercalibration programme (TIP) for the diagnosis of relevant histopathological liver lesions, as detailed in the report of the ICES Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants (see Section 4.3.3, above), should be conducted. This quality assurance plan should take account of the generic and specific quality assurance requirements detailed in the report of the ICES Special Meeting, and should be developed intersessionally and considered by WGPDMO in 1998.

Considerations of WGPDMO on the design and implementation of such a programme included the following:

1) A reference laboratory should be established to take the lead in coordinating the Training and Intercalibration Programme (TIP). The CEFAS Weymouth Laboratory, UK, has been proposed for this in the ICES Special Meeting report. This proposal was endorsed by WGPDMO.

2) Sets of reference slides with representative histopathological liver lesions should be prepared and circulated among participants in the TIP. In the first instance, they will be relevant participants from the ICES Special Meeting.

3) For training purposes, this set of reference slides should be accompanied by detailed comments and interpretations of the lesions. For the future, CD-ROM representation of reference material might be useful as a supplement to the slides.

4) Intercalibration exercises should be conducted on a regular basis, and intralaboratory calibration standards should be established so that several individuals in one laboratory can discuss and review slides.

5) Reference material for the TIP not available through the reference laboratory will be provided from laboratories in the Netherlands and the USA.

6) The requirement for funding of the TIP was emphasized. It was suggested that the reference laboratory should explore the possibility for financial support through the European Union (EU) and/or ICES.

The ACME emphasized that the TIP as proposed by WGPDMO should be integrated into the scheme for a QA programme for biological effects techniques proposed by WGBEC for funding by the EC, as detailed in Section 5.3.1, above.

Recommendations

ICES ACME recommends that the Oslo and Paris Commissions take note of the revised version of the tables in Technical Annex 1 of the draft Guidelines for General Biological Effects Monitoring with regard to liver histopathology, macroscopic liver nodules, and externally visible fish diseases contained in Table 5.3.2.1 and consider it for inclusion in the final JAMP Guidelines.

Reference

Bucke, D., Vethaak, A.D., Lang, T., and Mellergaard, S. 1996. Common diseases and parasites of fish in the North Atlantic: Training guide for identification. ICES Techniques in Marine Environmental Sciences, No. 19. 27 pp.

5.4 Quality Assurance of Chemical Measurements in the Baltic Sea

Request

Item 2 of the 1997 requests from the Helsinki Commission.

Source of the information presented

The 1997 report of the ICES/HELCOM Steering Group on Quality Assurance of Chemical Measurements in the Baltic Sea (SGQAC) and ACME deliberations.

Status/background information

The ACME reviewed the report of the ICES/HELCOM Steering Group on Quality Assurance of Chemical Measurements in the Baltic Sea (SGQAC) and noted that final guidelines have now been agreed for quality assurance procedures for chemical measurements for the HELCOM Baltic Monitoring Programme (BMP) and the HELCOM Coastal Monitoring Programme (CMP), which will be integrated into one monitoring programme (COMBINE) in the near future.

The guidelines prepared by SGQAC, and reviewed and commented on by the Marine Chemistry Working Group, are entitled 'Guidelines on quality assurance of chemical measurements in the Baltic Sea'. They comprise both general guidelines on the establishment of quality assurance procedures for chemical components to be measured for COMBINE, as well as a series of annexes covering specific details which also include technical

notes on the determination of specific substances in sea water and biota. The information in the annexes will be updated when appropriate, according to new developments in methodology, and new annexes on additional topics will be prepared.

The general quality assurance guidelines contain the following sections:

1) establishment of a quality system (organization, management, staff, documentation, laboratory testing environment, equipment, and quality audit);
2) determining analytical requirements (types of samples, concentration ranges, permissible tolerances in analytical error);
3) validation of analytical methods (selectivity, sensitivity, range, detection limit, accuracy, estimating systematic and random errors);
4) procedures for routine within-laboratory quality control (X-charts, control charts, CUSUM charts);
5) procedures for external quality assessment.

The annexes contain (a) information on the principal components of a quality manual, (b) information on important subjects for a quality audit, (c) examples of reference materials for internal quality control, (d) guidance on validation of an established analytical method, and (e) general information on sampling. They also contain technical notes that describe QA procedures in association with:

- sample handling;
- storage of samples;
- sample pretreatment;
- appropriate chemical analytical methods;
- calibration and the blank.

The technical notes cover the following topics:

a) the determination of nutrients in sea water;
b) the determination of the trace metals Cd, Pb, Cu, Co, Zn, Ni, and Fe in sea water;
c) the determination of chlorinated biphenyls, organochlorine pesticides, and metallic trace elements in fish tissues;
d) chemical analyses of anoxic waters.

The ACME expressed its appreciation for the development of these comprehensive QA guidelines on chemical measurements and agreed that they should be transmitted to HELCOM for incorporation in its monitoring manual.

Need for further research

The ACME noted that the guidelines for quality assurance of chemical measurements in the Baltic Sea will be updated according to methodological progress and the needs of the HELCOM monitoring programme.

Recommendations

ICES ACME recommends that HELCOM BMP/CMP laboratories regularly participate in an external quality assessment (proficiency testing) scheme in order to ensure long-term control of the accuracy and comparability of their analytical results.

ICES ACME agreed that the Guidelines on Quality Assurance of Chemical Measurements in the Baltic Sea, including the annexes and Technical Notes that have been completed, should be forwarded to HELCOM for use in its monitoring programme.

5.5 Results of the Seventh Intercomparison Exercise on Measurements of Trace Metals in Coastal Sea Water

Request

There is no specific request; this is part of the continuing ICES work of coordinating quality assurance activities and reporting on the results and their implications for monitoring programmes.

Source of the information presented

The 1997 report of the Marine Chemistry Working Group (MCWG) and ACME deliberations.

Status/background information

The 1996 ACME report described plans and preparations for the Seventh Intercomparison Exercise on Measurements of Trace Metals in Coastal Sea Water (7/TM/SW). This intercomparison exercise was conducted as planned in 1996 and a draft report on the results was discussed at the 1997 MCWG meeting. Prior to the exercise, the response to a letter to prospective participants had revealed that there was not sufficient interest to justify the additional cost of including mercury as an analyte. The exercise involved the analysis of two sea water samples of different salinities collected in the Öresund from a Danish vessel. The following is a brief summary of the outcome of the exercise.

Thirty-nine laboratories (71 % of those receiving samples) submitted data. About 25 % of the respondent

laboratories demonstrated an ability to competently analyse a majority of the trace metals of interest in this exercise. Six laboratories analysed for only a small number of trace metals, but produced good results. Only about one-third of the laboratories appeared to be quite competent regarding the analysis of the samples for all three trace metals (copper, zinc, and cadmium) that had been mandatory in the former OSPAR Joint Monitoring Programme (JMP). A disappointing 45 % of the respondent laboratories did not demonstrate the ability to adequately analyse both samples for these three trace metals. There are a number of competent procedures for the extraction of trace metals from sea water. The study could not discern significant differences in the efficiency of these separation methods. Also, there are a number of competent instrumental methods for the measurement of trace metal concentrations after extraction from sea water. The study could not discern significant differences in the efficacy of these instrumental procedures. Many laboratories do not appear to be using procedures of adequate sensitivity for the analysis of metals in sea water. However, their reported procedures are often not much different from those of laboratories producing good quantitative results.

The ACME noted that laboratory contamination and/or poor control of reagent blanks and/or improper calibration procedures appear to be major sources of error in many laboratories. Clean facilities, equipment, and reagents are prerequisites for the successful analysis of trace metals in sea water. Good laboratory practices are essential.

Recommendations

ICES ACME recommends that the final report on the results of the Seventh Intercomparison Exercise on Measurements of Trace Metals in Coastal Sea Water be published in the *ICES Cooperative Research Report* series.

Additional Comments

ICES ACME expresses its gratitude and appreciation for the high quality work and organization conducted by the laboratories which took responsibility for this exercise, namely, the Danish National Environmental Research Institute (Roskilde, Denmark), the Institute for National Measurement Standards, National Research Council (Ottawa, Canada), and the Scottish Office Agriculture, Environment and Fisheries Department (Aberdeen, UK).

5.6 Interlaboratory Study on Organotin Analysis

Request

There is no specific request; this item is for information on a quality assurance activity.

Source of the information presented

The 1997 report of the Marine Chemistry Working Group (MCWG) and ACME deliberations.

Status/background information

The ACME noted that MCWG discussed the requirement for an interlaboratory study on the measurement of organotins in the marine environment, and agreed that it was timely that an initiative on these compounds was undertaken. However, MCWG noted that the QUASIMEME scheme for 1997–1998 includes a development exercise for organotins. This exercise will begin with the distribution of a series of standard solutions and a mussel tissue. The compounds that will be studied are the mono-, di- and tributyl and phenyltins. In addition, the ethyl and pentyl derivatives will be available as a quality check on the derivative techniques that many laboratories use in their methods for the determination of these species. It was noted that, in the near future, QUASIMEME plans to develop the organotin programme to include sediment and water matrices.

The ACME agreed that this was a welcome development and that, in view of the QUASIMEME initiative in this area, there is no need for an ICES interlaboratory study on organotin analysis. However, the ACME requested MCWG to follow the development and outcome of this QUASIMEME exercise.

5.7 Protocols for Quality Control Procedures on Nutrients Data

Request

There is no specific request; this item is of relevance in the overall quality assurance of nutrients data.

Source of the information presented

The 1997 report of the Marine Chemistry Working Group (MCWG) and ACME deliberations.

Status/background information

The ACME reviewed the MCWG consideration of this topic and noted that there are three distinct stages in the overall quality assurance of nutrients data.

Stage 1: Sample handling and analytical chemistry

This covers all operations from the arrival of the hydrocast bottle on board the research vessel to the production of concentration data for individual nutrients. The most evident drawback in this area is the continued lack of Certified Reference Materials (CRMs) for

nutrients in sea water. Nutrients chemists are, therefore, unable to demonstrate their level of quality control by the use of Shewart and CUSUM charts, etc., in the manner that is customary for measurements in sediments and biota where suitable CRMs are available.

The nearest approach available to the nutrients chemist is to check on the consistency of autoanalyser performance by keeping a record of absorbance/concentration data for calibration solutions by way of Shewart and CUSUM charts. This can be a very effective early warning system for instrumental and chemical malfunction. It is hoped that the new EC Quality Assurance of Sampling and Sample Handling (QUASH) programme will contribute quantitatively and significantly to the understanding of how sample handling prior to the actual analytical chemical measurement can affect data quality.

Stage 2: Critical examination of the data

The oceanographic consistency of data can be examined in a variety of ways. There is no substitute for the well-trained eye and, combined with computer-generated property/property plots, anomalies can be readily identified. Nitrogen/phosphorus (N/P) ratios and nutrient/salinity plots are particularly useful. MCWG concurred that if no specific and satisfactory evidence can be found for rejecting outlying data points they must be retained, yet flagged in some way, otherwise phenomena such as 'The Great Salinity Anomaly' and certain Baltic Sea events, for example, the reduced silicate concentration in the water, might be overlooked. There are several ways of flagging such data.

MCWG had the view that, with the access to state-of-the-art computers, there would be no need to store data which are interpolated or extrapolated. Such data should be created only when they are needed.

Fully computerized quality control of data submissions is possible in the more straightforward cases, for example, nitrite cannot be greater than the sum of nitrate plus nitrite; likewise, total-N should be not less than the sum of nitrate, nitrite, and ammonia.

Specific combinations of data are important, for example, appropriate salinity data are vital to the interpretation of nutrients data. Therefore, no nutrient data should be considered acceptable without accompanying salinity data.

Stage 3: Suitability of data for their intended purpose

At the third stage, the emphasis is on the user of the data. The user has to be familiar with the data sets, the requirements under which they were collected, and the quality assurance information available in support of the data sets. Based on this, the user must decide whether the data meet the requirements of his intended study, e.g., a time trend assessment. Thus, the actual use and evaluation of the data are likely to reveal quality problems, if there still are any.

The use of the data should, therefore, be seen as the final check of their quality.

5.8 Quality Assurance of Measurements of Dissolved Oxygen in Sea Water

Request

There is no specific request; this is part of continuing ICES work on the development of quality assurance procedures for variables included in marine monitoring programmes.

Source of the information presented

The 1997 report of the Marine Chemistry Working Group (MCWG) and ACME deliberations.

Status/background information

The ACME noted that MCWG reviewed and accepted a paper on the determination of dissolved oxygen in sea water and associated quality assurance procedures, prepared by A. Aminot (France). The information in this paper is clearly presented and it can provide important guidance to the monitoring community. The paper is attached as Annex 3.

It is of interest to note that the Unesco Joint Panel on Oceanographic Tables and Standards recommended an algorithm and produced oxygen saturation tables in 1973; in 1986 the Panel made a new recommendation for an improved algorithm, but no new tables were produced.

Recommendations

It is recommended that ICES pursues with Unesco the production of new oxygen saturation tables.

5.9 Performance Indicators and Laboratory Performance in Recent NOAA and QUASIMEME Interlaboratory Studies of Metal Analyses

Request

There is no specific request; this issue is of relevance to the comparison of results from marine monitoring programmes.

Source of the information presented

The 1997 report of the Marine Chemistry Working Group (MCWG) and ACME deliberations.

Status/background information

The ACME took note of the MCWG consideration of the key developments and statistical outcomes of the recent U.S. National Oceanic and Atmospheric Administration (NOAA) and QUASIMEME interlaboratory performance studies. The details of the results of these studies can be found in the respective reports of these programmes; the NOAA studies will be published by NOAA, and the results of the QUASIMEME project will be published in a special issue of *Marine Pollution Bulletin* expected in spring 1998.

From these results, it was clear that the following conclusions can be made:

1) Regular laboratory performance studies are an important tool for establishing the quality of data from participating laboratories.

2) Most laboratories which participated in either exercise have improved their performance over the years. Because the NOAA programme has been in operation for a longer period, there is clearer evidence in the NOAA exercise for this improvement in analyses of trace metals in both biota and sediment.

3) The requirements and the original aims of the two programmes are different, as are the methods of data analysis and assessment. Therefore, it is considered that there is no real value in making a detailed comparison of the results of the two schemes.

4) The QUASIMEME information for the laboratories concerning their performance gives the mean, the Z-score (see also Section 5.10, below), and the P-score from the laboratory performance studies. The Z-score and P-score can easily be calculated from the NOAA data if required. The scores are based on a declared level of acceptable bias (currently at 25 % for a Z-score of 2). The required level of laboratory performance for a particular monitoring programme can be set by the programme and the new Z-score calculated on the basis of that performance criteria, if different from that set by QUASIMEME. The assessments can then be made according to the level required by the monitoring programme.

5.10 Developments within QUASIMEME and QUASH

Request

There is no specific request; the ACME addressed this item because of the long-standing ICES involvement in quality assurance matters.

Source of the information presented

The 1997 report of the Marine Chemistry Working Group (MCWG), QUASIMEME Bulletin Issue No. 4 and ACME deliberations.

Status/background information

The ACME noted that the EC-funded QUASIMEME project was completed in March 1996. A full report on the project will be published as a special issue of *Marine Pollution Bulletin* in spring 1998. The achievements of the project include:

- the establishment of a coherent system for the evaluation of laboratory performance based on Z-scores (ISO 43);
- a clear identification of 'problem' determinands (low levels of ammonia and phosphate in sea water, lead in biota, and CB29 in biota and sediment);
- establishment of a level of comparability and the best between-laboratory performances;
- establishment of realistic targets for bias and precision based on constant and proportional errors.

The ACME further noted that the QUASIMEME project has continued on a subscription basis for participating laboratories. Membership in the new QUASIMEME Laboratory Performance Studies (QUASIMEME II) is open to all institutes. Institutes in the Baltic Sea countries have also been invited to participate and have been supplied test materials according to their selected requirements. About 120 laboratories worldwide have joined the scheme. ICES is represented on the Advisory Board, along with representatives from OSPAR, HELCOM, and MEDPOL.

The ACME was informed that a consortium (mainly the same partners as in QUASIMEME) has put together a successful bid to the EC to provide funding for a new project, Quality Assurance of Sampling and Sample Handling (QUASH). The QUASH project will run for three years and will address problems related to sampling and sample handling, since they were only briefly considered by QUASIMEME. Laboratories from the fifteen EU countries have been invited to participate. The project is based on the following six components:

1) sampling and preservation of nutrients in sea water;
2) monitoring contaminants in biota: lipids and water as cofactors;
3) sampling of biological tissues;
4) sample handling and cofactors in relation to normalization procedures for sediments;

5) preparation of test materials for laboratory and field performance studies;

6) laboratory and field performance studies.

Additional comments

The ACME will continue to follow the progress of QUASIMEME II and QUASH.

Reference

QUASIMEME. 1997. QUASIMEME Bulletin, Issue No. 4.

6 ENVIRONMENTAL STUDIES IN THE BALTIC SEA

6.1 Baseline Study of Contaminants in Baltic Sea Sediments

Request

Item 3 of the 1997 requests from the Helsinki Commission.

Source of the information presented

The draft report on the results of the 1993 ICES/HELCOM Baseline Study of Contaminants in Baltic Sea Sediments, the 1997 reports of the Working Group on Environmental Assessment and Monitoring Strategies (WGEAMS) and the Working Group on Marine Sediments in Relation to Pollution (WGMS), and ACME deliberations.

Status/background information

The ACME reviewed a draft (Version 6) of the report 'Contaminants in Baltic Sea Sediments', edited by M. Perttilä, which describes the main findings of the 1993 ICES/HELCOM Baseline Study of Contaminants in Baltic Sea Sediments. The ACME agreed that the organization and execution of the Baseline Study was well done. It had proceeded in accordance with the ICES guidelines in the selection of sedimentation areas, sampling, radiological dating of the core samples, and quality assurance procedures.

In the report, the results are presented of the analyses of sediment cores from the 25 stations sampled. The report contains information on a number of topics, as described in Table 6.1.1, below.

After examination of the sediment cores, it became apparent that not all 25 stations are suitable for trend monitoring purposes. Based on ^{210}Pb profiles, fourteen stations were identified and recommended for use in future monitoring activities.

The ACME noted that, at the time of its meeting, the report was still not complete, and that important data are missing, which reduces the ability to make a complete assessment. For instance, the chapter on organic contaminants includes only an introduction and a few pictures, without further information. A comparative discussion of the dating methods seems to be necessary to explain some discrepancies between estimates of sedimentation rates. The interpretation of the results from the cores is only made by class of parameter (metals, nutrients, organics, etc.). The ACME is of the opinion that a multiparameter discussion could significantly improve the interpretations that could be made of this important data set. It was mentioned, for example, that the difference in the behaviour of metals and organics could be useful in the interpretation of the anthropogenic contributions to the observed concentrations.

Table 6.1.1. Topics covered in the report 'Contaminants in Baltic Sea Sediments' (Version 6.0).

Chapter 1	Introduction	• contains general information about the Baseline Study
Chapter 2	1993 ICES/HELCOM Baseline Study of Contaminants in Baltic Sea Sediments	• describes the overall objectives and summarizes the results of the Baseline Study
Chapter 3	Mineralogical composition and granulometry of the recent muds of the Baltic Sea	• contains information about the mineralogical composition of sediment samples and results of grain size analyses • results are presented in tables and maps
Chapter 4	Distribution of trace elements in Baltic Sea sediments	• describes the horizontal distribution of trace elements in Baltic Sea sediments using bar diagrams • spatial trace element surveys show certain areas with increased concentrations, in particular for As and Hg • down-core profiles indicate that natural redox processes can have a marked influence on the accumulation of Mn and Cd at certain stations, which makes it difficult to distinguish between natural and anthropogenic enrichments
Chapter 5	Carbon and nutrients	• contains results of analyses of carbon, phosphorus, and nitrogen in sediments
Chapter 6	Distribution of organic contaminants in Baltic Sea sediments	• describes the distribution of extractable organic chlorine (EOCl), ΣDDT, and ΣPCB in sediments
Chapter 7	X-ray studies of sediment cores	• contains X-ray radiographs showing the vertical variation in content of solids

The ACME appreciated that the results have been used to prepare proposals as to which of the sampling stations should be included in a future monitoring exercise. Nevertheless, the ACME did not favour the recommendation that this programme should continue in the same way before a more comprehensive interpretation of the core profiles is available. In its conclusion, the report states that "The main utility, however, of the sediments is to indicate areal variations rather than variations in time". This statement has been made about a study in which core sampling has played a central role and which was supposed to be used as a time trend tool, and also as the basis for repeated (temporal trend) studies. The main problems with the study are that the participants have largely been unable to interpret the core profiles with sufficient confidence so as to be able to identify the effects of changes in inputs of contaminants, changes in water column chemistry (e.g., stagnation, deoxygenation) and changes in the levels of primary productivity (eutrophication), and sediment diagenetic processes. The inability to quantify the effects of these processes on the observed sediment chemistry greatly limits the objectives that can be met by the present study and which might be addressed through the proposed repeat study, on a smaller scale. The ACME is of the opinion that, if a study were to be conducted under a similar sampling scheme in five years (as is proposed), it is highly probable that the participants would still be unable to adequately interpret the observed core profiles, and that no new information would be obtained on the explanation of the time trends potentially preserved in the sediment as described in the present study.

The ACME therefore recommends that the next baseline study, in the framework of the HELCOM Baltic Monitoring Programme, should be designed in a manner that reflects the conclusions and uncertainties identified in the 1993 Baseline Study. Sampling should include a much reduced number of sediment cores, selected after full interpretation of the 1993 study, and a more extensive sampling of surficial sediments. The surface sediment sampling locations should be selected to investigate the effects of the various processes that are recognized as having influenced the profiles observed in the 1993 study, i.e., water column chemistry, sediment physico-chemical conditions, primary production, input rates of contaminants, etc. The new data should be combined with the results of other components of the HELCOM programme, such as hydrochemical monitoring, primary production monitoring, and pollution load (input) monitoring. In this way, it may be possible to understand the influence of these processes on sediment chemistry and to move towards a fuller understanding of the meaning of the sediment analyses. The mechanism used to organize the 1993 Baseline Study, through a Steering Group responsible for all aspects of the planning, execution, analyses, and reporting of the study, should be retained, as it has proved to be efficient. The ACME noted, however, that it is essential that the report of the 1993 Baseline Study be completed as soon as possible to properly form the basis for planning for any follow-up programme.

Thus, after examination of the draft report on the Baseline Study of Contaminants in Baltic Sea Sediments, the ACME concluded that this report requires further elaboration. Bearing in mind that monitoring of contaminants in Baltic Sea sediments is not an urgent issue, the ACME decided that suitable recommendations will be developed after final completion and review of the Baseline Study report.

The ACME looked forward to reviewing the final report at its next meeting. The ACME noted that data from the study will be submitted to the ICES Environmental Data Centre, using the data collecting system provided by the Centre, and felt that this represents a very valuable data set.

6.2 Causes of the M-74 Syndrome in Baltic Salmon and Progress in Understanding Relevant Environmental Factors

Request

Item 5 of the 1997 requests from the Helsinki Commission.

Source of the information presented

The 1997 reports of the Working Group on Pathology and Diseases of Marine Organisms (WGPDMO), the Working Group on Biological Effects of Contaminants (WGBEC), and the Baltic Salmon and Trout Assessment Working Group (WGBAST), and ACME deliberations.

Status/background information

The ACME reviewed the relevant sections of the above-mentioned reports providing information on the progress in understanding the environmental factors that influence the occurrence of the M-74 syndrome and on the geographical extent of the distribution of this syndrome.

The ACME noted that there has been no significant progress in understanding the role of environmental factors in the aetiology of the M-74 syndrome. The research in this field still focuses on the thiamine/thiaminase kinetics in fish and thiamine/thiaminase dynamics in the ecosystem, factors which are considered major contributors to the development of the syndrome. At present, M-74-induced mortality in the Baltic stock of Atlantic salmon (*Salmo salar*) produced under aquaculture conditions is prevented effectively by thiamine treatment of brood fish, eggs or yolk-sac fry. However, the ACME endorsed the view of WGPDMO that this is a temporary solution for maintaining the smolt production programme, while the

problem still persists in wild salmon and sea trout populations.

Although a clear link between the M-74 syndrome and environmental contaminants has not been confirmed, Finnish studies suggest an involvement of dioxin-like organochlorines. These substances seem to disturb thyroid hormones, and retinoid and thiamine metabolism and functions in mammals and birds. The concentration levels of these substances in female Baltic salmon, eggs and yolk-sac fry are under investigation. It has been observed that yolk-sac fry dying from M-74 seem to be retarded in their development and have a lower oxygen consumption than healthy specimens. This indicates some impairment of thyroid function. Additional facts that indicate the involvement of thyroid metabolism are that:

- incubation of yolk-sac fry in thyroid hormone solution seems to prevent the outbreak of the M-74 syndrome; and
- incubation of healthy yolk-sac fry in a solution of phenylthiocarbamate (an anti-thyroid drug) results in the appearance of M-74-like symptoms.

Research collaboration between scientists in Sweden, Finland, and Latvia has been established to elucidate the occurrence and pathways in the ecosystem of thiamine in salmon caught on their feeding grounds.

The role of the carotenoid astaxanthin is currently being investigated, but results of these studies are not yet available.

With regard to the geographical distribution of the M-74 syndrome, the ACME noted that, except for the first observation of the syndrome in Estonia in 1996, there is no new information available. Results from Finland and Sweden indicate that the M-74 syndrome has stabilized at a high level. The prevalence of female salmon giving offspring affected by the M-74 syndrome was 68 % in 1996 in Sweden.

In its 1996 report, the ACME expressed its concern that the M-74 syndrome in Atlantic salmon might be more widespread than previously expected. This concern was mainly based on the observation of a M-74-like condition in Atlantic salmon in Galicia, Spain in 1995, reported by WGPDMO in 1996. However, the ACME emphasized that there is no new information available confirming this finding and there is, therefore, no clear evidence that the M-74 syndrome of Atlantic salmon occurs outside the Baltic Sea.

Nevertheless, it has been confirmed that the M-74 syndrome also occurs in Baltic sea trout (*Salmo trutta*) populations in Swedish and Finnish waters, although with less severe consequences. According to information provided in the 1997 report of WGBEC on the M-74 syndrome, other European fish species in which an abnormal development of offspring has been observed are Arctic charr (*Salvelinus alpinus*) and cod (*Gadus morhua*). However, the ACME emphasized that there is no indication so far that the abnormal development recorded in these species is identical with the M-74 syndrome.

Need for further research

The ACME noted that there is increasing evidence from various studies carried out in recent years that changes in the thiamine/thiaminase metabolic system are involved in the aetiology of the M-74 syndrome of Baltic salmon and, probably, also in sea trout, as well as in M-74-like conditions recorded in North American salmonids. However, it was emphasized that further research is still required in order to identify environmental factors responsible for these changes.

In addition, the ACME noted that there is a continuing need for studies investigating the occurrence of the M-74 syndrome and similar conditions in wild Atlantic salmon stocks and other fish species outside the Baltic Sea. In order to design and coordinate appropriate studies, the ACME endorsed the proposal of WGPDMO to elaborate and distribute information among ICES Member Countries on how to detect and diagnose the M-74 syndrome.

Recommendations

ICES ACME recommends that ICES Member Countries be made aware of the possibility of the occurrence of the M-74 syndrome outside the Baltic Sea and also in other fish species than Atlantic salmon, and conduct appropriate studies to evaluate this possibility.

6.3 Effects of Extraction of Marine Sand and Gravel on the Baltic Ecosystem

Request

Item 8 of the 1997 requests from the Helsinki Commission.

Source of the information presented

The 1997 report of the Working Group on the Effects of Extraction of Marine Sediments on the Marine Ecosystem (WGEXT) and ACME deliberations.

Status/background information

The ACME reviewed and accepted the section of the report of the Working Group on the Effects of Extraction of Marine Sediments on the Marine Ecosystem (WGEXT) containing information and discussions on the effects of extraction of marine sand and gravel on the Baltic ecosystem. This task was requested by the

Helsinki Commission and required the provision of information on the extent and volumes of sand and gravel extraction in the Baltic Sea, and on known impacts on, e.g., benthos, diving seabirds, and bottom-spawning fish and invertebrates.

In undertaking this task, WGEXT was aware that:

1) certain historical activities in the Baltic Sea had not conformed with the ICES Code of Practice for the Commercial Extraction of Marine Sediments (published in ICES, 1992) and that observed deleterious impacts associated with these activities were a feature of this departure from good practice rather than illustrative of particular risks in the Baltic Sea from extraction operations;

2) the sources of environmental disturbance associated with sand and gravel extraction in the Baltic Sea, providing that good extraction practice is followed, would be similar to those from extraction operations in other locations; but that

3) the specific composition and structure of Baltic ecosystems are substantially different from those in the North Sea and Northeast Atlantic, where extraction activities are undertaken by other countries.

The consequence of this is that WGEXT, in addressing potential effects, has assumed that good extraction practice would be followed and that the sources of environmental disturbance arising would be similar to those noted elsewhere (see, for example, ICES, 1992). The consideration of potential environmental impacts has been limited by the need for more detailed information on the Baltic ecosystem, notably in areas of very low salinity, and the requirement for further evaluation involving specialists in these areas of Baltic Sea ecology.

WGEXT did not specifically address the potential physical effects of extraction operations on coastal processes. Certain extraction operations in the Baltic Sea have led to coastal erosion and have altered sediment transport patterns. These risks would usually be evaluated as part of the environmental impact assessment for any project (see ICES Guidelines for the preparation of an Environmental Impact Assessment evaluating the effects of seabed aggregate extraction on the marine environment, ICES, 1994) and have been discussed in detail elsewhere (ICES, 1992).

Extraction of Marine Sediments in the Baltic Sea, by Country

The following paragraphs provide information on the reported aggregate extraction activities in Baltic Sea countries. It is known that aggregate extraction operations occur in other areas, but data on such activities were not available to WGEXT.

Denmark

The extraction of marine sand and gravel in the Danish Exclusive Economic Zone of the Baltic Sea represents 30–50 % of the total marine production of materials in Denmark for construction and reclamation. Most of the material dredged comes from areas along the east coast of Sjælland, Møn and Falster and from the Adler Ground-Rønne Bank and Kriegers Flak. The amount of materials dredged for construction has increased slightly since 1992.

During the construction of the fixed link between Denmark and Sweden, 3×10^6 m^3 of sandfill will be dredged from the Kriegers Flak in the Baltic Sea. The dredging started in January 1996 and is expected to continue for four years. To date, some 350,000 m^3 have been dredged in this area.

Table 6.3.1. Extraction of marine sediments from the Danish Exclusive Economic Zone of the Baltic Sea.

Year	Sand 0–2 mm	Gravel 0–20 mm	Gravel/Stones 6–300 mm	Sand fill
1990	0.2×10^6 m^3	0.1×10^6 m^3	0.2×10^6 m^3	0.1×10^6 m^3
1991	0.3×10^6 m^3	0.2×10^6 m^3	0.4×10^6 m^3	0.2×10^6 m^3
1992	0.3×10^6 m^3	0.1×10^6 m^3	0.7×10^6 m^3	0.2×10^6m^3
1993	0.3×10^6 m^3	0.1×10^6 m^3	0.6×10^6 m^3	
1994	0.5×10^6 m^3	0.1×10^6 m^3	0.7×10^6 m^3	0.1×10^6 m^3
1995	0.6×10^6 m^3	0.1×10^6 m^3	0.5×10^6 m^3	
1996*	0.6×10^6 m^3	0.1×10^6 m^3	0.5×10^6 m^3	0.6×10^6 m^3

* The figures for 1996 are preliminary.

Estonia

No data were available from Estonia.

Finland

The volume of sand and gravel extraction varies from year to year and there are no official statistics available. The annual average extraction of sand and gravel is, however, estimated to be less than 0.5 x 10^6 tonnes. The principal areas where extraction operations have occurred in recent years are in coastal regions near the cities of Helsinki, Kotka, and Pori (in the Gulf of Bothnia).

Germany

In 1995, approximately 1 x 10^6 tonnes of sand and gravel were dredged in the Baltic Sea and the coastal areas. It is expected that dredging, particularly for coastal protection, will increase in 1996. Sand is dredged from areas near the coast.

Latvia

No data were available from Latvia.

Lithuania

No data were available from Lithuania.

Poland

From 1985 to 1989, about 1.4 x 10^6 tonnes of aggregates were dredged from the Slupsk Bank. In 1990, exploitation was stopped for economic reasons. Test dredging was carried out from the Southern Middle Bank and in Koszalin Bay during 1987–1989. Approximately 4,000–6,000 tonnes were removed from each deposit. During the 1990s, a few thousand tonnes of aggregates were extracted from the eastern part of Pomeranian Bay without any formal controls.

A 1 km^2 sand extraction field located 4 km northeast of Jastarnia on the Hel Peninsula was used in 1993 and 1995 for beach nourishment needs (the total amount of sand extracted was approximately 200,000 m^3). Sand is currently being extracted in a 5 km^2 area northeast of Cape Rozewie for the needs of artificial beach nourishment. It has been dredged since 1995 at a rate of 100,000 m^3 per year.

Since 1989, sand has been extracted at four sites in the Puck Lagoon for sand nourishment on the Hel Peninsula. Two sites have been closed since 1993. From the remaining two areas, sand is presently being extracted at a rate of 150,000 to 300,000 m^3 per year, but this is planned to be stopped by 1998, except in instances of coastal catastrophy caused by storm activity. The total amount of sand extracted from the Puck Lagoon is about 6 x 10^6 m^3.

Sand is also extracted from approach channels to ports and from sand traps at ports within the operation of artificial sand by-pass systems; such extraction has taken place since about 1990 at the ports of Kolobrzeg (about 60,000 m^3 per year), Darlowo (about 80,000 m^3 per year), Ustka (80,000 m^3 per year), Leba (30,000 m^3 per year), and Wladyslawowo (about 200,000 m^3 per year).

Russia

No data were available from Russia.

Sweden

No commercial dredging has taken place in the Swedish Exclusive Economic Zone of the Baltic Sea in recent years.

Overview of Data on the Baltic Sea Environment

Benthic communities in the Polish marine area

The distribution of the bottom macrofauna depends strictly on the type of the bottom in a given area. In the Baltic Sea, sand is the dominating type of bottom sediment down to 50 m. In deeper parts of the sea, the bottom is composed mainly of muddy sediment. The composition and spatial variability of benthic species also reflect a whole range of diversity of environmental factors such as temperature, salinity, and oxygen content in both the water and bottom sediments.

In terms of species composition and macrofauna abundance, six bottom macrofauna communities have been identified in the Polish marine area (see Figure 6.3.1). The communities described below (J. Warzocha, pers. comm.) are named according to the occurrence of the most characteristic species (the most frequently found, dominating in terms of total biomass or abundance during the whole year). While WGEXT was only in a position to consider such data for Poland, it was of the view that this provided an important illustration of the types of benthic communities likely to occur in many areas of the Baltic Sea. In the northern and eastern parts of the Baltic Sea, however, benthic communities more characteristic of freshwater environments will occur; no data on such communities were available to WGEXT.

A) A ***Macoma balthica - Mya arenaria*** **community** occurs on sandy bottoms down to 20 m to 25 m (the limit of warm water during the summer). This is a very diverse community composed of twenty macrofauna species, mainly of Atlantic or Atlantic-Boreal origin. Some freshwater species can be also found in this zone.

In terms of abundance, the most dominant species in this community is the sedentary polychaete *Pygospio elegans*. The bivalves *Mya arenaria*, *Cerastoderma lamarcki*, *Macoma balthica*, and the amphipod *Corophium volutator* are common as well. Bivalves make up about 90 % of the total biomass. The supralittoral zone (splash zone) is a hostile habitat for benthic species due to wave action. Only a few species (*Bathyporeia pilosa*, *Eurydice pulchra*) are able to tolerate these conditions.

B) A *Mytilus edulis - Gammarus salinus* community occurs in the sandy-stony bottom of the Slupsk Bank at depths ranging from 14 m to 20 m.

Algae-covered stones create a diversified habitat, enhancing the faunal diversity: about eighteen species have been recorded, including eleven crustacean species. In terms of both abundance and biomass, *Mytilus edulis* is the dominant species, constituting 72 % and 96 % of the total macrofauna, respectively. Among the other species, only *Gammarus salinus* accounts for more than 1 % of the total biomass.

C) A *Macoma balthica - Saduria entomon* community occurs in sandy as well as sandy-muddy bottoms at a depth range of 25 m to 60 m.

Approximately twenty benthic macrofauna species can be found in this community. In terms of biomass, *Macoma balthica* is the dominant species; however, the most abundant species are the crustaceans *Pontoporeia affinis* and *Pontoporeia femorata*. *Macoma balthica* and the crustaceans *Saduria entomon*, *Pontoporeia affinis*, and *Pontoporeia femorata* are the most frequently occurring species in this community.

D) An *Astarte borealis - Astarte elliptica* community occurs in the clay, sand and gravel bottom of the slopes and sills of the Slupsk Furrow at depths between 60 m and 90 m.

The community consists of twenty species, with *Astarte* sp., *Saduria entomon*, *Scoloplos armiger*, and *Terebellides stroemi* predominating. The clams *Astarte* sp., found in this community only, predominated both in terms of abundance and biomass, contributing about 66 % and 87 %, respectively.

E) A *Scoloplos armiger - Macoma balthica* community occurs in the least populated, mainly muddy bottom area below 60 m.

This community comprises species which are able to tolerate extensive changes in oxygen concentration, such as the polychaete species *Scoloplos armiger*, *Bylgides sarsi*, *Arcidea suecica* and also the most ubiquitous benthic species occurring in the Baltic Sea, *Macoma balthica*. In this community, the most frequent and abundant species, which also comprises a large part of the biomass, is *Scoloplos armiger*. In this zone, it is possible to observe a temporary disappearance of some species due to oxygen deficit as well as recolonization of the bottom after refreshed oxygen conditions (inflow of oxygen-saturated water from the North Sea).

F) Lack of bottom macrofauna—the muddy bottom region below 70 m is practically uninhabited by benthic organisms due to the long-term oxygen deficit. Only a few small organisms, such as nematodes, exist at this depth. Recolonization in this area has been observed; however, it has usually been short term.

The significant dominance of bivalves is a characteristic feature of almost all communities except *Scoloplos armiger - Macoma balthica*, where polychaetes contribute 50 % of the total biomass.

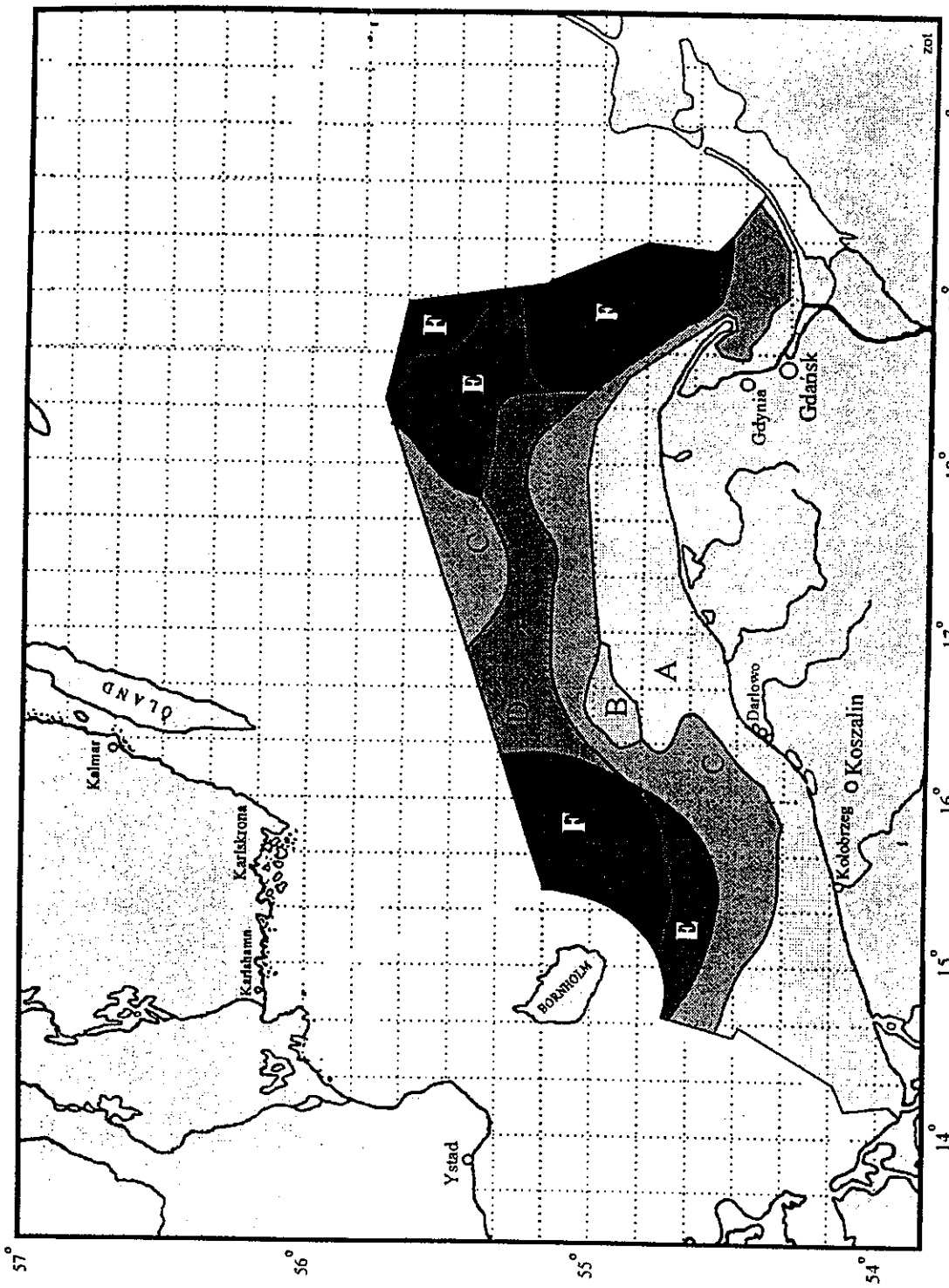
Macrophytes in the Baltic Sea

The Baltic macrophyte community is composed of marine and limnic plants and algae. Most of the marine algae can also be found in the North Sea. Due to the specific hydrodynamics and geomorphology, both the limnic and marine species have to cope with physiological stress caused by the very large range in salinity, the annual temperature fluctuations, and the seasonal changes in the intensity of the light in northern areas. The substrate type is also of great importance; because the substrate in the southern Baltic and the Baltic Proper consists mainly of sand and finer sediment, macroalgae have few surfaces on which to attach themselves. These areas are dominated by perennial higher plants such as eel grass, pond weed, and reed.

Since the 1960s, growing evidence of a massive decline in macrophytes has been reported. Climatic and hydrological changes, and eutrophication have been identified as the most important reasons for this decline. The increasing nutrient (phosphorus and nitrogen) concentrations stimulate particularly the primary production of planktonic algae and fast-growing epiphytic green algae. As a result, the community composition, production, and depth contour of the Baltic flora have changed. The most objective changes are the decline in perennial macrophytes (e.g., *Fucus* and *Zostera*) and an increase in algal blooms (Schramm, 1996).

From the 1950s to the end of the 1980s (Vogt and Schramm, 1991; Schwenke, 1965), a tremendous loss in abundance and biomass of *Fucus* was observed in Kiel Bay due to a shift in the lower depth contour of its

Figure 6.3.1. Benthic macrofauna communities inhabiting the southern Baltic Sea area (Warzocha, 1997).



A) *Macoma balthica -Mya arenaria* community
B) *Mytilus edulis - Gammarus salinus* community
C) *Macoma balthica - Saduria entomon* community
D) *Astarte borealis - Astarte elliptica* community
E) *Scoloplos armiger - Macoma balthica* community
F) Lack of bottom macrofauna

distribution from 10 m to 2 m. During the same period, the lower depth distribution of the red algae community in Kiel Bay shifted from 20 m to 18 m, while the contour of maximum biomass moved from 14–16 m to 8 m. The community changed from a *Furcellaria* sp. dominated community to a *Coccotylus* sp. and *Phycodrys* sp. community (Schramm, 1996).

Fish in the Baltic Sea

There are around 200 species of fish found in the North Sea, but this number decreases in the brackish waters of the Baltic Sea, with many of the marine species being replaced by purely freshwater species in the northern and eastern areas of the Baltic Sea (Table 6.3.2). No detailed information on freshwater fish populations was available.

In addition to commercially important species, there are several species that are protected by the Bern Convention, or which appear on the three protected fauna lists of the EU Habitats Directive. The marine species in this category are shown in Table 6.3.3, but it should be noted that there will be several other freshwater species on these lists. Several of these species also spawn on seabed substrates or have close associations with the seabed environment.

Table 6.3.2. Numbers of fish species and relative abundance of marine and freshwater types in the various areas of the Baltic Sea (Thiel *et al.*, 1996).

Area	Marine species	Migratory species	Freshwater species	Total species
Baltic Sea (without the Kattegat) (total)	97	7	40	144
Belt and Arkona Seas (southwestern Baltic)	97	7	22	126
Bornholm Sea, Gotland Sea, and Gulf of Riga (Baltic Proper)	41	7	23	71
Åland Sea, Archipelago Sea, Gulf of Finland, and Bothnian Sea (eastern and northern Baltic)	27	5	33	65
Bothnian Bay	10	5	25	40

Table 6.3.3. Marine fish species in the Baltic Sea that are protected by the Bern Convention or appear in the protected species lists of the EU Habitats Directive.

Species	Endemic/non-endemic	Geographical distribution
Alosa alosa	non-endemic	southern Baltic
Acipenser sturio	non-endemic	all
Alosa fallax	non-endemic	all
Coregonus albula	endemic	Gulf of Finland, northern Gulf of Bothnia
Coregonus lavaretus[1]	non-endemic	all
Cottus gobio	non-endemic	all
Cottus poecilopus	non-endemic	southern and northwestern Baltic
Lampetra fluviatilis	non-endemic	all
Triglopsis quadricornis	non-endemic	all
(*Myoxocephalus*)		
Petromyzon marinus	non-endemic	southern Baltic
Pomatoschistus microps	non-endemic	middle and southern Baltic
Pomatoschistus minutus	non-endemic	middle and southern Baltic
Salmo salar	non-endemic	all

(1) Subspecies:
C. lavaretus lavaretus: gillrakers 22–29 (mean 25), usually smooth; Gulf of Finland and Gulf of Bothnia.
C. lavaretus mediospinatus Pravdin: gillrakers 27–40 (mean about 35), mostly with denticulations; Gulf of Finland.
C. lavaretus pallasi Valenciennes: gillrakers 39–48 (mostly 42–44), usually with minute denticulations; Gulf of Finland.
C. lavaretus oxyrinchus Linnaeus: gillrakers 35–44 (usually 40), snout pointed; southwestern Baltic Sea and southeastern North Sea.

Although it is less productive than the North Sea, the Baltic Sea supports a productive fishery, with the marine species of herring, sprat, and cod being the most important fish. Catches of these species peaked in the late 1970s and early 1980s, declined until the early 1990s, and have recently increased due to the development of an industrial fishery for sprat. Cod, the most valuable of these species, has suffered from overfishing and poor environmental conditions for reproduction. While less important in commercial terms, there are also several fisheries for freshwater species.

Importance of the Baltic Sea to Seabirds

The seabird fauna of the Baltic Sea is primarily characterized by the almost ten million birds estimated to over-winter in the region (Durinck *et al.*, 1994). The winter bird fauna of the Baltic Sea is numerically dominated by benthivorous species, especially seaducks which comprise about 80 % of the total number of birds. Eight species occur in the Baltic Sea in numbers representing more than half the number in Western Europe (Rose and Scott, 1994); hence, these species are most likely to become detrimentally affected at the population level: red- and black-throated diver (*Gavia stellata, G. arctica*), mute swan (*Cygnus olor*), long-tailed duck (*Clangula hyemalis*), black scoter (*Melanitta nigra*), velvet scoter (*Melanitta fusca*), smew (*Mergus albellus*), razorbill (*Alca torda*), and black guillemot (*Cepphus g. grylle*).

Sensitive and Protected Areas and Habitats

It was noted that the proposed 62 Baltic Sea Protected Areas (BSPAs) will provide a network of protected sites of conservation importance; in addition, red book data are being compiled, and there is on-going work on habitats and threatened habitats.

Environmental Impacts and Impact Assessment

WGEXT noted that several of the dredging operations in the Baltic Sea on which it had information had not conformed to good dredging practice. Departures from agreed good practice (such as dredging fine material or dredging deep pits) may result in deleterious environmental effects, such as the release of nutrients into the water column or the development of anoxic conditions. WGEXT stressed the importance of the conduct of dredging operations according to the ICES Code of Practice and generally seeking to follow good dredging practice.

ICES ACME recommends to HELCOM the use of the ICES Code of Practice for the Commercial Extraction of Marine Sediments (published in ICES, 1992) and the Guidelines on environmental impact assessment (EIA) for the extraction of sand and gravel (ICES, 1994). In addition, there will be requirements under the Espoo Convention (not yet in force) for impact assessments to be made where projects may give rise to transboundary effects and, additionally, there are requirements of the European Union with regard to EIA. It is assumed that extraction projects would be subject to environmental impact assessments in the Baltic Sea area, which is the case in most North Sea and Northeast Atlantic areas.

The information below also highlights the importance of a full assessment of risks to seabirds, fish, and benthos and to special habitats, in particular, the Baltic Sea Protected Areas (BSPAs).

Chemical impacts on the seabed and water column

Seabed disturbance of sediments may result in mixing of the sediment with the overlying water. Additional inputs arise as a result of the discharge of fine sediments from the dredger overflow, which may give rise to localized turbidity.

The disturbance of sediment with a significant content of fine material will result in the mixing of interstitial water with overlying sea water and, potentially, the release of chemical components from the sediments. The composition of the interstitial water is likely to be most strongly affected by organic matter within the sediments. For example, the decomposition of this material can lead to, *inter alia*, nutrient and metal releases from particulate to dissolved phases.

Decomposition of organic matter, desorption of components from organic matter and clay minerals, and dissolution of soluble material may also occur when sediment particles and water are mixed by disturbance, during uplift or discharge. The effects of mixing on the water column may include increased consumption of oxygen by decomposing organic matter and the release of nutrients and metals. Equally, suspended clay minerals, with a high surface activity, may act as adsorbents of some dissolved species (e.g., trace metals).

It should be emphasized that the chemical effects arising from aggregate dredging are likely to be minor on account of the very low organic and clay mineral content of the sediments suitable for commercial extraction. In addition, dredging operations are very localized and transient, which further limits such effects.

Impacts on benthos

The Baltic Sea environment is potentially much less stable than that of the North Sea and English Channel. For example, in addition to the effects of natural sediment disturbance, caused primarily by wave action, there are also effects arising from spatial and temporal variations in salinity and temperature which, over the Baltic Sea, can range from 2–30 and −1 °C–22 °C, respectively. Such conditions favour the development of a benthic fauna more typical of an estuarine environment, and deposits of sand and gravel in the Baltic Sea tend to be dominated by species such as *Macoma balthica, Mya*

arenaria, Mytilus edulis, Cerastoderma glaucum, and *Hydrobia ulva*. Research into the effects of sand and gravel extraction in the Baltic Sea indicates that, where these animals occur, they quickly return to their pre-dredged status. Three examples provide information on the impact on and the recolonization of macrofauna after dredging:

1) In 1988 the area of Slupsk Bank, off Poland, at a depth of 17 m and a salinity of 7 (Okolotowicz, 1991), was extensively dredged. An examination of the macrobenthos after the dredging ceased indicated that the total number of taxa returned to the pre-dredged value within one year.

2) In 1985 near the town of Kotka in Finland (Winterhalter, 1990), 100,000 cubic metres of sand were dredged; this led to a significant deepening of the site down to 17 m, resulting in the total elimination of macrofauna. After one year, the number of species had returned to the baseline level, but the abundance and biomass remained at a low level, suggesting that the community would need several years to recover fully.

3) Between July 1987 and March 1988, 3 x 10^6 m^3 of sand were dredged in Køge Bay, off Denmark, creating 10 m deep pits with anoxic conditions that had significant effects on the benthos below 7 m depth (Norden Andersen *et al.*, 1992). In addition, trailing suction dredging removed up to 2 m on the sea floor, leaving a pattern of furrows 1.5 m wide and 0.5 m deep on the seabed. In this area, the benthic macrofauna recovered in numbers of taxa, abundance and biomass within 17 months after dredging. However, the settlement of mussels on boulders (exposed by the dredging operation) changed the composition of the former community.

There are a number of habitats supporting benthic species and communities which have particular sensitivity. These include:

- habitats which support large slow-growing invertebrates, namely, *Arctica islandica, Astarte* spp.;
- areas with macrophytes which provide important habitats for many invertebrates, such as species of gastropoda (*Rissoidae*), isopoda (*Cyathura*) and amphipoda (*Gammarus* sp.);
- spawning areas for fish;
- areas of seabed which are important as feeding grounds for wintering sea ducks, such as eider, scoter, and long-tailed ducks.

Effects on macrophytes in the Baltic Sea

Parts of the macrophyte distribution in the Baltic Sea overlap with candidate areas for sand and gravel extraction. As there are only a few studies that address the growth and recolonization of macrophytes, dredging of sand and gravel in areas stressed by eutrophication is of particular concern. As a consequence, shallow coastal sandy areas to 8 m depth and boulder areas to 20 m depth should be treated as particularly sensitive.

Effects on fish and fisheries

In ICES (1992), WGEXT stressed the importance of careful evaluation of seabed spawning habitats when the licensing of gravel extraction operations is being considered. The report stressed, in particular, the requirement for herring spawning grounds and whitefish (*Coregonus*) spawning grounds to be assessed when extraction operations were planned in the Baltic Sea area.

There are a number of ways in which aggregate extraction operations may affect populations of fish. These include:

- localized avoidance of any disturbance caused by extraction activities (notably of suspended material in the plume, e.g., Westerberg *et al.*, 1996);
- localized alteration of the benthos and possible reduction in food resources for certain fish species;
- localized alteration of seabed habitat caused by removal of sediment and settling of suspended material (identified as of particular importance for bottom-spawning fish with discrete spawning grounds occurring in candidate extraction areas);
- localized effects of suspended sediments on egg and larval stages (important also for pelagic-spawning fish, e.g., Westerberg *et al.*, 1996).

As with marine mammals, turbidity in the water column may result in avoidance behaviour by fish. The environmental assessment should evaluate potential impacts, particularly for any fish species with critical migrations through any areas likely to be affected in this way. Given the size of extraction areas, any effects of altered benthic communities on fish stocks are likely to be small and not detectable against natural variation in the fish stock. It is, however, important that all licensed extraction operations retain the nature and type of the original surface sediment layer and that no areas are dredged to a depth which results in an altered sediment type. Particular attention should be given to seabed spawning species and this aspect of the biology of freshwater species requires further attention.

Recent work in the Baltic Sea has highlighted the possible effects that suspended sediments may also have on the buoyancy of fish eggs and on the survival rate of ichthyoplanktonic stages (Westerberg *et al.*, 1996). The adhering of particles to cod eggs causes loss of buoyancy and the eggs sink to the bottom. Given the avoidance reaction of fish to suspended sediments, it is difficult to extrapolate from this work the likely effects on a stock,

but again critical spawning areas for pelagic-spawning fish should also be reviewed in any environmental assessment.

Conclusions on fish

The ACME accepted the view of WGEXT that the sources of environmental disturbance arising from extraction operations that might potentially have effects on fish and fisheries would be similar in the Baltic Sea to those recorded for other areas. The fish species and their ecology and populations are, however, much more specific to this sea area, and this feature would benefit from further investigation involving scientists with specialized knowledge of this aspect of Baltic Sea ecology. The principal means of ensuring that extraction operations do not have deleterious effects on fish and fisheries is good management. In this regard, any impingement on either critical spawning seasons or critical migrations can be avoided by selectively halting extraction operations at certain times of the year, if necessary. Similarly, as elsewhere, licensed extraction operations may not be permitted on known critical seabed spawning habitats (e.g., herring spawning grounds). The general view of WGEXT was that, while the Baltic Sea ecosystem is unique and different from the truly marine areas where extraction operations occur, extraction activities could be properly managed so as to ensure that no detrimental effects occur to fish or fisheries.

Impacts on Seabirds in the Baltic Sea

General

Only in a very few recent assessments have the extent and nature of the impact of extraction activities on seabirds been investigated. The populations and distributions of seabirds in the Baltic Sea may be affected in the following ways:

a) reduction of feeding conditions for plunge and pursuit diving birds through reduction of water clarity;

b) reduction of food resources for herbivorous species through negative impacts from sediment dispersal on vegetation;

c) reduction of food resources for piscivorous species through negative impacts from sediment dispersal on vegetation and fish larvae;

d) reduction of food resources for benthivorous species through removal of benthic communities and negative impacts from sediment dispersal on the settling of mussels;

e) avoidance of any disturbance caused by extraction activities.

WGEXT regarded all such potential effects as being localized and confined to the vicinity of any extraction operation.

Potential impact on benthivorous seabirds

Assessments of the impact of dredging activities associated with the construction of the fixed links across the Great Belt in Denmark and across the Öresund between Denmark and Sweden support the general concept that impacts on seabirds are local and site specific (Tasker *et al.*, in press). No impact has been experienced thus far in relation to the earth works in the Öresund (Miljø- og Energiministeriet, 1996), whereas a strong local (< 5 km radius from the source) effect on the number of wintering eiders (*Somateria mollissima*) has been reported in the Great Belt (Jensen and Skov, 1997). The overall distribution of birds wintering in the Baltic Sea is characterized by a large number of spatially discrete areas with distinct populations, with strong gradients in densities of birds occurring over short distances. This characteristic, along with the variation in the response of prey species to sediment loads, and the type of extraction make extrapolation of results from site to site very difficult.

Sensitive areas and habitats

Due to the heterogeneity of the distribution of sea ducks in the Baltic Sea, future research should be directed to the core feeding areas in the coastal lagoons and on the offshore banks, where densities above 1000 birds per km^2 may be found. All available information on seabird distribution and numbers in the Baltic Sea has been put together in a geographical information system and published in Durinck *et al.* (1994). Based on this information, 39 areas of international importance for seabirds were determined, of which only ten areas held about 90 % of the total estimated number of seabirds wintering in the Baltic Sea. Clearly, in these key areas, it is important that birds need to be given due consideration in the preparation of environmental impact assessments in relation to future extraction activities.

Conclusions on birds

There are likely to be only limited and very local effects on bird populations from marine aggregate extraction activities. Major concentrations of seabirds can readily be avoided by selectively choosing certain locations and seasons.

The information necessary for an assessment includes the following:

1) the proportion of the total population represented by any specific area (here the 1 % threshold is noted as representing a frequently used threshold of significance);

2) whether the population is of regional or national importance;

3) whether it is a rare or protected species and/or it is endemic to the Baltic Sea;

4) the depth range for feeding (over-wintering migrants and those staking);

5) breeding sites, etc., for resident species.

Marine Mammals

It was noted that effects on marine mammals had not been considered in connection with the fixed link project in the Great Belt. Populations of marine mammals seem to be increasing. Haul-out sites are coastal and not likely to be affected. There was a view that any indirect effects (loss of food chain production) would be insignificant. Perhaps localized avoidance behaviour might be observed as the only direct effect.

Conclusions on marine mammals

Marine mammals should be considered in any environmental assessment, but the view was that any effects on them were not likely to be of significant concern.

Conservation and Protected Areas

As a general rule, dredging operations would not be permitted in conservation areas. WGEXT felt that any effects of dredging or extraction were likely to be localized. However, specific local conditions should be appraised and potential effects on any nearby protected or sensitive areas addressed in the environmental impact assessment. WGEXT noted that during dredging in the Öresund, a plume of suspended materials up to 40 km long has been observed. Such factors clearly require proper evaluation prior to the licensing of an extraction operation, but in many areas of the Baltic Sea such turbidity would be far more localized, perhaps only extending to 1000 m or so.

Conclusions on Protected Areas

Clearly, any licensed extraction activities should ensure the integrity of protected and sensitive sites and, for species, their favourable conservation status.

Recommendations

ICES ACME recommends that Member Countries follow the ICES Guidelines for Environmental Impact Assessment (ICES, 1994) and the ICES Code of Practice on Commercial Extraction of Marine Sediments (published in ICES, 1992).

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7 FISH DISEASE ISSUES

7.1 Results of the Analysis of Data on Disease Prevalence in Wild Fish Stocks

Request

There is no specific request. However, the ACME considered this topic to be of importance for OSPAR since studies on fish diseases have been recommended for integration in the biological effects monitoring component of the OSPAR Joint Assessment and Monitoring Programme (JAMP) and since an assessment of fish disease data held in the Environmental Data Centre could be incorporated into the OSPAR Quality Status Report 2000.

Source of the information presented

The 1997 reports of the Study Group on Statistical Analysis of Fish Disease Data in Marine Fish Stocks (SGFDDS) and the Working Group on Pathology and Diseases of Marine Organisms (WGPDMO), and ACME deliberations.

Status/background information

The ACME reviewed the progress made with regard to the statistical analysis of fish disease prevalence data in the ICES Environmental Data Centre. According to decisions taken during 1996, data were submitted to ICES intersessionally by laboratories in selected ICES Member Countries (Denmark, Germany, the Netherlands, and the UK), compiled by the ICES Secretariat, and statistically analysed at the 1997 SGFDDS meeting.

The data used by SGFDDS for analysis were restricted to diseases of dab (*Limanda limanda*) covering the period 1981–1996 and stations in the North Sea, English Channel, and the Irish Sea. Four diseases of dab were considered: lymphocystis, epidermal hyperplasia and/or papilloma, acute and/or healing skin ulcerations, and liver nodules < 2 mm. Analyses of spatial and temporal trends in disease prevalence were carried out using generalized linear models taking into account the following factors: area (ICES statistical rectangles), time (month or quarter of the year), sex, and size group. Interaction terms between these factors were left aside due to imbalances in the data structure.

The ACME noted that the results of the preliminary spatial analyses are presented in the SGFDDS report as maps showing the estimated disease prevalence as circles with different diameters. In general, areas with high disease prevalence include the central North Sea and the east coast of the UK. However, as a considerable amount of fish disease data are still under preparation for submission to the ICES Environmental Data Centre, WGPDMO considered it premature to conduct a full interpretation in terms of the biological significance of the findings and the role of environmental contaminants.

Temporal trends in the estimated prevalence are shown according to year and ICES statistical rectangle. Only preliminary results for lymphocystis, epidermal hyperplasia/papilloma and acute/healing skin ulcerations were included, whereas data on liver nodules were considered too limited and, therefore, excluded from the analyses. For lymphocystis and epidermal hyperplasia/papilloma, pronounced trends were identified with the highest prevalences in the mid-1980s. However, as for the spatial trends, a conclusive interpretation of the findings is still not possible.

Need for further research or additional data

The ACME endorsed the conclusion of WGPDMO that the tasks of the Study Group have been fulfilled, and appreciated the successful efforts of the Study Group as well as of the ICES Secretariat. The completed ICES fish disease databank, as well as the standardized methodologies developed for data submission and statistical analysis, will facilitate future analyses combining fish disease data with environmental and fisheries data. It will also contribute to the OSPAR Joint Assessment and Monitoring Programme and the Quality Status Report 2000. The statistical analysis of fish disease data will be continued by WGPDMO as part of its regular work.

The ACME noted that the fish disease data set is still not complete with respect to spatial coverage and species and, therefore, encouraged ICES Member Countries to submit their data to the ICES Environmental Data Centre.

7.2 BMB/ICES Sea-going Workshop in the Baltic Sea

Request

There is no specific request; this is part of the continuing ICES work aiming at standardization and coordination of fish disease studies in the ICES area.

Source of the information presented

The 1997 report of the Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) and ACME deliberations.

Status/background information

The ACME noted that the BMB/ICES Sea-going Workshop on Fish Diseases and Parasites in the Baltic Sea was held on board the German R/V 'Walther Herwig III' under the co-convenership of T. Lang (Germany) and S. Mellergaard (Denmark) with twelve scientists attending, representing eight of the nine Baltic Sea countries. Practical work was carried out at eleven sampling sites along a transect from the southwestern (Mecklenburg Bight) to the northeastern (Gulf of Finland) Baltic Sea. The region covered represented the largest area of the Baltic Sea ever studied for the occurrence of fish diseases and parasites during a narrow time-window and using identical, intercalibrated methods.

The objectives of the Workshop were:

a) to provide scientific background data on the prevalence and spatial distribution of fish diseases and parasites in the Baltic Sea to be used as baseline information for further research and monitoring programmes,

b) to intercalibrate methodologies used for fish sampling, disease/parasite diagnosis, reporting and analysis of disease data,

c) to assess the applicability of the standard methodologies recommended by ICES for fish disease surveys and, if necessary, to recommend modifications to be followed in fish disease/parasite studies in the Baltic Sea, and

d) to enhance international cooperation and coordination between Baltic Sea countries with regard to research and monitoring of fish diseases/parasites in the Baltic Sea.

The scientific background data on diseases and parasites of fish species examined during the course of the Workshop and the main conclusions and recommendations on methodologies to be used in future fish disease surveys have been compiled in ten scientific papers; it is intended that these papers be submitted for publication in the *ICES Journal of Marine Science*. Issues covered are:

- externally visible diseases and parasites of flounder (*Platichthys flesus*);
- liver histopathology of flounder;
- metazoan parasites of flounder;
- enzyme induction in flounder;
- externally visible diseases and parasites of cod (*Gadus morhua*);
- externally visible diseases and parasites of herring (*Clupea harengus*) and sprat (*Sprattus sprattus*);
- bacteriology of the skin ulcer disease in Baltic fish;
- recommendations for the design of fish disease surveys in the Baltic Sea.

The ACME endorsed the view of WGPDMO that, due to the joint assessment and the practical exercises carried out during the Workshop, methodologies used for fish disease studies in the Baltic Sea can now be considered intercalibrated to a large extent and have reached a level comparable to that achieved for North Sea studies.

It was pointed out that the Baltic Sea constitutes a unique environment characterized by strong gradients of abiotic factors, such as temperature, salinity and oxygen content, which have a marked impact on species abundance, diversity and physiological performance. The BMB/ICES Workshop has shown that, owing to these factors, not all of the methodological guidelines developed by ICES based on the results from two workshops in the North Sea and Kattegat (Dethlefsen *et al.*, 1986; ICES, 1989; Bucke *et al.*, 1996) are directly applicable for studies in the Baltic Sea. The ACME emphasized that the design of fish disease monitoring programmes in the Baltic Sea should make use of the guidelines developed on the basis of the experience obtained during the BMB/ICES Workshop.

The ACME noted with appreciation that cooperation among Baltic Sea countries conducting fish disease surveys has improved considerably during recent years, mainly due to BMB and ICES activities, and that the BMB/ICES Workshop further strengthened these contacts.

The ACME endorsed the conclusion of WGPDMO that the objectives of the BMB/ICES Workshop can be considered fulfilled. Future fish disease research and monitoring programmes in the Baltic Sea will benefit from the scientific baseline data obtained during the Workshop as well as from the intercalibration and standardization of methodologies achieved.

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ICES. 1989. Methodology of fish disease surveys. Report of an ICES Sea-going Workshop held on U/F 'Argos', 16–23 April 1988. ICES Cooperative Research Report, No. 166. 33 pp.

8.1 Overviews of Contaminants in the Marine Environment

Request

There is no specific request; this is part of the continuing ICES work to keep under review contaminants of interest in a marine environmental context.

Source of the information presented

The 1997 report of the Marine Chemistry Working Group (MCWG), the review notes submitted by MCWG, and ACME deliberations. MCWG coordinates the preparation of overviews on contaminants that may be of interest in a marine environmental context. The papers that pass this review process are transmitted to ACME for further consideration.

Status/background information

At its meeting in 1997, MCWG examined review notes on several compounds and classes of compounds for which there is interest in a marine context, in order to evaluate their significance as contaminants in the marine environment. The following describes the current status of this work.

Irgarol 1051

The 1996 ACME report described the status of the work on a review of the environmental occurrence and significance of the antifouling agent Irgarol 1051. At the 1997 MCWG meeting, an overview paper on Irgarol 1051, prepared by R. Law (UK), was presented. This paper has been accepted and is attached as Annex 4. A brief summary is given below.

The herbicide Irgarol 1051 belongs to the same family of products as atrazine and simazine. The major application of Irgarol is in antifouling paints, to which it is added to prevent the formation of algal slimes. In these products, Irgarol is formulated with copper, copper and zinc oxides and thiocyanates, and zinc pyrithione as biocides. Several studies have quantified Irgarol in marine environmental samples. The highest reported concentrations of Irgarol in water (up to 1700 ng l^{-1}) have been observed at marinas where pleasure craft were moored, but port areas showed lower concentrations (< 5 to 280 ng l^{-1}), and coastal areas still lower concentrations.

Current discussions within the International Maritime Organization (IMO) may lead to an extension of restrictions on the use of TBT-based antifouling paints to include larger vessels, which is likely to further increase the usage of copper- and zinc-based paints that now often incorporate Irgarol 1051 in their formulation. New information provided in the review includes results from a study of Irgarol 1051 concentrations at a marina in Sweden, and data on the usage of Irgarol 1051 in Ireland, Belgium, Norway, and Denmark. Information on the usage of Irgarol 1051 in Germany is not available. While Irgarol 1051 is the only *s*-triazine herbicide which is used directly in marine systems, others (such as atrazine and simazine) are used far more abundantly in agriculture and are subsequently transported to estuarine and coastal locations. There is little information available as to the levels which will cause harmful biological effects, but the mode of action is likely to be additive with other triazines, such as simazine and atrazine. The algal toxicities of atrazine, simazine, and Irgarol 1051 seem to be broadly similar and marine animals appear to be much less sensitive than algae or plants. Irgarol bioaccumulates only slightly in fish and depurates rapidly when the fish are transferred to clean water.

Need for further research or additional data

Further information is needed on concentrations of Irgarol in coastal and estuarine waters before the environmental significance of its use in antifouling paints can be fully assessed, and the need for further controls evaluated. As new information on the ecotoxicological effects of Irgarol 1051 is now becoming available, this overview paper needs to be updated in the near future.

Toxaphene

A paper entitled 'Toxaphene in the marine environment', prepared by J. de Boer (The Netherlands), was reviewed and accepted by ACME. It is attached as Annex 5, and a summary is given below.

Toxaphene is an insecticide used primarily in cotton growing. Due to atmospheric transport, toxaphene is found even in remote marine areas. Toxaphene is mutagenic, potentially carcinogenic, and possibly weakly estrogenic.

Toxaphene is a very complex mixture primarily consisting of chlorinated bornanes. Although 32,768 congeners are theoretically possible, the total number of congeners in the environment is considerably smaller.

The bioconcentration of toxaphene is expected to be relatively high, based on the estimated octanol-water partition coefficient (log K_{ow}) of 6.44. High levels of toxaphene have been found in aquatic organisms from all over the world. For example, high toxaphene levels, up to 28 mg kg^{-1}, have been found in fish from the St. Lawrence River, Canada, and in Canadian cod liver oil. Toxaphene concentrations in North Sea fish are at least one order of magnitude lower than those in fish from Arctic and Canadian waters and vary from 1–600 µg kg^{-1}

wet weight. Very high toxaphene concentrations have been found in marine mammals, e.g., 23 mg kg^{-1} in St. Lawrence beluga whales.

Fish consumption is anticipated to be the main source of toxaphene exposure to humans. Tolerance levels with regard to fish consumption are only known from the USA (5 mg kg^{-1} wet weight) and Germany (0.1 mg kg^{-1} wet weight). Several fish species from the North Sea and the Baltic Sea may exceed this level.

The comprehensive discussion paper on toxaphene in the marine environment (Annex 5) describes the different analytical techniques required to determine total toxaphene and individual congeners. In addition to the analytical techniques, the use of bioassays to directly determine the toxicity of toxaphene appears promising.

Need for further research or additional data

Additional information is needed to assess the carcinogenicity of toxaphene. Additional information is also needed on the distribution of toxaphene and its degradation products, particularly in marine fish.

Formation of hexachlorobenzene metabolites

Hexachlorobenzene (HCB) is a ubiquitous contaminant in the environment from its use as a pesticide, as an intermediate in chemical processes, and given its formation as a combustion product. An examination of the formation of metabolites of HCB is of interest as an example of the complexity of behaviour of organochlorine compounds in the marine environment.

Major and minor metabolites of HCB have been detected in anaerobic sediments (see Figure 8.1.1), in fry of steelhead trout (*Oncorhynchus mykiss*), in *Saccharomyces cerevisiae* expressing human cytochrome P450 3A4, and in excreta from rats (see Figure 8.1.2). At least 42 different metabolites have been reported, including chlorobenzenes, chlorophenols such as pentachlorophenol (PCP), and compounds containing thiol, methylthioether, methyl sulphone and methyl sulphoxide groups. Some metabolites are lipophilic and can bioaccumulate, with some being toxic to fish.

These studies are generally carried out under experimental conditions in the laboratory. The difficulty in assessing the formation of HCB metabolites in the environment was recognized, particularly as PCP and its metabolites, as well as some lower chlorinated benzenes, occur independently of HCB.

Need for further research or additional data

More research on HCB and its metabolites in the marine environment is required if the environmental toxicity and effects of this complex array of compounds, and their implications for monitoring programmes, are to be understood.

References for further reading

Frankovic, L., Khan, M.A.Q., and Ghais, S.M.A., 1995. Metabolism of hexachlorobenzene in the fry of Steelhead trout, *Salmo gairdneri (Oncorhynchus mykiss)*. Archives of Environmental Contamination and Toxicology, 28: 209–214.

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Koss, G., Reuter, A., and Koransky, W. 1986. Excretion of metabolites of hexachlorobenzene in the rat and in man, pp. 261–266. International Agency for Research on Cancer (IARC), Oxford University Press.

Mehmood, Z., Williamson, M.P., Kelly, D.E., and Kelly, S.L. 1996. Metabolism of organochlorine pesticides: The role of human Cytochrome P450 3A4. Chemosphere, 33: 759–769.

Renner, G. 1988. Hexachlorobenzene and its metabolism. Toxicological and Environmental Chemistry, 18: 51–78.

Susarla, S., Masunaga, S., and Yonezawa, Y. 1996. Reductive chlorination pathways of chloro-organics under anaerobic conditions. Water Science and Technology, 34: 489–494.

WHO. 1997. Environmental health criteria for hexachlorobenzene. World Health Organization.

Polychlorinated diphenyl ethers

A paper entitled 'Polychlorinated diphenylethers: origin, analysis, and distribution in the marine environment and toxicity' was prepared by J. de Boer and M. Denneman (The Netherlands), and reviewed and accepted by ACME. It is attached as Annex 6, and a summary is given below.

Polychlorinated diphenylethers (PCDEs) are present as impurities in chlorophenol preparations, which are often used as wood preservatives. Other sources of PCDEs, such as municipal waste incinerators, seem to be of minor importance. The relatively limited data currently

Figure 8.1.1. Major (> 50 %) pathways (indicated by ⇒) and minor (< 50 %) pathways (indicated by →) for the transformation of HCB under anaerobic conditions. Half arrows show slow processes (Susarla *et al.*, 1996).

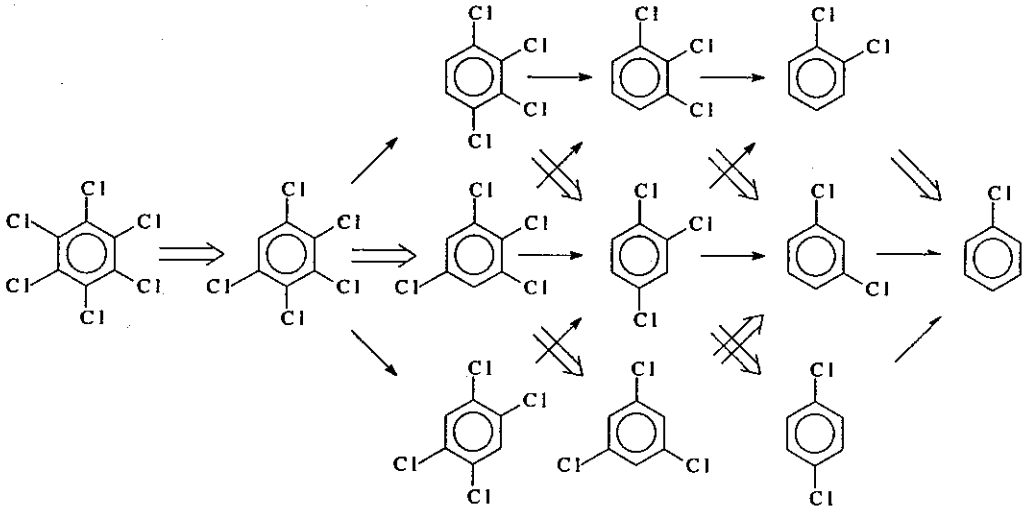
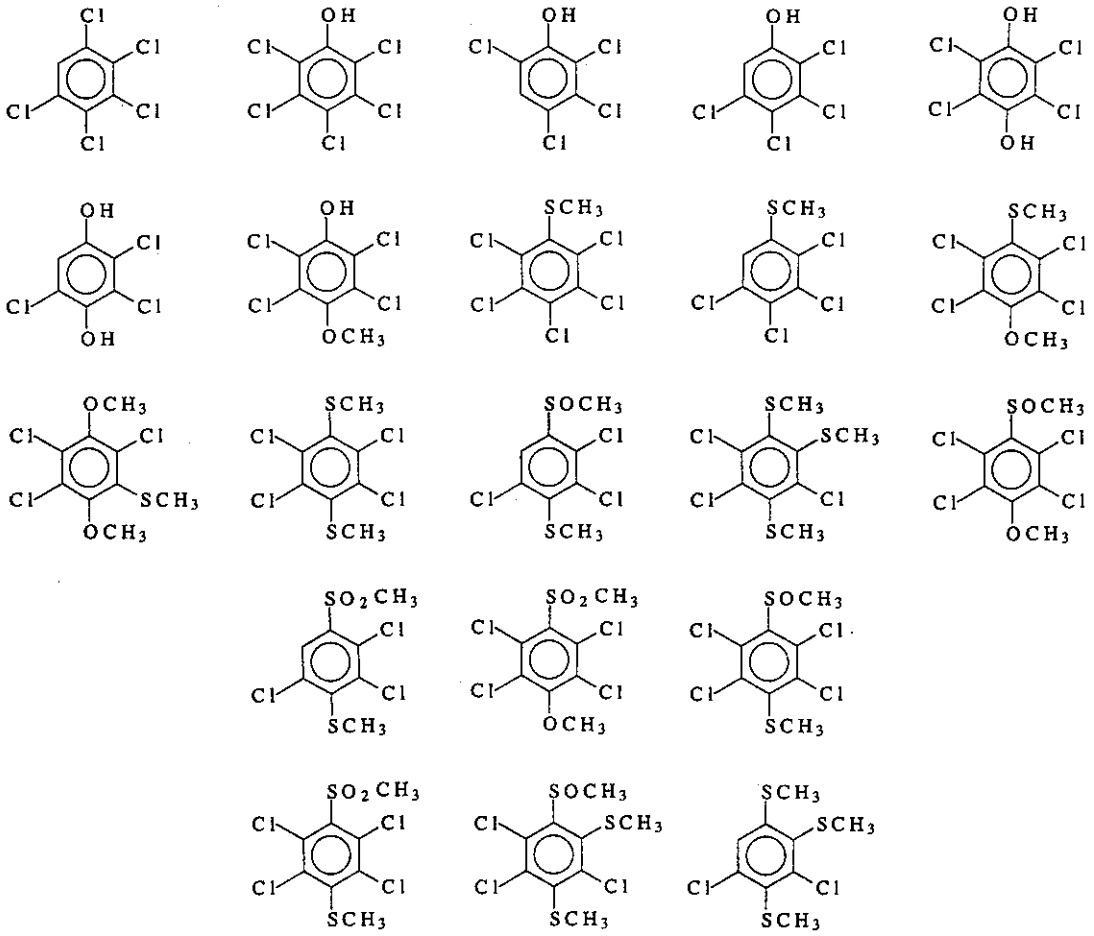


Figure 8.1.2. HCB metabolites found in rat urine (Koss *et al.*, 1986).



available suggest that PCDE concentrations in marine organisms are lower than concentrations of polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs) or polychlorinated naphthalenes (PCNs).

Many data are available on PCDE concentrations in freshwaters in Finland. High levels of PCDEs were found in Finnish rivers and lakes, which is clearly related to the use of chlorophenols in wood preservatives. Salmon, pike and bream from the Simojoki and Kymijoki rivers in Finland contained ca. 25–800 $\mu g\ kg^{-1}$ total PCDE on a lipid weight basis. High concentrations of PCDEs were found in Baltic white-tailed sea eagles (up to 13 $mg\ kg^{-1}$ lipid weight per congener).

The toxicological information available on PCDEs shows that they are immunotoxic and also have potential to induce hepatic microsomal aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-*O*-deethylase (EROD) activities.

Recommendations

In view of the limited information on PCDE concentrations in marine organisms, further research on levels of PCDEs in marine fish and mammals is recommended.

8.2 Transfer of Halogenated Organic Compounds through the Pelagic Food Chain

Request

Item 6 of the 1997 requests from the Helsinki Commission.

Source of the information presented

The 1997 report of the Marine Chemistry Working Group (MCWG), the paper 'Bioaccumulation: Chemical and biological factors governing the transfer of organic compounds in food chains', and ACME deliberations.

Status/background information

The ACME considered a review paper entitled 'Bioaccumulation: Chemical and biological factors governing the transfer of organic compounds in food chains', which had been prepared by A. Abarnou and V. Loizeau (France) in collaboration with M. Lebeuf (Canada) and A. van der Zande (The Netherlands). The ACME agreed that this paper provides a comprehensive introduction to the subject of biomagnification of halogenated organic contaminants in pelagic food chains. This paper is attached as Annex 7 to this report and a summary is given below.

The factors which govern the degree of transfer of halogenated organic compounds in food chains (i.e., their biomagnification) are fairly well understood, although a precise prediction of the degree of biomagnification which will occur under a given set of circumstances can only be made if a large amount of chemical and biological information is available.

Thus, the main chemical factors which govern uptake into aquatic organisms relate to a compound's hydrophobicity (as defined by its octanol–water partition coefficient, K_{ow}) and its resistance to biological degradation. If these are high, accumulation may also be high, although beyond a certain molecular size a compound becomes too big to pass from the water phase through biological membranes. Both hydrophobicity and resistance to biodegradation are essentially determined by structural characteristics of the molecule, but whereas quantitative structur–-activity relationships (QSARs) for K_{ow} are well established for halogenated organics, those for biodegradation are less so. Empirical measurements of K_{ow}, and more especially of biodegradation, are therefore still widely employed. Another important chemical factor is the degree of ionization of the molecule, which is very low in chemicals which are able to accumulate in biota. A summary of the main chemical characteristics which act as predictors of bioaccumulation is shown in Table 8.2.1.

As a general rule, chemical properties are most useful for predicting the bioconcentration factor (BCF), which is the degree to which aquatic organisms are able to accumulate a chemical directly from the water phase in which they live. To be precise, the BCF is the ratio between the contaminant concentrations in the water and the organism, at equilibrium. This is a particularly important mode of uptake for pelagic organisms that are fairly low in the food chain, such as plankton, filter feeders, and planktivorous fish. BCF values in algae, bivalves, and fish can be reliably predicted from log K_{ow} for a variety of chlorinated hydrocarbons (including those of concern to HELCOM). Details of these QSARs are given in Annex 7, including specific information on polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs and PCDFs).

On the other hand, there are many biological characteristics which modify the transfer of chlorinated organics through food chains, including respiration rate, growth, reproduction, and metabolic competence. These factors are highly species-specific, and depend in part on the position of an organism in the trophic web and its feeding habits. For example, the respiration rate will govern the amount of water pumped across the gills, which in turn affects the amount of dissolved organics to which an organism is exposed. Furthermore, absorbed residues will tend to become diluted in rapidly growing

Table 8.2.1. Characteristics of organic chemicals which predict the potential for bioaccumulation.

Characteristic	Predictors for high bioaccumulation
Chemical structure	High capacity: high proportion of C–C, C–H, and C–halogen bonds Few functional groups
Molecular weight	> 100 Da., with a maximum at about 350 Da., then declining to very low at about 600 Da.
Molecular dimensions	Cross-sectional width: < 9.5 Å Molecular surface area: 208–460 $Å^2$ Molecular volume: 260–760 $Å^3$
Stability	Resistance to degradation reflected in soil persistence in the order of years
Log K_{OW}	> 2, with a maximum at 6, and a decline to very low at about 10–12
Water solubility (mole l^{-1})	< 18, with a maximum at about 0.002
Degree of ionization	Very low

Table 8.2.2. The major biological factors governing bioaccumulation.

Factors tending to maximize bioaccumulation	Factors tending to minimize bioaccumulation
Small size of species (leads to high diffusion rates)	High excretion rate via faeces and urine, especially for relatively soluble compounds
Low trophic levels (especially algae)—species experience strong adsorption	Strong degradation mechanisms in tissues
High respiration rate in gill breathers	High growth rate, leading to dilution
High feeding rate on lipid-rich prey in consumers at high trophic levels	Low lipid content
Deposit feeding (especially for hydrophobic compounds)	Maturation of gonads and spawning
Suckling in newborn marine mammals	Transfer *in utero* and via milk

organisms, and the fat-soluble halogenated organics will tend to be transferred out of the organism when it spawns or suckles young. Finally, bioaccumulation will be less marked in those species which possess the biochemical apparatus that can efficiently metabolize the contaminant, although in many cases halogenated compounds are metabolized to substances which are themselves retained in the body (e.g., DDE, a metabolite of DDT). The major biological factors governing bioaccumulation are summarized in Table 8.2.2.

In broad terms, biological processes affect the dynamics of uptake, and therefore have a major influence on bioaccumulation and biomagnification. They are particularly important for predicting accumulation in organisms at higher trophic levels, but the large number of biological variables limits the generic usefulness of computerized models; they only tend to be precise and reliable for the species on which they are based. Thus, the most reliable generic predictions of bioaccumulation, based essentially on chemical properties, can only be derived for organisms occupying relatively low levels in pelagic food chains, although the propensity of a compound to biomagnify can certainly be predicted on a qualitative basis. Thus, of the compounds referred to in the HELCOM request, most of the DDT family and many of the PCBs are highly biomagnified in many pelagic food chains, whereas HCB and the dioxins and dibenzofurans are biomagnified to a lesser or negligible extent.

Recommendations

ICES ACME recommends that HELCOM consider the paper by Abarnou *et al.* (Annex 7) as a comprehensive introduction to the subject of biomagnification of halogenated organic compounds in pelagic food chains.

8.3 Effects of Contaminants on the Immunology of Marine Organisms

Request

There is no specific request; the ACME considered this topic as being relevant for the development of new techniques for the assessment and monitoring of biological effects of contaminants in marine organisms.

Source of the information presented

The 1997 reports of the Working Group on Pathology and Diseases of Marine Organisms (WGPDMO), the Working Group on Biological Effects of Contaminants

(WGBEC), the Working Group on Environmental Assessment and Monitoring Strategies (WGEAMS), and the Working Group on Seals and Small Cetaceans in European Seas (WGSEAL), and ACME deliberations.

Status/background information

The ACME reviewed the relevant sections of the above-mentioned reports on the status and usefulness of techniques measuring changes in the immune system of marine invertebrates and mammals, as indicators of early contaminant-induced biological effects.

The ACME noted that, during the past two decades, a large variety of experimental and *in situ* studies on effects of contaminants on the immune functions of marine organisms have been carried out and a number of new techniques utilizing molecular methods have been developed in this field. These studies have shown that a broad range of environmental contaminants have the potential to cause immunomodulation, possibly leading to changes in disease susceptibility. However, it has also been demonstrated that the specific and non-specific immune systems of marine organisms are highly complex, involving different components, some of which are not well understood or, possibly, not even identified so far. Therefore, the ACME considered that techniques measuring immunomodulation may be promising for future biological effects monitoring programmes. At present, however, investigations are still at the research stage and an immediate incorporation of these techniques into existing monitoring programmes is not advisable.

The ACME endorsed the conclusions of WGBEC that:

- contaminants can cause immunomodulation;
- relating immunological reactions to causality is still problematic;
- higher level consequences, such as those relating to disease susceptibility, are uncertain since the immune system is a complex adaptive system (i.e., an ecosystem in its own right);
- it is recommended that this issue be revisited regularly in the future, as the science is now advancing rapidly and better understanding of immune responses in invertebrates, fish, and marine mammals, as well as new tests, are likely to emerge in the near future.

Need for further research or additional data

The ACME emphasized that more basic experimental and *in situ* research is needed for understanding the immune system of marine organisms and the effects of environmental contaminants on components of these systems before the usefulness of techniques measuring immunomodulation for monitoring purposes can be assessed.

Recommendations

ICES ACME recommends that further studies on the effects of environmental contaminants on the immune systems of marine organisms be carried out and that progress in this field be reviewed by relevant ICES Working Groups.

8.4 Reproductive Effects of Contaminants in the Marine Environment

Request

No specific requests have been made on this subject, but it is felt by ACME to be topical in view of the concern about such effects in freshwater systems.

Source of the information presented

The 1997 report of the Working Group on Biological Effects of Contaminants (WGBEC) and ACME deliberations.

Status/background information

The ACME took note that WGBEC had discussed this issue with a view to stimulating research on this topic.

One example of reproductive problems in the marine environment involves the M-74 syndrome in Baltic salmon which impairs the survival of fry (see Section 6.2, above). It is similar to the swim-up syndrome found in the fry of Lake Ontario lake trout (and a number of other species), and is almost certainly caused by thiamine deficiency passed on from the parents. The reasons for this thiamine deficiency are still unknown, but it is suspected that there is a direct or indirect relationship with anthropogenic contaminants. Further research on this subject is urgently required. There are also possible links between contaminants and reproductive abnormalities in some seal populations (see Section 11.4.1, below).

There are now some limited data which show that male fish in certain estuaries are being feminized by oestrogenic contaminants, perhaps as a result of exposure to sewage discharges (see, e.g., ICES, 1996). The implications of this feminization for fish population survival are not yet understood, although sparse experimental data suggest that a variety of deleterious effects (e.g., reduced egg production, increased larval mortality) are to be expected. This subject should be investigated further in order to establish whether effects like those seen in some rivers also occur in the sea.

Almost nothing is known about the effects of these so-called endocrine disruptors on invertebrates, whether freshwater or marine, although there are some reports of abnormal sex ratios in crustacea exposed to sewage

effluents. The only well-understood example concerns tributyltin-induced imposex and intersex in gastropod molluscs, which is mediated by elevated testosterone titres. Invertebrate hormonal systems are poorly understood compared with those in vertebrates and there is, therefore, a need for both basic and applied research in this area.

WGBEC agreed that there is a need to conduct laboratory, field, and mesocosm studies which link the responses of biomarkers of endocrine disruption (e.g., vitellogenesis in male fish) to deleterious changes at higher organizational levels (e.g., reduced reproductive fitness, population survival). Without such research, it will not be possible to draw conclusions about the possible effects of endocrine disruptors and other reproductive toxicants on the ability of marine life to reproduce itself.

In order to guide researchers, WGBEC compiled lists of biomarkers and bioassays currently used to investigate the reproductive/endocrine-disrupting effects of contaminants in the aquatic (mainly freshwater) environment, reproduced here as Tables 8.4.1.a and 8.4.1.b. WGBEC also compiled lists of research needs in the fields of biomarker and bioassay development, as indicated in Tables 8.4.2.a and 8.4.2.b.

Need for further research

ICES ACME recognizes the potential importance of anthropogenic substances in the marine environment with the ability to interfere with normal reproduction via hormonal and other routes, and recommends that further research of the type discussed by WGBEC be undertaken.

Reference

ICES. 1996. Report of the ICES Advisory Committee on the Marine Environment, 1996. ICES Cooperative Research Report, No. 217: 91–95.

Table 8.4.1.a. Currently used biomarkers relevant to the reproductive effects of contaminants in the marine environment.

Biomarkers currently used	Specificity	Comments
Vitellogenin	oestrogen mimics	oestradiol-inducible hepatic protein
Oestradiol receptor	oestrogen mimics	hepatic levels increase in response to oestrogens
Egg shell proteins	oestrogen mimics	serum levels increase in response to oestrogens
Testicular and ovarian maturation	non-specific stressors	histological staging of germ cells in gonads
Intersex	oestrogen mimics and aromatase inhibitors	identification of hermaphrodites
Imposex/Intersex	tributyltin	morphological observations
Gonadosomatic indices	non-specific stressors	measures relative changes in gonadal weight
Secondary sex characteristics	hormone mimics and anti-hormones	morphological observations
Serum and tissue hormone concentrations	endocrine disruptors	generally measured by radioimmunoassay (RIA)
Aromatase inhibitors	specific inhibitors (e.g., tributyltin, flavanoids)	measures conversion of testosterone to oestradiol by ovary (fish) or adipose tissue (mammals)
Sex ratio	substances which alter sex steroids	morphological observations of populations/communities
Behaviour	non-specific	observation
Atresia and apoptosis	non-specific stressors	measures cell death in ovary
Vitamin A (retenoic acid)	organic contaminants	measured in liver (consequences for fish are not yet established)

Table 8.4.1.b. Biomarkers that require development to assist the study of reproductive effects of contaminants in the marine environment.

Biomarker research needs	Comments
Androgen biomarkers	to measure effects of contaminants on androgen action
Invertebrate biomarkers	to measure effects of contaminants on invertebrate reproduction
Gonadotropin receptor	as an indicator of gonadal maturation and a possible site of action for contaminants
Thyroid hormone action	may be an important biomarker for PCBs
Insulin and insulin-like growth factors	stressors and contaminants which act on metabolism

Table 8.4.2.a. Currently used bioassays relevant to the reproductive effects of contaminants in the marine environment.

Bioassays currently used	Specificity	Comments
E-screen	oestrogens and anti-oestrogens	human breast cancer cell line whose proliferation is regulated by oestrogens
Yeast cell genetically engineered to contain the human oestradiol receptor	oestrogen mimics	oestrogens activate a reporter gene which initiates a colour reaction
Fish ovarian follicular cell culture	inhibitors of steroid biosynthesis	ovarian follicles are stimulated with gonadotropin; steroid synthesis is measured in culture medium

Table 8.4.2.b. Bioassays that require development to assist the study of the reproductive effects of contaminants in the marine environment.

Bioassay research needs	Comments
Androgen bioassay	needed to identify androgenic substances and anti-androgens
Whole fish bioassay	marine species which can be used for life cycle studies
Invertebrate bioassays	marine species needed for life cycle studies

9 MARINE BIOLOGICAL PROCESSES AND RESPONSES

9.1 Responses of the Marine Environment to Anthropogenic Nutrient Inputs in some Example Areas

Request

There is no specific request; this issue is of interest to ICES for the scientific aspects, and to the Oslo and Paris Commissions and the Helsinki Commission for the regulatory aspects.

Source of the information presented

The 1997 report of the Working Group on Phytoplankton Ecology (WGPE) and ACME deliberations.

Status/background information

The ACME took note that nutrient levels on a global scale have been increasing in many marine coastal waters. Examples of this include coastal waters of the United States and the North Sea. Changes in the ratios of essential nutrient salts, such as in N:P, N:Si and Si:P in marine waters, also accompany nutrient loading. The significance of such changing ratios is that riverine delivery of nutrients not only influences the primary production responses to nutrient fluxes, but also resource-competition effects among groups of algae in coastal waters.

This pattern of long-term increases in nutrient loading has been accompanied by increases in phytoplankton biomass and primary production and changes in species composition with occurrences of novel, unusual and/or toxic phytoplankton blooms.

These changes have occurred in areas such as Asian coastal waters (Japan, China, Korea), the Black Sea, the inner Adriatic Sea, the North Sea, and embayments within the Skagerrak, the Kattegat, and the Baltic Sea. The specific processes involved are still being elucidated, but both site-specific and basic ecophysiological processes influence the outcome of the nutrient-stimulated responses of the phytoplankton community. One response common to several regions (e.g., the Kattegat, the German Bight, and the New York Bight) is the development of *Ceratium* blooms, which are largely ungrazed events, and which eventually become nutrient limited, sink to bottom sediments, and decompose leading to hypoxia and anoxia. Widespread die-offs of benthic and pelagic populations often result. A complex series of interactive biological and physical (such as water mass mixing characteristics) processes influence both the occurrences and consequences of such *Ceratium* blooms.

The Baltic Sea

In the Baltic Sea there are marked regional differences in phytoplankton growth conditions. Nevertheless, the results of the HELCOM Third Periodic Assessment of the Marine Environment of the Baltic Sea suggested that the increase in inputs of nutrients has increased phytoplankton production and biomass in the major gulf areas during the last decades; especially plankton blooms have intensified. Enhanced sedimentation of organic matter has caused anoxia in the bottom water layers also in shallow areas. Nitrogen or nitrogen and phosphorus together are proved to be the limiting nutrients for primary production. Silicate limitation is also reported. This is expected to be the main reason for the observed dominance of dinoflagellates in spring. Low N:P ratios are promoting the development of N_2-fixing cyanobacterial blooms. These blooms are not directly related to eutrophication, but the increased inputs of phosphorus are also supposed to have intensified these blooms.

The model calculations made for the Gulf of Finland indicate that only when both nitrogen and phosphorus inputs are reduced by approximately 50 % all around the sea area, will the phytoplankton biomass concentrations be reduced by 10–40 % in a few years.

The Kattegat Region

In the autumn of 1984, diarrhetic shellfish toxin (DST) suddenly became a threat to the growing Swedish mussel industry and harvesting was prohibited during the entire winter due to high values of DST. This was not the first time Swedes became ill by eating blue mussels (*Mytilus edulis*), but the problem was relatively unknown and very little was known about the mechanisms behind the intoxication. This situation was not unique for Sweden, and a similar pattern has been observed in a number of countries in Europe during the 1990s. It is now known that the *Dinophysis* species or *Prorocentrum lima* may contain DST and that DST-toxic mussels have become a more or less annual problem.

The apparent increase in DST occurrence took place at approximately the same time as large dinoflagellate blooms and oxygen deficiencies in bottom water were recorded in many areas, e.g., the German Bight, the Kattegat, and Scandinavian coastal waters. There is a general conviction among many scientists that the blooms and the oxygen problems were a result of eutrophication which, in addition to increased macronutrient inputs, also involved an increased nitrogen-to-phosphorus (N:P) ratio in many areas. Laboratory experiments have shown that many dinoflagellates, which have a potential to become

toxic, may increase their toxicity when grown under nutrient-deficient conditions or under unbalanced nutrient conditions. In addition to the effects of eutrophication, changes in the micronutrient composition of the runoff in areas affected by acidification may also have influenced the development of toxic *Dinophysis*. Thus, it can be concluded that the increase of DST in blue mussels during the 1990s in some European coastal waters, followed by the recurrent closure of harvesting of blue mussels, serves as an example of the possible effects of anthropogenic inputs to the marine ecosystem.

Norwegian coastal waters

In Norway, a national group of experts has carried out assessments of the state of eutrophication in the outer Oslofjord and in the waters along the Norwegian Skagerrak and west coasts (Anon., 1996, 1997). The assessments have been done using time series data from monitoring programmes and model calculations. A time series of observations on hydrography, oxygen, and sublittoral fish fauna, based on a standardized autumn survey, exists from the 1930s and 1940s. A more comprehensive set of eutrophication variables has been collected by sampling every two weeks at selected coastal stations in the Skagerrak since 1990. A model for water exchange, nutrients, plankton, and oxygen concentrations in fjords has been developed over the last ten years based on theoretical and empirical relationships (Aure and Stigebrandt, 1989a, 1989b). This model was used to provide quantitative estimates taking into account the dynamics of fjords and coastal waters. A 3-D coupled physical-chemical-biological model for water circulation, nutrients, and phytoplankton (NORWECOM) was also used as a tool in the assessments.

It was concluded that there was evidence of regional eutrophication of the coastal water mass in the Skagerrak. This was mainly due to the large nutrient inputs to the coastal waters of the southern North Sea and to the Baltic Sea and the Kattegat. Eutrophication was evidenced by an approximate doubling of the concentration of nitrate and an increased N:P ratio in winter and spring, increased concentration of total nitrogen, decreased oxygen concentration in autumn, and an approximately 50 % increase in the rate of oxygen consumption in fjord basins along the Skagerrak coast. The available data did not present conclusive evidence for biological effects in the coastal waters due to the difficulty in separating possible eutrophication effects from natural gradients. Observed gradients which could be related to eutrophication were reduced maximum depth of growth of red algae and greater density and size of polychaetes in the eastern than in the western part of the Skagerrak. Eutrophication in the Skagerrak has possibly increased the risk for the occurrence and blooming of harmful algae.

Local nutrient inputs from Norwegian sources were found to have a significant effect on the nutrient budget and plankton biomass in the inner part of the outer Oslofjord. In the more open waters of the Norwegian Coastal Current, the effect is, however, very limited due to the large dilution.

The German Bight

An approximately three-fold increase in phytoplankton biomass could be attributed to an increase in flagellates (which in this case are all non-diatoms). Within the flagellates, only nanoplankton < 20 µm was responsible for major changes, as this group of mainly minute flagellates showed a sudden increase at the end of the 1970s. The reasons for this are still unclear. A correlation exists with a sudden increase in nitrate concentrations, which in turn was negatively correlated with the salinity in the German Bight and, hence, with river water influence.

The flagellates without the nanoplankton component, mainly consisting of larger dinoflagellates, did not show a clear trend. Large interannual variations in dinoflagellate biomass (about one order of magnitude) tend to mask potential smaller trends, e.g., due to eutrophication. Those dinoflagellates grow mainly in summer and prefer vertically stratified water, where nutrient concentrations are reduced and motile flagellates have an advantage over diatoms. Such stratified water masses are regularly found within the convergence zone between the continental coastal and North Sea waters. This zone extends in the outer German Bight in a northwestern direction; its changing distance to the Helgoland monitoring station seems to be a major source of interannual variation in dinoflagellate populations measured there.

The Dutch and Belgian Coasts

In a recent series of papers, several aspects of the eutrophication status along the Dutch and Belgian coasts are described. These papers are the results of the North-West European Shelf Programme (NOWESP), an EU MAST Project, and will be published in the German Journal of Hydrography. The main topics of these papers include: long-term trends in the seasonal cycles of chlorophyll, zooplankton, and nutrients; variability in the long-term advective nutrient (N, P, Si) fluxes to the North Sea (1976–1995); and time series analyses of monthly mean data in the NOWESP area. They show the importance of long-term data sets for the analysis of long-term variation even on a decadal scale (cf. van Leussen *et al.*, 1996).

References

Anon. 1996. Outer Oslofjord. Eutrophication status, development, and expected effects of reduced input of nutrients. Report from group of experts on assessment of eutrophication in fjords and coastal waters. State Pollution Control Authority, Norway. 148 pp. (In Norwegian.)

Anon. 1997. The Norwegian coast from Jomfruland to Stad. Assessment of eutrophication status. Report from group of experts on assessment of eutrophication in fjords and coastal waters. State Pollution Control Authority, Norway. 129 pp. (In Norwegian.)

Aure, J., and Stigebrandt, A. 1989a. On the influence of topographic factors upon the oxygen consumption rate in sill basins of fjords. Estuarine Coastal Shelf Science, 28: 59–69.

Aure, J., and Stigebrandt, A. 1989b. Quantitative estimates of the eutrophication effects of fish farming on fjords. Aquaculture, 90: 135–156.

Van Leussen, W., Radach, G., Van Raaphorst, W, Colijn, F., and Laane, R. 1996. The North-West European Shelf programme (NOWESP): integrated analysis of shelf processes based on existing data sets and models. ICES Journal of Marine Science, 53: 926–932.

9.2 Progress in Understanding the Dynamics of Harmful Algal Blooms

Request

There is no specific request; this is part of the continuing ICES work to support research and collect information on this issue, owing to the health and economic problems associated with the worldwide occurrence of harmful and/or toxic phytoplankton blooms.

Source of the information presented

The 1997 report of the Working Group on Harmful Algal Bloom Dynamics (WGHABD) and ACME deliberations.

Status/background information

The ACME noted that a key parameter for interpreting harmful algal bloom (HAB) case studies and/or modelling of HABs is the species' growth rate. For several species, this parameter can be easily determined under laboratory conditions, but in actual blooms in nature, the laboratory information is not always relevant. For some species (e.g., *Dinophysis* spp.) attempts to determine the growth rate have not been successful even under laboratory conditions. In order to address this problem, WGHABD organized a Workshop on *In Situ* Growth Rate Measurements for Dinoflagellates in Kristineberg, Sweden, from 9–15 September 1996. The aim of the Workshop was to compare a number of available methods, both newly developed and traditional. A preliminary compilation of the results has been made and the final report is expected by February 1998.

While the majority of toxic events can clearly be related to the presence of toxic phytoplankton species, e.g., *Alexandrium* spp., *Dinophysis* spp., several 'unexplained toxic events' have been noted in recent years. These events, particularly those reported from France and Ireland, occurred in the absence of known toxic phytoplankton species and no known algal toxins were detected in the shellfish. Work is going on to isolate and identify the toxins involved. The data suggest that organisms other than phytoplankton can produce toxins which can accumulate in shellfish and lead to human illness. Studies to identify the source of these toxins are necessary if management methods are to be developed.

WGHABD continued to collate national reports on HAB events. It was evident that harmful algal toxic events were quite mild in ICES Member Countries during 1996. In addition, national representatives provided maps on the various HAB events in their national waters during the past ten years. On the basis of these maps, C. Belin (France) compiled twelve decadal maps covering the entire ICES area (see Annex 8). An initiative by IOC Science and Communication Centres to establish an information database based on national reports was supported by WGHABD and the centres were encouraged to prepare the databases for the 1998 meeting.

The problems associated with the transfer of harmful species through ship ballast water were discussed. Major concerns identified by WGHABD included that:

1) in addition to dinoflagellate cysts, motile dinoflagellates as well as other taxonomic groups (diatoms, flagellates, cyanobacteria) may be of concern and should also be considered;

2) in addition to studying the transport of dinoflagellate cysts in ballast tank sediments, the relative importance of water column versus sediment origin of cysts should be addressed in relation to dinoflagellate life cycles.

Liaison with the ICES/IOC/IMO Study Group on Ballast Water and Sediments (SGBWS) and the ICES Working Group on Introductions and Transfers of Marine organisms (WGITMO) was considered necessary for defining the relevant treatment options and to address the issue of transfer or movement of shellfish stocks, respectively.

The bloom of algal species, i.e., accumulation of biomass above normal levels, implies a relaxation or thwarting of grazing pressure. Therefore, understanding bloom dynamics is not possible without taking into account the impact by grazers, both pelagic and benthic. The studies needed for the evaluation of pelagic grazer impacts include experimental estimation of the grazing rates of individual animals, quantification of the abundance and composition of the grazer community and toxic and non-toxic phytoplankton in the natural assemblage, as well as direct impacts of toxins on grazers and their fecundity. Information on interactions between HABs and pelagic larvae of bivalve molluscs is rare, and more attention should be paid to benthic grazing as a phytoplankton production loss-term.

Mixotrophy is the ability of an organism to be both phototrophic and heterotrophic, in the latter case utilizing either organic particles (phagotrophy) or dissolved organic substances (osmotrophy). Mixotrophy may give substantial flexibility to a HAB species in its nutritional demands and should therefore be considered as a potential factor providing a competitive advantage over strict autotrophs. An overview of this subject revealed that very little is known about the quantitative importance of mixotrophy in HAB dynamics. It is evident that many phytoplankton species benefit from organic substances present in sea water or reaching coastal zones in river water. Quantification of several processes involved in mixotrophy is urgently needed if realistic models of nutrient transfer in HABs are to be achieved.

The need for agreement about a taxonomic coding system has recently been recognized in ICES. It is recommended that any decisions on adopting an existing system or developing a new system should be carried out as a cooperative activity among WGHABD, WGPE, and the Working Group on Marine Data Management (WGMDM) (see also Section 17.3, below).

In order to facilitate progress in elucidating the role of physical-biological interactions in HABs, WGHABD decided to begin compiling scenarios based on existing HAB case histories in the ICES area. A joint description of the basic systems will form a common basis for a discussion of modelling.

Need for further research or additional data

The ACME noted that there is a need to integrate experiments on the grazing pressure by zooplankton into the studies of HAB dynamics. These include experimental estimation of the grazing rates of individual animals, quantification of the abundance and composition of the grazer community and toxic and non-toxic phytoplankton in the natural assemblage, as well as direct impacts of toxins on grazers and their fecundity. Furthermore, clarification and quantification of the nutritional modes (autotrophy, mixotrophy) of HAB species requires further research. The establishment of an information database on HAB events may require that an agreement be made regarding the taxonomic coding system to be used.

Recommendations

ICES ACME welcomed the initiative to establish a HAB information database and considered it to be a useful tool for facilitating the search for information by several potential groups of users. ICES ACME considered that the maps of HAB events are informative and recommended that they be published annually in the ICES Environmental Status Report available on the ICES website. The establishment of different scenarios leading to the development of HABs will make it easier to distinguish functional groupings and to identify gaps in knowledge. It will also serve as a conceptual framework for modelling.

9.3 Effects of Seabed Disturbance on Benthic Communities

Request

There is no specific request; this is part of the continuing ICES work on studies of benthos in the marine environment.

Source of the information presented

The 1997 report of the Benthos Ecology Working Group (BEWG) and ACME deliberations.

Status/background information

The ACME was informed that the final report of the EU-funded IMPACT II project on the effects of different types of fisheries on North Sea and Irish Sea benthic ecosystems is in its last phase of completion. The contents of this report have been presented along with detailed results of a subproject concerned with the comparison of historical data with data collected during the North Sea Benthos Survey in 1986 by the ICES Benthos Ecology Working Group.

The main chapters of the IMPACT II final report cover the following topics:

- collection and analysis of historical data on benthic fauna;
- collection of historical and recent data on national fishing fleets and effort;
- investigation of mortality introduced by different intensities and types of fishery;
- scavenger responses to trawling;
- comparison of fished and unfished areas.

This report will include a comprehensive account of the various impacts of fishing on different benthic species and communities, as well as on the North Sea ecosystem. A reporting symposium, also covering other related aspects from EU-funded projects, is planned for 1998.

Another detailed report, which provided the results of the Gareloch Disturbance Project, was noted. In Loch Gareloch (a closed sea loch with no fishery for several years), the effects of trawling on the benthic community were studied by experimental trawling which was carried out during nine months. Benthic surveys were made at three-month intervals. Epifaunal densities were estimated using underwater television, while the infauna was sampled by means of a 0.1 m^2 Day grab. Samples were also collected for organic carbon and sediment particle size analysis.

Trawling disturbance had a clear effect, increasing the number of species and individuals relative to the reference area while reducing diversity and evenness. The fact that the measures of diversity decreased while the number of species and individuals increased suggests that there was a disproportionate increase in the abundance of a few dominant species in the treatment area.

The densities of some species declined when compared to the reference area *(Nucula nitidosa, Scoloplos armiger, Nephtys cirrosa* and *Terebellides stroemi)*, suggesting that these species are sensitive to physical disturbance.

Community structure measures of disturbance indicated that, relative to the reference area, the community at the treated area became more disturbed during the trawling period and only became comparable to the reference area eighteen months after the experimental trawling ceased. Measures of the numbers of species, the numbers of individuals, and the species diversity indicated that the sites were indistinguishable after twelve months of recovery, but multivariate analysis of the community data found significant differences between the areas after eighteen months of recovery. No long-term effects on epifaunal species were noted.

Request

There is no specific request, but given the current need to understand more thoroughly the various interactions taking place in marine ecosystems, the examination of the role played by seabirds is of great interest for ICES Member Countries and regulatory commissions. The issue of seabird/fisheries interactions has also been raised by subsidiary bodies of the Oslo and Paris Commissions.

Source of the information presented

The report 'Seabird/Fish Interactions, with Particular Reference to Seabirds in the North Sea' (ICES, 1996), the November 1996 report of the Working Group on Seabird Ecology (WGSE), and ACME deliberations.

Status/background information

The ACME noted that much information and data on seabirds and their interactions within marine ecosystems have been produced within ICES in the past several years. The following account provides highlights of the various studies and major findings.

Seabird/fish interactions

The compiled reports of the 1993 and 1994 meetings of the former Study Group on Seabird/Fish Interactions (SGSFI) were published in November 1996 in the *ICES Cooperative Research Report* series (ICES, 1996). This report should be of interest to all managers and scientists working in marine fisheries or involved with the marine environment.

The objective of the report was to evaluate the interactions that have been identified between seabirds and fish, and between seabirds and shellfish, in the North Sea and other nearby regions. Over four million marine birds breed on the islands and along the coast of the North Sea. In winter, similar numbers forage there, but the species composition differs from that in summer due to seasonal migrations. Additionally, particularly in autumn and winter, half a million seaducks forage in coastal waters and several million migrant waders forage in the intertidal zone. Topics covered in the report include the following:

a) introduction to seabird/fish interactions;

b) estimation of food consumption by seabirds in the North Sea;

c) analysis of fish consumption by seabirds by age class of prey fish;

d) effect of fisheries for small fish on seabirds in the Northeast Atlantic;

e) consumption of shellfish by seaducks and oystercatchers;

f) spatial and temporal variability in the breeding success of seabirds around the British Isles, and evidence for distinct sandeel stocks; and

g) relationships between fish populations and reproductive biology of common terns in the Wadden Sea.

Some of the major conclusions of the report are:

1) Seabirds (excluding seaducks and waders) in the North Sea are estimated to consume 600,000 tonnes of food per year, one-third of which is sandeel.

2) Seabird consumption of prey is unevenly distributed across the North Sea.

3) There is temporal variation in the consumption of sandeels by seabirds in the North Sea.

4) There is relatively little spatial overlap in sandeel harvest by seabirds and sandeel fisheries.

5) Discards and offal represent 30 % of the total food consumed by seabirds in the North Sea.

6) The consumption of shellfish by seaducks in the North Sea is concentrated in the German Bight and the Wadden Sea: annual consumption is estimated to be 100,000 tonnes of bivalves.

7) There appears to be significant spatial correlations in the interannual variability of the reproductive success of seabirds breeding around the British Isles. This result suggests that the birds breeding within regions with similar interannual patterns are responding to changes in the availability of the same fish stocks.

First ICES Seabird Symposium

An International Symposium on 'Seabirds in the Marine Environment' was held in Glasgow, UK, from 22–24 November 1996. This event was co-sponsored by ICES, the Joint Nature Conservation Committee (JNCC), and The Seabird Group. The Symposium focused on the role of seabirds as an integral component of marine ecosystems. The proceedings of the Symposium have been published in the *ICES Journal of Marine Science* (ICES, 1997).

Among aspects of particular interest to the ICES community, there were reports on the role of seabirds as scavengers of fisheries discards. However, the importance of discards in seabird diet should not be overestimated in the absence of data on natural food resources. Furthermore, a study of kittiwakes on the Isle of May showed that breeding success is linked to the amount of sandeel in the diet of the chicks. Birds from

the colony forage in an area, Wee Bankie, where there is a commercial sandeel fishery. In years when sandeel landings were at their maximum, the breeding success was lowest for the kittiwakes. It was also emphasized that a thorough comprehension of the energetics of seabirds is required in order to understand seabird/fisheries interactions.

Seabirds can also be used as indicators of marine contamination. For instance, since birds expel mercury through their feathers, seabirds can be used as an indicator of relative mercury levels in the oceans. Pelagic seabirds have been found to have the highest levels, as their mesopelagic prey accumulate mercury following its methylation in deep waters. In addition, the use of seabird eggs as a matrix for monitoring contaminants has been advocated by ACME (ICES, 1995).

Recent progress on seabird ecology issues

The Working Group on Seabird Ecology (WGSE), successor to the Study Group on Seabird/Fish Interactions (SGSFI), discussed the following major topics at its November 1996 meeting:

a) evaluation of the role of discards in supporting bird populations and their effects on the species composition of seabirds in the North Sea;

b) short-term and medium-term consequences of a reduction in the quantities of fish discarded;

c) causes, and consequences at the population level, of mass mortalities of seabirds;

d) seabird predation on fish by size group;

e) issues related to seabird consumption of fish and shellfish stocks, discards, and mariculture as well as the trophic role and ecology of seabirds and waders.

The main conclusions were:

1) Discards form only a proportion of the diet of seabirds in the North Sea. Furthermore, the percentage of discarded fish consumed by seabirds varies depending on the sub-region. Around 70–90 % of discarded roundfish (206,000 t) in the North Sea is consumed by seabirds, whereas only 10–40 % of discarded flatfish (38,000 t), 12 % of discarded elasmobranches (2,100 t), and 1–23 % of discarded benthic invertebrates (9,000 t) are consumed by seabirds. Around 55–99 % of discarded offal (55,000 t) is consumed by seabirds. About 5,500 t of discards from shrimpers in Niedersachsen (German Wadden Sea) are also consumed. Consumption rates are highest in winter and in the northwestern North Sea.

2) Numbers of seabirds in the North Sea have increased. Increases in recent decades have been most pronounced among scavenging seabird species. Several studies show the importance of discards in maintaining high breeding success and population growth of scavenging seabirds, even though discards may be less suitable for chick food than are sandeel, sprat, or juvenile herring. Scavenging seabirds now represent a much higher proportion of North Sea seabird communities than used to be the case. Reductions in discarding can be anticipated to have impacts, particularly on the smaller scavenging seabird species since they are less able to compete with larger seabirds, especially for larger discards.

3) Mass mortalities (wrecks) are a natural feature of seabirds, and can be due to a variety of causes. The most frequent causes of wrecks appear to be storms, food shortage, or oil pollution. Weather and food shortage may often interact. Few wrecks occur in summer. Most seabird species are subject to occasional wrecks, but some species are more susceptible than others.

4) The size of fish (excluding discards) eaten by seabirds varies with seabird size. Sandeels from 70–150 mm are taken by most seabirds in the North Sea, but terns tend to take sandeels of 30–80 mm.

Need for further research or additional data

Research and data are needed on the following priority topics: seabirds as indicators of variation in prey stocks as well as of the prevalence of chemical contaminants in the marine ecosystem; ecological processes affecting seabird feeding; interactions with mariculture; seabird impacts on recruitment of fish stocks; wrecks and fishery-induced mortality of seabirds; and influences of discards and offal on seabird populations and community structure.

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11 ISSUES REGARDING MARINE MAMMALS

11.1 Status of Marine Mammal Populations in the Baltic Sea

Request

Item 1 of the 1997 requests from the Helsinki Commission.

Source of the information presented

The 1997 report of the Working Group on Seals and Small Cetaceans in European Seas (WGSEAL) and ACME deliberations.

Status/background information

Information based on the report of the Working Group on Seals and Small Cetaceans in European Seas (WGSEAL) relevant to an assessment of the status of the marine mammal populations in the Baltic Sea is presented here. This section should be considered in conjunction with Section 11.2, which deals with the impact of fisheries on marine mammals, and Section 11.4, which deals with contaminant levels in marine mammals and their biological effects.

WGSEAL discussed the definition of the Baltic Sea as stated in the terms of reference, and agreed that this should comprise ICES Area IIId (see Figure 11.1.1). However, it was pointed out that the responsibilities of the Baltic fisheries and environmental commissions also include ICES Areas IIIb and IIIc (inner Danish waters and the German area of the Kiel and Mecklenburg Bays) and at least part of Area IIIa (Kattegat/Skagerrak). Therefore, although WGSEAL focused on ICES Area IIId, information from these adjacent areas was also discussed.

Harbour porpoise *Phocoena phocoena*

Two working papers have provided a comparative analysis of skull morphometrics and mitochondrial (mt) DNA of porpoises from the Baltic Sea, Kattegat/Skagerrak, and western Norway. Baltic porpoises were sufficiently different to suggest that they formed a discrete population. Similar differences have been observed in other recent analyses of mtDNA and microsatellite DNA, and allozyme frequencies in samples from porpoises collected in the Baltic Sea, inner Danish waters, and the North Sea (Andersen *et al.*, 1995; Tiedemann *et al.*, 1996). Earlier studies by Kinze (1985; 1990) also suggested that Baltic porpoises were distinct from those in the North Sea. WGSEAL noted that Walton (1997) had demonstrated significant differences in mtDNA haplotype frequencies between female porpoises from the southern and northern parts of the North Sea, but not between males. WGSEAL therefore recommended that sex, and possibly age, should be included as explanatory variables in statistical analyses of data on harbour porpoise population structure. A preliminary comparison of the frequency of tooth anomalies also supported the recognition of a distinct Baltic population.

The nature and level of contaminant burdens which are related to the diet of porpoises and the contaminant levels of their prey can indicate which area the animal comes from and can also provide insights into population structure. WGSEAL reported significant differences in levels of DDTs, chlorinated biphenyls (CBs), and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs/PCDFs) between porpoises from western Norway, the Kattegat/Skagerrak, and the Baltic Sea. Levels of mercury and methylmercury in porpoises from the North Sea are higher than those in porpoises from the Baltic Sea (Siebert, 1995). Siebert reported that porpoises from West Greenland, the North Sea, and the Baltic Sea could be separated on the basis of their organochlorine pattern; animals from the Baltic Sea and the North Sea also showed severe lesions in internal organs. A more detailed description of contaminant levels in marine mammals and their biological effects is given in Section 11.4, below.

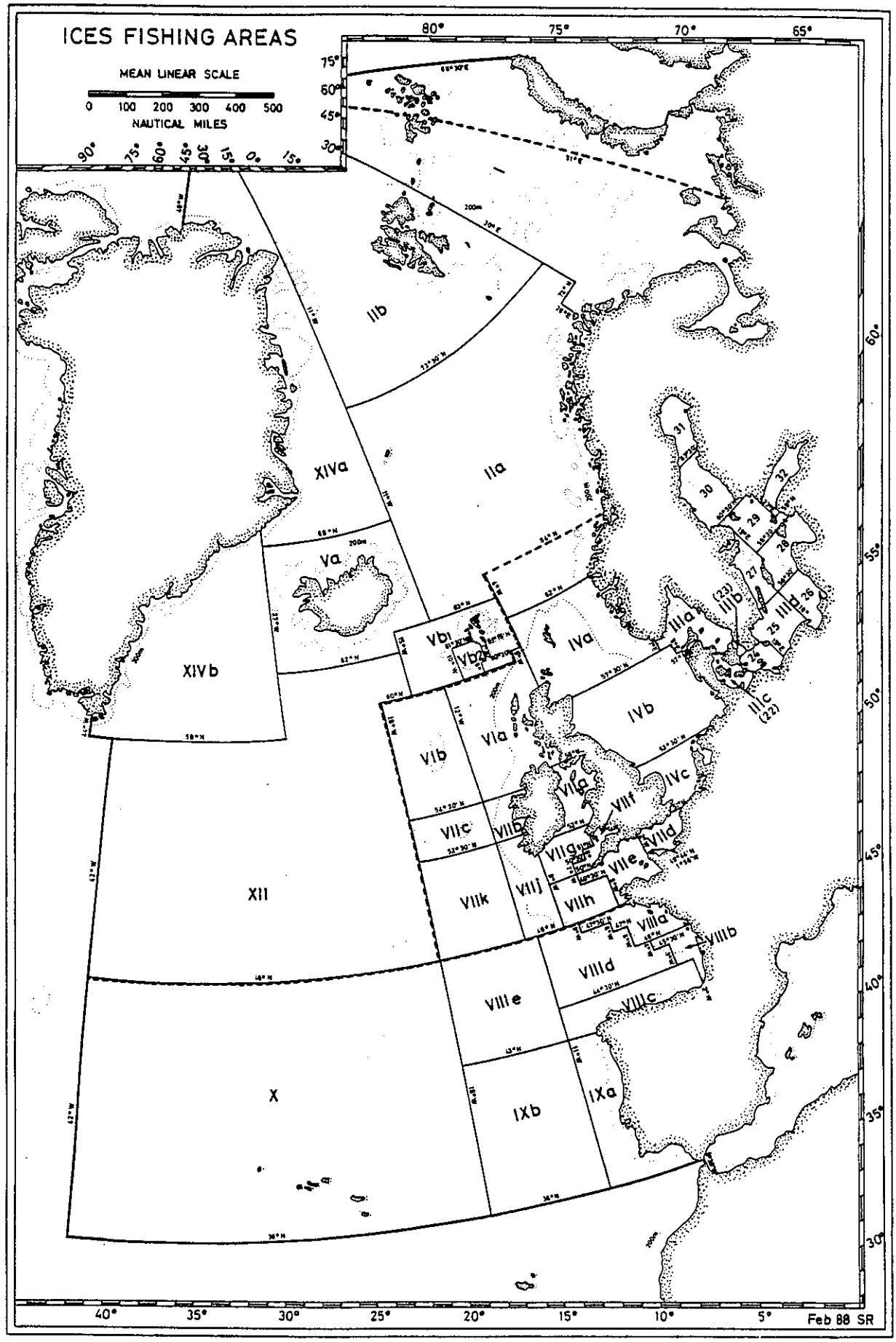
Therefore, WGSEAL concluded that the Baltic porpoises should be treated as a separate population from those in the Kattegat/Skagerrak and the North Sea. However, the ACME noted that this conclusion was based on relatively small sample sizes.

A survey for porpoises in the southwestern part of ICES Area IIId was conducted in the summer of 1995. The Polish coast, where porpoises are known to occur, was not included in this survey. It was estimated that this region contained 600 (95 % confidence limits, 200–3300) pods, i.e., social groups of porpoises. A German survey of the Kiel and Mecklenburg Bays (ICES Area IIIc) in 1996 provided an estimate of 950 (95 % confidence limits, 300–2100) pods in this area.

The porpoise population in the Baltic Sea has been classified as 'vulnerable', i.e., likely to become endangered in the near future if factors responsible for recent declines continue to operate, by the IUCN (IWC, 1994). Historical data on catches in the Baltic Sea and inner Danish waters, as well as anecdotal information, suggest a population decline during this century, but it is impossible to estimate the extent of depletion.

The ACME expressed concern for the status of the Baltic porpoises because of the lack of an estimate of total population size, the lack of accurate information on

Figure 11.1.1. ICES Fishing Areas in the Northeast Atlantic.



levels of by-catch, and because of the number of reported by-catches in ICES Area IIIc (see Section 11.2, below). At its 1995 meeting, the Scientific Committee of the International Whaling Commission (IWC) considered what levels of by-catch can be sustained for particular cetacean populations. It concluded that by-catches exceeding half of the estimated maximum growth rate for the population might not be sustainable (IWC, 1996). This approach was applied to harbour porpoise populations in the North Atlantic, where the Scientific Committee concluded that if the recorded by-catch exceeded 1 % of the total population size (which is one-fourth of the estimated maximum rate of increase of 4 % per year), then this should trigger urgent research (see also ICES, 1996). Based on this criterion, the reported annual by-catch of porpoises in ICES Area IIIc, which is 1.2 % of the abundance, requires urgent investigation.

Grey seal *Halichoerus grypus*

During summer, grey seals haul out at a large number of localities in the Baltic Sea. There are no haul-out sites in the southeast, but the number of sightings of seals in Poland has increased in recent years. The standard technique for monitoring grey seal numbers is to count the number of pups which are born each year. However, Baltic grey seals usually breed on ice, although they will pup on land when ice distribution is limited, and in most years it is impossible to obtain reliable counts of pups. Instead, the maximum number of seals hauled out during the moulting period is used. The maximum count for the whole of the Baltic Sea in 1995–1996 was 5250–5550. When there is limited ice coverage, as there was in 1992, pup counts can be performed in the entire Baltic Sea. Total pup production in 1992 was estimated to be 1400. Harwood and Prime (1978) have suggested that total population size is around 3.5 to 4.5 times pup production. If this is the case in the Baltic Sea, the total population in 1992 was 4900–6300 animals.

More than 1000 pups have been tagged along the west coast of Estonia, and recoveries have been reported from all Baltic countries, except Germany. This shows that pups wander widely within the Baltic region. A sub-adult seal fitted with a satellite-linked radio transmitter also traveled extensively within the Baltic Sea (Sjöberg *et al.*, 1995). Seasonal changes in the abundance of seals in different regions of the Baltic Sea also imply that there may be large-scale migrations of seals from the Baltic Proper to the Bothnian Sea and the Bothnian Bay.

South of 58°N, where seals are found at several small localities, numbers have increased by around 5 % per year since 1984. On the central and southern coasts of the Baltic Proper, the annual increase between 1975 and 1996 has been less than 5 %. In the area from northeast of Stockholm to Umeå, numbers have increased by 12 % per year over the period 1982–1996. However, the haul-out behaviour of grey seals may also have changed over this period. Seals may have become less shy during recent years because of the end of hunting. This is supported by a study at the locality of Tihällan, where counts made at night show an annual increase of 9 %, compared to an increase of 17 % in morning and day-time counts.

The ACME is concerned about the weak development of the grey seal stock in the southern part of the Baltic Sea, where the annual rate of increase is 3.3 %. This figure could be an overestimate due to changes in haul-out behaviour after the end of seal hunting. Despite the low numbers in the south, the total Baltic population has shown a strong increase because of the high growth rate in the north. Both pup counts and investigations at autopsies show that the previously reported reproductive disorders have a less pronounced effect on population development at present. However, the increased prevalence of colonic ulcers also in young specimens as well as the high prevalence of leiomyomas in females are still of concern. Another reason for the low rate of increase in the south could be the high frequency of by-catches of grey seals in the Baltic Sea (see Section 11.2, below).

Future development could be affected by the limited ice areas available for pupping during the last decade, when a large proportion of the pups was born on land. It is indicated that initial pup mortality rates could be as high as 25–30 % when grey seals are confined to breeding on land, whereas considerably lower mortality is recorded among pups born on the drift ice. This could result in relatively weaker cohorts during years when a large proportion of the stock reproduces on land. If limited ice coverage during winter continues, this could lead to a lower rate of increase in the decades to come.

Ringed seal *Phoca hispida*

WGSEAL reviewed earlier counts of ringed seals from the Gulf of Riga and the Gulf of Finland and found that published population estimates in these regions could not be justified. This conclusion is confirmed by results from the first comprehensive surveys in the entire area carried out during 1994–1996. The estimated hauled-out Baltic population of ringed seals in 1996 was about 5510 ± 45 %. Approximately 70 % (3945 ± 1778) was found in the Bothnian Bay, 25 % (1407 ± 590) in the Gulf of Riga, and less than 5 % (150) in the Gulf of Finland. Although colonies of ringed seals have not been reported along the Polish and German coasts, single live and dead animals have been observed.

The local population in the Bothnian Bay is constant or increasing. However, pathology studies have shown that 30 % of the females are sterile. Uterine occlusions occur in 10 % of the young animals sampled in recent years, which indicates that the population may still be affected by pollution from organochlorines (see Section 11.4, below).

The ACME expressed special concern about the situation in the Gulf of Finland, where only 150–200 ringed seals were reported in 1996. Half of the population may have died in the winter of 1991/1992 and, as most of the 150 dead animals found were adults, the effect on the population is enhanced. Additional adult mortality of 2–10 seals per year has been observed up to 1996. Females with occluded uterine horns are also reported from this area.

No data are available on the long-term trends in numbers of ringed seals in the Gulf of Riga. However, the early break up of ice in several years during the last decade has led to high pup mortality when pups have drifted ashore.

Harbour (common) seal *Phoca vitulina*

Harbour seals in the Baltic Proper are dispersed among seven localities in the southwestern region comprising Swedish and Danish waters. Occasional specimens are observed and found dead in German and Polish areas. Genetic studies suggest that the Baltic population consists of two separate groups, of which the southern group (Måkläppen and the Danish localities—also referred to as the 'west Baltic') is closely related to the Kattegat population, while the seals in the Kalmarsund area (also known as the 'east Baltic') are genetically divergent from all other western European stocks of harbour seals (Stanley *et al.*, 1996). The limited contact between these two groups may also explain why the northern ('east Baltic') group escaped the 1988 seal epizootic, while the southern ('west Baltic') stock suffered a similar mortality to that of harbour seals in the Kattegat/Skagerrak area. In addition to these indications of isolation, long-term studies of movements in the Skagerrak have shown that harbour seals are very sedentary (Härkönen and Heide-Jørgensen, 1997).

The first comprehensive aerial survey of the entire Baltic Sea was carried out in 1996. The maximum count in the 'east Baltic' totaled 269 seals compared with 315 in the 'west Baltic', giving a grand total of 584 seals. Population estimates based on maximum counts provide 15 to 25 times higher precision than those based on mean counts.

Regular ground surveys have been conducted at certain Swedish localities since 1989, and seal numbers in the area have been documented by use of a reporting system since 1982. Danish localities have been surveyed from the air since 1990. These data, plus the results of the comprehensive survey in 1996, show that the population in the 'east Baltic' has increased steadily since 1989. The mean proportion of pups was 23.2 % of the annual maximum counts of seals in the area during the period 1989–1996; this is similar to the proportion observed in other populations. Mortality from by-catches has declined in recent years due to the establishment of protected areas. The rate of increase in the 'west Baltic' has been weaker, mostly due to high pup mortality and the effects of the 1988 seal epizootic. The scanty data from Måkläppen and Saltholm on pup production suggest that this is lower than in the 'east Baltic' and more variable from year to year. The few older animals whose pathology has been studied have shown organ lesions similar to those reported earlier in grey seals.

Recommendations

ICES ACME recommends that an aerial survey of the known distribution of the harbour porpoise in the Baltic Sea be conducted as a matter of urgency. This survey should be stratified on the basis of existing information on incidental observations and by-catches so as to minimize the variance of the estimate of abundance. Greater coordination between countries in recording porpoise by-catches throughout the Baltic region is also required.

ICES ACME also recommends that samples for genetic and morphological analysis be collected from all stranded and by-caught porpoises in the Baltic Sea using a standardized methodology (Kuiken and Hartmann, 1991). This material is required to confirm the distinct nature of the harbour porpoise population in the Baltic Sea and may also be used to investigate the existence of sub-populations in the Baltic area.

ICES ACME supports the recommendation of WGSEAL that future studies for tracking the individual movements of animals be undertaken.

ICES ACME also encourages HELCOM to recommend the establishment of additional protected areas in the southern Baltic Sea, which will allow grey seals to recolonize their former ranges in Germany, Poland, and Russia.

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11.2 By-catches of Marine Mammals in Baltic Fisheries

Request

This topic is relevant to item 4 of the 1997 requests from the Helsinki Commission.

Source of the information presented

The 1997 reports of the Working Group on Seals and Small Cetaceans in European Seas (WGSEAL) and the Baltic Salmon and Trout Assessment Working Group (WGBAST), and ACME deliberations.

Status/background information

Data on by-catches of marine mammals in Baltic fisheries were available to WGSEAL from Estonia, Germany, Poland, and Sweden. The first results from an independent-observer programme in Denmark will not be available until 1998. WGSEAL had received no information from Finland, Latvia, Lithuania, or Russia.

The ACME noted that fishing gear and fishing practices and strategies vary considerably throughout the different parts of the Baltic Sea area and that information on effort in the various fisheries is often not available, making extrapolations of estimated by-catch rates to other fisheries or areas very difficult.

Harbour porpoise *Phocoena phocoena*

Records of by-catches and strandings in Polish waters are maintained at the Hel Marine Laboratory, University of Gdansk. There has been an average by-catch of five porpoises per year during the period 1987–1996. March and December were the peak months for by-catches, reflecting fishing effort, mostly in the salmon fishery. Most by-catches were reported from the Gulf of Gdansk. There were no official reports of by-catches in Estonian or Finnish waters. Estonia reported about one porpoise sighting per decade; Finland about one per year. By-catches of 3–5 porpoises per year in bottom-set gill nets and drift nets were recorded from the Swedish Baltic coast throughout the year. These were reported to the Natural History Museum in Stockholm and to the University of Stockholm. An estimated 111 porpoises were by-caught in German fisheries in ICES Area IIIc during the period 1987–1995 (Kock and Benke, 1996), and six were caught in 1996; of these, 112 animals were from the Schleswig-Holstein region and five were from Mecklenburg Bay. The animals were typically young and healthy. Records are maintained by the University of Kiel and the German Museum for Marine Research and Fishery in Stralsund.

These data suggest that reported annual by-catches of porpoises are 0.5–0.8 % of abundance in the southwestern part of ICES Area IIId (Baltic Sea), and 1.2 % of abundance in ICES Area IIIc. The ACME believes that these reports underestimate the magnitude of by-catches in both areas.

Seals

About 200 seals were estimated to have died per year in Estonian waters, predominantly in fyke nets. This is of a similar magnitude to the by-catch reported previously. Grey seals appear to be more vulnerable to being caught than ringed seals and constitute more than 80 % of the by-catches in Estonian waters. Most of the seals were caught in April–June and September–November. In Poland, a total of nine seals, of which a majority was grey seals, was reported being caught in 1996, predominantly in salmon nets. In German parts of the area, one harbour seal was caught by trawl and one in a fyke net in 1995; no cases were reported in 1996.

Salmon drift nets are also responsible for some seal mortalities in Swedish waters, but data are limited. Available Swedish data for the period 1974–1990 show that 29 of 216 (14 %) fishing net mortalities of grey, ringed and harbour seals were caused by salmon drift nets (ICES, 1995). A survey along the Swedish coast north of Åland showed that a minimum of 182 grey seals were by-caught in 1996. The total by-catch in Swedish areas was estimated to exceed 200 animals.

Data are also available from the Finnish fishery. They show that grey seals were by-caught in 1986–1995 in larger numbers in Baltic fisheries as they are more abundant than other seals. Of the 338 by-caught seals reported, 113 (33 %) were from the drift net fishery in the open sea.

In the Bothnian Bay, most of the seals were caught in traps set for salmon and whitefish, while nets set for salmon and whitefish were more important in the Bothnian Sea. In the Baltic Proper, most of the seals were caught in eel traps and turbot nets. Pups of the year made up about half of the total by-catch. In the mid-1980s, a large proportion of seals were by-caught in cod nets, but this problem has diminished with the decrease of the Baltic cod stock.

Effort for the various gear types was not included in the analyses presented; thus, it was not possible to determine whether the differences in by-catch among the three areas reflected the distribution of fishing effort within each area or whether they were caused by differences in susceptibility.

The recorded annual by-catch of about 400 grey seals represents about 29 % of the estimated pup production in 1992, and 7.5 % of the maximum count of seals in 1995–1996. These records almost certainly underestimate total by-catch and may not be sustainable.

WGSEAL noted that the information on by-catches of ringed seals and harbour seals is insufficient to evaluate their impact on populations.

Need for further research or additional data

The ACME stressed the importance of obtaining high quality data on by-catches according to the reporting scheme outlined in the 1996 report of SGSEAL (see ICES, 1996). Whenever possible, this scheme should be implemented and used as a guideline by independent observers. However, the use of independent observers is not applicable to a large proportion of the Baltic fleet, where numerous small open boats dominate the coastal fishery. Therefore, alternative reporting systems must be used to ensure that some information on by-catches becomes available. One possibility is to interview fishermen engaged in various types of fishery, as has been done in Estonia and Sweden. One of the major problems with this approach is that some fishermen are reluctant to provide the requested data, because of suspicions concerning how these data are going to be used. Interviews must therefore be complemented with information campaigns and carried out by personnel whom the fishermen trust. Where independent observers can be used, interview surveys should also be conducted to test the reliability of this technique.

Recommendations

ICES ACME recommends that all ICES Member Countries be requested to provide estimates of the by-catches of marine mammals per unit of effort in individual fisheries, with a clear description of how the estimates were obtained.

ICES ACME noted that fishing gear and fishing practices and strategies vary considerably throughout the different parts of the Baltic Sea area and that information on effort in the various fisheries is often not available, making extrapolations of estimated by-catch rates to other fisheries or areas very difficult. ICES ACME therefore recommends that a comprehensive review be conducted of the fishing gear and fishing practices and strategies employed in the different fisheries in the Baltic Sea, including an assessment of the effort expended in the various fishery operations, and the potential threat they pose to marine mammal populations.

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11.3 Seal Behaviour in relation to Fishing Gear

Request

Item 7 of the 1997 requests from the Helsinki Commission.

Source of the information presented

The 1997 report of the Working Group on Seals and Small Cetaceans in European Seas (WGSEAL) and ACME deliberations.

Status/background information

The ACME took note of the following information from WGSEAL on this topic.

Behavioural Responses of Marine Mammals to Fishing Gear

Results were reviewed from an experimental study of factors affecting by-catches in pelagic trawls, which is co-funded by the European Commission. The response of a three-year old female porpoise in a 20 m x 30 m captive facility at Neeltje Jans (The Netherlands) to nets with mesh sizes ranging from 1.8–10 m, both without and with deterrent noise, was investigated. The aim was to determine the largest mesh size that would act as a barrier. The animal hesitated at all mesh sizes, but it sometimes voluntarily passed through 4.8 m and 7.2 m meshes. It could be scared into passing through a 3.6 m mesh by an acoustic alarm (70 kHz sweeps with a source level of 117 dB re 1 µPa at 1 m). The study concluded that acoustic alarms could be used to manipulate and alter the swimming path of porpoises in the vicinity of nets. A second series of trials was designed to identify what characteristics of acoustic alarms might deter harbour porpoises from entering nets. Broad-band sweeps (3-octave bandwidth centering on 17.5, 35, 70, and 140 kHz) evoked the greatest response. The SCANMAR acoustic distance sensor (110 kHz with source level 158 dB re 1 µPa at 1 m), currently used in industrial fisheries, caused the porpoise to move the furthest possible distance away from the source.

Studies of grey seal behaviour around static fishing gear in Swedish and Estonian waters show that the seals cause considerable damage. The seals appear to be able to enter and leave the fish traps easily, and only occasionally get entangled and drown. Traps (bound nets) used in Estonian waters have inner compartments which are not covered with nets. Seals can enter and leave these inner compartments by jumping over the sides. This very rarely results in damage to the gear, but damage to fish caught in the trap is often extensive. Fish caught in fyke nets are attacked by seals from the outside, causing damage to both the fyke and the fish. It was noted that most of the damage at static gear was caused by grey seals and, to some extent in southern areas, by harbour seals. Ringed seals were rarely involved, probably because of their different distribution, behaviour and diet.

Methods for Protecting Fishing Gear and for Repelling Seals

In Sweden and Estonia, the main method for protecting fish traps is the use of stronger net material. However, this has only a minor effect on damage to the fish catch, and the stronger material increases the risk of drowning for seals getting entangled in the nets. Additional protection is attempted in Estonian waters by covering the inner compartments of bound nets. Attempts at designing a gate that will prevent seals from entering the trap without reducing the catch are being made in Sweden.

Protecting other types of gear is more difficult. The use of acoustic alarms (pingers) has had very limited success, especially since seals appear to habituate very quickly to the signals. A recent review by Jefferson and Curry (1996) of the use of pingers to protect fishing gear from seal attacks concluded that pingers are not effective. Acoustic harassment devices (seal scrammers) have been used with some effect around aquaculture facilities, but their use is limited by their high energy requirements. It was suggested that more effort should be put into developing fishing gear that better sustains seal attacks, but this may increase entanglements.

The ACME took note of plans in both Sweden and Finland to study whether limited hunting in close proximity to fish traps can limit damage by seals. In Sweden these studies will be accompanied by photo-identification studies around control traps to determine whether particular individuals specialize in exploiting fishing gear. The ACME took note of the conclusion of WGSEAL that baseline data on seal behaviour and damage to fishing gear in these areas in the absence of hunting were essential for assessing the effects of these experimental hunts.

Technical Modifications for Reducing By-Catch

A number of studies on the effect of acoustic signals on harbour porpoise behaviour have been conducted in recent years under controlled conditions with animals in captivity, under semi-controlled conditions in the wild, and in commercial fishery operations. A worldwide

review of the effects of pingers has been conducted by Reeves *et al.* (1996). In some fisheries, the use of pingers has resulted in a considerable reduction of the by-catch of harbour porpoises, whereas in other apparently similar fisheries there has been no effect. It is not known why pingers work in some cases but not in others. There is, therefore, a need to study basic harbour porpoise behaviour in the presence of nets, as well as to conduct more large-scale experiments with pingers in different commercial fisheries in different areas. The ACME was informed that a test of the effectiveness of pingers was conducted in the Swedish fishery for cod and pollock in the Skagerrak in March–April 1997. A large-scale test, with a similar design, is planned for the Danish cod fishery in the North Sea in September 1997.

The ACME noted a number of potential problems with the more widespread use of pingers in commercial fishery operations, including habituation of harbour porpoises to pinger signals, increased noise levels in densely fished areas, and the additional cost to the fishermen of using pingers. Enhancing the detectability of nets by using passive reflectors is free of some of these problems, but comparatively little effort has gone into this area of research. It was noted, however, that passive reflectors will only work if the harbour porpoise is using its sonar when approaching the nets.

Other ways of reducing by-catch include modifying existing fishing gear or introducing new fishing gear that does not have by-catch problems. In Denmark, the effect of reducing the height of bottom-set gill nets for turbot is being studied, and in Sweden, the use of baited fish cages as an alternative to bottom-set gill nets is being explored in a pilot study in the cod and pollock fishery in the Skagerrak.

At the 1997 ICES Annual Science Conference in Baltimore, Maryland (USA), a Theme Session is scheduled on 'By-Catch of Marine Mammals: Gear Technology, Behaviour, and Kill Rates' (Co-Conveners: A. Bjørge, G.T. Waring, and G. Massart). This Theme Session is designed to bring together the multi-disciplinary researchers involved in marine mammal by-catch problems, and to discuss and evaluate various mitigation measures. The following topics are being covered:

- gear technology designed to reduce marine mammal by-catches;
- behavioural interactions between marine mammals and commercial fishing operations;
- modification of fishing practices aimed at reducing by-catches;
- marine mammal kill rates (estimation problems and techniques, spatial and temporal scales, population impacts, and conservation measures); and non-lethal by-catch (methods of estimating levels and effects of injuries).

Recommendations

ICES ACME recommends that more research be conducted into ways of reducing the by-catch of marine mammals, including studies of the behaviour of porpoises in the vicinity of nets, the effects of pingers (and potential habituation to them), the effects of passive reflectors, and alternative fishing gear.

ICES ACME encourages Member Countries to report on research into technical measures to reduce by-catch before the next meeting of WGSEAL.

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11.4 Contaminant Levels in Marine Mammals and their Prey

11.4.1 Contaminant levels in marine mammals

Request

Item 1 of the 1997 requests from the Helsinki Commission. The issue of contaminant levels in marine mammals is also of interest to OSPAR.

Source of the information presented

The 1997 report of the Working Group on Seals and Small Cetaceans in European Seas (WGSEAL) and ACME deliberations.

Status/background information

Contaminant Levels

The ACME noted recent information collated by WGSEAL for marine mammals from European seas. Szefer *et al.* (1995) analysed Hg, Cd, Pb, Ag, Zn, Cu, and Mn in tissue samples of harbour porpoises collected along the Polish coast. Levels and patterns were similar to those found in other studies of porpoises from northern European waters and Greenland waters, and in Baltic seals. Recent data on mercury concentrations in harbour porpoises collected in German waters in the North Sea and the Baltic Sea showed slightly higher concentrations

in the animals from the North Sea (Benke *et al.*, 1997). There are significant differences in levels of chlorinated organic contaminants (DDTs, *ortho*- and non-*ortho* CBs, and PCDDs/PCDFs) in samples from harbour porpoises collected in the Baltic Sea, the Kattegat/Skagerrak, and off the west coast of Norway (Berggren *et al.*, 1997). The highest concentrations of DDTs and PCBs were found in the Baltic Sea and the lowest along the Norwegian coast. A similar gradient from the Baltic Sea to the North Sea was found previously in seals (Blomkvist *et al.*, 1992). The concentrations of DDTs and PCBs increased with the age of the animal (Berggren *et al.*, 1997). A decline in concentration levels from 1978–1981 to 1988–1990 was found in the Kattegat/Skagerrak for all contaminant groups, except PCDDs/PCDFs and planar CBs. There was significant spatial variation in contaminant patterns, as revealed by principal component analysis (PCA). The most obvious difference was between the Norwegian animals and the other two groups.

WGSEAL reported that German studies of concentrations of PCBs, PAHs, pesticides, and PCDDs/PCDFs have been conducted since 1994 on blood, brain, liver, and blubber tissue samples from harbour porpoises collected in the North Sea, the Baltic Sea, and a limited number from Greenland waters. The results from these studies showed that it was possible to distinguish between the various geographical populations by their contaminant patterns. In Baltic animals, activities of P450IA and P450IIB enzymes were also measured. Levels of planar CB77, CB126, and CB109 were under the detection limits (1–2 $\mu g\ kg^{-1}$ lipid weight). A report on the results of these studies will be available by the end of 1997. BCPS (bis-*p*-chlorophenyl sulfone), a new contaminant, has been shown to accumulate in the tissues of Baltic fish and grey seals; this is probably a discharge from polymer industries (Olsson and Bergman, 1995). Studies on Baltic biota have also indicated that concentrations of polybrominated compounds have been increasing over time.

Biological Effects

Studies on harbour porpoises collected in the North Sea and the Baltic Sea (Benke *et al.*, 1997) have shown that the most prominent pathological lesions were caused by parasites, especially in the respiratory tract. The parasite infestation was often associated with various degrees of pneumonia caused by secondary bacterial infections. None of these lesions could be related to changes known to result from mercury poisoning in other marine mammals or described for domestic animals or man. WGSEAL reported that an on-going study of harbour porpoises from the Baltic Sea, the North Sea, and Greenland waters showed a significantly lower incidence of pathological lesions of the respiratory system in animals from Greenland.

The prevalence of uterine occlusions in ringed seals from the Gulf of Bothnia has been studied since the end of the 1960s (Helle, 1997). The prevalence increased between 1965 and the end of the 1970s, but has now declined. The prevalence of uterine occlusions increases with the age of the animal. At present, fewer than 20 % of the animals 4–11 years old have one or both uterine horns occluded. These animals were born more than a decade after organochlorine levels in Baltic biota began to decline. The fact that occlusions are still observed indicates that contaminants continue to cause problems, although at a lower level than in the past.

The prevalence of pathological changes in Baltic grey seals has been followed since the middle of the 1970s (Bergman, 1997). A disease complex which has been diagnosed as hyperadrenocorticism (with secondary reactions in other organs, including pathological changes in skin, claws, bone tissue, intestine, arteries, kidneys, female sex organs) has been observed in these animals. The prevalence was compared between samples collected during 1977–1986 and 1987–1996, and among animals of different age groups. The prevalence of adrenocortical hyperplasia, uterine occlusions, and severe bone lesions in the skull has declined and the number of pregnant females has increased. Diseased and starved grey seal females have higher concentrations of PCBs and DDTs (but not PCDDs/PCDFs) than healthy females. The extent to which these high concentrations are implicated in the development of the diseases and reproductive impairment cannot be evaluated.

The prevalence of leiomyomas (benign tumours in the uterus) has remained at a high level and the prevalence of colonic ulcers (associated with *Corynosoma* sp.) has increased in young animals. The high prevalence of leiomyomas, as well as the high incidence of metaplasia in renal tubuli, might indicate a hormonal disturbance, probably oestrogen excess (Bergman, 1997). The aetiology of the moderate to severe colonic ulcers (which may be fatal) is unknown. It is still unclear to what extent contaminants are involved, but contaminant-induced immunosuppression might be an important factor.

The decline in contaminant levels in Baltic biota documented above, the increases in seal numbers which have been observed in some areas in recent years (Härkönen and Heide-Jørgensen, 1997; Härkönen *et al.*, 1997; Helander and Lundberg, 1997), and the decline in the overall prevalence of uterine occlusions and symptoms of hyperadrenocorticism suggest that there has been an improvement in environmental conditions for marine mammals in the Baltic Sea. However, the high incidence of uterine occlusions in young Baltic ringed seals in recent years (Helle, 1997), the increasing prevalence of colonic ulcers in young Baltic grey seals, and the high prevalence of leiomyomas (Bergman, 1997)

all indicate that problems still remain. In addition, there is concern about the contaminant levels which have been measured in harbour porpoises from the Baltic Sea and this points to the need for detailed pathology studies of these animals to investigate their possible effects.

Need for further research or additional data

Concern was expressed about the lack of progress on research on the immune system of marine mammals. Applicable methods need to be developed, and marine mammals from non-polluted or less polluted geographical areas need to be examined in order to establish baseline information on the normal status of these animals. At present, there is a heavy reliance on samples from necropsied or live-captured animals in order to improve our knowledge.

The ACME noted that some future investigations will concentrate on the development of methods to study the lymphatic organ systems of harbour porpoises. It also noted that there is on-going cooperation between Estonia and Norway to study contaminant concentrations and immunological parameters in grey seal pups from the Estonian and Norwegian coasts.

Finally, in relation to the development of biological effects techniques to be applied to marine mammals, the ACME reiterated the need for progress in the four priority areas of research identified in its last report (see ICES, 1996), i.e., toxicokinetic markers, reproduction and early development, immunosuppression, and cancer induction and mutagenic effects.

Recommendations

ICES ACME recommends the continuation of long-term studies in the Baltic Sea aimed at following the health of the marine mammal populations as well as the development of the population status.

ICES ACME also recommends that data available on contaminant concentrations in marine mammals be submitted to the ICES Environmental Data Centre, which already holds such data for the AMAP area, reiterating (see ICES, 1996) that only contaminant data for which information is available on the age, sex, reproductive state, and nutritional condition of the animal sampled, and on the quality assurance and quality control procedures used by the analytical laboratory, should be included in a data bank.

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11.4.2 Effects of contaminants on the quality of cetacean prey

Request

In recommendation 19 of the 1995 Report of the International Whaling Commission (IWC) Workshop on Chemical Pollution and Cetaceans, ICES was asked to

provide an evaluation of the effects of contaminants on the abundance and quality of cetacean prey (mainly crustaceans, cephalopods, and fish).

Source of the information presented

The 1997 report of the Working Group on Environmental Assessment and Monitoring Strategies (WGEAMS) and ACME deliberations.

Status/background information

In Section 13.3 of its 1996 report (ICES, 1996), the ACME only briefly commented on this request. At its 1997 meeting, the ACME considered this subject again but, in the absence of information on the diet and feeding locations of cetaceans in the ICES area, was only able to comment on one aspect of the question, namely, the effects of contaminants on the quality of cetacean prey, as indicated by the presence of persistent organic contaminants in prey species. It is known that there is potential for persistent organic contaminants (e.g., CBs, dioxins, dieldrin, DDT compounds) to adversely affect a wide range of marine predators, for example, through the inhibition of reproduction in seals or the thinning of egg shells in some bird species.

The primary uptake route for these compounds is through the diet. The resultant concentrations and patterns of compounds in the predators, including cetaceans, are therefore a function of intake (species composition of diet, concentrations of contaminants in dietary species, variations of diet and its contaminant burden in space and time, age, etc.) and loss mechanisms (e.g., condition of the animal, reproductive status, excretion rate, metabolic abilities and activity). For example, the loss of lipophilic contaminants during lactation has been shown to lead to considerable differences in contaminant concentrations between mature males and females of some species. Different species show different abilities to metabolize or degrade organic contaminants.

Consequently, there are often very large differences (e.g., factors of 1000) in the concentrations of organic contaminants among individuals of the same species (e.g., differences between old mature males and newly born young). The patterns of CB congeners found in marine mammals show greater consistency than the absolute concentrations, and species-specific patterns can be recognized which have resulted from the factors indicated above, for example, differences in diet and metabolic capability.

Data from Wells *et al.* (in prep.) demonstrate species-specific patterns of CBs in marine mammals from northern European waters. Marine mammals feeding on similar food in localized areas show similar congener patterns, but species feeding at different trophic levels (i.e., on different prey species) are likely to have distinctly different CB patterns, reflecting different uptake and metabolic processes. Broad divisions can be drawn between fish feeders, mixed feeders, and cephalopod feeders.

CB congener patterns in prey species, e.g., fish, also show geographical differences, which appear to be linked to proximity to coasts and the mode of transport of the contaminants (e.g., atmospheric versus aquatic mechanisms). There are, for example, differences in CB patterns between fish from the northern and western coasts of Scotland (Wells *et al.*, in prep.).

While the pattern of congeners in the prey species clearly influences the pattern in the predator species, the quality of the prey is also dependent on the absolute concentrations of contaminants in the prey and their potential to induce adverse effects in the predators. These concentrations vary geographically; for example, concentrations of CB congeners in cod liver are a factor of ten higher in the Southern Bight of the North Sea than in the northern North Sea (North Sea Task Force, 1993) and differences have been reported between various areas of the Scottish coast (Kelly and Campbell, 1994). There is very little information available on the acceptable concentrations of organic contaminants in cetacean prey. However, criteria have been set by several agencies for concentrations of selected organic contaminants in fish tissue to protect fish-eating wildlife, with particular reference to the Great Lakes. These criteria are summarized in Table 11.4.2.1.

It must be noted that there can be considerable differences between the values given by different agencies for the same contaminant group, particularly for ΣDDT. This may be due to the fact that these values are derived using different methods. Furthermore, it must be remembered that laboratory data used may not always reflect the complexity of situations found in the real environment. Therefore, caution is warranted in the use of such values. The values for mink (and other terrestrial mammals) are given because they are well supported by experimental data and mink have been shown to be relatively sensitive to these contaminants in their diet.

In the absence of detailed information on the diets and feeding locations of the cetacean species of concern within the ICES area, only very general comparisons can be made between the values in Table 11.4.2.1 and the concentrations of contaminants found in fish liver in the ICES area. Data for North Sea fish species are available for eleven Sub-Regions as a map in the North Sea Quality Status Report (see Figure 3.7, North Sea Task Force, 1993). Data for Swedish coastal waters were provided at the 1997 WGEAMS meeting. The ranges of concentrations are summarized in Table 11.4.2.2.

It would appear from these data that the concentrations of CBs and total DDTs in fish in the ICES area can exceed the environmental guidelines given in Table 11.4.2.1. This suggests that there is a potential for the chemical

Table 11.4.2.1. Guidelines for the concentrations (wet weight) of selected organic contaminants in fish tissue as food for piscivorous animals.

Contaminant	(a) IJC (fish tissue)	(b) EQG (fish tissue)	(c) USEPA (fish tissue)	(d) Dietary NOAEC values (mink food)
2,3,7,8-TCDD		1.1 pg g^{-1}	0.5 pg g^{-1}	2 pg g^{-1}
DDT	1.0 µg g^{-1}	0.0063 µg g^{-1}	0.039 µg g^{-1}	100 µg g^{-1}
Total PCBs	0.1 µg g^{-1}	0.06 µg g^{-1}	0.16 µg g^{-1}	0.072 µg g^{-1}
Aldrin/Dieldrin	0.3 µg g^{-1}			5 µg g^{-1}
Toxaphene				(e) 4 µg g^{-1} (rat and dog food)

a) Objectives for protection of piscivorous aquatic life and wildlife (concentrations in whole fish) (IJC, 1978).
b) Draft Canadian Environmental Quality Guidelines (EQG) for protection of animals that consume aquatic biota (Environment Canada, 1996).
c) USEPA guideline values for assessment of hazards to fish-eating wildlife (USEPA, 1995).
d) Dietary No Adverse Effect Concentrations (NOAEC) for reproductive effects on mink (Giesy *et al.*, 1994).
e) NOAEC for thyroid effects in rats and dogs (Chu *et al.*, 1986).

Table 11.4.2.2. Summary of the concentration ranges (wet weight) of selected organic contaminants in fish tissue in the North Sea and Swedish coastal waters.

Contaminant	North Sea	West Sweden		Baltic Sea	
	Fish liver (µg g^{-1})	Cod liver (µg g^{-1})	Herring muscle (µg g^{-1})	Cod liver (µg g^{-1})	Herring muscle (µg g^{-1})
Σ7 CBs	0.2–6	0.18–0.23	0.004–0.009	0.4–0.6	0.004–0.014
ΣDDT	0.1–1.2	0.06–0.08	0.002–0.006	0.6–0.8	0.002– 0.014
Dieldrin	0.01–0.16				

quality of potential prey species (fish) to be adversely affected by current (1990–1996) organic contaminant concentrations in these fish in the North Sea and the Baltic Sea. However, considerable caution is required in attempting to use these comparisons to obtain some guidance on levels of contaminants which may be safe for cetaceans (see also ICES, 1995). This is due to the reasons already stated above, and also because the values in Table 11.4.2.1 were derived for piscivorous animals of the Great Lakes area, and not with cetaceans in mind. Indeed, one must take into account the particularities of the physiology and metabolism of cetaceans, the different susceptibility of various mammal species, the variation in bioavailability of contaminants in various prey of different cetacean species, and possible additive or synergistic effects of complex mixtures of contaminants. Furthermore, some of the environmental guidelines in Table 11.4.2.1 clearly refer to whole fish, whereas the chemical analyses summarized in Table 11.4.2.2 are of defined organs or tissues. Cetaceans will consume entire prey, or at least are unlikely to effectively separate and selectively consume particular organs. As these contaminants are lipophilic, it is likely that the data for fish liver will be an overestimate of the concentrations in whole fish, whereas the data for herring muscle may be an underestimate of the concentrations in whole herring.

No information was available concerning the feeding locations or preferred prey species of the cetaceans, or their susceptibility to contaminants. If a more complete analysis, taking these factors into account, tends to confirm that there may be some potential for effects, it would be necessary to undertake programmes of appropriate observations on the target organisms (the cetaceans) themselves in an integrated biological and chemical programme.

Recent compilations of data under the Arctic Monitoring and Assessment Programme (AMAP) have emphasized the occurrence of high mercury and cadmium concentrations in the kidney and liver of a range of Arctic marine mammals and birds. In the Iceland/Greenland area (Stange *et al.*, 1996), high concentrations have also been found in redfish (*Sebastes marinus* and *Sebastes mentella*), and there are suggestions that some other species of deep-water fish may accumulate high concentrations of these elements. There may be some potential for these or other deep-water species to act as prey species for cetaceans. The significance of these metals to the fish species (and to their predators) is not yet understood.

Finally, it should be noted that the methodology used in this section to assess the potential for biological effects from contaminant concentrations is akin to that which was used by AMAP. The AMAP procedures relied heavily on direct relationships between contaminant concentrations in organisms and the biological effects observed in the same organisms. In some cases, use had been made of experimental data relating dietary contaminant burdens to biological effects in predators. The ACME feels that, despite the need to approach it very cautiously, this type of methodology holds promise to identify species potentially at risk. It is also in line with current ICES advice on monitoring strategies, in that it seeks to combine chemical and biological effects measurements in sensitive species.

Need for further research or additional data

Information on the diets and feeding locations of cetaceans in the ICES area would allow an examination of the other part of this request, i.e., the effects of contaminants on the abundance of cetacean prey. Such information would have to be obtained by ICES from the IWC.

Recommendations

ICES ACME recommends that more emphasis be placed on the 'protection of marine life' purpose in current monitoring programmes, in order to contribute to the databases required for carrying out assessments of possible effects of contaminants on marine animals at higher trophic levels. This implies using an approach with integrated chemical and biological effects measurements.

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Request

Item 4 of the 1997 requests from the Helsinki Commission; this request is to evaluate the impact of different fishing practices (e.g., drift nets, gillnets, bottom trawling) in the Baltic Sea on target and non-target species, including, in addition to fish, invertebrates, marine mammals, and birds.

Source of the information presented

The 1997 reports of the Baltic Salmon and Trout Assessment Working Group (WGBAST), the Baltic Fisheries Assessment Working Group (WGBFAS), the Working Group on Seals and Small Cetaceans in European Seas (WGSEAL), and ACFM and ACME deliberations. This section represents a joint report from ACFM and ACME; accordingly, the name ICES is used instead of that of a single Advisory Committee.

Status/background information

The effect of fishing practices on fish, invertebrates, marine mammals, and birds is poorly known for the Baltic Sea. There are few directed studies of these effects in the Baltic area, and even much incidental information is not systematically documented. Therefore, it is necessary to draw from experience in other areas.

The ICES Study Group on Ecosystem Effects of Fishing Activities was established in 1990 and became a working group in 1992. This group has produced several reports on relevant investigations, primarily in the North Sea (ICES, 1994, 1995a, 1996a). Because many of the results contained in these reports are thought to reflect general ecological processes, ICES, in preparing this response, has drawn heavily from these reports. There are, however, distinct differences in the hydrography and bathymetry of the Baltic Sea which are reflected in, e.g., low species diversity. Therefore, there are likely to be important differences between ecosystem effects of fishing activities in the Baltic Sea compared to the effects which ICES has reported for the North Sea. Direct studies in the Baltic Sea will be required before it will be possible to advance the scientific understanding of ecosystem effects of fishing activities in the Baltic Sea area.

Description of the fisheries

The commercially most important fisheries are for cod, herring, sprat, and salmon.

The main fisheries for cod in the Baltic Sea are those using demersal trawls, high opening trawls (operating both pelagically and demersally), and gillnets. There has been an increase in gillnet fisheries in the 1990s and the share of the total catch of cod taken by gillnets has been about 50 % in recent years. Baltic herring is exploited mainly by pelagic trawls, demersal trawls and, during the spawning season, by trap nets and/or pound nets in coastal areas. The main part of the sprat catch is taken by pelagic pair trawling and used for industrial purposes. The sprat catches have been increasing in recent years. Baltic salmon is exploited by drift net, gillnet, trap nets, and long-line fisheries.

While feeding in the sea, salmon are caught by drift nets and long-lines, and during the spawning run they are caught in coastal fisheries mainly by trap nets and fixed gillnets. Where fisheries are allowed in the river mouths, set gillnets and trap nets are used.

The coastal fishery targets a variety of species with a mixture of gears including fixed gears (e.g., gill-, pound and trap nets and weirs) and Danish seines. The main species exploited are herring, flounder, turbot, cod, and freshwater and migratory species (e.g., pike, pikeperch, smelt and whitefish). In addition, there are demersal trawling activities for herring, cod and flatfishes in some parts of the Baltic Sea. Coastal fisheries are conducted along the entire Baltic coastline.

Direct effects of fishing on target fish populations and fish communities

Although there have been many studies of growth, reproduction, and mortality of target species in the Baltic Sea, different analyses would be required to relate observed patterns of change in biological characteristics of populations to impacts of fishing. In other areas and with a variety of species, studies have shown that fishing at exploitation rates which have been observed on some species in the Baltic Sea can induce changes in growth rate, sex ratios, maturity ogives, and genetic composition of fish populations (Jørgensen, 1990; ICES, 1996b).

In addition, the removal of large quantities of fish from the population may represent a major loss of reproductive potential. However, ICES notes that management is prepared to keep the fish stocks inside safe biological limits with the purpose of protecting reproduction potential. In the North Sea and Northwest Atlantic, it has been argued that the loss of reproductive potential due to excessive fishing mortality has become so severe that the viability of a few populations has been placed at biological risk. The cod population in the eastern Baltic Sea is thought to be outside safe biological limits.

It has been argued that fishery harvests can alter the structural and functional characteristics of fish assemblages. For example, between 1973 and 1993 the North Sea showed a net loss of biomass and numbers of

large size classes of fish (> 70 cm) and an increase in species diversity in intermediate size classes of fish (ICES, 1996b; Rice and Gislason, 1996). These changes were caused at least partly by fisheries reducing the abundance of dominant target fish species. However, investigations in the North Sea did not reveal direct relationships between trends in the abundance of individual species and effects of fishing; fluctuations in the abundance of these species could be attributed to natural variability of the environment and/or anthropogenic activities.

Again, although there are some partial survey data for the Baltic Sea, they would have to be analysed in new ways to explore the impacts of fishing on Baltic fish assemblages. Analyses of experimental bottom trawl surveys carried out in the Gulf of Riga during 1974–1986 and 1993–1996 indicated many changes in abundance and species composition (Ojaveer, in prep.). These changes are governed by a complex of causes, i.e., changes in salinity, temperature and oxygen levels, pollution, fisheries, and predation (Hilden, 1985). It is very difficult to separate the effects of these influences. Moreover, it is expected that these impacts on fish communities in the Baltic Sea will be different from those observed in the North Sea, because, for example, beam trawling and purse seining are not allowed in the Baltic Sea area.

In general, there is evidence that trawling activity can potentially alter trophic relationships through increasing the availability of various prey species for predators. Trawling can increase potential food for bottom-feeding predators by exposing benthic organisms and damaging hard-shelled organisms. There is some evidence from the Baltic Sea that small herring escaping from trawl cod ends die within a few days of escapement. These herring may be a source of prey for cod or for benthic consumers (Suuronen, 1995).

Ghost fishing

In the open sea and coastal gillnet fishery, some nets may occasionally get lost. These gillnets continue to fish (ghost fishing) for shorter or longer periods, causing unaccounted mortality of various fish, benthos, and marine mammal species. However, the impact from ghost fishing is not known.

Effects due to by-catch and discarding

The total by-catch of fish is unknown for the Baltic Sea fisheries. Reports generally contain few data on this topic, so quantitative estimates cannot be derived. It is expected that some new information will be obtained as part of an on-going EU project and a new EU project from August 1997 onwards.

Another type of discarding activity is the return of fish offal to the sea. For cod, 15 % or more of the whole body weight is discarded as offal. If it is assumed that all cod landed has been gutted at sea, then the amount of cod offal discarded in the whole Baltic Sea reached the highest values during the first half of the 1980s, with a minimum estimate of 58,000 tonnes per year. Some offal is consumed by seabirds (ICES, 1997). The sinking of unconsumed fish offal results in inputs of organic material to the sea floor, particularly in deep basins where the cod fishery and gutting activity are intense. There, the decomposition of offal may further reduce oxygen concentrations in the bottom waters of the basins.

Data on by-catches in the salmon drift net fisheries were presented in detail in ICES (1995b). Observations suggest that the species affected in the long-line and trap net fisheries are the same, i.e., guillemots, razorbills, and seals. There are no quantitative data available to estimate the precise impact.

Impact of fishing gear on benthos

All towed demersal fishing gears cause disturbance of the sea bottom surface and, thus, may impact on the structure and processes of the sea floor. Some of the potential impacts that have been identified in other areas, particularly in the North Sea, include:

1) damage to and mortality of benthos due to direct contact with mobile fishing gear;
2) increased predation due to exposure of infauna by trawls (some data indicate that much of the increased predation can be on benthos, accounted for in (1), above);
3) altered chemical or textural properties of sediments that change the suitability of the sea bottom for adult and young life history stages of organisms;
4) reduced food quality for filter feeders and smothered spawning areas due to sediment resuspension;
5) increased bioavailability of toxic materials (e.g., heavy metals) in marine sediments;
6) increased rates of nutrient flux between the sediment and water column, which can stimulate phytoplankton production (in some parts of the Baltic Sea this could reduce oxygen levels, thus adding increased stress to fish and benthos);
7) removal or displacement of boulders.

Most of these effects have been demonstrated in association with beam trawling, which does not take place in the Baltic Sea.

In general, towed gears that penetrate the sediment considerably, especially bottom trawls, impact both benthic infauna and epifauna, whereas other types of gears (e.g., gill- and trap net, Danish seine) may have effects on some epifauna in some circumstances. Local studies are often needed to quantify impacts of specific

fisheries. Studies in the North Sea estimate that benthic mortalities caused by beam trawls appeared to be at least one order of magnitude higher than those caused by otter trawls.

There are some specific case studies of the impact of trawl gears on benthos and benthic habitats in the Baltic Sea:

a) nutrient response to trawling activity

   In the Kiel Bight, there was evidence of sediment disturbance. Remobilization of nutrients and increased release of nutrients and organic material were followed by an increase in oxygen consumption (Krost, 1990).

b) biological response of the invertebrate community to trawling activity in Kiel Bight

   There were obvious biological impacts on thin-shelled bivalves, and starfish suffered heavy damage, whereas little or no damage occurred to the solid-shelled bivalves. However, it was demonstrated that trawling reduced the mean population size of the solid-shelled *Arctica islandica*. An increase in the proportion of damage with increasing body size was found for several species of mussels. Many epibenthic organisms suffered dislocations, but lesser levels of mortality. An increase in predatory and scavenging species feeding on dying and dislocated fauna was also documented in this area (Rumohr and Krost, 1991).

Effects on seabirds

It is unlikely that fishing activity for cod, herring, or sprat is responsible for the direct mortality of seabirds in the Baltic Sea. However, the drift net fishery for Baltic salmon is responsible for some guillemot (*Uria aalge*) and razorbill (*Alca torda*) mortalities (ICES, 1995b). Nevertheless, there are considerable mortalities recorded for the Kiel Bight area, where diving ducks have been caught in gillnets (Kirchhoff, 1982).

Fishing activities for cod, herring, and sprat will indirectly affect the seabird community in other ways. These include the effects of discarding of unwanted catch and offal, which then become an important source of food. In the North Sea, several studies indicate that 70–90 % of the offal will be consumed by seabirds (ICES, 1994). This food source is believed to be responsible for increases in seabird populations over time (ICES, 1994, 1996b). It is difficult to extrapolate these North Sea results to the Baltic Sea because of possible differences in seabird communities, fishing patterns and discarding activities.

Effects on marine mammals

In Polish waters, there has been an average by-catch of five harbour porpoises per year during the period 1987–1996, mostly in the salmon fishery. By-catches of 3–5 porpoises per year in bottom-set gill nets and drift nets were recorded from the Swedish Baltic coast throughout the year. An estimated 111 porpoises were by-caught in German fisheries in ICES Area IIIc (the Kiel and Mecklenburg Bays and inner Danish waters) during the period 1987–1995 (Kock and Benke, 1996), and six were by-caught in 1996 (see Section 11.2, above). These reports suggest that annual by-catches of porpoises are 0.5–0.8 % of abundance in the southwestern part of ICES Area IIId (Baltic Sea), and 1.2 % of abundance in ICES Area IIIc. ICES believes that these reports underestimate the magnitude of by-catches in both areas.

About 200 seals were estimated to have died per year in Estonian waters, predominantly in fyke nets. Grey seals appear to be more vulnerable to being caught than ringed seals and constitute more than 80 % of the by-catches in Estonian waters. In Poland a total of nine seals, of which a majority was grey seals, was reported being caught in 1996, predominantly in salmon nets. In German parts of the area, one harbour seal was caught by trawl and one in a fyke net in 1995; no cases were reported in 1996. See also Section 11.2, above, and ICES (1996a).

Salmon drift nets are also responsible for some seal mortalities in Swedish waters. Available Swedish data for the period 1974–1990 show that 29 of 216 (14 %) fishing net mortalities of grey, ringed and harbour seals were caused by salmon drift nets (ICES, 1995b). A survey along the Swedish coast north of Åland showed that a minimum of 182 grey seals were by-caught in 1996. The total by-catch in Swedish areas was estimated to exceed 200 animals. In the Finnish fishery, 338 seals were reported by-caught in the period 1986–1995, of which 113 (33 %) were from the drift net fishery in the open sea. Most of these were grey seals.

In the Bothnian Bay, most of the seals were caught in traps set for salmon and whitefish, while nets set for salmon and whitefish were more important in the Bothnian Sea. In the Baltic Proper, most of the seals were caught in eel traps and turbot nets. Pups of the year made up about half of the total by-catch.

The recorded annual by-catch of about 400 grey seals represents about 29 % of the estimated pup production in 1992, and 7.5 % of the maximum count of seals in 1995–1996. These records almost certainly underestimate total by-catch and may not be sustainable.

Preventive measures

The ecological impact of various fishing gears is under investigation as well as ways to reduce adverse effects on fish, invertebrates, marine mammals, and seabirds by changes in the design of gears and/or additional stimuli to avoid unwanted captures. Many EU-funded projects are being carried out. The results are still premature, but in some fisheries, potential for improvement was demonstrated and put into legislation (e.g., *Nephrops* trawling square mesh and Swedish Exit Windows in the UK, Swedish Exit Windows in the Baltic cod trawl fishery, and sorting grids in the Norwegian shrimp fishery).

Summary

The low and variable salinity in the Baltic Sea has resulted in an ecosystem which has a relatively low species diversity and a relatively simpler food web. Fluctuating hydrographic conditions, together with the fisheries, determine to a great extent the distribution and abundance of organisms in the Baltic Sea. It appears that there is very little documentation available which allows separation of the consequences of fishing activities from other causes of change in the Baltic marine ecosystem.

Nonetheless, two broad patterns of fishing impact on the Baltic ecosystem seem likely. First, offshore fishing activities will mainly have an impact on the fish and benthic communities. This impact will be due primarily to direct removals and the impacts of bottom trawling and, secondarily, to escapee mortalities and the discharge of by-catch and offal. Second, the coastal fisheries will mainly impact the Baltic ecosystem via by-catch of non-target species and, to a small extent, marine mammals and seabirds. The capture of these species will affect coastal fish biodiversity and species interactions. These impacts need to be quantified in future studies.

Need for further research or additional data

ICES noted that there was a lack of specific knowledge on the effects of fishing practices on fish, invertebrates, marine mammals, and seabirds in the Baltic Sea. Baltic countries are encouraged to conduct impact studies using the experience of the Working Group on Ecosystem Effects of Fishing Activities (WGECO) gained from investigations in the North Sea.

Recommendations

ICES recommends that Baltic Sea countries initiate studies on the impact of different fishing practices on non-target species in the Baltic Sea.

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13 INTRODUCTIONS AND TRANSFERS OF MARINE ORGANISMS

13.1 Status of On-going Introductions

Request

ICES Member Countries may request ICES to review proposed introductions and transfers of marine organisms for mariculture purposes. These proposals receive in-depth review by the Working Group on Introductions and Transfers of Marine Organisms (WGITMO), with final review by the ACME. WGITMO also keeps under review the progress of such introductions and reports the outcome to the ACME.

No new requests for review of proposed introductions were received in 1997, but the status of on-going and proposed introductions and transfers was reviewed.

Source of the information presented

The 1997 report of the Working Group on Introductions and Transfers of Marine Organisms (WGITMO) and ACME deliberations.

Status/background information

ICES ACME reviewed the WGITMO report and agreed to present the information and advice contained in the following section.

Status of existing controlled introductions

Japanese seaweed (Nori) *Porphyra yezoensis* in the USA

The ACME noted that the outcome of the regular monitoring programme in the Gulf of Maine, USA, for Japanese seaweed *Porphyra yezoensis* was the discovery of monospores of sexual reproduction outside the farms' physical growth structures. Electrophoretic techniques have been used to distinguish between introduced *P. yezoensis* and the native *Porphyra* species. From eighteen plants that were collected from outside the culture area, only three were *P. yezoensis*. Although monospores can recruit, *P. yezoensis* does not outcompete the native species and monospores do not survive in winter. Therefore, the risk of natural spreading of this alga from designated culture sites would appear to be low.

The ACME noted that future developments of the culture programme include domestication of native species of *Porphyra* for culture in contained land-based culture facilities.

In Canada, the federal Department of Fisheries and Oceans authorized the importation of a maximum of 100 nori (*P. yezoensis*) nets from an approved facility in Maine to a private aquaculture site in New Brunswick. Interest has also been expressed by aquaculturists from Prince Edward Island and Newfoundland in the possibility of nori culture in their provinces, but no decisions have been made.

Japanese seaweed *Undaria pinnatifida*

Attached plants of this species have been found at two additional locations along the south coast of England in Devon and the Isle of Wight. Plants have also been found on the Jersey and Channel Isles. Owing to the distance between sites, these represent new introductions rather than natural spreading from the original site.

In France, natural sublittoral *Undaria* populations have decreased significantly, especially in the northern part of Brittany. In southwestern France, limited cultures using long-line techniques still exist but, while small algae were found on the nearby mussel long-lines, no overwintering occurred and no significant sightings of *Undaria* were made on the adjacent coast.

Red King crab *Paralithodes camtschatika*

Investigations into the distribution and abundance of the Red King crab *P. camtschatika,* introduced from the north Pacific into the Barents Sea, were carried out jointly in 1996 by the Norwegian and Russian authorities. Results from trial fishery surveys and catch rates from the commercial fishery suggest a doubling of abundance estimates in the distribution area. Tagging recapture experiments indicate a westward migration, possibly enhanced by the proportion of very large egg-carrying females in the population.

It was reported to WGITMO that the Russian authorities were interested in introducing the blue crab *Paralithodes platypus* to the Barents Sea. This species was evaluated for introduction to the Atlantic in the 1930s and was considered to have high potential for adaptation success compared to the Red King crab. Such new introduction plans should, however, be evaluated by international scientific organizations including ICES to assess potential implications of the proposed introduction.

Accordingly, ICES ACME encourages the Russian Delegates to ICES to submit information to ICES concerning the proposed introduction of blue crabs (*Paralithodes platypus*) from the Kamchatka region

to the Barents Sea, including information regarding potential ecological impacts and other information relative to the standard ICES procedures for the evaluation of proposed new introductions.

Status of existing accidental introductions

The ACME noted the following information on the status of accidentally introduced species.

Japanese brown algae *Saragassum muticum*

This species has extended its range in a number of ICES Member Countries. It is now well established in the southern part of the Norwegian coast (in the Skagerrak) and has also been observed occasionally on the western coast as far north as Hordaland. In summer 1996, dense aggregations were found in a number of shallow bays in western Norway. The northern movement of established populations of this species is still continuing.

A coastal survey of the distribution of *S. muticum* carried out in Sweden in 1996 compared results with areas surveyed in 1993. The distribution of attached plants has expanded about 100 km south during this three-year period. It is now found growing in large quantities in the northern province of Halland (in the northern Kattegat) and also further south in the eastern Kattegat. By forming large and dense populations, the plants act as barriers for water movements, leading to water stagnation and growth enhancements of ephemeral algae.

S. muticum was found in Strangford Lough, Northern Ireland during summer 1996. To date, no attached plants have been seen, but sea water temperatures have been too low for it to grow extensively.

Asian Shore Crab *Hemigrapsus penicillatus*

This crab is a Northwest Pacific Ocean species. It was previously unknown in European fauna, but was discovered in La Rochelle, France in 1994. It appears to be spreading rapidly. Its known range now extends from Laredo, Spain (43° 25′N, 03° 20′W) to Fromentine, France (46° 53′N, 02° 09′W). It is typically found in sheltered areas under stones in the mid-littoral zone. It is locally abundant, with densities of up to twenty individuals per square metre, particularly in estuaries and ports where it tolerates muddy zones. Being tolerant to both high and very low temperatures, it has a wide potential range from Norwegian to North African coasts.

13.2 Ballast Water Activities: Cooperation with other International Organizations

Request

There is no specific request; however, this issue is of interest to ICES Member Countries and several international organizations

Source of the information presented

The 1997 reports of the ICES/IOC/IMO Study Group on Ballast Water and Sediments (SGBWS), the Working Group on Introductions and Transfers of Marine Organisms (WGITMO), and the Working Group on Harmful Algal Bloom Dynamics (WGHABD), and ACME deliberations.

Status/background information

The ACME noted that the first meeting of the new ICES/IOC/IMO Study Group on Ballast Water and Sediments (SGBWS) was held in La Tremblade, France in April 1997. The meeting was co-chaired by J. Carlton (ICES), M. Nauke (IMO), and T. Wyatt (IOC). A representative of the International Chamber of Shipping (ICS) also attended.

International Cooperation

There was general agreement that increased levels of cooperation and coordination are extremely important, given the number of on-going and proposed studies on the issue of the potential introduction of alien species via ballast water and sediments. Critically important is the need for coordination and calibration of sampling techniques, in particular, specific sampling methods (nets, pumps, traps and other devices), sample analysis methods and identification procedures, and the standardization of data sheets. It was noted, however, that considerable difficulties may arise in the identification of holoplankton (such as copepods) from foreign waters and even greater difficulties in identifying meroplankton (the larvae of marine invertebrates and fish). These identification problems lead to problems in risk assessment modelling.

Australia's Centre for Research on Introduced Marine Pests (CRIMP) is presently conducting a world-wide survey of ballast sampling methods with the aim of producing an internationally comparative database.

An EU-funded project, known as the 'Concerted Action Plan: Testing Monitoring Systems for Risk Assessment of Harmful Introductions by Ships to European Waters', is due to begin in late 1997. The project includes participants from Germany, Ireland, Lithuania, Finland, Sweden, and the UK. Invited experts from Australia, Canada, Israel, Japan, and the USA will also participate.

Inoculations and Invasions

Many studies are under way to assess the biotic diversity of incoming ballast. These range from broadly based studies on a range of different taxa to specific studies of certain taxa. It was noted, however, that to date few studies have focused on bacteria or viruses. Studies are also being conducted to examine the relationship between the volume of incoming ballast and the number of successful invasions, the relationship between the source of ballast water and the diversity and/or number of invasions, survival potential during transport, and post-colonization potential with an emphasis on examining details of the environmental match between donor and recipient environments. It was acknowledged that there is often difficulty in recognizing which species were introduced. The results of the above studies may lead to the possibility of identifying regions from which successful, obvious exotic invasions could occur.

Management and Control

The ACME noted that the favoured ballast water management tool at present is the open-sea exchange of ballast water. This involves deballasting on the high seas and then reballasting with oceanic water. The result is that the neritic organisms die in the open ocean and the oceanic organisms die when released in coastal waters, particularly estuaries. Open-water exchange is mandatory for trans-oceanic ships entering the Great Lakes in North America. IMO issued Resolution A774 (18) in 1993 calling for open-ocean ballast exchange when safe and practicable. This resolution is due to be revised in late 1997, with a new section on ship safety and ship stability added. The representative of the ICS stated that, while desiring to respond to the issues of ballast water transport of alien species, the shipping industry faces certain constraints and challenges to open-water ballast exchange. These include the issues of ship and crew safety, the added costs of exchange, the time element involved, and the impracticability of having to stop and exchange water in coastal zones prior to coming into port if the vessel has been unable to do so in the open sea

Extensive research is being conducted on the effectiveness of open-ocean exchange of ballast water, focusing on the extent to which both water and sediment may be released during the exchange process, the use of ballast exchange within coastal zones compared to open-ocean exchange, and understanding exactly what individual vessels can accomplish in the exchange process. Studies are also under way on how ballast exchange can be monitored and the cost of such monitoring.

Note was taken of recent US legislation on ballast water—the National Invasive Species Act of 1996—which, for an initial three-year period, requests vessels arriving at US ports to exchange their water on the high sea (given safety considerations). At the end of this period, the US Coast Guard will assess the level of compliance and, if found to be insufficient, ballast exchange at sea will become mandatory.

There are a variety of experimental approaches being pursued to supplement and/or replace open-ocean ballast exchange. These include:

- heat treatment (sea trials have been and are being conducted in Australia);
- treatment with ozone (a desk study in Australia);
- chlorination (desk and trial studies in Canada and Australia);
- hydrogen peroxide treatment (studies in Australia);
- organic acid treatment (desk study in Canada);
- centrifugation combined with UV irradiation (desk study in Norway);
- filtration—sea trials are under way in the USA to test the effectiveness of high speed (1500–2000 gallons/per minute) microfiltration. The smaller mesh size scheduled to be tested is 40 µm (note that, in the case of toxic dinoflagellates, the cysts of *Alexandrium* spp. are 35–45 µm long and those of *Gymnodinium catenatum* are 40–60 µm long).

Risk Assessment and Decision Systems

Research is proceeding on risk assessment and decision systems relating to ballast management. It was noted that it is crucial to include economic models in such assessments, and that more rapid advances may be made when policy-setting and decision-making bodies include joint representation from the shipping industry, government, and scientific institutes.

In reviewing the work of SGBWS, WGITMO noted that other ship-associated transport mechanisms, or vectors other than ballast, can and do play a role in the transport of non-indigenous species, e.g., through fouling on the ship's hull. There may, therefore, be difficulties in distinguishing between different ship-associated vectors. Some species may be transported by more than one vector, which makes risk assessment more difficult. The 'ICES Handbook of Human-mediated Dispersal Vectors', being prepared by WGITMO, will provide detailed descriptions of such vectors, which may assist in the management of this problem.

The ACME noted that WGHABD also discussed the issue of ballast water, specifically in relation to the transport of potentially harmful algal species. Concern was expressed that most of the information available relates to the transport of dinoflagellate cysts in ballast sediment and that little information was available on other species such as diatoms, flagellates, and cyanobacteria, or on the transfer of motile cells in addition to resting stages. Also, while studies of the transport of dinoflagellate cysts in ballast tank sediments should continue, the relative importance of water column versus sediment origin of cysts should be addressed in relation to dinoflagellate life cycles.

Need for further research or additional data

The focus on potentially harmful algal species in ships' ballast should be broadened to include toxic diatoms, cyanobacteria, and flagellates. Further research is also required on the transport of bacteria and viruses. Furthermore, research should cover the transfer of motile cells in addition to resting stages.

13.3 Marine Biocontrol of Invasive Species

Request

There is no specific request; however, this issue is of interest to ICES Member Countries and several international organizations.

Source of the information presented

The 1997 reports of the Study Group on Marine Biocontrol of Invasive Species (SGMBIS) and the Working Group on Introductions and Transfers of Marine Organisms (WGITMO), and ACME deliberations.

Status/background information

The ACME considered the report of the new Study Group on Marine Biocontrol of Invasive Species (SGMBIS), which was established to "review information relevant to the potential biological control of marine invasive species, taking into account recent interest in this issue in the Black Sea, France, Australia and elsewhere. Emphasis should be placed initially upon the control of the invasive seaweed *Caulerpa taxifolia* in the Mediterranean Sea by the proposed introduction of exotic herbivorous seaslugs because of the potential for this alga (and its herbivores) to spread into ICES regions".

The ACME noted that SGMBIS set out the theoretical strategies for the control of invasions of marine and brackish water organisms. These strategies include:

a) mechanical control: removal of individuals, *in situ* destruction of individuals;

b) chemical control: use of toxic chemicals, development of species-specific chemicals;

c) genetic control: genetic engineering of introduced species to reduce environmental tolerance or fitness;

d) physiological control: chemical interference with feeding, locomotion or life cycle;

e) ecological control by habitat modification: environment is physically/chemically modified so that the target species is affected;

f) ecological control by species introduction or enhancement (biocontrol): introduction of one or more exotic species or enhancement of one or more native species.

Biocontrol may be defined as 'the intentional release of an organism (a predator (including herbivores), pathogen, parasite or parasitoid/virus) that will consume or attack the pest species (the target organism) in order to decrease the population size of the target organism'.

The usual goal of a biocontrol programme is to reduce the population size of the target organism. It is generally accepted that complete eradication is not practical.

A mandatory requirement of a biocontrol organism is that it is species-specific, remains species-specific, and keeps the pest species in check by balancing its population size with the population size of the pest species. There should be minimal to no risk that the biocontrol species itself will become a pest. It is clear that there are no universal criteria which define when a biocontrol organism will 'always' be effective.

SGMBIS pointed out that the basic prerequisites of a biocontrol programme are a thorough understanding of:

1) the population biology and ecology of the organisms being impacted by a pest species;

2) the proposed biocontrol organism;

3) any native taxa that did or could operate as a biocontrol organism;

4) the range of variation in the critical environmental variables that affect the population size of 1), 2), and 3), above.

In addition, a genetic characterization of the biocontrol organism would be useful before it is released, as the potential may exist for the biocontrol organism to hybridize with native species or undergo local genetic change over time.

If a decision is made to proceed with a research programme on marine biocontrol, SGMBIS suggested that the following guidelines should be followed:

1) Data must be available on the biology, ecology, physiology, diseases, parasites, pathogens, and genetics of the biocontrol organism. Similar data must also be available for the target species and any potential non-target species.

2) Data must be available to adequately describe the target ecosystem.

3) Data must be available on all possible post-release dispersal mechanisms that could transport the biocontrol organism out of the target site.

4) It is crucial that 'before' and 'after' data on the population size and distribution of the target species and potential non-target species are available so that the effectiveness and impacts of the control programme can be assessed.

5) A formal risk assessment study must be undertaken.

6) Consideration should be given to a rigorous cost-benefit analysis.

7) It would be advisable to submit any proposed biocontrol programme to an independent group of experts, preferably from outside the country in which the programme is to take place. To this end, a formal process should be established to permit and encourage such a review process. (Note that the 1997 meeting of GESAMP has also proposed such a system.)

8) Political, community, and conservation/environmental groups should participate in the decision-making process.

9) There should be an integrated pest management system. Reliance for control of a pest species should never be placed on one method alone.

Current biocontrol programmes

At present, there are no proposed biocontrol programmes within the ICES area. There are, however, several programmes proposed for other areas. These include the following:

Control of the green alga *Caulerpa taxifolia* in the Mediterranean Sea. The proposal is for the possible introduction of herbivorous predators to control the invasion in the northwestern Mediterranean Sea of the green alga *Caulerpa taxifolia.* This seaweed was first found in the area in 1984 and has since spread to cover an area of 3000 hectares. The proposed biocontrol organism is a tropical benthic seaslug mollusc (sacoglasson), *Elysia subornata* (Verrill, 1901), from the Caribbean. This organism is presently held in culture under quarantine at the University of Nice and discussions are under way in France concerning a preliminary release in the open sea. WGITMO is concerned about the spread of *Caulerpa taxifolia* to ICES areas. The continuously expanding range of *Caulerpa taxifolia* in the Mediterranean Sea and the cold tolerance of this population may enable it to colonize other areas in the North Atlantic.

Control of the American comb jellyfish (ctenophore) *Mnemiopsis leidyi* in the Black Sea. Consideration is being given to the possible introduction of predators to control the invasion in the Black and Azov Seas by *Mnemiopsis leidyi.* This organism was first found in 1982, again in 1986, and continuously thereafter. The invasion by *Mnemiopsis leidyi* is considered to be a major factor in the decline of the endemic anchovy and other fisheries in the region. The predators being considered are carnivorous fish (including cod from the Baltic Sea and butterfly fish or chum salmon from North America) or the American comb jellyfish *Beroe* sp. No immediate action is scheduled at this time.

Control of the European shore crab *Carcinus maenas* in Australia. Under consideration is the introduction of parasites to control the invasion in Tasmania by the European shore crab *Carcinus maenas.* It has only been discovered in Tasmania in the last decade and, as it is a well-known voracious omnivore, it may have impacts on the shellfish industry. *Sacculina carcini,* a parasitic barnacle, is being considered as a possible biocontrol organism.

Recommendations

ICES ACME recommends that the GESAMP proposal for the establishment of a permanent Working Group on the Control of Marine Pest Invasions be supported in principle. This Working Group could be formed by a possible consortium of GESAMP, ICES, and other international agencies.

13.4 Risk of Disease Transfer via Movements of Stocks

Request

There is no specific request; however, it is of interest to ICES Member Countries to assess the risk of disease transfer via movements of stocks which are tolerant to the agents responsible for significant diseases.

Source of the information presented

The 1997 reports of the Working Group on Introductions and Transfers of Marine Organisms (WGITMO) and the Working Group on Pathology and Diseases of Marine Organisms (WGPDMO), and ACME deliberations.

Status/background information

The ACME noted that the Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) has discussed progress in the development of resistance to commercially significant diseases in American oysters (*Crassostrea virginica*), European oysters (*Ostrea edulis*), and Pacific oysters (*Crassostrea gigas*). While resistance has not been clearly demonstrated for any oyster species to date, there have been clear cases of the development of tolerance to infectious agents, e.g., *Haplosporidium nelsoni* and *Bonamia ostreae*. Only one case has demonstrated the complete disappearance of clinical infection, i.e., Malpeque disease of *Crassostrea virginica*.

Naturally developed tolerance has been demonstrated by *Crassostrea virginica* to the agent of Malpeque disease (aetiology unknown), *H. nelsoni* (MSX disease), and possibly *Perkinsus marinus* (Dermo disease). There is also evidence of the natural development of tolerance to *B. ostreae* by *O. edulis*. In the Netherlands, outbreaks of bonamiasis in 1988 killed > 90 % of the oysters in Lake Grevelingen. Surveys of surviving oysters demonstrated a parallel decline in the prevalence of *B. ostreae*. Since oyster populations are beginning to build up again, it is important to know whether the decline of *B. ostreae* infection is due to acquired tolerance by the oyster or inhibition of parasite proliferation by low host densities.

Selective breeding is commonly used to accelerate the production of oysters tolerant to specific infectious pathogens. The method of selection has led to inbreeding in certain stocks. This was particularly notable in *C. virginica* stock selected for tolerance to *H. nelsoni* infection, which showed enhanced susceptibility to *P. marinus*. Tolerant oysters frequently show no evidence of infection. Thus, they may be healthy carriers of infectious agents.

In response to a request by WGPDMO, WGITMO reviewed this issue and concluded the following:

1) Tolerance or resistance in oysters can be developed naturally, but under specific conditions (modifications of the environmental conditions, or of the pathogenicity of the pathogen, or of the physiology and/or immune system of the host that enables it to build up natural immunity). Before it can be claimed that tolerance or resistance is permanent and transmissible (i.e., from F1 to F2 to F3, etc.), it must be shown through challenge trials using experimental infections of the pathogen. If the tolerance is confirmed, the second step is to confirm this new character of tolerance and then to check whether the character is transmitted to the progeny. It must also be demonstrated that the tolerance or resistance is real (e.g., as a result of strong immunity) and not due to the absence of sensitivity to the pathogen (i.e., that the oyster is a vector of the pathogen without any disease being evident). In the latter case, the pathogen can be carried alive in the tolerant individuals and if the individuals are moved to a pathogen-free area, the pathogen can be spread to this new area.
2) Tolerance or resistance can be acquired through genetic selection. If an effective resistant or tolerant strain is developed, then the strain should be managed in a similar way to the naturally tolerant or resistant individuals described in 1), above.

WGITMO recognized that tolerant individuals can still be the vector of pathogens. Therefore, it recommended that only eyed larvae or juvenile stages produced in a hatchery from broodstock with a demonstrated (through challenge trials) tolerance or resistance to a pathogen and reared under quarantine conditions should be transferred to pathogen-free areas.

Furthermore, the ACME continues to express concern in relation to the transfer of fish and shellfish stocks within the EU and other countries relative to the potential for the accidental introduction of exotic species and particularly those that could lead to disease and pest problems.

Recommendations

ICES ACME recommends that the ICES Code of Practice on the Introductions and Transfers of Marine Organisms (ICES, 1995) be followed in all cases involving the transfer of fish or shellfish stocks.

Reference

ICES. 1995. ICES Code of Practice on the Introductions and Transfers of Marine Organisms 1994/Code de conduite du CIEM pour les introductions et transferts d'organismes marins 1994. 12 pp.

14 ISSUES RELATED TO MARICULTURE

14.1 Escape of Fish from Mariculture Operations: Potential Impacts

Request

Item 4.1 of the 1997 Work Programme from the Oslo and Paris Commissions.

Source of the information presented

The 1997 reports of the Working Group on Pathology and Diseases of Marine Organisms (WGPDMO), the Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM), the Working Group on North Atlantic Salmon (WGNAS), the report of the Workshop on the Interactions between Salmon Lice and Salmonids (Edinburgh, UK, 11–15 November 1996), and ACME deliberations.

Status/background information

The OSPAR request was to provide quantitative information on the escape of fish from mariculture operations in the context of disease transfer, genetic composition in relation to wild stocks, and competition for food and habitat, and means by which these impacts can be controlled.

Accordingly, the ACME reviewed the relevant sections of the above-mentioned reports providing information on fish escapes from mariculture in relation to disease transfer to wild stocks (WGPDMO), information on genetic aspects of interactions between escaped farmed salmonids and wild populations (WGAGFM), and information on salmon escapes from fish culture operations (WGNAS). In addition, information on the impact of salmon farming on wild stocks of Atlantic salmon (*Salmo salar*) in the Canadian Maritime Provinces was provided in the report of the Workshop on the Interactions between Salmon Lice and Salmonids, along with information on the catch of salmon escapees north of the Faroe Islands.

Fish escapes from mariculture operations in relation to disease transfer to wild stocks

The ACME noted that the WGPDMO review indicated that very few data were available on fish escapes from mariculture and none of the information was quantified. However, data from the Faroe Islands presented during the ICES Workshop on the Interactions between Salmon Lice and Salmonids indicated that 30 % of the salmon caught by the long-line fishery conducted 200 nautical miles north of the Faroes was salmon escaped from marine fish farms. Information from British Columbia, Canada showed that the annual escapes of Atlantic salmon during the period 1992 to 1996 were approximately 42,000 fish per year. However, catches of Atlantic salmon escapees accounted for only 0.004 % of the annual catch of Pacific salmon in 1996.

In terms of the potential of disease transfer from farmed to wild stocks, the highest risk appears to be with local endemic diseases and the subsequent magnification of them through mariculture back into the wild population. A review in progress on disease implications of farmed/wild salmonid interactions indicates that there is limited evidence of significant disease transfer from farmed to wild fish. A possible exception to this may be the sea lice situation, but the degree of interaction and consequences have not been quantified. No diseases in sea bass, sea bream, or turbot are known to present a risk to locally occurring species. There is, however, a potential for disease transfer to wild shrimp stocks by escapes of cultured shrimp. Unfortunately, no information is available on this subject. The escape of molluscs from aquaculture production is not regarded as a problem in relation to the spread of exotic diseases because of the limited mobility of these species.

The ACME emphasized that mechanisms to prevent disease introduction and the transfer of exotic diseases from farmed or escaped fish and shellfish are listed in the Office International des Epizooites (OIE) Aquatic Animal Health Code and in the 1994 ICES Code of Practice on the Introductions and Transfers of Marine Organisms (ICES, 1995). Proper implementation of these codes should reduce the risk of the spread of new diseases.

Genetic aspects of interactions between escaped farmed salmonids and wild populations

Information from WGAGFM refers mainly to Atlantic salmon (*Salmo salar*), which has a farmed production of more than 400,000 tonnes around the North Atlantic. There are possible effects of escapes on intra-population variation as well as on inter-population variation. In this context, wild catchment groups are referred to as 'populations' and farmed escapees as 'strains'. Most of the effects discussed result from interactions between non-native reared fish and their wild conspecifics.

Effects on intra-population variation can be either indirect or direct. Indirect effects (without interbreeding) could result from farmed escapees physically damaging the spawning beds (redds) of a wild population or spreading a disease which causes high mortality among the native salmonids. Reduction in wild population size could be so severe that reduction in genetic variability would result. There appear to be no documented cases of this phenomenon, but since escapees often ascend rivers to spawn later than wild populations, such effects could occur, particularly if the number of wild spawners is small (< 100) and the incursion is large.

A recent field experiment in Ireland simulated an interaction situation in a spawning stream. In the study, wild families survived approximately twice as well as reared families during the freshwater phase. Relative fitness in the ocean was not investigated, but results from Norway suggest that the overall performance of escaped farmed salmon is substantially lower than that of native salmon. In field experiments in northern Spain, significantly better performance was observed from native salmon in fresh water than from introduced fish from Scotland. It was further noted that the genetic evidence of farmed fish diminished rapidly over the next two years, when no further escapes seemed to have occurred. Because of the fitness differentials and the empirical evidence of farmed influence over time, it might be predicted that farmed progeny would decline rapidly in number. It might also be predicted that such declines would be more rapid in situations where the farmed strain is comparatively low in genetic variability or where the reared strain originates from a geographically distant area. It is not clear how much genetic variation there is in the most popular farmed strains in Europe, when compared with wild populations.

In the case of effects on inter-population variation, it is generally recognized that Atlantic salmon occur in a large number of relatively isolated riverine (or sometimes tributary) populations. These populations are maintained by accurate natal homing and there is thought to be limited gene flow between them. Farmed escapees in Europe now usually consist of a very limited number of strains. Hence, escapees which enter several rivers in a particular area are likely to be of a single strain, and thus greatly increase artificially induced gene flow. There have been few genetic studies directed to local adaptation, though there is abundant proof of the importance of this effect from other studies.

The ACME supported the findings of the WGAGFM which point out that repeated incursions must be regarded as much more serious than single incidents. If incursions continue on a regular basis, a situation is anticipated whereby the current complex population structure is replaced by a single aggregation of considerably reduced fitness. Evolutionary potential would also be considerably reduced, if such a scenario were to prevail.

The ACME further noted an important example demonstrating the genetic impact of farmed escapees on wild populations. In the Bay of Fundy, comparisons of DNA in tissue samples from Magaguadavic salmon collected in the mid-1970s prior to the development of salmon farms in nearby Passamaquoddy Bay, and from salmon sampled between 1992 and 1994, have provided evidence of genetic compromise of the wild stock. This clearly demonstrates that the principle of preventing escapes from mariculture facilities must be followed.

Two genetic methods to minimize genetic compromise of farmed and wild stocks have been repeatedly cited in debates concerning the alleviation of problems caused by farmed escapees. One method is the use of sterile salmon (by triploidizing all-female stock). The second method is the use of native salmon, or near-native salmon, in farming. The triploidizing method is neither 100 % effective nor fully acceptable to the farming industry, since triploids often perform less well in certain aspects of the culture cycle. In terms of using a native strain, it should be recognized that, even if a native strain were to be utilized in farming, it would have undergone domestication and thus would have reduced fitness in the wild. Therefore, it can be concluded that, at present, there are no reliable genetic methods for reducing the impact of farmed escapees on wild populations and that the principle of preventing escapes from mariculture facilities must be followed.

Escapes from fish culture operations

The ACME reviewed the information by WGNAS concerning salmon. It was noted that the techniques used to distinguish mariculture fish from wild fish include the assessment of morphological defects, scale patterns, otolith reading, and carotenoid pigment or genetic analyses. None of the methods are totally discriminatory and, in general, fish that escape very early in life cannot be recognized.

After escape, fish appear to resume a form of the natural life cycle. Thus, fish that escaped from sea cages and fish that can be demonstrated to have been reared in hatcheries until near smolting have been shown to be present among wild fish on open ocean feeding grounds in the Norwegian Sea and at West Greenland. These and further examples from field studies strongly indicate that escaped salmon have the potential to exert permanent genetic effects where they spawn.

The ACME noted the information, presented below, from the national reports for countries around the North Atlantic with major mariculture operations; this information summarizes recent salmon production and incidences of escape.

The proportion of farmed salmon caught in Norwegian coastal fisheries has varied between 34 % and 54 % of the total catch during the period 1989 to 1996. These proportions are significantly higher than in fjord fisheries, where 10–21 % of the catch has been of farmed origin.

No considerable numbers of escaped fish were detected in the UK (Scotland) in any of the fisheries examined before 1986. After 1986, reared salmon were detected regularly and were observed at the highest frequencies

(ranging to 38 %) in fisheries in the coastal areas closest to, or shared with, marine aquaculture operations (i.e., Redpoint, Achiltibuie, Culkein, and Strathy). At sites most distant from aquaculture facilities, reared fish were detected only irregularly (< 0.5 %).

In 1996, the aquaculture production of Atlantic salmon in Ireland was 13,500 tonnes. As a condition of their license, all fish farmers must report the occurrence of escapes to the licensing authority (Department of the Marine). Three major escapes from fish farms were recorded in 1996. These included the escape of 5000–8000 fish, 11,200 fish under one kilogram, and 9500 smolts from farms in the southwest, west, and northwest of the country, respectively. Overall, the rate of escapees in Irish commercial catches is very small (< 3 %).

No systematic data have been collected for in-river frequencies of escaped fish in the rivers of the Republic of Ireland. However, the Bush River (Northern Ireland, UK) is monitored for escapees. Escaped fish have been detected annually between 1991 and 1996. The frequency of escapees detected each year has been < 1 %, with the exception of 1994 when the frequency was 2.6 %.

The number of aquaculture-escaped Atlantic salmon in Canada has increased in recent years following the trend of increasing salmon production (almost 17,000 tonnes in 1996 in eastern Canada). In 1994, escapes of Atlantic salmon in the Bay of Fundy area were estimated at 20,000 to 40,000 salmon, an amount greater than the total returns of wild and hatchery origin salmon (both small and large) (13,000 to 21,000 fish) to the entire Bay of Fundy in the same year.

Salmon originating from farming operations in the USA have been observed in eight Maine rivers to date. The first documented incidence of farmed salmon in Maine rivers occurred in 1990, when a minimum of 17 % (14 of 83 fish) of the rod catch in the East Machias River was of farmed origin.

The ACME emphasized that farmed salmon tend to differ genetically from local wild stocks because of their non-local origin and/or because of deliberate and inadvertent selection during culture. Concern has been expressed that the spawning of escaped farmed fish and the assimilation of their progeny (and genes) into wild populations will result in losses of natural genetic diversity in wild populations. Further, the scope for escaped fish to exert effects on wild populations will depend on the relative numbers of escaped and wild fish in the group and patterns of incursion. Small populations that are at risk of large incursions are likely to be the most susceptible to change. Large populations may be robust to low rates of straying by escapees, as they are to low-level straying among wild populations. The persistence of initial effects will be determined by the relative fitness of the progeny of escaped fish, native fish, and hybrid progeny.

It was noted that the outcome of any interaction between wild and escaped stock will be dependent on:

a) the number of escaped fish;

b) the genetic constitution of the escaped fish, as it reflects source and selection;

c) the character of escaped fish, as it reflects their culture environment prior to release;

d) the size of the wild population group;

e) the genetic constitution of the wild population group and the individuals of which it is composed;

f) the character of the wild fish, as reflected in the nature of their interactions with escaped fish.

Need for further research or additional data

The ACME noted that there is sufficient information available to indicate that considerable escapes of fish from mariculture operations occur. However, these data are still too limited to allow a comprehensive quantification of escapes. Furthermore, there was no information available on how escapes are controlled. Therefore, the ACME pointed out that there is a need to conduct more quantitative studies based on standardized methodologies for data collection that have yet to be defined.

Due to the lack of comprehensive quantitative data on fish escapes, the ACME considered that an overall assessment of the consequences of escapees on wild stocks is not yet possible. However, the ACME expressed its concern that escapes might have adverse effects on disease transfer, genetic composition in relation to wild stocks, and competition for food and habitat, depending on the extent of escapes and the vulnerability of wild stocks in affected areas. Therefore, the ACME emphasized that there is a precautionary requirement to minimize escapes from mariculture operations by applying appropriate technical measures.

Recommendations

ICES ACME encourages ICES Member Countries to conduct studies aimed at quantifying the escapes of fish and shellfish from mariculture operations.

ICES ACME recommends that technical measures be developed and applied that aim at minimizing escapes of organisms from mariculture operations in order to reduce the potential risk of adverse effects of escapes on disease transfer, genetic composition in relation to wild stocks, and competition for food and habitat.

ICES ACME further recommends that, in order to prevent disease transfer from farmed and escaped farmed organisms to wild stocks, the guidelines provided in the OIE Aquatic Animal Health Code and in the 1994 ICES

Code of Practice on the Introductions and Transfers of Marine Organisms (ICES, 1995) should be followed.

Reference

ICES. 1995. ICES Code of Practice on the Introductions and Transfers of Marine Organisms 1994/Code de conduite du CIEM pour les introductions et transferts d'organismes marins 1994. 12 pp.

14.2 Chemicals Used in Mariculture

Request

There is no specific request; this is part of continuing ICES work on mariculture issues.

Source of the information presented

The 1996 report of the Working Group on Environmental Interactions of Mariculture (WGEIM), the 1997 report of the Working Group on Marine Sediments in Relation to Pollution (WGMS), the report of the Workshop on the Interactions between Salmon Lice and Salmonids (WKSLAS), and ACME deliberations.

Status/background information

In its 1996 report, the ACME summarized new information on chemicals used in mariculture and decided to continue updating and providing information on new compounds and the results of associated research (ICES, 1996). For that purpose the ACME reviewed the relevant sections of the above-mentioned reports.

In 1994, a technical report entitled "Chemicals Used in Mariculture" was published (ICES, 1994). This report, prepared by members of WGEIM during the period 1992 to 1994, provides information on the chemical and biological properties of a range of substances used in mariculture in the ICES area at that time, and contains extensive bibliographic lists. However, in many cases there was relatively little information on the environmental implications of the use of the substances and their subsequent release to the environment, either directly with waste feed or treatment baths, or after excretion/depuration from treated fish.

Very small amounts of chemicals are used in shellfish farming, usually at some shellfish hatcheries which use recirculating water systems. In finfish farming, the situation is different. Chemicals are widely used. Of the various types of chemicals used in mariculture, two primary groups which have the potential to give rise to measurable concentrations in seabed sediments or around fish farms are antimicrobial compounds and chemicals used for the control of external or internal parasites.

Antimicrobial compounds

There are a number of antimicrobial chemicals authorized for use in the ICES area (see ICES, 1994). Not all compounds are authorized in all countries; for example, only oxytetracycline, oxolinic acid, potentiated sulphonamids, and amoxycillin are authorized for use in the UK, whereas Ireland also allows the use of flumequine. The list is undergoing continuous change as compounds become available and new compounds are considered or brought forward for licensing. As an example, chloramphenicol, which was permitted in a few situations in aquaculture until recently, has been placed on Annex IV of EC Regulation 2377/90. Compounds in that Annex are prohibited for use with food animal species.

There is a consistent pattern of reductions in the use of antimicrobial agents in well-established salmon farming industries. In Norway, Ireland, and Scotland, the absolute amount of antibiotics has decreased in recent years, even though the production of fish has increased considerably. In mariculture there is increased awareness of the need to ensure that disease-free smolts are used, and that vaccination against furunculosis, vibriosis, and other diseases is applied wherever possible.

Chemicals used for the control of external or internal parasites (chemotherapeutants)

One of the more controversial aspects of salmonid cultivation in the sea has been the need to control infestations by ectoparasitic sea lice. Until recently, the only licensed chemical treatment has involved the use of the organophosphorus compound dichlorvos as a bath treatment, with the compound subsequently released into the marine environment. Dichlorvos (now licensed as the medicine Aquagard) is widely used in Norway, France, the UK, Ireland, etc. The compound is fairly soluble in sea water and is not found in marine sediments. However, there is evidence that some sea lice populations have developed resistance to it. Sea lice are still the greatest threat to the health of farmed salmon in most of the ICES area and there is a large demand to identify new chemicals which can be licensed for salmon treatment.

Recently, other treatments which do not accumulate in sediments have become available, notably, a hydrogen peroxide bath at 1500 ppm. This substance seems to degrade rather rapidly in the sea, presumably through a combination of reaction with organic matter and, perhaps, catalytic decomposition on contact with salts of transition metals such as manganese. It is, however, relatively expensive to use and, therefore, of limited commercial interest.

There are other products under development, under consideration for authorization, or undergoing field trials

that contain active ingredients, some of which are more readily associated with sediments. These are the organophosphate azamethiphos, the synthetic pyrethroid cypermethrin, the anthelminthic ivermectin, and the chitin inhibitors diflubenzuron and teflubenzuron, among others.

a) Azamethiphos

Azamethiphos, sold as Salmosan by Ciba-Geigy, is more widely used as an experimental or emergency bath-type treatment for sea lice than the other unlicensed organophosphates, such as malathion and carbaryl. Like dichlorvos, this compound kills mobile lice stages but not larvae. Its effects on non-target species are still being assessed. Information from the St. Andrews Biological Station, New Brunswick, Canada, suggests that azamethiphos had no effect on lobster survival or primary production in the vicinity of mariculture sites. Further, it is considered to have little if any impact on seabed sediments.

b) Cypermethrin

The use of cypermethrin as a bath treatment for sea lice is relatively new. Reports on its possible toxicity are inconclusive. It has been reported to kill both adults and larvae of sea lice. Preliminary consideration of the bath treatment system and concentration suggests that cypermethrin should present only a small environmental risk in sediments, although it is strongly adsorbed onto particulates. It has been licensed for control of midge larvae in drinking water. However, it is not registered for use in Canada, where it is considered to be highly toxic to aquatic invertebrates. There, it has been tried for years to limit aerial application for terrestrial uses as a means of reducing input to aquatic systems.

c) Ivermectin

Ivermectin has been an effective anthelminthic for agricultural animals. It will reduce sea lice infections on fish if administered in feed in doses ranging from 0.02–0.2 mg kg^{-1} fish weight, once or twice a week. It has been reported to have negative effects on fish and is not recommended for use in fish by the manufacturer. Although its use is ecologically questionable, many salmon farms are using ivermectin as an off-label prescription drug. Recent studies (Davies *et al.*, in prep.) show that acutely toxic concentrations of ivermectin might arise in sediments below and immediately surrounding salmon farms.

d) Chitin inhibitors

Chitin inhibitors such as diflubenzuron and teflubenzuron inhibit chitin synthesis and thus prevent moulting of arthropods. They have been, and perhaps are still being, considered for use against sea lice. They can persist for some time in the environment and thus could negatively affect marine crustaceans. Indeed, the use of diflubenzuron in agriculture has been known to damage estuarine crustacea.

Other chemicals are being tested for their effectiveness against sea lice. All compounds which eventually obtain full licensing are rigorously tested for environmental and user safety. There is a need for monitoring data to ensure that these licenses are adequate to protect the marine environment from contamination and the effects of these chemicals or their degradation products.

Need for further research or additional data

The ACME noted that many of the compounds considered here are poorly soluble in water, readily associate with particles, and are incorporated into marine sediments. One main area of concern is that antimicrobial compounds in sediments might affect and reduce the activity of the natural populations of bacteria responsible for the degradation of organic matter, particularly the organic waste from the fish farms. There is a need for more information and validated models on the dispersion of the chemicals after application in order to assess exposure and to predict impacts.

It should be noted that although this section focuses largely on the risks to sediment dwellers, substances marketed for use in mariculture are generally well-characterized with respect to their toxicity to water-column fauna. Furthermore, the environmental risks of officially approved substances to water-column organisms are usually well established before these products reach the market.

The ACME noted that there is still continuing pressure from the mariculture industry for additional substances to be added to the range of antimicrobial compounds that can be prescribed for use on farmed fish. The environmental effects of these substances have to be clarified before they are applied in mariculture.

References

Davies, I.M., Gillibrand, P.A., McHenery, J.G., and Rae, G.H. In prep. Environmental risk of ivermectin to sediment-dwelling organisms. (Submitted to Aquaculture.)

ICES. 1994. Chemicals used in mariculture. ICES Cooperative Research Report, No. 202. 100 pp.

ICES. 1996. Report of the ICES Advisory Committee on the Marine Environment, 1996. ICES Cooperative Research Report, No. 217: 81–82.

15 ASSESSMENT TOOLS

15.1 Background Concentrations of Contaminants in the Marine Environment

Request

The Oslo and Paris Commissions requested ICES to co-sponsor a workshop on background concentrations of contaminants in the marine environment.

Source of the information presented

The report of the OSPAR/ICES Workshop on the Overall Evaluation and Update of Background/Reference Concentrations for Nutrients and for Contaminants in Sea Water, Biota and Sediments (Hamburg, 22–25 October 1996), the 1997 reports of the Working Group on Environmental Assessment and Monitoring Strategies (WGEAMS), the Marine Chemistry Working Group (MCWG), the Working Group on Marine Sediments in Relation to Pollution (WGMS), and ACME deliberations.

Status/background information

The report of the OSPAR/ICES Workshop gives an update of the background/reference concentrations for different compounds in different compartments of the marine environment as a follow-up to the North Sea Task Force Workshop on this topic held in 1992. The ACME felt that the best had been done with the material available and that this report was an improvement over the 1992 Workshop report. It was recognized that this report is likely to be used by the OSPAR Ad Hoc Working Group on Monitoring as a first step to gain experience in the usefulness of background concentrations as assessment criteria in the framework of the assessment of data from the OSPAR monitoring programmes.

It is important to recognize the limitations of the operational definition of the term 'background concentration' before applying such concentrations in an assessment because the term 'background' has different meanings in different disciplines. The assessment work, therefore, must be undertaken only by experts who have a clear awareness of the quality and limitations of the data. The interpretation of the field observations will not be straightforward, nor will the conclusions be unambiguous.

It is not possible to establish background concentrations of nutrients independently of the water mass considerations because the marine environment is highly dynamic. Physical processes such as upwelling in coastal areas cause rapid changes in the nutrient concentrations by bringing in, e.g., Atlantic waters enriched in nutrients by natural biogeochemical processes. In these cases, it may be difficult to distinguish between natural and anthropogenic sources. The inclusion of nutrient data in a report entitled 'background concentrations of natural compounds' has great potential to be misused.

The background concentrations developed by the Workshop, as pointed out in the report, have been derived from data representing different time frames and on different geographical bases. Such factors are the origin of many of the technical and conceptual criticisms that have been directed at the report, such as:

- It could be argued that for man-made compounds such as PCBs the background concentration is zero. If this is not acceptable, then it is better to speak of present minimum concentration values in surface sediments and biota due to the long-range atmospheric distribution of, e.g., PCBs.
- In comparing data with the background values, uncertainties must be taken into account. At present, it is unclear what levels of uncertainty apply to the specific boundaries of the background ranges, how analytical and other variances are to be handled, and whether comparison should be made using means, medians or upper quartiles.
- It is recommended that background values from additional areas, e.g., Iceland and Greenland, be incorporated in the report to make it usable for more regions.
- It is not possible to define only one background concentration for a given substance for the entire OSPAR Convention Area since large regional variability can occur. Background concentrations must therefore be defined on a regional basis.
- Errors during sampling, handling, and analysis can always occur due to contamination problems when samples contain low concentrations. This can influence the concentration ranges in areas often thought of as being pristine.
- It is recommended that data for trace metals in sea water be used from the winter season and from depths between 10 m and 100 m.

15.2 Methods for Assessing Temporal Trend Monitoring Data

Request

There is no specific request; this is part of continuing ICES work to provide advice on the development of effective methods for designing monitoring programmes and assessing temporal monitoring data.

Source of the information presented

The 1997 report of the Working Group on Statistical Aspects of Environmental Monitoring (WGSAEM) and ACME deliberations.

Status/background information

At its 1996 meeting, the ACME discussed various statistical objectives for monitoring (ICES, 1996). In particular, objectives which incorporated environmental target values were shown to lead to better-informed decisions and simple protocols for re-allocating monitoring effort when these decisions were taken. The ACME noted that OSPAR intends to include reference values in the assessments of trends of contaminant concentrations in biota, due to take place early in 1998.

WGSAEM reported further developments showing how reference values coupled with appropriate statistical objectives can be exploited.

One approach looked at ways of optimizing sampling effort when monitoring at multiple sites. The heart of this method is some procedure for deciding whether current contaminant levels are above or below the reference value, with some penalties (loss) for incorrect decisions. Different sampling strategies can then be compared on the basis of the loss averaged over a large number of years. A dynamic strategy, where a part of the effort was allocated to sites most likely to benefit, appeared to offer an improvement over a fixed-effort strategy.

WGSAEM also reported on two methods whereby reference values are exploited in compliance monitoring. These methods are both **Cu**mulative **Sum** (CUSUM) techniques, whereby some test statistic is updated and tested as each new data point is collected. This approach can be particularly useful for assessing long-term monitoring data. Explicitly recognizing that a significance test will be carried out in every year also leads to tests which correctly control the error rate of false trend detection.

The first method consists of computing d_i on each sampling occasion, where d_i is the proportion of, e.g., 25 fish for which the concentration of the contaminant of interest exceeds a given reference value. Then the CUSUM defined by

$$S_i = \sum_{j=1}^{i} (d_j - k)$$

is plotted against i and compared with a decision value, h. Values below h are considered 'acceptable' and values above h are considered 'unacceptable'. To demonstrate this, an example is shown in Figures 15.2.1 and 15.2.2 using data from the WGSAEM test data sets for mercury in cod muscle.

A reference value of 0.08 mg kg^{-1} mercury has been used, selected from the range reported by OSPAR (1997). The numbers of fish exceeding this reference value are shown along the Year axis. The decision value is taken as $h = 5$. The permissible values of S_i have a lower (zero) and an upper ($h + h' = 10$) limit. When passing through h from below (acceptable to unacceptable), S_i is reset to ($h + h' = 10$). When passing through h from above (unacceptable to acceptable), S_i is reset to zero. Note that S_0 is not an observed value, but defined by the user. Choosing a starting value of S_0 = lower limit (0) assumes that the results are initially acceptable; S_0 = upper limit ($h + h'$) assumes that the results are initially unacceptable.

The two figures demonstrate the effect of starting the CUSUM from an optimistic ($S_0 = 0$) or a pessimistic ($S_0 = 10$) viewpoint.

The values of the parameters (h, h' and k) are chosen to make the CUSUM have desirable statistical properties, i.e., to control the length of time before reaching a wrong decision, e.g., the average number of years starting from $S_0 = 0$ that elapse before correctly concluding that results are unacceptable. A more theoretical discussion of this method is given in ICES (1997).

The second type of CUSUM plot is based on the Smoother used in the current OSPAR methodology for assessment of temporal trends in contaminants in biota (Nicholson *et al.*, 1998). The fitted Smoother in the final year of the time series can be represented as a partial, weighted CUSUM of the annual contaminant level. Hence, the display used in the routine assessments can be interpreted in terms of a fixed-period test or as an annual test by simply overlaying the appropriate reference lines.

Need for further research or additional data

The ACME noted that these are new approaches and that the results are very preliminary. However, incorporating reference values clearly offers many benefits and potentially more-informative analyses. The ACME is aware of the merits of these new ways of thinking about the design and assessment of monitoring programmes and the way they integrate the assessment of temporal data, environmental reference values, and decision making. Practical methods for optimizing the value of monitoring efforts are potentially useful and should be developed further.

The two CUSUM methods emphasize different features of monitoring data. Together, they provide complementary information about changes in both average contaminant levels and compliance rates for individual fish. The ACME encouraged further development in order to demonstrate how the methods could be integrated into an effective management tool.

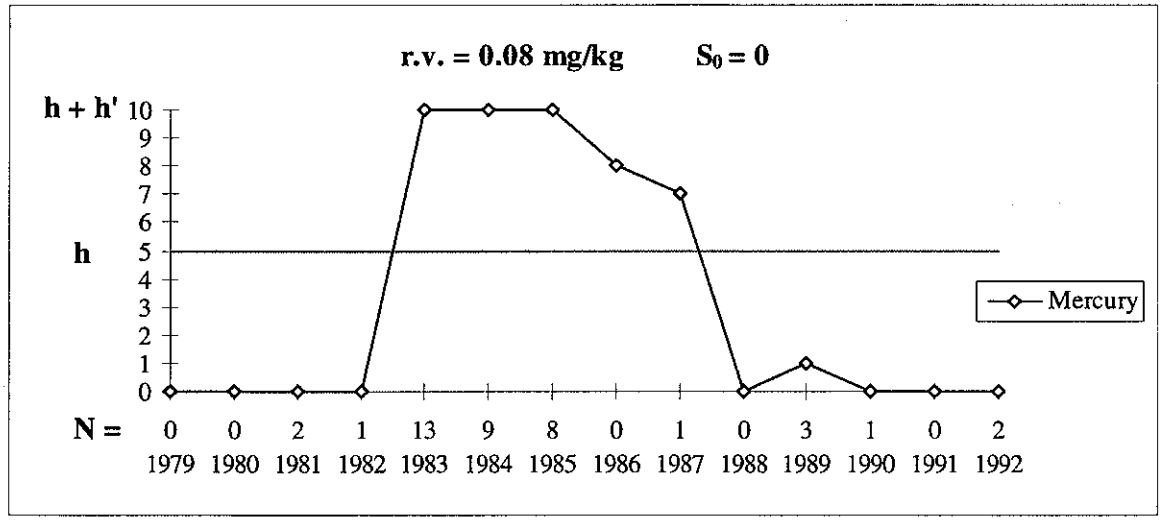

Figure 15.2.1. Cumulative Sum Plot of the proportion of cod exceeding the reference value of 0.08 mg kg^{-1} Hg in muscle. The centre line divides the graph into Acceptable (0–5) and Unacceptable (5–10). The sample size is 25 fish per year; N is the number of fish per year exceeding the reference value. The starting value ($S_0 = 0$) assumes that before monitoring began, Hg levels were Acceptable.

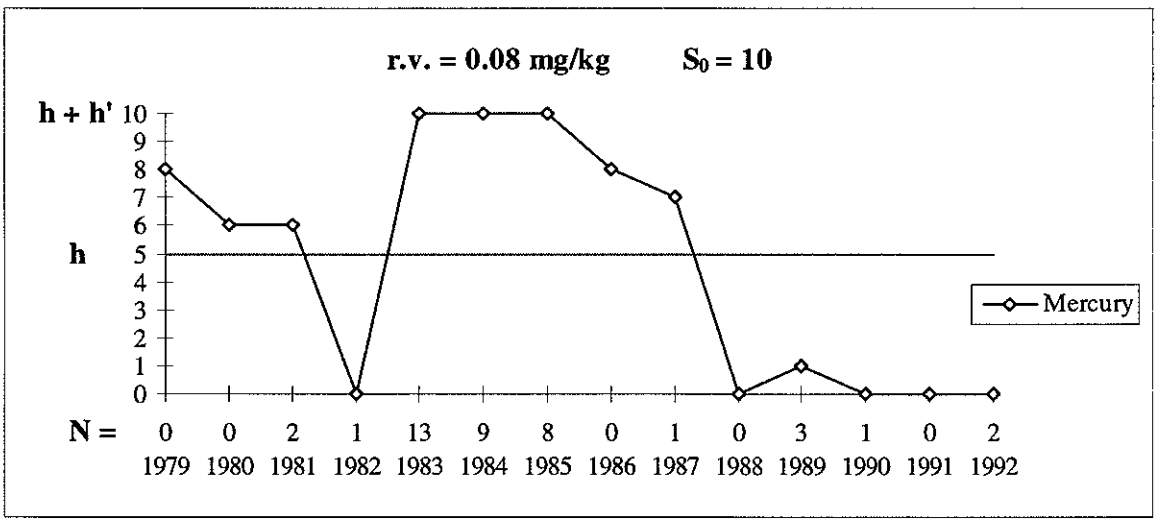

Figure 15.2.2. The same Cumulative Sum Plot as Figure 15.2.1 but with a more precautionary assumption, i.e., that before monitoring began, levels of Hg were Unacceptable. Within four years, the two plots are the same.

References

ICES. 1996. Report of the ICES Advisory Committee on the Marine Environment, 1996. ICES Cooperative Research Report, No. 217: 6–14.

ICES. 1997. Report of the Working Group on Statistical Aspects of Environmental Monitoring. ICES CM 1997/Env:1.

Nicholson, M.D., Fryer, R.J., and Larsen, J.R. 1998. Contaminants in marine organisms: A robust method for analysing temporal trends. ICES Techniques in Marine Environmental Sciences, No. 20. 21 pp.

OSPAR. 1997. Report of the Third OSPAR Workshop on Ecotoxicological Assessment Criteria. SIME(1) 97/7/1-E. Oslo and Paris Commissions, London.

Request

There is no specific request; this is part of continuing ICES work on marine environmental issues.

Source of the information presented

The 1997 report of the Working Group on Shelf Seas Oceanography (WGSSO) and ACME deliberations.

Status/background information

In 1996, the ACME noted that the Global Ocean Observing System (GOOS) will ultimately serve as the overall framework for many programmes, including marine environmental monitoring programmes. The ACME felt that a discussion should be pursued within ICES concerning 'operational fisheries oceanography on the time scale of fish stock assessment'.

Fisheries oceanography

Data on fish stocks used in fish stock assessments come from two main sources: fish catch statistics and surveys with research vessels. Generally, environmental data (e.g., on hydrographic conditions, nutrients, plankton) are also collected during fish stock surveys. They constitute a major portion of the data on the spatial distribution of environmental properties in coastal and oceanic waters of the ICES area.

The time scale of fish stock assessments is generally the annual cycle, with milestones of survey cruises, working group meetings, and meetings of the Advisory Committee on Fishery Management (ACFM). The challenge for ICES and its Member Countries, and participating national institutions which comprise the ICES network, is to assemble, assess, and use environmental data within this annual fish stock assessment cycle. Since much of the environmental data are collected during fish stock surveys, the requirement is to process the environmental data in parallel with the fish data, with a data handling requirement at a time scale of months.

To meet the requirement to handle environmental data with some months between their collection and use requires the involvement of national institutes, national data centres, and the ICES Secretariat Data Centre in a coordinated and cooperative effort. There are four main elements in this work: 1) data submission, 2) data compilation, 3) data assessment, and 4) assessment of the environmental conditions.

National institutes must submit their quality assured data as quickly as possible after their collection. Data from different institutes must be compiled into larger sets of data which provide a description of the environmental conditions in parts of or the entire ICES area. The ICES Secretariat will serve as a regional data centre in this process, but should work in close cooperation with national data centres to facilitate their work. Much of the data compilation and storage could be handled by national data centres working together with the ICES Data Centre.

ICES should play a dual role in this process. In addition to being a regional data centre, ICES can also provide the cooperative framework for work by national experts involved in the data assessment and environmental assessment. In order to achieve this, and to accommodate the needs of operational fisheries oceanography, the goals and remits of some working groups may need to be adapted. For example, environmental working groups (such as the Working Group on Oceanic Hydrography, the Working Group on Shelf Seas Oceanography, the Working Group on Phytoplankton Ecology, and the Working Group on Zooplankton Ecology) and fish stock assessment working groups may need to work more closely together in order to describe and quantify environmental impacts on fisheries. There may also be a need for mixed environmental/fish stocks working groups with a regional or ecosystem focus.

The principle of using a 'lead country' to delegate responsibility for given tasks or topics to one or more countries has been employed in organizations such as OSPAR and AMAP. For preparing Environmental Assessment Reports for subregions within the OSPAR area, Regional Task Teams are used with participants from the OSPAR Contracting Parties interested in the region under the leadership of a 'lead country'. There is a dual benefit in such a system. First, there is a clear separation between regional issues and issues of general interest for the whole OSPAR region. Second, extra costs are covered by the interested parties at the subregional level. The principles of lead country and regional task teams should be considered by ICES as possible mechanisms for carrying out some of the work in the ecosystem-based working groups.

Automated data collection from buoys, ships of opportunity, and remote sensing

In addition to survey data collected from research vessels, there is another major line of data collection from moored buoys, ships of opportunity, and remote sensing. Automated systems can provide measurements with high temporal resolution when deployed at oceanographic buoys or ships of opportunity. Such data can be stored locally or transmitted to shore via satellite in near real time. Data collected by remote sensing from satellites or aircraft can provide repeated synoptic pictures of large areas of the ocean surface.

There is much interest for such data to be used in GOOS, particularly in the Ocean Services module. This is related to the importance of meteorological conditions as driving forces for ocean dynamics. To be of optimal use, data from automated systems and remote sensing should be used along with spatially distributed data from surveys. Data handled within the ICES system related to operational fisheries oceanography would, therefore, be of relevance to the Ocean Services module of GOOS. Conversely, the data flow from, e.g., buoys and remote sensing is a source of information which is potentially useful in the operational fisheries oceanography work of ICES.

The flow and storage of data from the two main avenues of data collection, research vessel surveys and automated systems and remote sensing, need to be harmonized and coordinated. National data centres should play a role in this along with the ICES Data Centre and possibly other regional data centres. There is scope for commercial activity from the private sector in GOOS, particularly within the Ocean Services module. There is a possibility that operational units may be developed for such activities, e.g., within EuroGOOS. The ICES community needs to consider its role and means of cooperation with such units to ensure orderly and cost-effective use of data for both governmental and private use.

Modelling

The use of mathematical models will gain increasing importance in describing and forecasting the state of the ocean. This will be the case for a range of applications including operational fisheries oceanography. ICES and the ICES community need to be actively involved in the development and use of such models.

Data and data assimilation will become more important in modelling activities. Continuous measurements from automated systems may provide updated information on the time scale of changes in the meteorological driving forces. On a larger scale, changes in physical oceanographic conditions are relatively slow. Survey data on, e.g., density fields and nutrient concentrations may provide important input to improve the model descriptions of currents and phytoplankton production. As one goes up the food chain to migratory fish and other higher trophic level organisms, it becomes increasingly more difficult to model them from first principles and the reliance on data input increases correspondingly. Data assembled, assessed, and used for the purpose of operational fisheries oceanography in ICES are, therefore, important data sources for improved model descriptions for other GOOS applications.

Applications modules of GOOS

GOOS is being planned with five applications modules:

1) Climate Monitoring, Forecast, and Prediction;
2) Coastal Zone Management;
3) Living Marine Resources;
4) Health of the Ocean;
5) Ocean Services.

The main activities of ICES are within the Living Marine Resources module, but with considerable activities also within the Health of the Ocean and, increasingly, the Coastal Zone Management modules.

The five modules are interlinked in that there is a high degree of commonality in the data needs and data handling and modelling tools required within each module. The data on ocean climate collected in operational fisheries oceanography within the framework of the Living Marine Resources module constitute a major component of data in climate monitoring. A long-term objective in fisheries oceanography is to be able to predict the ocean climate at a time scale of months to years in order to be better able to predict the development of the state of the ecosystem and the fish stocks.

The data requirements and modelling capabilities for applications within the Ocean Services module are similar to the requirements for describing events such as algal blooms, oil spills, and fish larvae transport for applications within the Health of the Ocean and the Living Marine Resources modules.

The ACME agreed that, due to the involvement of the ICES community in GOOS-related and relevant activities, ICES should take an active and leading role in the further development and implementation of GOOS at the North Atlantic regional level.

Recommendations

ICES ACME recommends that ICES seek a central role in the networking of GOOS activities in the North Atlantic. For that purpose, ICES should take a more active role in GOOS and EuroGOOS by contributing directly to relevant meetings.

ICES ACME recommends that the ICES representative to the IOC Assembly should present the case for ICES involvement in GOOS as described above.

ICES ACME recommends that ICES take steps to implement an involvement in GOOS by requesting the new ICES Oceanography, Marine Habitat and Living Resources Committees to prepare an action plan for the conduct of activities to contribute to GOOS.

17 DATA HANDLING

17.1 Activities of the ICES Environmental Data Centre

17.1.1 Databases on contaminants in marine media and biological effects of contaminants

The ACME took note of information presented by the ICES Secretariat on the handling of data from the Joint Monitoring Programme (JMP) on contaminants in biota, sediments, and sea water and on biological effects of contaminants. In this context, the ACME noted that, at present, the ICES Environmental Data Centre includes the following components:

- contaminants in marine invertebrates, fish, birds, and mammals (approximately 275,000 records);
- contaminants in sea water (approximately 280,000 records);
- contaminants in marine sediments (approximately 80,000 records);
- biological effects (approximately 4000 records).

The annual flow of data submitted to the ICES Environmental Data Centre is approximately 25,000 records for each of the first two components and 5000 records for the third component. The flow of new data into the biological effects database is small. The data flow is illustrated in Figures 17.1.1.1 to 17.1.1.4.

The ICES Environmental Data Centre is preparing for the assessment of temporal trends in contaminants in biota to be conducted by the OSPAR Ad Hoc Working Group on Monitoring in February 1998. This activity is expected to increase the flow of data, especially for the most recent years.

Major data products

The ICES Environmental Data Centre provided information for the ICES/OSPAR Workshop on Background/Reference Concentrations for Nutrients and for Contaminants in Sea Water, Biota and Sediments (see Section 15.1, above) and for the OSPAR Workshop on Ecotoxicological Assessment Criteria, both of which were held during autumn 1996. For both workshops, the information supplied included data and univariate statistics on data on contaminants in biota and sediments.

Quality Assurance Database

The ICES Quality Assurance Database consists of the following components:

1) composition of reference materials;
2) written documentation from the laboratories on monitoring activities, analytical methods, etc;
3) data generated via intercomparison exercises.

The first component currently consists of descriptions of approximately 150 materials with their referenced and/or recommended composition. The second component consists of approximately 250 documents. Countries and/or laboratories reporting data to the ICES Environmental Data Centre are requested to supply additional written information about, e.g., sampling and analytical procedures, but major gaps have been identified in this compilation. The OSPAR procedure has recently been strengthened in this respect and this is expected to result in a more stable flow of information.

The component on intercomparison exercises is based on two major sources of information: the ICES intercomparison exercises and exercises carried out under QUASIMEME I and II. The development of the component based on the ICES intercomparison exercises is hampered by the fact that, in most cases, only paper versions of the reports of the exercises exist. These paper copies are being digitized at the speed that staff resources permit. At present, five of 49 exercises have been digitized.

Data generated under the QUASIMEME I exercise are confidential, and no solution has been found for the direct access to these data. However, individual laboratories are free to submit these data to ICES as a voluntary contribution. For data generated under QUASIMEME II, a mechanism has been established that allows individual laboratories to report their data to the ICES Environmental Data Centre. Thus far, no data have been reported by this mechanism.

Handling of data for the Arctic Monitoring and Assessment Programme (AMAP)

The ICES work as the Thematic Data Centre for the marine component of AMAP covering the years 1993 to 1996 has been completed. This project was described in detail in the 1996 ACME report (ICES, 1996). A final report was presented to the AMAP Working Group in February 1997.

A new contract for ICES to continue to serve as the Thematic Data Centre for the marine component of AMAP until the end of 1998 is in the final stages of negotiation.

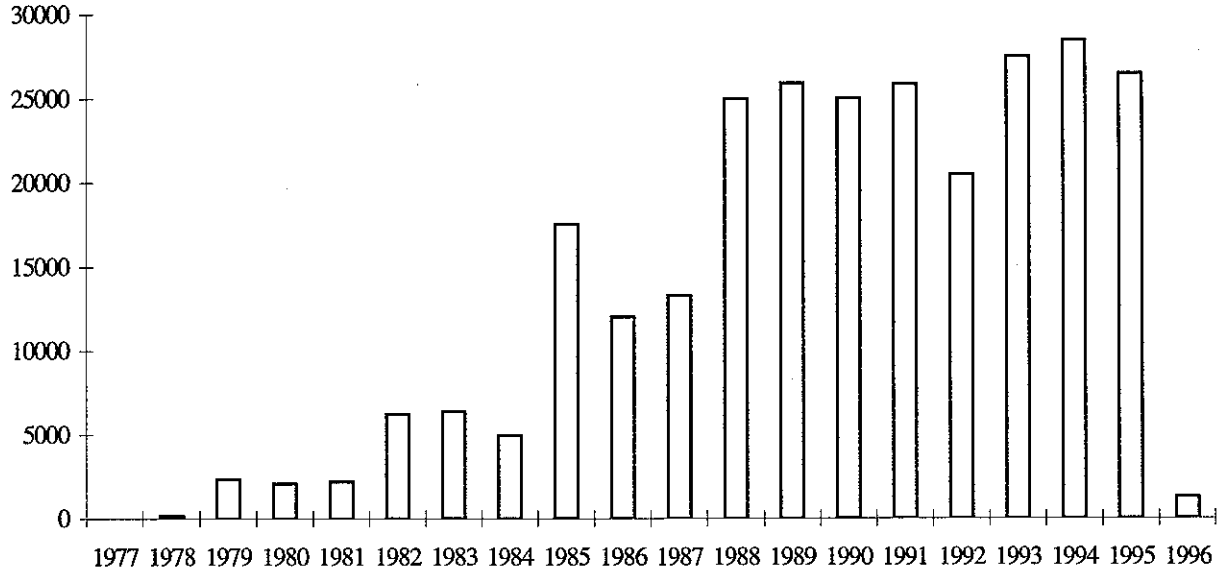

Figure 17.1.1.1. Annual flow (number of records) of data on contaminants in biota submitted to the ICES Environmental Data Centre.

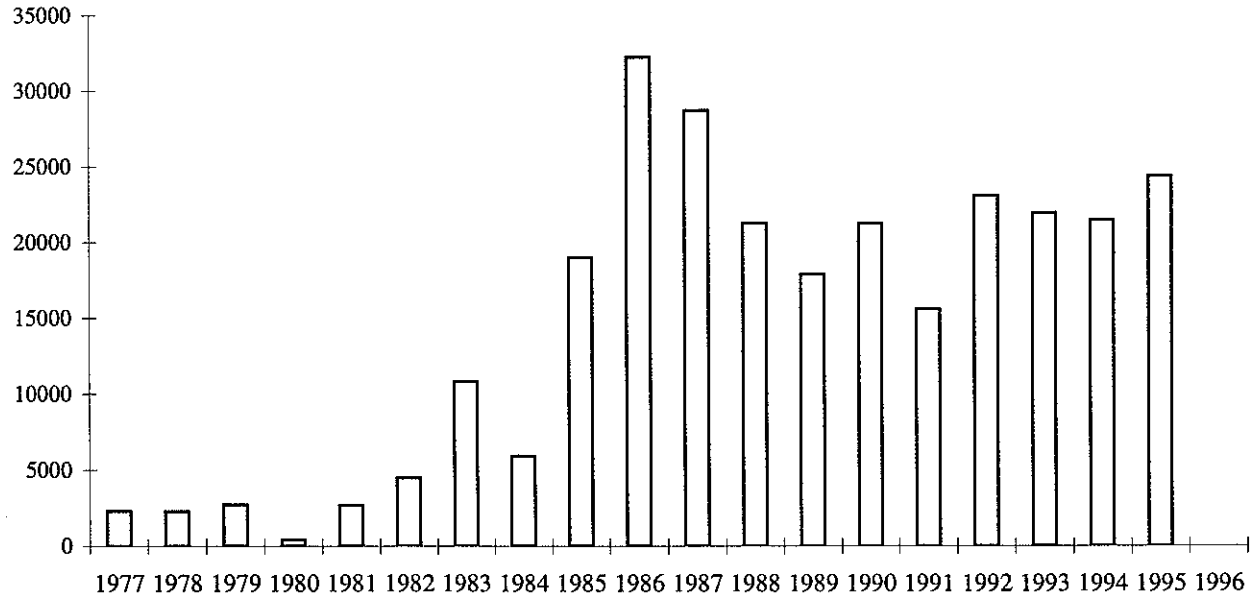

Figure 17.1.1.2. Annual flow (number of records) of data on contaminants in sea water submitted to the ICES Environmental Data Centre.

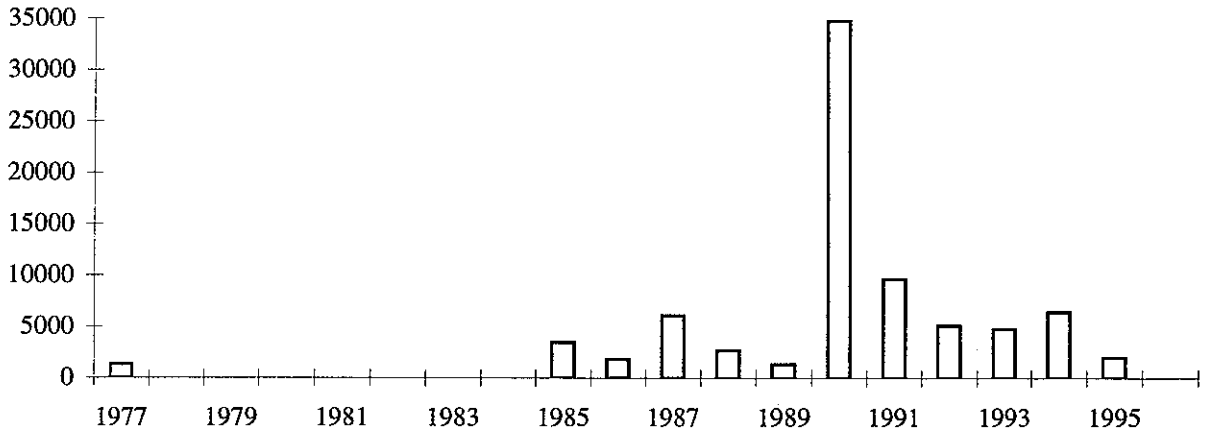

Figure 17.1.1.3. Annual flow (number of records) of data on contaminants in sediments submitted to the ICES Environmental Data Centre.

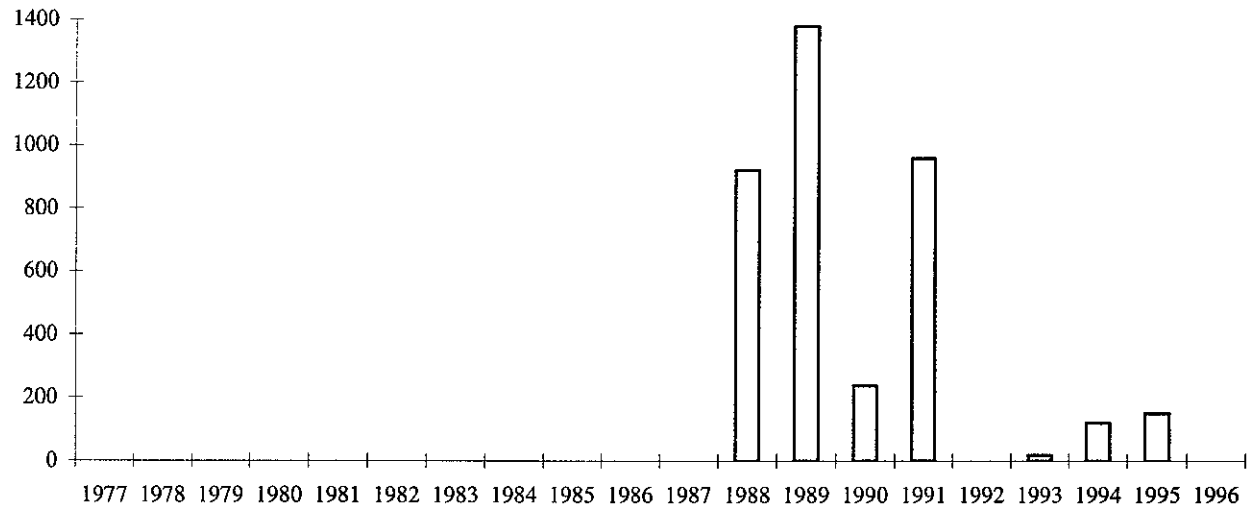

Figure 17.1.1.4. Annual flow (number of records) of data on biological effects of contaminants submitted to the ICES Environmental Data Centre.

European Environment Agency

The ICES Environmental Data Centre has received, and is processing, requests for the provision of data on contaminants in biota and sediments to be used in the EEA Dobris+3 report that is being prepared during 1997.

Reporting formats and data handling software

An update of the annexes to the 'ICES Environmental Data Reporting Formats' was released in July 1996, together with version 1.31 of the associated screening software. Data validation software was released simultaneously. This software facilitates the data validation procedure by allowing the conversion of the standard data structure into a more readable form.

Beginning in April 1997, the annexes to the 'ICES Environmental Data Reporting Formats' are gradually being moved to the World Wide Web, with the ultimate aim of phasing out the production of printed versions of the annexes.

On-line access to data inventories

Access to dynamic/query inventories of the data in the ICES Environmental Data Bank has been established through a World Wide Web interface. The interface allows the user to retrieve information about present holdings of contaminants and biological effects data. Although the interface gives no access to the actual data, it was felt that the data originators should be asked for permission prior to the inclusion of their data in the inventory. Forty laboratories have been asked to give their permission; thirteen have replied, all positively.

17.1.2 Fish Disease Database

The ICES Environmental Data Centre organized the compilation and analysis of data for the 1997 meeting of the Study Group on Statistical Analysis of Fish Disease Data in Marine Fish Stocks (SGFDDS) (see Section 7.1, above). Approximately 80,000 observations from Denmark, Germany, the Netherlands, and the UK were analysed. Use of the World Wide Web allowed participants to have access to the data and products prior to the meeting.

17.1.3 Marine Mammals By-catch Database

No new data were submitted to the database in 1996/1997.

Reference

ICES. 1996. Report of the ICES Advisory Committee on the Marine Environment, 1996. ICES Cooperative Research Report, No. 217: 85.

17.2 Handling of Nutrient Data for the Oslo and Paris Commissions

The ACME noted that the OSPAR Environmental Assessment and Monitoring Committee (ASMO) has agreed to a more flexible reporting of nutrient data submitted as part of their Nutrients Monitoring Programme. Previously it had been mandatory that institutes submitted nutrients data using the ICES Environmental Data Reporting Format, but now data may be submitted using more general guidelines but using established vehicles for the delivery of supporting information. This agreement reflects what has been happening in practice, particularly as ICES has long-established suppliers of oceanographic data who did not wish their routines to be disrupted. The Data Centre will now be able to work more efficiently since in many cases data sets submitted using the mandatory format were often quite incomplete both in the range of parameters and the number of stations. For quality assessment reasons, it is requested that all data, in addition to data labelled 'OSPAR', should be submitted.

The ACME took note of information describing the work of the ICES Oceanographic Data Centre with OSPAR in terms of the development of quantitative assessment criteria to implement the 'Common Procedure for Identification of the Eutrophication Status of the Maritime Area of the Oslo and Paris Conventions'; this is contained in Annex 9.

The ACME noted with concern the rapidly deteriorating position with regard to the delivery of nutrient data. It has not been established why this is so, but the root of the problem may lie in the difficulties a number of institutes are having in establishing relational databases of combined CTD and chemical data. These difficulties are not a problem for the databases *per se*, but may arise from incorrect procedures 'on station'. For example, it is the routine of one country to perform a CTD survey on one day, then return one or more days later to collect the water samples.

Concern was also expressed about the deterioration in the quality of the submissions, which was placing an unanticipated demand on the Secretariat's resources. In one case, a sixth resubmission of the same data set is currently being handled. The ACME recognized this problem as arising from inadequate resources at the institute level.

The ACME noticed that OSPAR were now recognizing the value of the total ICES nutrient database, and were recommending its use in many activities in support of eutrophication issues. The ACME also recognized that such data sets will increase their value significantly when initiatives on operational fisheries oceanography such as in the context of the Global Ocean Observing System (GOOS) materialize (see Section 16, above).

17.3 Taxonomic Code Systems

Request

This is relevant to item 2.1 of the 1997 Work Programme from the Oslo and Paris Commissions, and is also of interest to the Helsinki Commission and ICES Member Countries.

Source of the information presented

The 1997 reports of the Benthos Ecology Working Group (BEWG), the Working Group on Zooplankton Ecology (WGZE), the Working Group on Phytoplankton Ecology (WGPE), the ICES/IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD), and the Working Group on Marine Data Management (WGMDM), and ACME deliberations.

Status/background information

During discussions at the 1996 meetings of the Benthos Ecology Working Group (BEWG) and the Ad Hoc Group of Database/GIS Practitioners (AGDATA), it became clear that many different taxonomic coding systems are being used by a variety of groups and projects. In addition, the ICES Secretariat databases utilize a number of different coding systems. The ACME agreed that a review should be made with a view to identifying a common coding system that may be of use across the various biological oceanography disciplines. Consequently, the ACME proposed that the Working Group on Phytoplankton Ecology, the ICES/IOC Working Group on Harmful Algal Bloom Dynamics, the Working Group on Zooplankton Ecology, and the Benthos Ecology Working Group work towards meeting this objective. The Working Group on Marine Data Management (WGMDM) also considered this topic, especially as its meeting took place at the location where the U.S. National Oceanographic Data Center (NODC) taxonomic code system was developed and is maintained.

The five Working Groups reviewed the problem, as requested by ACME, and all came to the conclusion that the NODC system was the preferred one. However, this particular system is currently being extensively revised and adapted for modern relational databases. The present state of the system was presented in detail for the WGMDM at its 1997 meeting at NODC in Washington, D.C. Details of the findings are summarized below.

Taxonomic names have synonyms and commonly used variant spellings; they are not unique and are hierarchically related. In the past, numeric coding systems have been developed as it was more difficult to manipulate text than numeric values. The present system is being developed in the USA by the Interagency Taxonomic Information System (ITIS). This is a collaborative effort among the Department of Agriculture, the Department of Commerce, the Department of the Interior, the Environmental Protection Agency, and the Smithsonian Institution. In the future, this will be extended to cover all US federal organizations as well as state organizations. International collaboration would also be valuable, but this is currently limited due to a lack of resources.

Version 7 of the US NODC Taxonomic Code comprised 'intelligent' keys, whereby the hierarchy can be determined from the code. However, in time, it is no longer possible to place codes in the correct place and thus the taxonomic code number cannot be translated back to the name. In Version 8, a non-intelligent taxonomic serial number is assigned. The advantage of this is that the number translates to a unique name and does not change even if the taxon is reclassified. The disadvantage is that the hierarchy cannot be seen from the number alone. Version 8 of the US NODC Taxonomic Code (released in May 1996) includes approximately 250,000 records, the final list of former NODC codes, hierarchy and synonymy, and some common names. Most of this can be viewed on the ITIS Web page (http://www.itis.usda.gov/itis).

The addition of new codes to Version 7 of the NODC Taxonomic Code has been suspended, and the ITIS system is currently undergoing testing. The system is expected to be operational in May 1997. The addition of new codes should be a simple matter if the name to be added is fully documented, but without the documentation it could take weeks to months. As the ITIS system is undergoing a testing phase and is not fully operational, it is premature to recommend its usage.

As taxonomic information is not necessarily received in a consistent way, software is available at NODC to take the string and convert it. This can then be edited as necessary, but it requires some expertise. However, only about 5 % of the incoming information is lost due to a lack of expertise being available. This is most problematic if the originator is no longer available. Much work is presently under way with plankton data, at the Ocean Climate Laboratory, as funding was obtained to include this type of data on the forthcoming World Ocean Atlas. This could be useful to JGOFS, and there has also been some interaction with GLOBEC.

In terms of other coding systems in use, there is a Dutch system mainly using codes based on an old system from the US NODC; 30,000 species have been coded, although there have been some problems with synonyms. In the UK, there is a system in use at the Plymouth Marine Laboratory, developed some time ago for use with Continuous Plankton Recorder data. A further system is in use at the SOAEFD Marine Laboratory, Aberdeen, but discussions held recently with the US NODC may result in the Marine Laboratory adopting the US NODC system. The other system discussed, which has been used in the Baltic Sea countries and for HELCOM, is the RUBIN code. This was developed partly because the US NODC taxonomic coding system

did not include many Baltic species. This coding system is still in use, but is no longer being maintained (i.e., no new codes are being added). ICES Working Groups have expressed concern about the lack of maintenance of the RUBIN code system.

The possibility has been considered of merging the RUBIN codes into the US NODC Taxonomic Code, together with a translation table. This has not happened yet due to a lack of resources, but it would be a very useful development. Considering the advances in computing during the last few years, the use of names is not the problem it once was. The ACME felt that the best solution would be to use the full Latin name, but that the US NODC should be the authority for the code list. In effect, the US NODC Taxonomic Code Version 8 is an indexing system rather than a coding system and, from the point of view of a scientist, this type of authoritative list is what is required.

Recommendations

ICES ACME agreed to the following recommendations:

1) The exchange of data should continue based on present systems until the situation is clarified. This includes the recommendation to continue to use NODC Taxonomic Code, Version 7.

2) It is premature to recommend use of the ITIS Taxonomic Serial Number System, since it is still under development, but the issue should be revisited when the system is operational.

3) ICES should contact ITIS to suggest an alternative prioritization of the work (see ICES, 1997).

4) The RUBIN species list should be transferred to ITIS with a recommendation that the inclusion of these species be given high priority.

Reference

ICES. 1997. Report of the Benthos Ecology Working Group. Annex 16. ICES CM 1997/L:7.

17.4 Development of Biological Databases

Request

Under item 2 of the 1997 data handling requests from the Oslo and Paris Commissions, ICES has been asked to begin the establishment of a databank for phytobenthos, zoobenthos, and phytoplankton species. In addition, the Helsinki Commission has requested ICES to update and further elaborate the ICES Biological Data Reporting Format (macrozoobenthos, phytobenthos, phytoplankton, zooplankton) to meet the revised HELCOM Baltic Monitoring Programme (BMP) Guidelines and recommendations on quality assurance procedures arising from the ICES/HELCOM Steering Groups.

Source of the information presented

ACME deliberations.

Status/background information

ICES has been requested by OSPAR to prepare the setup of databases to support the biological components (phytobenthos, zoobenthos, phytoplankton) of the monitoring programmes of the Commissions. In addition, there is an ACME and Consultative Committee recommendation for ICES to establish a benthos database (ICES, 1995). Furthermore, HELCOM has requested ICES to prepare an updated Biological Data Reporting Format and associated data entry software for its monitoring programme covering macrozoobenthos, phytobenthos, phytoplankton, and zooplankton.

The ACME noted that work has begun on the preparation/review of reporting formats, and will be followed by the development of data entry software and data screening software. This work will run over two years, starting in 1997, and will include an e-mail conference where present procedures and experience will be identified and reviewed.

The preparations for the databases will also be based on the work of all relevant ICES working groups, including recommendations from the ICES/HELCOM Steering Group on Quality Assurance of Biological Measurements in the Baltic Sea (SGQAB) on the revision of the present ICES biological data reporting formats, and the incorporation of QA procedures.

Reference

ICES. 1995. Report of the Mid-Term Meeting of the ICES Consultative Committee 1995, pp. 99, 101. *In* ICES Annual Report, 1995. ICES, Copenhagen.

ANNEX 1

DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN SEDIMENTS: ANALYTICAL METHODS

1 INTRODUCTION

This Technical Annex provides advice on PAH analysis in total sediment, sieved fractions, and suspended particulate matter. The analysis of polycyclic aromatic hydrocarbons (PAHs) in sediments generally includes extraction with organic solvents, clean-up, and high performance liquid chromatography (HPLC) with ultraviolet or fluorescence detection or gas chromatographic (GC) separation with flame ionization (FID) or mass spectrometric (MS) detection (e.g., Fetzer and Vo-Dinh, 1989; Wise *et al.*, 1995). All steps in the procedure are susceptible to insufficient recovery and/or contamination. Quality control procedures are recommended in order to check the performance of the method. These guidelines are intended to encourage and assist analytical chemists to critically reconsider their methods and to improve their procedures and/or the associated quality control measures, where necessary.

These guidelines are not intended as a complete laboratory manual. If necessary, guidance should be sought from highly specialized research laboratories. Whichever procedure is adopted, each laboratory must demonstrate the validity of each step of its procedure. In addition, the use of a second (and different) method, carried out concurrently to the routine procedure, is recommended for validation. The analyses must be carried out by experienced staff.

2 SAMPLING AND STORAGE

Plastic materials (except polyethylene or poly-tetrafluorethene (PTFE)) must not be used for sampling or storage owing to possible adsorption of the PAHs onto the container material. Samples should be transported in closed containers and a temperature of 25 °C should not be exceeded. If the samples are not analysed within 48 hours after collection, they must be stored at 4 °C (short-term storage). Storage over several months is only possible for frozen (i.e., below –20 °C) and/or dried samples (Law and de Boer, 1995).

As PAHs are sensitive to photo-degradation, exposure to direct sunlight or other strong light must be avoided during storage of the samples as well as during all steps of sample preparation, including extraction and storage of the extracts (Law and Biscaya, 1994). The use of amber glassware is strongly recommended.

3 BLANKS AND CONTAMINATION

The procedural detection limit is determined by the blank value. In order to keep the blank value as low as possible, PAHs or other interfering compounds should be removed from all glassware, solvents, chemicals, adsorption materials, etc., that are used in the analysis. The following procedures should be used:

- Glassware should be thoroughly washed with detergents and rinsed with an organic solvent prior to use. Further cleaning of the glassware, other than calibrated instruments, can be carried out by heating at temperatures > 250 °C.
- All solvents should be checked for impurities by concentrating the amount normally used to 10 % of the normal end volume. This concentrate can then be analysed by HPLC or GC and should not contain significant amounts of PAHs or other interfering compounds.
- All chemicals and adsorption materials should be checked for impurities and purified (e.g., by heating or extraction), if necessary. Soxhlet thimbles should be pre-extracted. Glassfiber thimbles are preferred over paper thimbles. Alternatively, full glass Soxhlet thimbles, with a G1 glass filter at the bottom, can be used. The storage of these supercleaned materials for a long period is not recommended, as laboratory air can contain PAHs that will be adsorbed by these materials. Blank values occurring despite all the above-mentioned precautions may be due to contamination from the air. The most volatile compounds will usually show the highest blanks (Gremm and Frimmel, 1990).

4 PRETREATMENT

Before taking a subsample for analysis, the samples should be sufficiently homogenized. The intake mass is dependent on the expected concentrations. For the marine environment, as a rule of thumb, the mass of sample taken for analysis can be equal to an amount representing 50–100 mg organic carbon.

PAHs can be extracted from wet or dried samples. However, storage, homogenization and extraction are much easier when the samples are dry.

Drying the samples at ambient or elevated temperatures as well as freeze-drying may alter the concentrations, e.g., by contamination or by loss of compounds through evaporation (Law *et al.*, 1994). Possible losses and contamination have to be checked. Contamination can be checked by exposing 1–2 g C18-bonded silica to drying conditions and analysing it as a sample (clean-up can be omitted) (Smedes and de Boer, 1998). Contamination during freeze-drying is reduced by placing a lid, with a

hole about 3 mm in diameter, on the sample container, while evaporation of the water is not hindered.

Chemical drying of samples can be performed by grinding with Na_2SO_4 or $MgSO_4$ until the sample reaches a sandy consistency. It is essential that at least several hours elapse between grinding and extraction to allow for complete dehydration of the sample. Residual water will decrease extraction efficiency.

5 EXTRACTION

Exposure to light must be kept to a minimum during extraction and further handling of the extracts (Law and Biscaya, 1994). Since photo-degradation occurs more rapidly in the absence of a sample matrix, first of all the standard solution used for checking the recovery of the procedure will be affected, allowing a proper detection of the influence of light. The most photo-sensitive PAH is benzo(*a*)pyrene, followed by anthracene.

5.1 Wet Sediments

Wet sediments should be extracted using a stepwise procedure by mixing with organic solvents. Extraction is enhanced by shaking, Ultra Turrax mixing, ball mill tumbling or ultrasonic treatment. Water-miscible solvents, such as acetone, methanol, or acetonitrile, are used in the first step. The extraction efficiency of the first step will be low as there is a considerable amount of water in the liquid phase. For sufficient extraction, at least three subsequent extractions are needed. The contact time with the solvent should be sufficient to complete the desorption of the PAHs out of the sediment pores. Heating by microwave or refluxing will accelerate this process.

When utilizing a Soxhlet, the extraction of wet sediments should be conducted in two steps. First, a polar solvent, such as acetone, is used to extract the water from the sediment, then the flask is replaced and the extraction continued with a less polar solvent or solvent mixture (e.g., acetone/hexane). Thereafter, the extracts must be combined.

For both batch and Soxhlet extraction, water must be added to the combined extracts and the PAHs must be extracted to a non-polar solvent.

5.2 Dry Sediments

Although all the methods mentioned above can also be used for dried sediments, Soxhlet extraction is the most frequently applied technique to extract PAHs from dried sediments. Medium-polar solvents such as dichloromethane or toluene, or mixtures of polar and non-polar solvents can be used. When using dichloromethane, losses of PAHs have occasionally been observed (Baker, 1993). Although toluene is not favoured because of its high boiling point, it should be chosen as solvent when it is expected that sediment samples contain soot particles. For routine marine samples, the use of a mixture of a polar and a non-polar solvent (e.g., acetone/hexane (1/3, v/v)) is recommended.

The extraction can be carried out with a regular or a hot Soxhlet (Smedes and de Boer, 1998). A sufficient number of extraction cycles must be performed (approximately 8 hours for the hot Soxhlet and 12 to 24 hours for the normal Soxhlet). The extraction efficiency has to be checked for different types of sediments by a second extraction step. These extracts should be analysed separately.

Supercritical fluid extraction (SFE) is a relatively new method for the extraction of organic compounds. The optimum conditions may vary for specific sediments (e.g., Dean *et al.*, 1995; Reimer and Suarez, 1995). Accelerated solvent extraction (ASE) or enhanced solvent extraction (ESE) are promising new techniques, applying high temperatures and high pressure (Höfler *et al.*, 1995; Richter *et al.*, 1995).

All the methods described, both for wet and dry samples, are in principle suitable for the extraction of PAHs from sediments. However, Soxhlet extraction is recommended over mixing methods, especially for dry samples. For naphthalene, which can easily be lost in several steps of the sample preparation, headspace or purge and trap analysis might provide a suitable alternative to extraction methods.

6 CLEAN-UP

The crude extract requires a clean-up to remove the many other compounds which are co-extracted (e.g., Wise *et al.*, 1995). Due to chlorophyll-like compounds extracted from the sediment, the raw extract will be coloured and will also contain sulphur and sulphur-containing compounds, oil, and many other natural and anthropogenic compounds. Selection of the appropriate clean-up method depends on the subsequent instrumental method to be used for analysis. Prior to the clean-up, the sample must be concentrated and polar solvents used in the extraction step should be removed. The recommended acetone/hexane mixture will end in hexane when evaporated because of the formation of an azeotrope. Evaporation can be done either using a kuderna danish or rotary evaporator. Especially for the latter, care should be taken to stop the evaporation in time at about 5 ml. For further reducing the volume, a gentle stream of nitrogen should be applied. The extract should never be evaporated to dryness.

For removing more polar interferences from the extract, deactivated aluminium oxide (10 % water), eluted with hexane, as well as silica or modified silica columns, e.g., aminopropylsilane, eluted with toluene or a semipolar solvent mixture such as hexane/acetone (95/5, v/v) or hexane/dichloromethane (98/2, v/v), can be used. Gel

permeation chromatography (GPC) can be used to remove high molecular weight material and sulphur from the extracts.

When using HPLC/fluorescence detection, for the majority of samples polar interferences can be removed from the extract using an aluminium oxide (deactivated with 10 % water) column that is eluted with hexane. If interferences appear to be present in the chromatogram, a clean-up combination of silica and a cyanopropyl phase, eluted with, e.g., hexane/acetone, is suitable. For GC/MS analysis, sulphur should be removed from the extracts, in order to protect the detector. This can be achieved by the addition of copper powder, wire or gauze during or after Soxhlet extraction. Ultrasonic treatment might improve the removal of sulphur. As an alternative to copper, other methods can be used (Smedes and de Boer, 1998).

Analysis by GC/FID or HPLC/UV requires a more comprehensive clean-up. Aliphatic hydrocarbons originating from mineral oil interfere with the flame ionization detection. They can be removed from the extract by fractionation over columns filled with activated aluminium oxide or silica. The first fraction eluting with hexane is rejected. The PAHs elute in a second fraction with a more polar solvent, e.g., diethylether or acetone/hexane. When applying fractionation, the elution pattern has to be checked frequently. This should be carried out in the presence of sample matrix, as that can partially deactivate the clean-up column, resulting in earlier elution of the PAHs than in a standard solution.

Alkylated PAHs are difficult to remove from extracts by column clean-up. When excessive amounts of these compounds are present, they may interfere with HPLC analysis and such samples are better analysed by GC/MS. An alternative could be preparative HPLC fractionation using a normal phase silica, cyanopropyl or aminopropyl column. After clean-up, the eluate or fractions must be concentrated to 1–2 ml.

HPLC and GC require different solvents for injection of the extract. The methods suggested above all result in an extract in which non-polar solvents are dominant. In HPLC, even small amounts of non-polar solvents result in a shift of retention time and broadening of the peaks (Reupert and Brausen, 1994). As for solvent exchange, evaporation to dryness must be avoided; hexane should be removed by the addition of 5 ml acetonitrile for each ml of extract and subsequent evaporation to 1–2 ml. Azeotropic evaporation leaves only acetonitrile. Although this also works with methanol, acetonitrile is preferred because PAHs show a better stability when dissolved in acetonitrile. Azeotropic exchange can also be applied the other way round. In that case, 5 ml hexane must be added for each ml of acetonitrile. For GC methods, iso-octane or toluene are suitable solvents for injection and can already be added, before evaporation to the required volume, as a keeper.

7 CHROMATOGRAPHIC DETERMINATION

The separation of PAHs should be optimized for at least the sixteen EPA PAHs (Keith and Telliard, 1979). Separation should not only be optimized for a standard solution but also for a sample, as samples often contain several non-target PAHs that should be separated from the target compounds, if possible.

7.1 High Performance Liquid Chromatography

For adequate HPLC analysis of PAHs, the equipment should meet some minimum requirements. At a minimum, a binary gradient is necessary to achieve proper separation. Solvents must be degassed in order to allow proper operation of the high pressure pump. Sample injection should be carried out with an autosampler.

7.1.1 Columns

The column specifications are:

- stationary phases: e.g., octadecylsilane (RP-18), or special PAH column material;
- length: 15–25 cm;
- inner diameter: 4.6 mm or less;
- particle size: 5 µm or less.

Columns with diameters smaller than 4.6 mm can be chosen in order to reduce the flow of the eluent and thus save solvents, if the dimensions of the detector cell and the tubings are appropriate. When using a smaller diameter column, the amount injected should also be reduced (e.g., 25–50 µl for a 4.6 mm column, 10 to 20 µl for a 3 mm column).

7.1.2 Elution

At a minimum, a binary gradient is necessary to allow for a proper separation. For elution, e.g., methanol/water or acetonitrile/water can be applied. Acetonitrile allows more rapid flow, but presents a greater health risk than methanol. A typical gradient (1–1.5 ml min^{-1} for a 4.6 mm column) starts at 50 % methanol/water or acetonitrile/water and runs to 100 % methanol or acetonitrile in 40 minutes, where it remains for 20 minutes and then returns to the initial conditions again for about 5 minutes. Prior to the next injection, equilibrium time should be about 5–10 minutes (3–5 times the dead volume).

100 % methanol or acetonitrile may not be sufficient to elute all non-target compounds from the column, resulting in peaks that disturb the baseline in the

subsequent chromatogram. To avoid this, a further elution step using acetone/methanol (1/1) or acetonitrile/acetone (1/1) can be applied. A ternary gradient is then necessary.

In order to obtain reproducible retention times, the equilibrium time after each run should be constant. Therefore, automatic injection is strongly recommended. In addition, a thermostated column compartment (10–30 °C) should be used. Not only retention times but also the resolution between some PAHs can be affected by varying the temperature.

7.1.3 Detection

For the detection of PAHs, the more sensitive and selective fluorescence detector is preferred to a UV detector. The excitation and emission wavelengths should be programmable to allow the detection of PAHs at their optimum wavelength (see Reupert and Brausen, 1994; ISO, 1995). However, when PAHs elute close to each other, wavelength switching cannot be carried out between these peaks and a wavelength pair appropriate for the respective compounds has to be chosen. The use of two detectors in series, or running the analysis twice with different wavelength programmes, can minimize the need for such compromises.

As the fluorescence signals of some PAHs can decrease by up to a factor of ten in the presence of oxygen, the eluents must be degassed thoroughly. This can be done either by continuously passing a gentle stream of helium through the eluents or using a commercially available vacuum degasser. In addition, after degassing the eluents, they should not pass PTFE tubings, as this material is permeable to oxygen and allows oxygen to enter the system again. The use of stainless steel or PEEK (polyetheretherketone) tubing is recommended.

Acenaphthylene is not detectable with fluorescence. A UV- or diode-array detector can be used for detection.

7.2 Gas Chromatography

7.2.1 Columns

Column dimensions for the determination of PAHs should be the following:

- length: minimum 25 m;
- inner diameter: maximum 0.25 mm;

  More resolution can be obtained by increasing the length and reducing the inner diameter to 0.20 mm or less. Below a diameter of 0.15 mm the carrier gas pressure rises to values greater than 500 kPa, which are not compatible with normal GC equipment. Also, the risk of leakages increases.
- film thickness: between 0.2 and 0.4 µm;
- stationary phases: A wide range of non-polar or slightly polar stationary phases can be used for the separation of PAHs, e.g., a 5 % phenyl-substituted methyl polysiloxane phase.

7.2.2 Carrier gas

Preferably helium should be used as the carrier gas for GC/MS and hydrogen for GC/FID. When using columns with very small inner diameters, the use of hydrogen is essential. The linear gas velocity should be optimized. Appropriate settings for 0.25 mm i.d. columns range from 20–40 cm s^{-1} and for 0.15 mm i.d. columns from 30–50 cm s^{-1}.

7.2.3 Injection techniques

An autosampler should be used for injection. The two systems commonly used are splitless and on-column injection. Other techniques such as temperature-programmed or pressure-programmed injection may have additional advantages, but should be thoroughly optimized before use. Due to their high boiling points, for PAHs on-column injection is recommended.

7.2.4 Temperature programming

The temperature program must be optimized for a sufficient separation of the PAH compounds. In addition to a reproducible temperature program, a fixed equilibration time is important for a correct analysis and constant retention times.

7.2.5 Detection

A frequently used detector for PAH analysis is a mass spectrometric detector, used in the Selected Ion Monitoring (SIM) mode. Electron impact ionization (EI) may be used as the ionization method. The selectivity of a mass spectrometric detector is excellent and the chromatographic noise of a standard is similar to that of a sample. However, major drawbacks are the matrix-dependent response and the convex calibration curves that both often occur and make quantification difficult. The use of a flame ionization detector (FID) is also possible. Since the selectivity of the FID is low, bias due to coelution of target or other interfering compounds can easily occur.

7.3 Identification

The individual PAHs are identified by comparing the retention time of the substance in a sample with that of the respective compound in a standard solution analysed under the same conditions. In case of doubt, it is recommended to confirm the results by using a different wavelength for UV-absorption or a different combination of wavelengths for fluorescence detection. Using a GC/MS system, the molecular mass or characteristic

mass fragments are a suitable way to prove the identification of the PAH compound.

7.4 Quantification

A multilevel calibration with at least five concentration levels is recommended. The response of the FID detector is linear. For UV and fluorescence detection, the linear range is also large. The working range should be linear and must be covered by the calibration curve.

Since the mass/spectrometric detector often has no linear response curve, the use of stable, deuterated isotopes is a prerequisite. Furthermore, the response of PAHs in standard solutions is often much lower than in sample extracts. Only a combination of different techniques, e.g., the use of internal standards and standard addition, might give reliable quantitative results.

The calibration curve can be checked by recalculating the standards as if they were samples and comparing these results with the nominal values. Deviations from the nominal values should not exceed 5 %.

When chromatograms are processed using automated integrators, the baseline is not always set correctly, and always needs visual inspection. Because in HPLC analysis the separation of the peaks is often incomplete, the use of peak heights is recommended for quantification. Using GC techniques, either peak heights or peak areas can be used.

Prior to running a series of samples and standards, the GC or HPLC systems should be equilibrated by injecting at least one sample extract, the data of which should be ignored. In addition, standards used for multilevel calibration should be regularly distributed over the sample series so matrix and non-matrix containing injections alternate. A sample series should include:

- a procedural blank;
- a laboratory reference material;
- at least five standards;
- one standard that has been treated similarly to the samples (recovery determination).

The limit of determination should depend on the purpose of the investigation. A limit of 2 ng g^{-1} (dry weight) or better should be attained for single compounds. The method for calculating the limit of determination should reflect QUASIMEME advice (Topping *et al.*, 1992). The limit of determination that can be achieved depends on the blank, the sample matrix, concentrations of interfering compounds, and the mass of sediment taken for analysis.

8 QUALITY ASSURANCE

A number of measures should be taken to ensure a sufficient quality of the analysis. Four main areas can be identified:

- extraction efficiency and clean-up;
- calibrant and calibration;
- system performance;
- long-term stability.

Internal standards should be added to all standards and samples either in a fixed volume or by weight. The internal standards should preferably be non-natural PAHs which are not found in sediment samples and do not coelute with the target PAHs. Several perdeuterated PAHs have proved to be suitable for GC/MS as well as for HPLC analysis. For example, for GC/MS it is recommended to add four internal standards representing different ring-sizes of PAHs.

The following compounds can be used (Wise *et al.*, 1995):

- for HPLC analysis: phenanthrene-d10, fluoranthene-d10, perylene-d12, 6-methyl-chrysene;
- for GC/MS analysis: naphthalene-d8, phenanthrene-d10, chrysene-d12, perylene-d12;
- for GC/FID analysis: 1-butylpyrene, *m*-tetraphenyl.

8.1 Extraction Efficiency and Clean-up

A check on extraction efficiency and clean-up can be performed by analysing a reference material. To determine the recovery rates of the clean-up and concentration steps of each sample series, a standard solution should be put through the entire procedure. Additionally, at least one internal standard should be added to each sample before extraction, to check for recovery during the analytical procedures. If major losses have occurred, then the results obtained should not be reported.

8.2 Calibrants and Calibration

PAH determinations should preferably be carried out using calibration solutions prepared from certified, crystalline PAHs. However, the laboratory should have the appropriate equipment and the expertise to handle these hazardous crystalline substances. Alternatively, certified PAH solutions, preferably from two different suppliers, can be used. Two independent stock solutions should always be prepared simultaneously to allow a

cross check to be made. Calibration solutions should be stored in ampoules in a cool, dark place. Weight loss during storage should be recorded for all standards.

After clean-up and before GC analysis, an additional internal standard is added for volume correction.

8.3 System Performance

The performance of the HPLC or GC system can be monitored by regularly checking the resolution of two closely eluting PAHs. A decrease in resolution indicates deteriorating HPLC or GC conditions. The signal-to-noise ratio yields information on the condition of the mass spectrometric (MS) detector. A dirty MS-source can be recognized by the presence of a higher background signal, together with a reduced signal-to-noise ratio. Additionally, the peak shape can be affected.

8.4 Long-term Stability

One laboratory reference sample should be included in each series of samples. A quality control chart should be recorded for selected PAHs, e.g., fluoranthene (stable results), pyrene (sensitive to quenching), benzo(*a*)pyrene (sensitive to light). If the warning limits are exceeded, the method including calibration solutions should be checked for possible errors. When alarm limits are exceeded, the results should not be reported. A Certified Reference Material should be analysed at least twice a year and each time the procedure is changed. Each laboratory analysing sediments should also participate in interlaboratory studies on the determination of PAHs in sediments on a regular basis.

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ANNEX 2

TOTAL, DISSOLVED, AND PARTICULATE ORGANIC CARBON IN CHEMICAL OCEANOGRAPHY: A LITERATURE REVIEW

1 INTRODUCTION

Scientific interest in organic carbon in the marine environment appears to have increased in recent years. In particular, the controversy about actual levels of dissolved organic carbon (DOC) in the oceans, a result of the later withdrawn data presented by Sugimura and Suzuki (1988), has encouraged healthy discussions on both the analytical methods and the measured amounts of organic carbon in the oceans. After Sugimura and Suzuki presented their high temperature catalytic oxidation (HTCO) method for the determination of DOC in the oceans, and claimed that they found 2–3 times higher levels than using the old analytical methods, researchers around the globe have attempted to confirm or dismiss their results. We now know that the original findings were not correct (Suzuki, 1993), and that the HTCO method produces results very similar to, though probably slightly higher than, earlier results.

2 LITERATURE REVIEW

A literature survey shows that a number of papers on different aspects of organic carbon have been published recently. A closer examination of the articles also shows that we still have a limited knowledge of both the nature of the organic carbon and the actual concentrations of the different fractions found in the oceans. Our knowledge about sources and sinks in the oceans, as well as the availability of organic carbon and nitrogen to biological processes, is also very limited (Allard *et al.*, 1994; Barber, 1968; Benner *et al.*, 1992; Coble *et al.*, 1990; Dhargalkar and Verlencar, 1992; Gearing *et al.*, 1994; Hedges, 1992; Hedges *et al.*, 1992; Henneke and de Lange, 1990; Hulth *et al.*, 1997; Ittekott, 1988; Kieber *et al.*, 1989, 1990; Kristensen and Blackburn, 1987; Meyers-Schulte and Hedges, 1986; Mopper *et al.*, 1991; Mopper and Stahovec, 1986; Pecherzewski, 1980; Saliot *et al.*, 1984; Skoog *et al.*, 1996; Tan and Strain, 1983; Williams and Druffel, 1987; Woodwell *et al.*, 1978; Zepp, 1988).

The main reason by far for measuring organic carbon has been to study the global carbon cycle (Craig *et al.*, 1994; Druffel *et al.*, 1992; Gordon and Cranford, 1985; Hedges, 1992; Meyers-Schulte and Hedges, 1986; Mopper *et al.*, 1991; Mopper and Stahovec, 1986; Woodwell *et al.*, 1978). A portion of the anthropogenic carbon dioxide released into the atmosphere disappears, and the removal mechanism has not been found (Paillard *et al.*, 1993; Sarmiento, 1991; Siegenthaler and Sarmiento, 1993). The pool of organic carbon in the sea is approximately the same size as the pool of atmospheric CO_2. Dissolved CO_2 is transformed to organic material by the photosynthetic plants that live in oceanic surface water. The export of biogenic carbon from the surface layer is responsible for maintaining the vertical gradient of CO_2 over the sea/atmosphere interface and, thus, for a part of the regulation of the atmospheric CO_2 level. The transport of carbon both as particulate organic carbon (POC) and DOC within the oceans can be of significant magnitude (Burdige and Homstead, 1994; Craig *et al.*, 1994; Wassmann, 1990). Some papers also discuss the availability of both the carbon and nitrogen contained in organic material to primary production and other biological processes (Carlsson and Granéli, 1993; Geller, 1986; Hung *et al.*, 1980; Lara *et al.*, 1993; Lindell *et al.*, 1995; Ogura, 1969; Postma and Rommets, 1984; Woodwell *et al.*, 1978).

Attempts have been made to use total organic carbon (TOC) and DOC as water mass tracers for mixing in large basins. TOC was successfully used for studying mixing in the Baltic Sea (Wedborg *et al.*, 1994; Wedborg *et al.*, in prep.). In an investigation in the Weddell Sea, Antarctica, it was shown that humic substances, which make up 10–30 % of the DOC, could be used as a tracer for water mass age, while DOC could not be used for the same purpose (Skoog and Wedborg, 1994). In other investigations, however, DOC has shown a conservative behaviour (Mantoura and Woodward, 1983).

A number of studies investigate complexation between metals and organic compounds. This is an interesting topic for many reasons, the most obvious one being that complexation of metals to other compounds strongly affects their role as micronutrients or toxins (Anderson and Morel, 1982; Haraldsson *et al.*, 1991, 1993). Only a small fraction of the total metals contained in the oceans appears to be directly available to marine organisms. Certain fractions of DOC (notably humic substances), with a strong tendency towards binding metals, are believed to play an important role in the transport of metals in the oceans (Gu *et al.*, 1995; Sholkowitz *et al.*, 1978; Tipping, 1981).

3 CONCLUSIONS

Organic carbon in the oceans is primarily measured because it plays a very important role in the global carbon cycle, with implications, e.g., for global warming through the elevated atmospheric carbon dioxide levels. The usefulness of TOC and DOC for oceanographic purposes is limited and very few successful attempts to use these parameters as tracers for ocean mixing processes have been reported. It is indeed even difficult to find papers describing any kind of correlation between organic carbon and other chemical or biological parameters. The main reasons are the problems of measuring TOC/DOC accurately in sea water and, more

importantly, that they are insufficiently characterized parameters with strongly varying composition and behaviour between different sea areas.

Important aspects of TOC/DOC, apart from the global carbon cycle, are their role in the complexation of metals within the oceans and their potential role as sources of carbon and nitrogen for primary production.

The ACME does not, in principle, recommend that organic carbon should be included in monitoring programmes. Data on organic carbon are only useful if monitoring programmes are targeted to ask specific questions, for which such data would be required.

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ANNEX 3

DISSOLVED OXYGEN IN SEA WATER: DETERMINATION AND QUALITY ASSURANCE

1 INTRODUCTION

Dissolved oxygen (DO), a parameter of primary interest in water quality investigation, is measured in almost every laboratory dealing with environmental studies and monitoring. This paper reviews the context of DO determinations, including aquatic importance, the main procedures, and quality assurance (QA) requirements.

Although QA in analytical chemistry covers all aspects that concur to give confidence in an analytical result (including an adequately equipped laboratory, well-trained analysts, good laboratory practice), this paper focuses only on specific analytical points that are important in the procedure and on good technical practices for producing accurate DO data.

The work on DO carried out by the Chemical Oceanography Subgroup of the ICES Marine Chemistry Working Group (MCWG) has also been taken into account and the conclusions of DO intercomparison exercises have been reviewed.

2 DISSOLVED OXYGEN IN AQUATIC MEDIA

It is postulated that QA is greatly enhanced when analysts understand the implications of the determinations they perform for the study of aquatic media.

Dissolved oxygen is the common term for dissolved 'dioxygen', which means that the corresponding determination will be restricted to the concentration of molecular O_2 exclusively, and no other oxygen form present in any inorganic or organic combination. This molecular form governs all oxic biological processes, i.e., most terrestrial and marine life.

The DO concentration in water results from several physical, chemical, and biological processes:

- exchange at the air-water interface (gain or loss);
- diffusion and mixing within the water body;
- photo-oxidation (loss);
- chemical oxidation (loss);
- respiration of aquatic organisms, including mineralization (loss);
- nitrification (loss);
- photosynthesis (gain).

In cases where only physical processes are involved, the DO concentration is governed by the laws of solubility, i.e., it is a function of atmospheric pressure, water temperature, and salinity. The corresponding equilibrium concentration (solubility) is generally called saturation. It is an essential reference for the interpretation of DO data. As an example, DO saturation under 1 atmosphere and at 20 °C is 6.4 ml l^{-1} in pure water and 5.1 ml l^{-1} in sea water at a salinity of 35. Precise saturation data, tables, and mathematical functions have been established (Carpenter, 1966; Murray and Riley, 1969; Weiss, 1970) and adopted by the international community (Unesco, 1973). However, Weiss (1981) drew attention to an error in the international tables in which the values are lower by 0.10 %. Later, the Joint Panel of Oceanographic Tables and Standards recommended that the oxygen saturation formula of Benson and Krause (1984), which incorporated improved solubility measurements, be adopted and the tables updated (Unesco, 1986). However, no official document has been published yet.

It should be clear that as far as purely physical processes are involved, dissolved oxygen concentrations will always tend to reach saturation. This has several implications. Firstly, when the pressure or temperature, for instance, changes, exchange of oxygen with the atmosphere will take place until a new equilibrium concentration is reached. As physical characteristics generally change slowly or little, they rarely induce significant divergence's from saturation. Secondly, and consequently, large differences from saturation prove that biological activity is taking place (chemical and photo-chemical processes remain of minor importance). Thirdly, purely physical action such as shaking and bubbling (waves, ship propeller mixing, etc.) always modifies DO concentrations towards saturation, regardless of whether the water was previously under- or over-saturated: apparent over-saturation obtained after a physical action must be attributed to micro-bubbles, not to truly dissolved oxygen.

Very low DO concentrations are lethal for higher animals, especially fish. According to Train (1979), a DO concentration of 5 mg l^{-1} (3.5 ml l^{-1}) seems to be the minimum for a typical population of fish in fresh water. Low concentrations are mostly generated by degradation of organic matter (for example, in case of eutrophication) or intense nitrification (mainly in estuaries). When a medium becomes anoxic, only microorganisms can survive and chemical conditions lead to the occurrence of particular processes (some metals can be trapped while others are dissolved). Such conditions can be encountered temporarily in estuaries, or permanently in the bottom water layer of semi-enclosed seas and basins with a sill (e.g., the Baltic Sea or Black Sea).

3 METHODS FOR THE DETERMINATION OF DISSOLVED OXYGEN

Two main methods are generally used for the determination of dissolved oxygen: chemical methods and sensors. Chemical methods are used to treat discrete water samples, leading to volumetric titration as the final step. Sensors are particularly interesting for *in situ* measurements (but some can be used for discrete samples).

3.1 Chemical DO Determination with Particular Reference to the Winkler Method

Chemical determination of DO must be performed on discrete samples and, therefore, it requires an adequate sampling procedure (see below). Very few chemical methods have been developed for DO measurement. For sea water, the so-called 'Winkler method', which is now more than one century old (Winkler, 1888), is commonly used.

In this method, Mn II and hydroxide ions (together with iodide) are added to the sample. Manganese hydroxide precipitates, and then reacts with the oxygen dissolved in the sample. Mn II is thus oxidized into Mn III and Mn IV. This first step must be performed as soon as the sample is taken. The fixing reaction takes some time, but once the oxygen is fixed the sample is stable, and the second step of the Winkler determination can be postponed. After the reaction is complete, acid is added to dissolve the precipitate. Iodide is then oxidized by Mn III/IV into iodine which can be titrated by thiosulfate; 4 moles of thiosulphate correspond to 1 mole of O_2 initially present in the water aliquot.

The Winkler method has been carefully re-examined for its accurate application to sea water (Carritt and Carpenter, 1966; Carpenter, 1965a, 1965b) and it still remains the reference method for calibrating oxygen sensors. Carpenter's (1965a, 1965b) version of the Winkler method has been adopted for use in the Baltic Sea (Carlberg, 1972) and was recently adopted for use in the World Ocean Circulation Experiment (WOCE) programme (Culberson, 1991). Unfortunately, Strickland and Parsons (1972) did not update the last edition of their manual, thereby depriving many users of more accurate DO data.

3.2 DO Sensors and Probes

DO sensors have been developed since the introduction by Clark (1956) of the membrane-covered DO electrode. The use of sensors offers several advantages: simplicity, few interferences from other solutes, rapid and *in situ* measurements, and the possibility of continuous measurements for real-time investigations. The sensors consist of a cathode and an anode immersed in an electrolyte separated by an O_2-permeable membrane from the medium in which DO has to be measured. The sensor exploits the reduction of O_2 at the cathode; the resulting current is measured and expressed in its DO equivalent. In polarographic sensors, a constant voltage is applied between a suitable reference electrode and the noble metal cathode; the electrolyte participates in the overall reaction. In galvanic sensors, the anode is made of a relatively basic metal and the cathode of a noble metal, thus the generated voltage is sufficient for the reduction of O_2; the electrolyte does not participate in the reaction (Lee and Tsao, 1979). Most oceanographic probes seem to be of the polarographic type. One of the main uses of *in situ* probes in oceanography is to obtain continuous vertical profiles.

4 QUALITY ASSURANCE

The following quotation from Carritt and Carpenter (1966) should be kept in mind: "to an uninitiated person, the Winkler procedure is likely to be deceptively simple, ... However, changes in procedure that may appear to be insignificant to the nonchemist may actually produce significant differences in the results". At many stages, such as sampling or subsampling in particular, quality assessment and traceability are difficult to establish, and therefore QA relies almost entirely on the application of good practice rules. Consequently, this section focuses on those technical points that are important for accurate DO measurements. However, it does not intend to replace original publications or handbooks in which detailed descriptions of the protocol are given.

4.1 MCWG Activities Relevant to Dissolved Oxygen Measurements

Problems concerning DO measurements are regularly dealt with in the MCWG. In recent years, the following topics have been examined:

- 1988—determination of DO at low concentrations;
- 1989—problems with the determination of DO;
- 1990—design of an intercomparison exercise for monitoring DO in Baltic waters (Visby intercomparison exercise);
- 1990—high precision measurements of DO;
- 1991—results of the Visby intercomparison exercise (preliminary data);
- 1992—final results of the Visby intercomparison exercise;
- 1993—the WOCE protocol for DO determinations;
- 1993—the WOCE intercomparison exercise for DO determinations.

Information from the corresponding documents or conclusions of the MCWG will be referred to in the following sections.

4.2 Sampling and Handling

This section is relevant to discrete samples and thus mainly to the Winkler determination. The entire sampling/subsampling protocol is reviewed here, since most points are somewhat rigid, and the few test results or comments available on this essential part of the protocol are given. The operations described in this section are normally undertaken onboard a ship ('deck work').

4.2.1 Sampling from a water mass

General sampling rules also apply to taking samples for the determination of dissolved oxygen. Conventional plastic or glass sampling devices are suitable, but metallic bottles should not be used if samples will be trapped in the bottles for long periods, for instance during deep-water sampling (Riley *et al.*, 1975; Worthington, 1982).

Note: The results of the Visby intercomparison exercise, in which subsampling was conducted without delay after sampling, led to the conclusion that there were no significant differences arising from the use of different sampling equipment (ICES, 1991, 1992; HELCOM, 1991).

4.2.2 Sample bottle

Only glass vials with rounded or tapered ground-glass stoppers meet the requirements for subsequent Winkler determination, for several reasons. First, glass—unlike plastic—is both inert towards oxygen and impermeable to it. Second, the ground-glass stopper is the only closing system which permits rejection of the water in the bottle neck in contact with air, without trapping air bubbles (caution: no grease should be present on the ground-glass joint!). Vials of 50–150 ml capacity are generally convenient.

Note: If the whole bottle procedure is used (as is strongly recommended, see below), bottles must be colourless and calibrated; stoppers and vials must be identified since they are not interchangeable.

4.2.3 Filling the sample bottle

As for all gases and highly reactive substances, samples for DO determination should be the first (or one of the first) samples to be drawn from the hydrocast bottle. Because exchange between the sample and air must be avoided, there is no better way to fill the sample bottle than to use a flexible, transparent plastic tube fitted to the hydrocast bottle tap and plunging down to the bottom of the sample bottle. After slow filling (avoiding bubbling and turbulence), sufficient overflow must be ensured: two to three times the content of the bottle is recommended but, obviously, saturated water requires less overflow than water that is not saturated.

Note:

- The tube used to transfer the water from the hydrocast bottle to the sample bottle should be stored in water prior its use for the first time in order to reduce the risk of trapping air bubbles inside the tube (WOCE intercalibration; ICES, 1993).
- In the Visby intercomparison exercise, no significant differences could be attributed to sampling staff (ICES, 1991, 1992; HELCOM, 1991).

At this stage, oxygen can be measured using an oxygen sensor. If an oxygen sensor is not used, reagents must be added.

4.2.4 Adding the reagents

When the bottle is filled to the brim, the reagents are added immediately (without intermediate stoppering), well below the water surface, by means of dispensers or automatic pipettes with a new tip for each pipetting (see Reagents in Section 4.3, below). The stopper is then put into place, care being taken not to trap bubbles, and the sample shaken vigorously to disperse the precipitate. Shaking has to be repeated when the precipitate has partly settled.

Note:

- Classical pipettes do not meet the requirements for QA because: i) dipped successively in samples and in a reagent, they are sources of contamination; ii) they are particularly difficult to handle in rough seas.
- Grasshoff (1962) showed that the reaction time is ~1 minute with continuous shaking.
- The diffusion of oxygen from the air into the sample between the end of subsampling and the adding of reagents does not appear to be too critical: Grasshoff (1962) showed that a water sample only 25 % saturated reached no more than 26 % saturation when left open for 10 minutes.

At this stage, samples are left some time, in order to allow the precipitate to settle to the lower third of the bottle volume; during this time the oxygen is completely fixed. Then determination can proceed, with acidification to dissolve the precipitate, followed by titration.

4.2.5 Storage of the samples

Storage of the fixed samples is possible (up to three days), provided that the temperature of the bottles is

maintained almost constant and the ground-glass surfaces do not become dry, in which case air will contaminate the sample (Grasshoff, 1962).

Note:

- For prolonged storage or if the temperature is likely to vary after the oxygen is fixed, Riley *et al.* (1975) suggested keeping the sample bottles under water.
- An acidified sample (= iodine solution) must not be stored (Grasshoff, 1962; Riley *et al.*, 1975); it should be titrated without delay.

4.3 Winkler Method

This section describes problems in handling the fixed samples. Treatment of the fixed samples can be done onboard ship or at the shore-based laboratory ('bench work'). According to Carpenter (1965a), the sources of error in the Winkler method are the following:

- air oxidation of iodide;
- volatilization of iodine;
- oxygen contributed by the reagents;
- iodate contamination of the iodide solution;
- consumption or production of iodide by reagent contaminants;
- difference between titration end point and the equivalence point.

Consequently, some conditions and critical steps in the procedure must be carefully controlled (Carritt and Carpenter, 1966); these are:

- the acid concentration;
- the iodide concentration;
- the blank determination;
- the transfer of iodine solutions;
- the cleanliness of sample bottles (titration flask).

4.3.1 Reagents

The composition and volume of the reagents optimized by Carpenter (1965b) fulfill the following criteria:

- a final pH of ~ 2 to minimize air oxidation of iodide and < 2.7 for rapid dissolution of the precipitate and reaction of iodide with manganese;
- formation of the triiodide complex (high iodide concentration) to minimize iodine volatilization.

The main reagents are accordingly:

Reagent 1: $MnCl_2 \cdot 4H_2O$ (3 mol l^{-1}),

Reagent 2: NaOH (8 mol l^{-1}) + NaI (4 mol l^{-1}),

Reagent 3: H_2SO_4 (5 mol l^{-1}).

The same volume of the three reagents is added in the sample, i.e., 1 ml for a sample of 130–140 ml.

Note:

- For solubility reasons, sodium iodide must be used, instead of potassium iodide, to reach a concentration of 4 mol l^{-1} of iodide in reagent 2.
- For proper pH control, Carpenter (1965b) stated that errors in the volumes of reagents 2 (alkaline) and 3 (acid) should not exceed 5 %; dispensers must therefore be well calibrated.
- The reagents contain some dissolved oxygen which must be subtracted from the final result. Murray *et al.* (1968) determined the oxygen content of the reagents precisely: a total amount of 0.0017 ml of O_2 for 1 ml of each of the reagents 1 and 2. For samples of ~130 ml, this would correspond to O_2 results in excess by 0.013 ml l^{-1}.

4.3.2 Whole bottle titration

As iodine volatilization is one of the main sources of inaccuracy, transfer of an aliquot of the acidified sample to another vessel for titration must strongly be discouraged. This led to the development of whole bottle titration (Carpenter, 1965b; Carritt and Carpenter, 1966). In this procedure, the whole volume of sample is titrated, preferably in the bottle itself. If necessary, some room can be made in the bottle, for addition of the titrating solution, by removal of a known volume of solution with a pipette.

Note:

- The volume of the sample bottle is determined by weighing: with stopper inserted, bottles are calibrated 'to contain' using distilled water.
- Removal of water to allow titrant addition can be done either before or after acidification. If done before, the solution is anoxic and no volume correction is required, but care should be taken to ensure that the precipitate does not come in contact with air during shaking for dissolution. If done after acidification, iodine solution is removed, therefore the volume titrated corresponds to the bottle volume minus the removed volume.
- Loss by transference is stated to be 1–2 % by Riley *et al.* (1975), and ~ 0.5 % was found by Aminot (1988) when using Carpenter's reagents. Strickland and Parsons' reagents generate a loss of ~ 1.6 % (Aminot, 1988). Variability is also generally increased.

- After titration, bottles must be carefully cleaned with tap and distilled water to remove all traces of Mn ions.

4.3.3 Manual titration (starch indicator)

Once the sample is acidified, the titration of iodine has to be undertaken without delay, for iodine can both volatilize and photo-oxidize. For this reason, most of the thiosulphate is added rapidly while gently stirring until the solution reaches a straw yellow colour. Then the starch indicator is added and the titration is continued slowly until the blue colour completely disappears.

Note:

- Starch should not be added from the beginning of the titration since it forms a strong complex with I_2.
- The starch solution is unstable and should be renewed frequently.

4.3.4 Automated titration

The use of electrochemical or spectrophotometric end-point detection is now widespread, especially when the iodine titration is performed using an automated titrator. The titrator must ensure sufficient time between final additions of the thiosulphate to enable the complete mixing of the solution and thus to avoid over-running the end-point.

4.3.5 Calibration

Routine DO determination cannot be calibrated directly with O_2 standards. An iodide standard is prepared by oxidation of iodide with iodate. A precise volume of the potassium iodate standard (usually 0.01 N) is introduced into a clean sample bottle and distilled water (not sea water) is added to fill the bottle as in the case of a sample. Then, while stirring, the acid reagent 3 is added, followed by the alkaline-iodine reagent 2, then reagent 1. The liberated iodine is immediately titrated with the thiosulphate solution as for the sample.

Note:

- In his protocol, Carpenter (1965b) adds no manganese (reagent 1), and mentions that bottles should be carefully rinsed to avoid the presence of Mn ions. Other authors (Grasshoff, 1983; WOCE manual) specify the addition of reagent 1 also, since it is added for the blank determination. It is clear that Mn must be absent during the hydroxide addition and that all hydroxide must have been neutralized by reagent 3 (including traces on the flask wall) before Mn is added.
- Although several oxidizing compounds have been proposed for calibration, potassium iodate is recommended as it is available commercially with a high purity, it is stable up to 180 °C (no risk of decomposition on drying as for biiodate) and it reacts rapidly with iodide; dichromate is not to be used since it leads to less reliable data (Riley *et al.*, 1975).
- The thiosulphate solution can deteriorate and must be controlled regularly. If control charts are used (see Section 4.5, below), this may be done less frequently.
- The concentration of the thiosulphate solution should be adapted to the burette used, in order that the delivered volume can be measured with sufficient accuracy.

4.3.6 Blanks

Either positive or negative blanks may be found for the reagents, therefore the procedure must be able to measure both kinds. The general procedure consists of titrating iodine liberated by a small, precise volume of iodate standard (typically 1 ml), following the calibration protocol exactly, then adding a second identical amount of iodate and titrating again. The second volume minus the first volume of thiosulphate represents the blank. Remarks concerning calibration are also relevant to the blank determination.

4.3.7 Expected performance

The DO measurements using the Winkler method as outlined by Carpenter (1965a) are expected to have an accuracy of 0.1 %. Standard deviations of 0.004 to 0.015 ml l^{-1} (coefficient of variation (CV) of 0.06 to 0.3 %) have been reported in ten papers, by either manual or automated titration, using starch, photometric, amperometric or potentiometric end-point detection (Graneli and Graneli, 1991).

4.3.8 High precision DO determinations

For the highest accuracy and precision in measurement, a number of minor corrections in volumetry and weighing have to be taken into account (ICES, 1990). This is beyond the scope of this paper. Analysts interested in these corrections can find detailed descriptions in the WOCE manual (Culberson, 1991).

4.3.9 Problems in areas with anoxic waters

This problem has been considered by the MCWG (ICES, 1989). Sometimes, samples contain hydrogen sulphide in very low concentrations. Such samples may yield erroneous positive values for oxygen. In case the presence of sulphide is suspected, several options can be considered:

- collect one sample for sulphide determination and another for oxygen; if the test is positive for sulphide, discard the other sample;

- accept the possibility that low oxygen and sulphide concentrations coexist in mixing zones, but view the oxygen data with reservation;
- add Winkler reagents as usual, allow the precipitate to settle, acidify and, just before titration, replace an aliquot of the supernatant with an exact amount of iodate solution; titrate and calculate the potential sulphide concentration according to Fonselius (1983).

The water may contain other reduced forms of sulphur.

4.4 Sensor Measurements

In the MCWG (ICES, 1988, 1989, 1993), attention has been paid to particular aspects of the use of sensors, specifically calibration of the sensors and problems encountered in areas with anoxic waters.

4.4.1 General maintenance

Accurate measurement using sensors requires, first of all, strict respect of the manufacturer's operating instructions. In particular, the membrane and the electrolyte have to be renewed regularly. Diffusion of oxygen through the membrane is temperature and pressure sensitive, consequently response time is, also. Between stations, the membrane should be kept damp and clean, particularly, free of oil contamination or fouling by, e.g., phytoplankton.

4.4.2 Calibration of sensors

Sensors should preferably be used in conjunction with conductivity-temperature-depth (CTD) devices in order to evaluate oxygen profiles from an oceanographic perspective (stratification, etc.) and simultaneously acquire information for the determination of saturation rate.

For calibrating the sensors, laboratory work does not appear to be successful in yielding useful field calibration parameters (WOCE manual from Millard, 1991 and ICES, 1993). Instead, discrete oxygen samples are collected at different depths for traditional Winkler determination. The results of repeated calibrations in this way will show how many samples are needed per profile and whether each profile should be sampled (ICES, 1989).

Note:

- The WOCE manual suggests that down-profile CTD oxygen data be merged with corresponding up-profile discrete DO samples (at corresponding pressure levels). As DO sensors are flow sensitive, down-profile data are indeed more reliable.
- With minor criticisms, the procedure in the WOCE manual can be recommended for CTD oxygen calibration (ICES, 1993).

4.4.3 Problems in areas with anoxic waters

Specific problems occur in areas with anoxic waters (ICES, 1989). Indeed, hydrogen sulphide can pass through the membrane and modify the response of the sensor to oxygen. Therefore, it has to be established:

1) whether or not the sensor can recover from exposure to sulphide-containing water;
2) what time is required to produce meaningful oxygen data after exposure to sulphide;
3) if the sensor does not recover rapidly, it must be protected (or not immersed at all) in anoxic water.

4.5 Use of Control Charts

Control charts are now widely used for quality control when a reference material is available. In the case of oxygen, a Laboratory Reference Material (LRM) can be readily produced by storing a large volume of fresh water in the laboratory. After a few days, and provided that the temperature of the water remains relatively stable, this water becomes saturated in oxygen. Its concentration can therefore be determined from the Oceanographic Tables or by computation using the recommended formulas, and it constitutes a satisfactory LRM. This LRM should be analysed with each series of samples, and control charts can be produced from percentage saturation data (rather than DO concentration).

4.6 Associated Determinands: Temperature and Salinity

Interpretation of DO data may require calculation of the saturation of oxygen in the studied water. For this, the corresponding temperature and salinity of the water need to be known. It is interesting to determine the precision needed for these two parameters in order not to introduce errors and misinterpret percentages of saturation.

Within the temperature range of 0–35 °C and the salinity range of 0–40, changes (hence errors) in saturation values are as follows:

- for a temperature change of 1 °C, the change in DO saturation varies from 0.05 ml l^{-1} (t = 35, S = 40) to 0.28 ml l^{-1} (t = 0, S = 0);
- for a salinity change of 1, the change in DO saturation varies from 0.02 ml l^{-1} (t = 35, S = 40) to 0.07 ml l^{-1} (t = 0, S = 0).

Assuming that it is possible to be accurate within 0.01–0.03 ml l^{-1} for reasonably routine determinations of DO, it follows that the temperature should be known within ±0.05–0.1 °C and salinity within ±0.1–0.2.

4.7 Learning from Intercomparison Exercises and Conclusions

Many oxygen intercomparison exercises have taken place since the Winkler method was re-examined by Carpenter (1965a). The main conclusion reported by Carritt and Carpenter (1966) from two exercises involving up to eleven institutes was that the accuracy of ±0.05 ml l^{-1} often stated for DO data was actually the precision achievable during certain standardization procedures. Recent exercises have been carried out under the auspices of the WOCE Hydrographic Programme (onboard the R/V Akademik Vernadsky) and HELCOM (in Visby). Five 'groups' (= laboratories) were involved in the WOCE exercise; four of them produced oxygen values in the range of 3–5 ml l^{-1} which were consistent within ±1 %, although WOCE requirements were ±0.5 %. Intragroup precision was generally in the range 0.1–0.4 %. As for the HELCOM exercise, although each laboratory produced consistent data, interlaboratory differences were significant (up to 7 %).

Intercomparison exercises show that biases are generally of the systematic kind and that calibration errors seem to be the cause of most of them.

In addition to a strict application of the procedure, analysts should replicate samples at regular intervals to assess their own repeatability. Good repeatability on samples, that is in agreement with what can be expected from a correct protocol, is the first point that must be achieved. Then, as accuracy strongly depends on correct calibration, high repeatability in titrating iodate standards must also be achieved, i.e., several standards must be titrated with each series of samples. Poor standard repeatability denotes some failure in the calibration process and its cause must be determined (e.g., it may be due to difficulty by the analyst to correctly handle the reverse addition of reagents 2 and 1, in which case reagent 1 (Mn solution) should not be added).

5 DISSOLVED OXYGEN UNITS

DO is usually expressed in the unit 'milligram per litre' in fresh water, while oceanographers are accustomed to use 'millilitre per litre', at standard pressure and temperature conditions (1 atmosphere, 0 °C). International tables use the SI unit 'cubic centimetre per cubic decimetre'. Noting that 'litre' is just another name for 'cubic decimetre' (1 l = 1 dm^3, exactly), 1 ml l^{-1} = 1 cm^3 dm^{-3}. Conversion of units may lead to erroneous data, therefore conversion factors are listed below:

concentration in	when multiplied by	is converted into
millilitre per litre	1.429	milligram per litre
milligram per litre	0.700	millilitre per litre
millilitre per litre	0.0893	millimole O per litre
millimole O per litre	11.20	millilitre per litre

The conversion of the amount of DO (mass, volume or mole) per litre into the amount per mass (kilogram) of sea water requires determining seawater density from temperature and salinity measurements (and possibly pressure for deep-sea *in situ* values) using international tables.

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ANNEX 4

REVIEW NOTE ON IRGAROL 1051 IN THE MARINE ENVIRONMENT

1 INTRODUCTION

The ban on the use of tributyltin (TBT)-based paints on boats of < 25 m, originally implemented in France and the UK in 1982 and 1987, respectively, was extended throughout the European Union (EU) in 1990 (Evers *et al.*, 1995). These paints were not banned from use on larger vessels, however, as field studies undertaken in 1986–1987 had shown that the amount of TBT released from these vessels was considerably less than from yachts (Waldock *et al.*, 1988). More recently, studies undertaken around a traffic separation scheme in the North Sea have demonstrated increased imposex (a phenomenon caused by exposure to TBT) in whelks (*Buccinum undatum*) (Ten Hallers-Tjabbes *et al.*, 1994) and the occurrence of imposex in *Nucella lapillus* due to large ships visiting the oil terminals in Sullom Voe (Shetlands) and Scapa Flow (Orkneys) (Davies and Bailey, 1991). Such indications of impact stemming from the use of TBT-based antifouling paints on large vessels will reinforce calls for stricter controls presently being pursued internationally through the International Maritime Organization (Evers *et al.*, 1995). Following the controls on TBT-based antifouling paints, there has been a move back to paints in which the active ingredients are compounds based on copper or zinc, but because these paints are generally not as effective at preventing fouling as those based on TBT, new alternatives are now being investigated and marketed. One group of compounds gaining acceptance contains an added herbicide, Irgarol 1051. If current restrictions on the use of TBT-based antifouling paints are extended to larger vessels, as seems possible, then the use of these alternatives is likely to increase and it is timely, therefore, to review what is known about the environmental distribution and effects of Irgarol 1051.

2 MANUFACTURE AND USE

The herbicide Irgarol 1051 (2-methylthio-4-*tert*-butylamino-cyclopropylamino-*s*-triazine, CAS Registry No. 28159-98-0) belongs to the same family of compounds as atrazine and simazine. It is manufactured by Ciba-Geigy Ltd. in Switzerland and in the USA. The scale of production and use is held as 'Commercial in Confidence' by the manufacturer and hence is not available to the public. Many, though not all, countries around the world have given approval for the use of formulated products containing Irgarol. Apart from a minor application in the preparation of paints used, for example, on concrete roofing tiles (Steen *et al.*, in prep.), the major application of Irgarol is in antifouling paints, to which it is added in order to prevent the formation of algal slimes. In the UK, approximately 100 antifouling products containing Irgarol are registered with the Health and Safety Executive. In these products, Irgarol is formulated with copper, copper and zinc oxides and thiocyanates, and zinc pyrithione as biocides. Other organic constituents include the fungicides dichlofluanid, thiram, and zineb; dichlorophenyl dimethylurea, 2,3,5,6-tetrachloro-4-(methylsulphonyl)pyridine, and 2-(thiocyano-methylthio)benzothiazole. Two of the formulated products also contain tributyltin as the methacrylate or oxide. Authorization for products containing Irgarol has not been granted in Belgium or Ireland, and no information could be found regarding the use of Irgarol in Germany. In Denmark, about 9 tonnes of Irgarol was sold for use in antifouling paints and approximately 48 tonnes was exported in 1995. In Norway, the annual use of Irgarol is approximately 2 tonnes in 49 products, 45 of which are antifouling paints, and most of which are exported. The structure of Irgarol 1051 is shown in Figure A4.1, below.

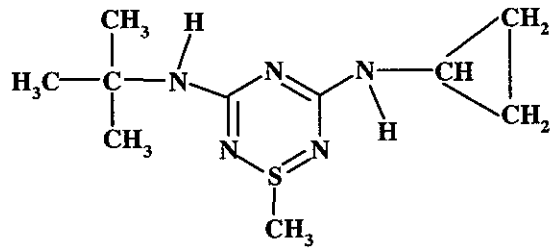

Figure A4.1. Structure of Irgarol 1051.

Irgarol 1051 has a solubility in water of 7 mg l^{-1}, and a log K_{ow} of 3.6.

3 ANALYTICAL METHODOLOGY

Four recently published studies quantify Irgarol 1051 in marine environmental samples. The first study presents data for water samples from the Côte d'Azur on the Mediterranean coast of France (Readman *et al.*, 1993b); the second study presents data for water and sediment samples from coastal waters and estuaries of southern England (Gough *et al.*, 1994); the third study presents data for water samples from the Humber estuary and adjacent coastal waters (Zhou *et al.*, 1996); and the fourth study presents data for water samples around a marina on the west coast of Sweden (Dahl and Blanck, 1996). The report of a fifth study, which utilizes a novel tandem mass spectrometric method, measured concentrations of Irgarol 1051 in the estuary of the Scheldt River (The Netherlands). Data suggest a riverine source of the compound, as concentrations declined in a linear fashion from 8–10 ng l^{-1} at low

salinity (1–4) to about 2 ng l^{-1} at the estuary mouth (salinity 25–30) (Steen *et al.*, in prep.).

3.1 Water Sampling and Extraction

Water samples (1 litre) were taken directly into pre-cleaned glass bottles and kept cool prior to extraction. In one case, they were filtered through a GF/F filter (study 3). Internal standards were added to samples (chlorthion in study 1; ametryn, an *s*-triazine herbicide, in study 2; atrazine-d_5 in study 3; and terbutryn and ethion in study 4), which were then extracted either by solid-phase extraction (C_{18}-silica cartridge eluted with ethyl acetate) (studies 1 and 3), or by direct liquid-liquid extraction with dichloromethane (studies 2 and 4). Analytical quality control (AQC) procedures were applied in studies 1, 2, and 3; the limits of detection given were 5 and 2 ng l^{-1} in studies 1 and 2, respectively, and 1 ng l^{-1} in study 3. The recoveries reported were 85% ± 15% for study 1, more than 80 % for study 2, and 91 % ± 5 % for study 3 (in this case only given for atrazine). In study 4, the recovery of Irgarol from field samples was not given.

3.2 Sediment Sampling and Extraction

Sediment samples were taken at low tide with a pre-cleaned stainless steel spatula and stored at −15 °C in pre-cleaned glass vials. Aliquots were dried at 60 °C for 24 hours, then extracted with dichloromethane by ultrasonic agitation. Ametryn was added before extraction as an internal standard. The limit of detection was 20 ng g^{-1}, with a recovery of 95 % ± 3 %.

3.3 Instrumental Analysis

In all studies, capillary gas chromatography was applied to sample extracts without prior clean-up. In studies 1 and 2, two columns of differing polarity and selectivity (e.g., DB-5 and DB-1701) were used in conjunction with nitrogen selective detection, and in selected samples the presence of Irgarol was confirmed by gas chromatography/mass spectrometry (GC/MS) analysis. In studies 3 and 4, analyses were conducted on a single column with detection by GC/MS in the multiple ion monitoring mode. Full-scan GC/MS confirmation was performed on selected samples in study 3.

4 ENVIRONMENTAL CONCENTRATIONS OF IRGAROL

A summary of reported concentrations of Irgarol 1051 in water (152 samples) is given in Table A4.1. The ranges of concentrations reported for Irgarol in water were < 5 to 1700 ng l^{-1} (study 1), < 2 to 500 ng l^{-1} (study 2), and not detected (nd) to 400 ng l^{-1} (study 4). The highest concentrations were observed in marina areas in which pleasure craft were moored. Port areas showed a lower concentration range, < 5 to 280 ng l^{-1} (study 1) and 9 to 14 ng l^{-1} (study 2), and coastal areas yielded relatively low concentrations (studies 1, 2, and 3). Irgarol was not detected in upstream riverine samples from the Rivers Itchen and Test (study 2). Irgarol would be expected to show a higher affinity for particulate material than other triazines such as atrazine and simazine, and three estuarine sediment samples from the River Hamble, which is a major yachting centre, yielded concentrations of 12 to 132 ng g^{-1} dry weight; this behaviour may be critical when evaluating the compound's fate in the marine environment (Gough *et al.*, 1994). Concentrations of Irgarol in filtered water from the Humber estuary and adjacent coastal waters were in the range < 1 to 39 ng l^{-1}: the concentrations typically peaked in April, then decreased in June before increasing again in September (Irgarol was not determined in the first series of samples taken in January 1995) (Zhou *et al.*, 1996).

Table A4.1. Concentrations of Irgarol 1051 in coastal and estuarine waters.

Location	Time Period	Marinas ng l^{-1}	Ports ng l^{-1}	Estuarines ng l^{-1}	Coastal areas ng l^{-1}	Reference
Côte d'Azur, France	June 1992	110–1700	< 5–280	not sampled	not detected	Readman *et al.*, 1993b
Western Sweden	June 1993–September 1994	nd to 400	not determined	not determined	not determined	Dahl and Blanck, 1996
Southern England	July–September 1993	52–500	9–14	4–18	< 2–11	Gough *et al.*, 1994
Eastern England	April 1995	682	not sampled	1–39	not determined	Zhou *et al.*, 1996
Eastern England	June 1995	536	not sampled	< 1–10	not determined	Zhou *et al.*, 1996
Eastern England	September 1995	169	not sampled	4–10	not determined	Zhou *et al.*, 1996
Plymouth, Southwestern England	July and August 1995	28–127	not sampled	1–24	< 1	T. Fileman (pers. comm.)

nd = not detected

5 SIGNIFICANCE OF IRGAROL 1051 CONCENTRATIONS

While Irgarol 1051 is the only *s*-triazine herbicide which is used directly in marine systems, others (such as atrazine and simazine) are used far more abundantly in agriculture and subsequently transported to estuarine and coastal locations (Readman *et al.*, 1993a, 1993b). In the UK, the Advisory Committee on Pesticides is currently reviewing the use of Irgarol 1051 in non-agricultural pesticides. An Environmental Quality Standard (EQS) for both fresh and saline waters has previously been set in the UK, for atrazine and simazine combined, with a maximum allowable concentration of $10\,\mu g\,l^{-1}$ and an annual average value of $2\,\mu g\,l^{-1}$ (ENDS, 1992). *Concentrations in estuarine and coastal waters are* generally lower than this (Law *et al.*, 1994). As *s*-triazines share a common toxic action in photosynthesizing organisms, it is likely that their effects on algae will be additive; thus, these EQS values could be applied to the sum of such herbicides present (Hedgecott, 1990). The highest values reported in environmental studies to date are below these critical values, but data are currently very limited. A recent microcosm study has indicated long-term effects on periphyton communities at Irgarol concentrations of 63–250 $ng\,l^{-1}$, which is within the range of reported environmental concentrations (Dahl and Blanck, 1996). Irgarol 1051 has been shown to be toxic to non-target marine algae and sufficiently persistent and mobile to reach toxic concentrations in certain areas of the marine environment; Dahl and Blanck concluded that Irgarol 1051 is likely to damage microalgal communities in contaminated coastal waters.

As the use of Irgarol may continue to increase in future years, some caution is clearly needed. The algal toxicities of atrazine, simazine, and Irgarol 1051 seem to be broadly similar, and marine animals appear much less sensitive than algae or plants (Hedgecott, 1990). Irgarol bioaccumulates only slightly in fish, and depurates rapidly when the fish are transferred to clean water. Further information is needed on concentrations of Irgarol in coastal and estuarine waters, particularly those which are poorly flushed, before the environmental significance of its use in antifouling paints can be fully assessed, and the need for further controls evaluated.

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ANNEX 5

TOXAPHENE IN THE MARINE ENVIRONMENT

SUMMARY

This paper gives an overview of recently available information on toxaphene in the marine environment. The toxicology of toxaphene and the analytical methods which are currently in use for the determination of toxaphene are described.

The use of gas chromatography with electron capture detection or gas chromatography/mass spectrometry with negative chemical ionization is recommended for the determination of total toxaphene, and the use of multidimensional gas chromatography techniques is recommended for the determination of congener-specific chlorinated bornanes (CHBs).

An alternative approach may be sought in the development and use of bioassays, such as the gamma-aminobutyric acid assay, which may provide a rapid measurement of toxaphene based on a specific effect.

Toxicological knowledge about toxaphene is still limited and more evidence is required on its carcinogenic potential. Information is also needed on toxic effects of environmentally modified toxaphene mixtures and of individual CHBs.

With regard to underpinning regulations and possible tolerance levels for toxaphene in relation to fish consumption and consumer safety, and to obtain a more complete overview of the distribution of toxaphene in the marine environment, it is recommended to investigate concentrations of total toxaphene and specific chlorobornanes (CHBs), in particular the two most persistent compounds, CHB26 and CHB50, in fish and shellfish samples. The inclusion of these congeners in marine monitoring programmes is suggested.

1 INTRODUCTION

The insecticide toxaphene is a complex mixture primarily consisting of chlorinated bornanes (CHBs) with an average elemental composition of $C_{10}H_{10}Cl_8$ (Casida *et al.*, 1974; Cairns *et al.*, 1981; Saleh, 1991). Prior to its ban in 1982 by the United States Environmental Protection Agency (Saleh, 1991), toxaphene was the most extensively used pesticide in the USA and many other parts of the world. One of the main applications has been its use in cotton growing (Saleh, 1991). It has also been used as a piscicide, to eradicate undesired fish stocks from various lakes in Canada (Saleh, 1991). The global production of toxaphene is estimated to be 1.3 million tonnes (Voldner and Li, 1993), which is higher than that of polychlorinated biphenyls (PCBs). Considerable amounts may still be placed in storage, although exact data are difficult to obtain (Voldner and Li, 1995). Toxaphene is still produced and used in several countries throughout the world (Saleh, 1991). In western Europe, toxaphene has been used only to a limited extent (Voldner and Li, 1993). Toxaphene has been detected as a contaminant in various environmental compartments and has a widespread distribution (Ballschmiter and Zell, 1980; de Boer and Wester, 1993; Hargrave *et al.*, 1993; Wideqvist *et al.*, 1993; Muir and de Boer, 1995). Due to aerial transport, toxaphene concentrations were detected even in very remote areas (Zell and Ballschmiter, 1980; Bidleman *et al.*, 1995). Condensation at low temperatures is supposed to result in elevated concentrations in polar regions (Wania and Mackay, 1995). It has been suggested that toxaphene concentrations in North Sea fish are also due to this cold condensation process taking place after aerial transport of toxaphene from more southern and western regions in Central and North America (de Boer and Wester, 1993).

Different chlorine substitutions can theoretically lead to 32,768 possible congeners (Vetter, 1993), of which a number also show chiral activity (Kallenborn *et al.*, 1994). However, this large number of congeners will not be found in either the technical mixture or in environmental samples. Technical toxaphene, also known as strobane, phenatox, or toxin 63, mainly consists of C_{17} and C_{18} congeners and therefore cannot contain more than 6,840 congeners. However, a number of these CHBs is unlikely to be present because of unfavourable substitution positions on ring and bridge carbon atoms (Hainzl *et al.*, 1994). Jansson and Wideqvist (1983) have separated 670 different CHBs from a technical toxaphene mixture. In environmental samples, the total number of CHBs will be smaller due to degradation in the environment (weathering effects) and biotransformation.

Toxaphene is highly mutagenic (Hooper *et al.*, 1979). Some studies have indicated that toxaphene is also potentially carcinogenic (Innes *et al.*, 1969; Reuber, 1979).

This paper gives an overview of the existing knowledge on the analysis of toxaphene, toxaphene concentrations in the marine environment, and the toxicology of toxaphene.

2 ANALYSIS

Toxaphene is a mixture of chlorinated bornanes and bornenes with a relatively high degree of complexity. The production process of the Hercules company in the USA, one of the main producers of toxaphene (Saleh, 1991), started with extraction of crude α-pinene from pine stumps, using methyl isobutylketone, heat and pressure. Isomerization of the α-pinene produced

camphene, bornylene, and α-terpineol. The camphene was subsequently chlorinated to produce toxaphene. Since chlorination of camphene can take place to varying levels and on various sites, at least 670 congeners exist (Jansson and Wideqvist, 1983; Saleh, 1991; Glassmeyer *et al.*, 1996).

The degree of complexity of toxaphene is about fourfold higher than that of polychlorinated biphenyls, of which 209 congeners are theoretically possible and approximately 150 occur in the technical mixtures (Mullin *et al.*, 1984). Although the sensitivity to weathering effects and metabolism is higher for toxaphene than for PCBs, also in biota the number of CHBs present is higher than that of PCBs in similar samples. In average fish samples, the number of PCB congeners present will be between 50 and 100 (de Boer and Dao, 1989), whereas 100–200 toxaphene congeners may be expected (de Boer *et al.*, 1996b). It is generally agreed that it is not possible to separate all PCB congeners present in biota samples by single-column high resolution gas chromatography (GC) with electron capture detection (ECD) (Duinker *et al.*, 1988; Larsen and Bøwadt, 1993; de Boer *et al.*, 1996c). Consequently, given the higher degree of complexity of the toxaphene patterns in biota, it is expected that a separation of some CHBs by single-column GC/ECD may be possible, but that the number of easily quantifiable CHBs will be considerably smaller than for PCBs. Because of a more efficient biotransformation, the chances for CHB separation are better in marine mammals, in which they may even be comparable to those of PCBs. However, because strong differences in biotransformation exist between different species of marine mammals, the chance for a reliable separation will be species-dependent (Boon *et al.*, 1996).

In conclusion, it will be very difficult to carry out a reliable congener-specific CHB determination by single-column GC/ECD. However, there are two other methods for determining toxaphene: (i) determination of the total toxaphene concentration without attention to individual CHBs, and (ii) congener-specific CHB determination using more advanced analytical techniques.

2.1 Determination of Total Toxaphene

Due to the analytical difficulties of a congener-specific approach and the lack of analytical standards for individual CHBs, most determinations of toxaphene carried out to date have been based on the measurement of total toxaphene. Obviously, there are a number of drawbacks associated with this approach. In the first place, due to weathering effects and biotransformation, the toxaphene patterns in biological samples are considerably different from that in the technical mixture. This difference causes an error in the final result because, normally, the total area of the peaks in the chromatograms of the technical mixture and of the sample are measured and compared to each other, assuming equal response factors for all CHBs. The latter will certainly not be true. The error is not known, but is expected to be considerable (Wester *et al.*, 1996). Such errors will occur with both electron capture and mass spectrometric (MS) detection. More sophisticated approaches, in which groups of isomers were measured (Swackhamer *et al.*, 1987), did not result in more accurate data. Additionally, with this approach it is not possible to obtain any information on the concentrations and behaviour of more toxic or more persistent congeners.

The extraction and clean-up procedures for total toxaphene determination are fairly similar to those used for PCB determination (de Boer and de Geus, 1995). Most toxaphene compounds normally elute in the second fraction after elution over silica gel columns, together with most organochlorine pesticides and chlordanes.

Two rounds of an international interlaboratory study on the determination of total toxaphene in a fish oil were recently carried out (Andrews *et al.*, 1993; Andrews *et al.*, 1995). The participants were allowed to use their own clean-up methods and detection systems. The mean, standard deviation, and range of results in a sample of cod liver oil in the first round were 3.99 ± 1.98 mg kg^{-1}, 0.79–6.8 mg kg^{-1} (n = 17), respectively. In the second round, also for cod liver oil, the results did not improve much and the mean, standard deviation, and range were 4.28 ± 2.01, 1.67–9.1 mg kg^{-1} (n = 13), respectively (Andrews, 1996). The results obtained by GC/MS using negative chemical ionization (NCI) were slightly better than those using GC/ECD. This is probably partly due to the better sensitivity of GC/NCI-MS for toxaphene. Several participants had difficulties with recoveries in the clean-up procedure, mainly due to the use of florisil or silica gel columns, which caused losses due to adsorption of specific CHBs. The broad range of results shows that most laboratories still have serious difficulties in analysing toxaphene.

In conclusion, the determination of total toxaphene may be used to obtain a rough estimation of the toxaphene concentration in biological samples. The uncertainty in this determination will, in principle, be larger than that in the determination of individual compounds, particularly in samples from biota with a high biotransformation rate for toxaphene, such as several marine mammals (Boon *et al.*, 1996), and in human milk. In case a total toxaphene determination is carried out, the use of GC/NCI-MS is recommended owing to its higher sensitivity and better accuracy. However, improvement in the comparability of laboratory results is needed.

2.2 Congener-Specific Determination of Toxaphene

For a reliable congener-specific analysis of CHBs, a higher resolution is required than can be obtained with single-column GC/ECD. This can be obtained by refining the detection system, for example, by applying high

resolution mass spectrometry (HRMS). The resolution remains similar to that of GC/ECD, but separation of co-eluting congeners is obtained by separating mass fragments with small mass differences. HRMS can be applied with two ionization techniques, electron impact (EI) and NCI. HREI-MS offers a higher selectivity due to a broad fragmentation pattern, but is not very sensitive (approximately 10- to 100-fold less sensitive than ECD) and is, therefore, not suitable for a CHB determination in most fish samples (Onuska and Terry, 1989; Andrews, 1996). HRNCI-MS combines selectivity with a high sensitivity (approximately ten-fold better than ECD) (Barrie *et al.*, 1993; Stern *et al.*, 1993). The selectivity is not as good as that of HREI-MS due to less fragmentation. Neither HRMS technique always offers a solution for the determination of co-eluting CHB-homologues (isomers), although HREI-MS may offer better possibilities in this respect than HRNCI-MS. Another MS technique which has been applied for a congener-specific CHB analysis is EIMS/MS. Buser and Müller (1994) used this technique to characterize a CHB in whale blubber, heptachlorobornanes in penguin and harbour seal extracts. The application of NCIMS/MS for CHB analysis is presently not known.

More separation, needed for a reliable congener-specific CHB analysis, can also be obtained by a more advanced GC technique such as multidimensional gas chromatography (MDGC). Two capillary GC columns, preferably installed in two independently controllable ovens, are used in this technique. Heart-cuts of target CHBs can be transferred by pressure switching from the first to the second column. By selecting a column combination with different characteristics, the resolution can be greatly enhanced. Separation of all PCB congeners was easily possible with MDGC/ECD (de Boer and Dao, 1991; de Boer *et al.*, 1995). Promising results have also been obtained for toxaphene (de Boer and de Geus, 1995; de Boer *et al.*, 1996a, 1996b). A congener-specific CHB determination was possible in various biota samples. Only in the very complex technical toxaphene mixture was an overlap of peaks still observed in the chromatograms of the heart-cuts. A combination of MDGC with ECD offers, in principle, sufficient sensitivity and selectivity for a reliable congener-specific CHB determination. A combination of MDGC with NCI-MS would be ideal because of the high sensitivity and the extremely high selectivity. However, such a combination has not yet been applied for the analysis of CHBs.

One drawback of MDGC/ECD may be a relatively long analysis time and, consequently, less sample throughput compared to single-column GC/ECD. The technique is sufficiently robust for the use of autosamplers, but their use in environmental applications has not yet been tried. The heart-cut technique offers a reliable determination of one or two specific CHBs per GC run. If heart-cuts of more CHBs would be combined per GC run, there is an increasing risk of co-elution because peaks in the heart-cuts would start to overlap again on the second column. A repeated run to enable the determination of, for example, four or maybe five CHBs solves this problem, but is obviously time consuming. It is very likely that a new technique called comprehensive multidimensional gas chromatography (CMDGC) will solve these problems (Phillips and Xu, 1995; de Boer *et al.*, 1996a; de Geus *et al.*, 1996). With CMDGC, all peaks of the first column are transferred to the second column without causing peak overlap. Currently, this technique can only be combined with flame ionization detection (FID), but combinations with other detectors are being studied. In addition to a very high selectivity, a high sensitivity is also expected because peaks can be narrowed considerably by using an advanced trapping technique.

Despite the problems with reliable congener-specific CHB analysis by single-column GC, such a method has been developed in Germany and is now used by several German laboratories (Alder *et al.*, 1995a, 1995b, 1997). The method, in which the sum of congeners CHB26, CHB50, and CHB62 is determined, consists of a Bligh and Dyer fat extraction and a clean-up with Bio beads SX-3, 2.5 x 40 cm, 200–400 mesh, cyclohexane/ethylacetate (1:1, v/v), 5 ml per minute. The CHBs eluted together with the PCBs, chlordanes, and other chlorinated pesticides between 95 and 150 ml. After removal of the solvent, the sample was dissolved in iso-octane and eluted over a 1 g silica gel column, with 1 cm sodium sulphate on top. The PCBs and *p,p'*-DDE were fractionated by elution with 8 ml hexane. This fraction also contained CHB26. The other CHBs, chlordanes, and organochlorine pesticides were collected in the second fraction, consisting of 8 ml hexane/toluene (65:35, v/v). The presence of CHB26 in the first fraction was also reported by de Boer *et al.* (1996a, 1997). Krock *et al.* (1997) have developed a fractionation procedure in which CHB26 was collected together with the other CHBs, but the separation was still not complete and the solvent volumes needed were relatively large. An interlaboratory study among German laboratories resulted in coefficients of variation between 10% and 50 % (Alder *et al.*, 1995a). Because of the risk of co-elution, use of this method may lead to positively biased results and, consequently, to a greater chance of exceeding tolerance levels. In the second round of the international interlaboratory study on toxaphene organized by Andrews (1996), participants were asked to determine four toxaphene congeners (CHB26, CHB32, CHB50, and CHB62) in cod liver oil. All results showed a broad range, with the following means and standard deviations: CHB26: 0.30±0.28 mg kg^{-1} (n = 13), CHB32: 0.03±0.04 mg kg^{-1} (n = 10), CHB50: 0.43±0.36 mg kg^{-1} (n = 13) and CHB62: 0. 22±0.15 mg kg^{-1} (n = 9).

Analytical standards of the most relevant CHBs are now available. Certified reference materials are not yet available for either total toxaphene or for CHBs.

Summarizing, it can be concluded that a congener-specific determination of CHBs has now become a

realistic possibility. The two most promising techniques are GC/HRNCI-MS and MDGC/ECD. The latter technique offers the best selectivity combined with a good sensitivity and, therefore, results in the most reliable congener-specific method. MDGC/ECD is considerably less expensive than HRMS techniques. Other promising techniques may be developed in the future, such as GC/NCI-MS/MS, MDGC/MS, and CMDGC/ECD.

3 DISTRIBUTION OF TOXAPHENE IN THE MARINE ENVIRONMENT

The octanol-water partition coefficient (log K_{ow}) of toxaphene is estimated to be 6.44 (Hooper *et al.*, 1979), which is somewhat lower than that of technical PCB mixtures, but higher than those of *p,p'*-DDT and its metabolites. This means that the bioconcentration of toxaphene is expected to be relatively high. Indeed, high levels of toxaphene have been found in aquatic organisms from all over the world. High toxaphene levels, up to 28 mg kg^{-1}, have been found in fish from the St. Lawrence River (Canada) and in Canadian cod liver oil (Musial and Uthe, 1983; Müller *et al.*, 1988). Baltic fish contained toxaphene concentrations up to 6 mg kg^{-1} lipid weight (Andersson *et al.*, 1988). Toxaphene concentrations in North Sea fish are at least ten-fold lower than in fish from Arctic and Canadian waters and vary from 1 to 600 µg kg^{-1} wet weight (Janssen and van Leeuwen, 1993). Recently, an extensive study on toxaphene levels in fish from the North Sea, the Baltic Sea, the English Channel, the Bay of Biscay and from waters south of Ireland, west of Norway, around Iceland, and east of Canada was carried out by Alder *et al.* (1997). In this study, only the sum of CHB26, CHB50, and CHB62 was reported, as determined by single-column GC/ECD. Confirmation with MS was carried out in some samples, in addition. A significant relationship between the toxaphene level and the fishing ground could not be established. Concentrations of 1–34 µg kg^{-1} wet weight were found in herring samples for the sum of the three CHBs. Concentrations of these compounds in halibut, redfish, sardine, and mackerel, and in farmed fish, such as salmon, trout, and eel, were comparable to those in herring, while concentrations in lean fishes such as plaice were lower, between not detected and 1 µg kg^{-1}. The daily intake of the total toxaphene calculated from the results of this study, based on a daily consumption of 20 g fish per person, was estimated at between 2.8 and 5.6 ng kg^{-1} body weight.

Very high toxaphene concentrations have been found in marine mammals, such as 23 mg kg^{-1} in St. Lawrence beluga whales (Muir *et al.*, 1990) and 19 mg kg^{-1} in dolphin blubber from the central North Sea (de Boer and Wester, 1993). Toxaphene has also been determined in human milk from Sweden, Finland, and the Netherlands (0.05–0.7 mg kg^{-1} lipid weight) (Vaz and Blomkvist, 1985; Pyysalo and Antervo, 1985; de Boer and Wester, 1993), and from Nicaragua (up to 68 mg kg^{-1} lipid weight) (Müller *et al.*, 1988; de Boer and Wester, 1993). Fish consumption is anticipated to be the main source of exposure for humans (Janssen and van Leeuwen, 1993).

4 TOXAPHENE AND FISH CONSUMPTION

Tolerance levels for toxaphene with regard to fish consumption by humans are only known for the United States and Germany. The USA tolerance level is 5 mg kg^{-1} wet weight and the German tolerance level is 0.1 mg kg^{-1} lipid weight for fatty fish (> 10 % fat) and 0.1 mg kg^{-1} wet weight for lean fish (< 10 % fat). There seems to be no sound toxicological basis for either standard. The German tolerance level will presumably be changed to 0.1 mg kg^{-1} on a wet weight basis for the sum of CHB26, CHB50, and CHB62, for all types of fish. According to a study by Alder *et al.* (1997), most fish samples analysed fall under this new German tolerance level. Other studies show, however, that several fish species from the North Sea and from the Baltic Sea may exceed this level. In Canada, instead of a tolerance level, an acceptable daily intake (ADI) value of 0.2 mg kg^{-1} is used.

5 TOXICOLOGY

A detailed overview on the toxicology of toxaphene has been given by Saleh (1991). A risk evaluation of toxaphene concentrations in fish was carried out by Janssen and van Leeuwen (1993).

Toxaphene is extremely toxic to fish. The lethal range of concentrations is 5–100 µg l^{-1} for most freshwater species (Saleh, 1991). The acute LD_{50} value for man is estimated at 60 mg kg^{-1} body weight, which corresponds with a lethal dose of approximately 4 g (Anon., 1969).

The majority of the studies reported some form of liver pathology in rats at dietary levels of 100 mg kg^{-1} or above. Only at the relatively high concentration of 1,000 mg kg^{-1} or higher does toxaphene exposure elicit central nervous system effects characteristic of acute human intoxication.

Toxaphene was shown to be mutagenic in the Ames test (Hooper *et al.*, 1979). However, the carcinogenicity of toxaphene is still under discussion. Reuber (1979) showed that toxaphene is highly carcinogenic in rats and mice, inducing malignant neoplasms of the liver. In a report published by the US Environmental Protection Agency (US EPA), the human carcinogenic potency of oral toxaphene doses was estimated at 1.13 mg kg^{-1} per day (USEPA, 1987). Janssen and van Leeuwen (1993) did not respond to the results of Reuber (1979). They discussed a test carried out with mice species (B3C6F1) and Osborne-Mendel rats which are both supposed to be very sensitive for liver tumours. Based on the sensitivity of these species and the formation of many benign

tumours, the authors expressed their doubts on the possible carcinogenicity of toxaphene to man.

With regard to the risk evaluation of toxaphene concentrations in fish, they recommended use of the no observable adverse effect level (NOAEL) value of 0.2 mg kg^{-1} body weight per day reported by Chu *et al.* (1986), based on a 13-week semi-chronic test with a dog. The above-mentioned difficulties and the very small database clearly show the gaps in knowledge on the possible carcinogenicity of toxaphene. An additional problem is that all data produced to date are based on *total toxaphene. Biotransformation of toxaphene results* in different CHB patterns in various organisms at different levels in the food chain. Saleh (1991) reported that some CHBs have much greater toxicities than toxaphene itself. Recent studies demonstrate that most of the toxaphene toxicity has been shown to be generally *attributed to only a few of its components, particularly* those of hepta- and octachlorobornanes (Saleh, 1996). It has been reported that toxaphene is a weak estrogenic compound, although, in combination with other weak estrogens such as dieldrin or endosulfan, it becomes 1000 times more potent in human estrogen receptor *transactivation (Arnold et al., 1996). However, it should* be noted that several expert laboratories have been unable to repeat these experiments and that great care should be taken in extrapolating *in vitro* receptor studies to the wider marine environment.

For a further and more solid underpinning of tolerance levels, it is necessary to obtain more knowledge on the toxicity of biotransformed toxaphene mixtures and of specific CHBs. The variety of other toxicity studies on toxaphene (genotoxicity, teratogenicity, liver toxicity, thyroid toxicity, neurotoxicity) does not take away the above-mentioned need for studies on the carcinogenicity of toxaphene and individual CHBs.

6 SUGGESTED APPROACH FOR FUTURE MONITORING AND TOXICOLOGICAL RESEARCH

To supply policy makers with tools to underpin regulations and possible tolerance levels for toxaphene in fishery products, it is essential that information becomes available on toxaphene levels in different fish and shellfish species, the spatial distribution of toxaphene in the North Sea and other relevant areas, and on differences in toxaphene levels between individual fishes. With regard to discussions on tolerance levels for toxaphene, which in Germany and the rest of Europe tend to go in the direction of congener-based tolerance levels, it is desirable that, in addition to total toxaphene concentrations, concentrations of individual CHBs are also determined. Information on CHB concentrations is of particular interest with regard to the German congener-specific method based on single-column GC/ECD, which includes a serious risk of producing positively biased results. The use of MDGC/ECD for the determination of CHBs is therefore recommended. In any case, the two most persistent CHB congeners, CHB26 (2-exo,3-endo,5-endo,6-endo,8b,8c,10a,10b-octachloro-bornane) and CHB50 (2-exo,3-endo,5-exo,6-endo,8b,8c, 9c,10a,10b-nonachlorobornane), should be selected for such a study. CHB32 (2,2,5-endo,6-exo,8b,9c,10a-hepta-chlorobornane) and CHB62 (2,2,5,5,8b,8c,9c,10a,10b-nonachlorobornane) could also be considered, but these two compounds are more easily biotransformed (Boon *et al.*, 1996). Other CHBs are also possible, but making a sensible further selection is difficult because information on their toxicity is not yet available. According to Saleh (1996), heptachlorobornanes and octachlorobornanes may be the most relevant CHBs with regard to toxicity.

It will be essential to improve the quality of analytical data on toxaphene; thus, an interlaboratory intercomparison exercise on toxaphene determination is needed. In such an exercise, sufficient guidance should be given to the participants on the choice of proper analytical conditions. In addition, there is a clear need for one or two certified reference materials for toxaphene, preferably a biological tissue and a sediment.

Obviously, information only on levels of toxaphene and CHBs is insufficient to make judgements on safety limits for consumers. Therefore, more information should be generated on the carcinogenicity of toxaphene and on toxic effects of biotransformed toxaphene mixtures and specific CHBs. A complete *in vivo* carcinogenicity study is complicated, time consuming, and expensive. Short-term *in vitro* assays may be used as a first step to obtain information on the carcinogenicity of toxaphene and CHBs. Such *in vitro* assays should also be carried out with biotransformed toxaphene mixtures which can be extracted from fish. If the *in vitro* tests show significant differences between the toxicity of environmentally modified toxaphene and the technical toxaphene mixture, it will be necessary to confirm these findings using *in vivo* animal studies. Tumour-promoting characteristics may also be confirmed by means of an altered hepato fico (AHF) carcinogenicity assay.

The combination of knowledge on toxaphene and CHB concentrations in fish and shellfish and knowledge on the toxicology of toxaphene and CHBs may offer sufficient information to policy makers. However, this approach, particularly the toxicity studies, may be very time consuming, so it may be useful to consider an alternative approach. There are indications that toxaphene concentrations can be measured using a bioassay in which toxaphene binds with a receptor. Based on the neurotoxicity of toxaphene, presumably of only a few congeners, Saleh and Blancato (1993) developed the gamma-aminobutyric acid (GABA) radioreceptor assay. The GABA receptor was prepared by a sequence of ultra centrifugation and dialysis of mammalian (rats, cows, catfish, and goats) brain homogenates. The receptor was treated with ^{35}S tertiary butylbicyclophosphorothionate (TBPS) and the assay was conducted by measuring the displacement of radioactivity following incubation with the sample containing the analytes. The assay is fast and

requires very little sample preparation prior to the analysis. The assay was applied for the determination of toxaphene in blood samples. The detection limit was 2 $\mu g\ kg^{-1}$. The activity of the receptor did not change for six months if it was stored at −80 °C. A disadvantage may be that only an indication of the total toxaphene content is obtained. Spike experiments showed an acceptable reproducibility (Saleh, 1996), but interlaboratory studies have not been carried out. Another drawback may be that in this assay only the neurotoxicity of toxaphene is considered, whereas other toxic effects may be more important. However, it may be possible to develop such tests based on other effects and perhaps also based on specific CHBs. A similar approach has been used in the development of the luciferase assays (CALUX) for planar PCBs (Denison *et al.*, 1996). Toxicologists should be consulted for advice on the development and use of such assays.

7 CONCLUSIONS

1) To underpin regulations and possible tolerance levels for toxaphene in relation to fish consumption, and to obtain a better overview of the distribution of toxaphene in the marine environment, it is recommended that more information on concentrations of total toxaphene and of at least two persistent CHBs in fish and shellfish from relevant areas be collected. Relevant areas are expected to be mainly in the North Atlantic. Including toxaphene in marine monitoring programmes should be considered, provided that the between-laboratory comparabilty of toxaphene results is acceptable.

2) The use of GC/NCI-MS is recommended for the determination of total toxaphene and the use of MDGC/ECD is recommended for congener-specific CHB determinations.

3) Information on toxaphene concentrations should be combined with information on the toxicity of toxaphene, in particular, on its carcinogenicity and on toxic effects of environmentally modified toxaphene mixtures and of specific CHBs.

4) There is a need for an interlaboratory intercomparison exercise on the determination of toxaphene in which the participants are guided to select proper analytical conditions.

5) There is a need for a certified reference material for toxaphene.

6) The use of bioassays may be considered as an alternative approach for a rapid determination of total toxaphene and possibly for specific CHBs, based on a specific toxic effect.

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ANNEX 6

POLYCHLORINATED DIPHENYLETHERS: ORIGIN, ANALYSIS, DISTRIBUTION IN THE MARINE ENVIRONMENT, AND TOXICITY

SUMMARY

This paper gives an overview of the origin, use, toxicity, and analysis of polychlorinated diphenylethers (PCDEs), as well as the distribution of PCDEs in the marine environment. PCDEs are present as impurities in chlorophenol preparations, which are often used as wood preservatives. Other sources of PCDEs, such as municipal waste incinerators, seem to be of minor importance. It is estimated that 250–2500 metric tonnes of PCDEs have been produced worldwide; this quantity is less than the production of polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs), or polychlorinated naphthalenes (PCNs).

The analytical methods for determining PCDEs, although complex, are comparable to the methods used for the determination of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Gas chromatography with low resolution or high resolution mass spectrometry or with electron capture detection is recommended for the final determination of PCDEs.

There is only limited information on the presence of PCDEs in the marine environment. High concentrations of PCDEs were found in Baltic white-tailed sea eagles (up to 13 mg kg^{-1} lipid weight per congener). Total PCDE concentrations of 50–300 µg kg^{-1} were reported in cod liver oil samples.

PCDEs are immunotoxic and show a clear potency as inducer of hepatic microsomal aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-*O*-deethylase (EROD) activities.

Although PCDE concentrations in marine organisms are expected to be lower than those of PCBs, PBDEs, and PCNs, the toxic properties, persistence, hydrophobicity, and bioaccumulative character of these compounds, in addition to the limited information on PCDE concentrations in marine organisms, strongly indicates that further research on PCDE concentrations in marine organisms is needed.

1 ORIGIN AND USE

Polychlorinated diphenylethers (PCDEs), sometimes called chlorinated diphenyloxides, have a structure that resembles that of polychlorinated biphenyls (PCBs) (see Figure A6.1). The difference between these compounds is the oxygen atom that connects the two phenyl rings, which is absent in the PCB structure. Although the numbering system of PCDEs is identical to that of PCBs, the two compound classes are essentially different. The widespread occurrence of PCDEs in the environment is mainly due to their presence as impurities in chlorophenol preparations (Becker *et al.*, 1991). The presence of PCDEs, in addition to polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), in commercially available chlorophenols of technical and analytical grade has been confirmed by infrared spectrometry and mass spectrometry (Becker *et al.*, 1991).

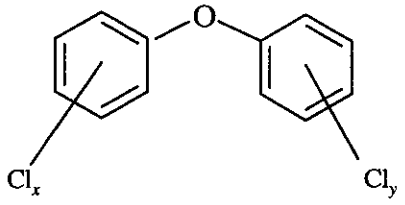

Figure A6.1. Molecular structure of polychlorinated diphenylethers.

The PCDEs identified in chlorophenols contain three to ten chlorine atoms (Stafford, 1983). Commercial chlorophenol preparations are widely used as wood preservatives, fungicides, and as key intermediates in the production of chlorinated phenoxyacetic acids, which are used as herbicides (Stafford, 1983; Becker *et al.*, 1991). The estimated levels of PCDEs in commercial chlorophenols were in the range of 100–1000 mg kg^{-1} (Nilsson and Renberg, 1974). There is considerable variation in PCDE concentrations in chlorophenols (Becker *et al.*, 1991). Kurz and Ballschmiter (1995) determined 106 individual PCDE congeners in Xyladecor (Decowag-Bayer Holzschutz GmbH, Germany, 1983) and Sadolins (Sadolins GmbH, Germany, 1984), two wood preserving formulations, each with a pentachlorophenol (PCP) content of 10 %. PCDE patterns were the same in both types of wood preservative. Total PCDE concentrations, based on the sum of 106 PCDEs, were 20.8 mg kg^{-1} in Xyladecor and 33.4 mg kg^{-1} in Sadolins. The PCDE concentration in PCP itself, derived from these concentrations, was estimated at 200–230 mg kg^{-1}, which corresponds with the range indicated by Nilsson and Renberg (1974). Most PCDEs present had seven to ten chlorine atoms. Total PCDE concentrations in 2,3,5,6-tetrachlorophenol and in Na-2,4,5-trichlorophenolate were 212.6 mg kg^{-1} and 4.4 mg kg^{-1}, respectively (Kurz and Ballschmiter, 1995). The chlorination degree varied between three and ten chlorine atoms, with larger amounts present of compounds with seven or eight chlorine atoms. Koistinen *et al.* (1995b) determined PCDEs in the wood preserving formulation Ky-5. The total PCDE concentration found was 16.5 mg kg^{-1}. Table A6.1 provides an overview of

the fourteen dominant PCDE congeners found by Koistinen *et al.* (1995b) and Kurz and Ballschmiter (1995). The Ky-5 formulation apparently has a lower chlorination degree than the other mixtures. For each PCDE congener, the chlorine substitution is exactly the same as for the corresponding chlorobiphenyl congener (Paasivirta and Koistinen, 1994).

PCDEs have also been detected at sawmill wood waste sites. Chemical treatment with sodium hypochlorite or caustic soda decreased the quantity of chlorophenols and phenolic dimers in waste products, but treatment with sodium hypochlorite increased the levels of PCDEs, PCDFs, and PCDDs (Paasivirta *et al.*, 1982).

In 1980, Rappe (1980) estimated the annual world production of all chlorophenols to be approximately 150,000 metric tonnes, of which 25,000 tons was PCP produced in the USA. Carron and Afghan (1989) reported a total use of 80,000 tons of chlorophenols in the USA in 1975 and 6,600 tons in Canada in 1980. Jones (1984) reported an annual production of 5,250 metric tonnes in Canada in 1981. The use of chlorophenols has declined since the late 1970s (Carron and Afghan, 1989). The use of pentachlorophenols in wood preserving formulations was banned in Sweden in 1978. Prior to the ban, 150 tonnes of PCP-containing wood preserving formulations had been used annually (Rappe, 1980). In the Netherlands all materials containing more than 5 mg kg^{-1} PCP were banned in 1994 (Anon., 1995). The European Commission decided to restrict the use of PCP-containing materials in 1992 (Anon., 1995).

Combining the cumulative production figures of chlorophenols with the percentage of PCDEs in chlorophenols yields a total quantity between 250–2500 metric tonnes of PCDEs which may have entered the environment. This is less than the amounts produced of PCBs (1.2 x 10^6 tonnes (Pearson, 1982), PBDEs, > 26,000 tonnes per year (Pijnenburg *et al.*, 1995), and PCNs, 5,000 tonnes per year (Pearson, 1982). Thus, assuming that sources of PCDEs other than chlorophenol mixtures are relatively small, PCDE levels in the marine environment are expected to be lower than the levels of PCBs, PBDEs, and PCNs. High PCDE concentrations will only be found near local sources of chlorophenols.

There are only a few reports in the literature on the use of PCDEs as industrial chemicals. The use of PCDEs in heat exchange is mentioned by Rappe *et al.* (1980) and the use of PCDEs in chemical synthesis is mentioned by Proctor and Hughes (1978). Nitro-substituted PCDEs are used as herbicides (Gara *et al.*, 1981) and hydroxy-substituted chlorinated diphenylethers are used as antimicrobial agents (Tulp *et al.*, 1979). Paasivirta *et al.*

Table A6.1. PCDE concentrations in technical chlorophenol mixtures and in wood preservatives.

PCDE No.	Structure	Na-2,4,5-tri-chlorophenolate[a] (µg kg^{-1})	2,3,4,6-tetra chlorophenol[a] (µg kg^{-1})	Xyladecor[a] (µg kg^{-1})	Sadolins[a] (µg kg^{-1})	Ky-5[b] (µg kg^{-1})
35	3,3′,4	39	50	6	< 1	-
91/99/115	2,2′,3,4′,6/					
	2,2′,4,4′,5/					
	2,3,4,4′,6	3	642	4	< 1	2400[c]
100	2,2′,4,4′,6	-	-	-	-	1300
147/153	2,2′,3,4′,5,6/					
	2,2′,4,4′,5,5′	42	3065	52	55	1900
154	2,2′,4,4′,5,6′	35	5719	40	8	2100
180	2,2′,3,4,4′,5,5′	72	3794	59	81	670
182	2,2′,3,4,4′,5,6′	-	-	-	-	1500
196	2,2′,3,3′,4,4′,5,6′	1124	68117	8100	9306	550
203	2,2′,3,4,4′,5,5′,6	83	1818	134	216	100
206	2,2′,3,3′,4,4′,5,5′,6	375	1563	1317	2572	< 100
209	2,2′,3,3′,4,4′,5,5′,6,6′	226	177	468	1072	< 100

[a] Kurz and Ballschmiter, 1995; [b] Koistinen *et al.*, 1995b; [c] concentration of PCDE99.

(1986) and Kurz and Ballschmiter (1995) have also mentioned the formation of hexachlorinated and heptachlorinated diphenylethers in municipal waste incinerators.

2 ANALYSIS

PCDEs are often determined together with PCDDs, planar PCBs, and PCNs. An example of such a combined method is that used by Koistinen *et al.* (1995a). The analytical procedure is shown in Figure A6.2. The biota samples are ground with sodium sulphate, air-dried, and extracted with a mixture of petroleum ether: acetone:hexane:diethylether (18:10:5:2, v/v/v/v) in a Soxhlet apparatus for six hours. The lipid content is determined gravimetrically after solvent evaporation. Internal standards, added before extraction, include one $^{2}H_{5}$-pentachlorinated diphenylether. The extracted fat is dissolved in 5 ml of hexane, which has been mixed with an equal amount of concentrated sulphuric acid by shaking in a test tube with a screw cap. PCBs, chlordanes, hexachlorocyclohexanes, and DDT and its metabolites were analysed in this purified hexane extract. One part of the purified extract was cleaned for planar CB and PCN analysis, using carbon and basic alumina micro-columns. Another part of the extract was fractionated on a florisil column and further cleaned on two carbon columns, resulting in two extracts, containing PCDEs and PCDDs and PCDFs, respectively. The final PCDE determination was carried out with low resolution mass spectrometry (LRMS) (HP 5970 mass selective detector). High resolution (HR) MS (resolution 10,000) was used for the verification of the PCDEs. The GC columns used were 25 m x 0.2 mm x 0.33 µm HP5 columns. The carrier gas was helium. Temperatures were: injector 250 °C, transferline 260 °C (HRMS) or 300 °C (LRMS), oven one minute at 100 °C, 20 °C per minute to 180 °C, 5 °C per minute to 280 °C or 290 °C. A splitless injection of one µl was used. Fifty chlorinated diphenylethers, all synthesized by the University of Jyväskylä (Finland), were determined.

The same method was used by Koistinen *et al.* (1995b) for the determination of PCDEs in sediments. The sediments were freeze-dried prior to Soxhlet extraction. Birkholz *et al.* (1995) used $^{13}C_{12}$-chlorinated diphenylethers as internal standards. Following Soxhlet extraction with dichloromethane:hexane (1:1, v/v) for sixteen hours and sulphuric acid treatment of the extract, they used a multi-layer silica column (elution with 50 ml 2 % (v/v) dichloromethane in hexane) and florisil columns (elution with 100 ml hexane and 300 ml dichloromethane). The dichloromethane fraction was used for PCDD/PCDF and planar CB determination. The hexane fraction was further fractionated over a 5 g basic alumina column (elution with 100 ml hexane and 100 ml dichloromethane). The first fraction contained the PCBs and the second, dichloromethane fraction contained the PCDEs. HRMS (resolution 10,000) in the selected ion mode was used for identification and quantification of the PCDEs.

Huestis and Sergeant (1992) developed a method for the removal of PCDEs as interferences during PCDD/PCDF analysis. This method was used by Niimi *et al.* (1994) for the determination of PCDEs in biota. Gel permeation chromatography (GPC) was used to separate the PCDEs from the lipids, which were extracted from the tissue with dichloromethane. GPC was followed by fractionation over silica columns. A VG HRMS (Autospec, resolution 10,000) was used for the identification and quantification of the PCDEs. The temperature program of the oven used was comparable to that used in the method of Koistinen *et al.* (1995a). An Rtx-5 column (60 m x 0.25 mm x 0.25 µm) was used, with helium as the carrier gas. Seventeen chlorinated diphenylether congeners were determined, which were synthesized by Zenon Environmental Inc., Burlington, Ontario, Canada, or purchased from Ultra Scientific, Kingston, RI, USA. The detection limits reported are 0.4 ng g^{-1}.

Kurz and Ballschmiter (1995) used a 2-(1-pyrenyl) ethyldimethylsilylated silica (PYE) high performance liquid chromatography (HPLC) column for the separation of PCDEs and PCBs from PCDDs and PCDFs. They determined PCDEs in cod liver oil which was dissolved in hexane, heated with sulphuric acid, and eluted over basic alumina columns (first fraction 50 ml hexane, discarded, second fraction 50 ml hexane/dichloromethane 1:2 (v/v)), prior to HPLC. The gradient used for the PYE column was 100 % hexane (5 minutes), 5 % dichloromethane (v/v) per minute to 100 % dichloromethane (15 minutes). The first fraction (0–8 minutes) contains the PCDEs, the PCBs, and the chlorinated pesticides, the second fraction (8–40 minutes) contains the PCDDs and the PCDFs.

Standards of individual PCDEs are commercially available. Nevalainen and Koistinen (1994) synthesized 54 chlorodiphenylethers (CDEs). Their structures were confirmed by MS and proton magnetic resonance spectroscopy. Retention times on SE-54 and OV-1701 capillary GC columns were determined relative to ^{13}C-CB77. The retention times for PCDEs increased with the increasing number of vicinal chlorines within a series of isomers. The chlorine substitution patterns of PCDEs were used to develop a method for predicting the relative retention times for congeners that were not synthesized. Certified reference materials (CRMs) are not available, although an indicative value for PCDEs in a NIST CRM for PCBs in cod liver oil was given by Kurz and Ballschmiter (1995).

Electron capture detection (ECD) is, in theory, suitable for the detection of PCDEs. The limited selectivity may, however, lead to ambiguous results (Paasivirta and Koistinen, 1994). For example, peaks of toxaphene

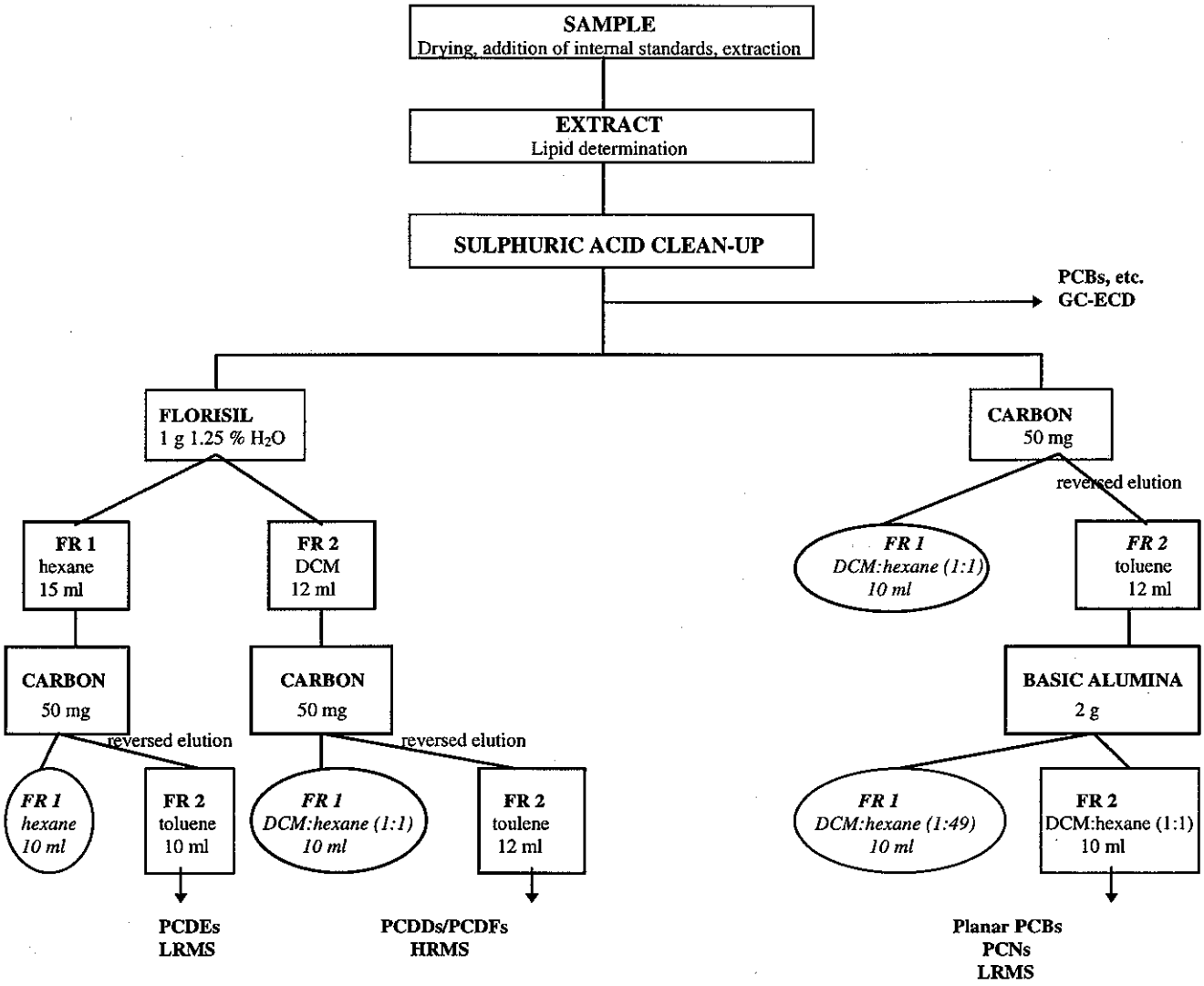

Figure A6.2. Analytical procedure for the determination of PCDEs (Koistinen *et al.*, 1995a).
DCM = dichloromethane
florisil = PR, 60–100 mesh
carbon = SK-4, 80/100, Alltech Assoc., Inc.
alumina = ICN alumina B, Akt. I, ICN Biomedicals

appear at the same retention area as tetra- to hepta-CDEs on a DB-5 column (Sergeant and Onuska, 1989). ECD is considered to be sufficiently sensitive for the analysis of PCDEs with three or more chlorine atoms. Detection limits are in the order of 1–10 ng g^{-1} wet weight (Paasivirta and Koistinen, 1994).

Misidentification of false positive results is reduced by the use of a dual-column GC system or (comprehensive) multidimensional GC (de Geus *et al.*, 1996; de Boer *et al.*, 1997).

3 DISTRIBUTION IN THE MARINE ENVIRONMENT

PCDEs are persistent hydrophobic compounds. Log K_{ow} values for some tri- and tetra-CDEs vary between 5.44 and 5.78 (Opperhuizen and Voors, 1987). These log K_{ow} values are very similar to the log K_{ow} values of PCBs with the same chlorine substitution. Neely *et al.* (1974) reported log K_{ow} values up to 7.62 for PCDEs.

Opperhuizen and Voors (1987) studied the uptake and elimination of 2,4,5-tri-CDE and 2,3,3′,4′-tetra-CDE in guppies (*Paecilia reticula*). They concluded that, in contrast to PCDDs and PCDFs, PCDEs accumulate at comparable rates and to comparable extents as similarly substituted PCBs. Niimi (1986) reported half-lives of PCDEs up to 167 days (for hexa-CDEs), which is somewhat lower than half-lives of PCBs with a comparable chlorine substitution (> 300 days). The value of the determination of these half-lives in combined uptake/elimination experiments in laboratories may be limited, however, given the results for PCBs in an

elimination experiment with eels in their natural environment (de Boer *et al.*, 1994).

Photodechlorination reactions have been reported for several PCDEs. PCDEs with chlorine substituents in the 2 or 6 positions gave rise to PCDFs (Choudry *et al.*, 1977). The latter reaction is suspected to occur in the environment under the influence of natural products which have photochemical characteristics similar to that of acetone (Choudry *et al.*, 1977).

Information on PCDE levels in marine fish and marine mammals is scarce. Many data are available on PCDE concentrations in freshwater biota from Finland. High levels of PCDEs have been found in Finnish rivers and lakes, which is clearly related to the use of chlorophenols in wood preservatives. PCDEs were first identified in the marine environment in 1990 by Koistinen *et al.* (1993a) in Baltic salmon. Since then, many marine organisms from waters around Finland have been analysed for PCDEs. Koistinen *et al.* (1993a, 1995a, 1995b) determined PCDEs in Baltic white-tailed sea eagles and black guillemot eggs. The white-tailed sea eagles from the Baltic Sea (Åland Islands) in particular, which are at the top of the food chain, contained high concentrations of PCDEs, 0.3–13 mg kg^{-1} lipid weight per congener, in the breast of one animal with a total PCDE content of approximately 50 mg kg^{-1} lipid weight. Other eagles analysed contained lower concentrations. Black guillemot eggs from the Baltic area contained <3–1400 μg kg^{-1} lipid weight per CDE congener, with a total PCDE content of about 1.7 mg kg^{-1} lipid weight. Kurz and Ballschmiter (1995) determined PCDEs in cod liver oil of unknown origin and found 49 μg kg^{-1} total PCDE, with a range of 1–19 μg kg^{-1} per congener. The standard reference material (SRM) for PCBs cod liver No. 1588 of the National Institute for Standards and Technology (NIST), Gaithersburg, Maryland, USA, contains about 350 μg kg^{-1} total PCDEs (Kurz and Ballschmiter, 1995). An overview of these data is given in Table A6.2.

Most other PCDE data are related to samples taken in the freshwater environment. Salmon, pike, and bream from the Simojoki and Kymijoki Rivers in Finland contained approximately 25–800 μg kg^{-1} total PCDE on a lipid weight basis (Koistinen, 1993a, 1995a, 1995b). Seals from Lake Saimaa contained approximately 500 μg kg^{-1} total PCDE on a lipid weight basis. Sediments from the Kymijoki River contained about 600 μg kg^{-1} total PCDE on a dry weight basis. The Kymijoki River is close to the city of Kuusankosi where the wood preservative Ky-5 is produced (Koistinen *et al.*, 1995b). Trout from the Great Lakes, Canada contained about 75 μg kg^{-1} total PCDE on a wet weight basis (Niimi *et al.*, 1994).

PCDEs have also been identified in human tissue. Williams and Lebel (1988) and Williams *et al.* (1991) studied nona- and deca-CDE concentrations in adipose tissue of men and women from England and Canada. The concentrations were in the range of 1.0–1.5 μg kg^{-1} and 0.3–0.4 μg kg^{-1} wet weight for nona- and deca-CDEs, respectively.

More information, particularly on PCDE concentrations in marine fish and mammals, is needed to obtain a more complete picture.

4 TOXICITY

The toxicity of PCDEs has been studied less intensively than that of PCBs, PCDDs, and PCDFs. Nevertheless, information is available on the acute toxicity, enzyme induction, and immunosuppression of PCDEs.

Acute toxicity

Chui *et al.* (1990) studied the acute toxicity of PCDEs in trout. Table A6.3 gives an overview of LC_{50} values for trout determined after 24, 48, 72, and 96 hours. The mono- and di-CDE congeners studied showed a higher acute toxicity than the tri- and tetra-CDE congeners studied. The acute toxicity of PCDEs for fish is somewhat lower than that of DDT or γ-HCH, for which harmful effects on fish are found in the range 0.02–0.1 mg l^{-1} and 0.03–0.2 mg l^{-1}, respectively (Liebmann, 1960).

Enzyme induction and immunosuppression

Harper *et al.* (1993a) studied dose–response effects of nona- and deca-CDEs on splenic plaque-forming cell (PFC) response to sheep red blood cells (SRBCs) and the induction of hepatic microsomal ethoxyresorufin-*O*-deethylase (EROD) in aryl hydrocarbon (Ah)-responsive C57BL/6 and less Ah-responsive DBA/2 mice. Their results suggested that the immunotoxicity of PCDEs was mediated through the Ah receptor. The results showed that the immunotoxicity of the nona-CDEs and deca-CDEs was unexpectedly high compared to that of lower chlorinated CDEs. Therefore, the immuno-suppressive effects observed for the nonachlorinated and decachlorinated diphenylethers may be Ah receptor-independent. Harper *et al.* (1993b) suggested that the suppression of the trinitrophenyl-lipopolysaccharide (TNP-LPS)-mediated immune response may be a more reliable indicator of the Ah receptor-dependent immunotoxicity of PCDEs, and of halogenated hydrocarbons in general. The dose–response effects of several PCDEs on the inhibition of PFC response to SRBCs and the induction of Ah hydroxylase (AHH) and EROD activities were studied in male C57BL/6 mice by Howie *et al.* (1990). The potencies of PCDEs as inducers of hepatic microsomal AHH and EROD activities were similar to their immunotoxicities and only 2,3′,4,4′,5,5′-hexa-CDE did not cause dose–response immuno-suppressive effects in mice. The coplanar 3,3′,4,4′-tetra-CDE and 3,3′,4,4′,5-penta-CDE were less potent than the mono-*ortho* 2,3′,4,4′,5-penta-CDE and 2,3,3′,4,4′,5-hexa-CDE, respectively. Similar results were observed

Table A6.2. PCDE concentrations in marine organisms.

Ref.[a]:		1	2	2	3	3
PCDE No.	**Structure**	**Salmon**	**Cod liver oil**	**Cod liver oil (SRM 1588)**	**Black guillemot eggs**	**White-tailed sea eagle**
		Finland[c] ng g^{-1} ww[b]	Germany ng g^{-1} lw[c]	USA ng g^{-1} lw[c]	Gulf of Bothnia ng g^{-1} lw[c]	Åland Islands ng g^{-1} lw[c]
9	2,5	-	-	-	-	-
14	3,5	-	-	-	-	-
35	3,3',4	-	19	35	-	-
47	2,2',4,4'	1.15	-	-	75	1300
99	2,2',4,4',5	0.47	-	9	1400	1400
138	2,2',3,4,4',5'	0.04	-	-	6.6	980
139	2,2',3,4,4',6	-	-	-	-	-
140	2,2',3,4,4',6'	0.03	-	-	14	600
147	2,2',3,4',5,6	-	-	4	76	4700
153	2,2',4,4',5,5'	0.21	-	4	76	4700
154	2,2',4,4',5,6'	0.16	2	3	15	7800
167	2,3,4,4',5,5'	0.04	-	-	14	600
170	2,2',3,3',4,4',5	0.04	-	-	< 3	560
180	2,2',3,4,4',5,5'	0.04	1	253	9.5	2200
181	2,2',3,4,4',5,6	-	-	-	9.5	2200
182	2,2',3,4,4',5,6'	-	-	-	4.7	1900
183	2,2',3,4,4',5',6	-	-	-	-	-
184	2,2',3,4,4',6,6'	-	-	-	< 3	490
196	2,2',3,3',4,4',5,6'	0.07	-	15	9.3	13000
197	2,2',3,3',4,4',6,6'	-	-	-	3.2	3600
198	2,2',3,3',4,5,5',6	-	-	-	-	-
203	2,2',3,4,4',5,5',6	-	3	24	3.3	1700
206	2,2',3,3',4,4',5,5',6	-	-	-	< 5	1300
209	2,2',3,3',4,4',5,5',6,6'	-	-	-	< 5	270

[a] References: 1. Koistinen *et al.*, 1993a; 2. Kurz and Ballschmiter, 1995; 3. Koistinen *et al.*, 1995a.
[b] ng g^{-1} wet weight (ww); [c] ng g^{-1} lipid weight (lw).
[c] Simojoki, Finland.

Table A6.3. LC_{50} values for PCDEs determined by Chui *et al.* (1990).

PCDE No.	Structure	LC_{50} 24 hours mg l^{-1}	LC_{50} 48 hours mg l^{-1}	LC_{50} 72 hours mg l^{-1}	LC_{50} 96 hours mg l^{-1}
3	4	1.40	1.00	0.84	0.73
7	2,4	1.24	0.90	0.70	0.66
28	2,4,4'	> SL[a]	> SL	> SL	> SL
74	2,4,4',5	> SL	> SL	> SL	> SL

[a] saturation level

for enzyme induction potencies. The study demonstrated that for the PCDEs, increasing mono-*ortho* substitution is less effective in reducing the activity of these congeners compared to the well-recognized *ortho* effects for PCBs. Chu *et al.* (1990) studied the effects of 2,2′,4,4′,5-penta-CDE, 2,2′,4,4′,5,5′-hexa-CDE and 2,2′,3,4,4′,6,6′-hepta-CDE in rats. An increase in EROD activity was found for the hexa- and hepta-CDE congeners. The hepta-CDE was also found to be immunosuppressive. Chui *et al.* (1985) reported that 2,4,4′-tri-CDE and 2,4,4′,5′-tetra-CDE appeared to be a phenobarbital-type inducer and a mixed-type inducer of rat liver mixed function oxidase activity (MFO), respectively. Pretreatment of rats with these two congeners also caused a proliferation of the smooth endoplasmic reticulum in the liver. No significant alterations in the MFO activities of rat and trout livers were found after administration of 100 mg kg^{-1} per day of 4-CDE and 2,4-di-CDE for three days. Iverson *et al.* (1987) reported that all twelve (tetra- to deca-) CDE congeners studied increased P-4501A levels or increased monooxygenase activities in a manner resembling 3-methylcholanthrene, phenobarbital, or a combination of both (mixed). The responses resembled those of PCBs.

Other biological effects

Chu *et al.* (1990) reported an increase in liver weight in both sexes of rats after a four-week diet of feed containing 500 mg kg^{-1} of 2,2′,4,4′,5-penta-CDE, 2,2′,4,4′,5,5′-hexa-CDE, or 2,2′,3,4,4′,6,6′-hepta-CDE. A decreased food consumption was also observed. The hepta-CDE congener also caused a significant reduction in circulating lymphocytes in male rats. All three CDEs produced mild and adaptive histological changes in the liver and the thyroid, but only the hepta-chlorinated congener elicited mild changes in the thymus, bone marrow, and spleen. Chu *et al.* (1990) concluded that all three CDEs should be considered to be moderately toxic in rats. The no observable effect levels (NOEL) appear to be between 5 and 50 mg kg^{-1} in the diets (0.36–3.0 mg kg^{-1} body weight). Kodavanti *et al.* (1996) studied neurotoxic effects of 4,4′-di-CDE, 2,4,4′-tri-CDE, 3,3′,4,4′-tetra-CDE, and 2,2′,4,4′,5-penta-CDE. They found an increase of the [^{3}H] phorbol ester ([^{3}H]PDBu) binding in cerebellar granule cells in a concentration-dependent manner, with 2,4,4′-tri-CDE as the most active congener. They also reported that all PCDEs studied inhibited $^{45}Ca^{2+}$ sequestration by microsomes in mitochondria. Two CDEs, 4,4′-di-CDE and 2,4,4′-tri-CDE, were tested for cytotoxicity and both were found to be cytotoxic at higher concentrations and for longer exposure periods. The *ortho*-substituted congener exhibited the greater cytotoxic potential of the pair (Kodavanti *et al.*, 1996).

Safe (1992) proposed a toxic equivalency factor (TEF) of 0.001 for all non-*ortho* and mono-*ortho* substituted PCDEs, similar to the TEFs reported by Safe (1992) for mono-*ortho* substituted PCBs. The proposed TEFs were derived from quantitative structure–activity relationships and were based on the tetrachlorodibenzo-*p*-dioxin (TCDD) activity of the PCDEs.

5 CONCLUSIONS

Although there is less toxicological information available for PCDEs than for PCBs, PCDDs, and PCDFs, the first indications show that at least for several enzymatic effects, PCDEs may be comparable to PCBs. Given this indication, and given the persistent, hydrophobic, and bioaccumulative character of PCDEs, further research on levels of PCDEs in marine fish and mammals seems to be justified. However, based on emission figures from the primary sources, PCDE concentrations in marine organisms are expected to be lower than concentrations of PCBs, PBDEs, or PCNs.

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ANNEX 7

BIOACCUMULATION: CHEMICAL AND BIOLOGICAL FACTORS GOVERNING THE TRANSFER OF ORGANIC COMPOUNDS IN FOOD CHAINS

1 BACKGROUND

Bioaccumulation, or the presence of high concentrations of a chemical compound in an organism, has been observed for a long time. Historically, the initial findings of potentially toxic chemical substances in wildlife tissues largely contributed to the general public's ecological awareness that began in the 1960s. This quite recent awareness, in some cases the fear of being exposed to harmful substances via food consumption, together with the availability of increasingly sensitive analytical methodologies, has encouraged a large amount of research on contaminants in biota.

Many early studies dealt with the identification and quantification of contaminants in biological tissues without regard to their sources or relevant mechanisms. It is in this context that aquatic organisms are used as natural 'bioextractors' which then allow for the determination of compounds that are present in the water column at very low levels. These measurements form the basis for the use of biological indicators and for the implementation of contaminant monitoring networks, such as 'mussel watch' programmes, that use fish or shellfish as bioindicators.

A sound interpretation of the data obtained in these programmes requires the ability to differentiate various factors acting on bioaccumulation processes. Intuitively, discrepancies between these measurements may be attributed to one or several of the following causes: poor quality of the data due to improper analytical methodology; biological variability due to many parameters such as species, food web interactions, growth, reproduction, etc.; chemical factors which are directly related to the nature of the compound concerned and its physico-chemical properties; and, lastly, environmental conditions which define the presence of the substance in the medium and, consequently, its availability to biota. Monitoring programmes are particularly interested in the latter aspects because the main objectives of such programmes are the observation of levels and trends of contaminants.

Bioaccumulation is also of great concern for risk assessment because it determines the exposure of fragile species or key organs to potentially toxic compounds. Bioactive compounds, presumed to be harmless for lower organisms, might become toxic for higher consumers due to food web enrichment. Moreover, during their trophic transport, innocuous compounds might be bio-transformed into more reactive toxic substances which in turn might be stored in biological tissues. On the borderline between environmental chemistry and ecotoxicology, bioaccumulation studies are required in hazard assessment since this process defines the exposure of biota to chemicals.

As far as the issues briefly stated above are concerned, the approach has been basically analytical with the aim to identify and quantify contaminants in biological tissues. Like many other disciplines, environmental chemistry has moved from measurement, which is not always the simplest task, to the understanding of mechanisms. In that respect, modelling has recently contributed to an increase in detailed information on processes to be included in models. The growing interest in ecological models is partly due to their capacity to integrate information on processes in a more comprehensive and conceptual framework. More and more knowledge on bioaccumulation has been acquired, leading to increasingly sophisticated models. The predictive capacity of these tools depends on the care taken to set the basic processes leading to bioaccumulation. In that respect, there is an important need to ascertain the main factors acting on bioaccumulation. At the same time, it appears important to propose a classification of the various factors according to their actual influence on processes leading to bioaccumulation.

Environmental managers and policy makers are very fond of such predictive tools. However, models of bioaccumulation will not answer every question and scientists must remain very prudent in using these tools. A perfect, precise model could be very specific for one group of particular compounds in relation to a limited species living under defined environmental conditions. Any new questions will probably need a preliminary assessment based on the limited information available. In the first stage, a more empirical evaluation of the question might be more appropriate using a few key parameters that take into account the main characteristics of the chemicals, as well as basic biological information.

Several international bodies including international conventions (for instance, UNEP, OSPAR, International Commission for the Great Lakes), international associations of chemical producers (EuroChlor), and non-governmental environmental organizations have started, each with their own motives, to debate and work on the question of persistent and bioaccumulative compounds. Different terms have been used to describe these threatening compounds: persistent, bio-accumulative, and toxic compounds (PBTs) or persistent organic pollutants (POPs). The scientific community has not remained absent from similar reflections on these bioaccumulative compounds.

In this context it appears, for a number of reasons, that there is a serious need to review the various aspects of

bioaccumulation, and that a contribution from the ICES Marine Chemistry Working Group (MCWG) is expected. This is the main objective of this review. Therefore, at the very beginning of this reflection, the need to clarify very common terms which, in some cases, might have been misunderstood or even misused, was identified. This is done in the next section. Starting from early observations of bioaccumulation to more recent models, the precise terms which are currently used in such studies are applied. In subsequent sections, the chemical and biological aspects involved in the transfer of organic contaminants in food chains are discussed. A few examples, from studies of PCBs and dioxins, will be given in order to further illustrate this brief and general presentation.

2 PROCESSES OF BIOACCUMULATION: FROM OBSERVATION TO MODELLING

2.1 Basic Definitions

Regardless of the sources of contamination and the detailed mechanisms, bioaccumulation occurs, for any organism, when the intake of a compound exceeds its elimination. Very simply, this increase of any chemical in biota may be expressed as the ratio between its concentration in the organism and its concentration in the external medium, whether water, sediment, or food.

For aquatic species, this ratio was first linked to the concentration in the surrounding water leading to the concept of bioconcentration and to the bioconcentration factor (BCF).

$$BCF = C_B/C_W = \frac{\text{concentration in biota}}{\text{concentration in water}}. \quad (1)$$

Other ratios have been used, such as the biota-sediment accumulation factor (BSAF), which links the concentration in biota to that in sediment. At this stage, the numbers which characterize accumulation should be considered only as ratios without any precise physical sense.

Bioconcentration is the accumulation of a chemical in an organism from water and it is admitted that water is the only source of contamination. In all cases, water is the primary source of contamination either by adsorption, in the case of smaller organisms like phytoplanktonic cells, or during respiration, or by direct exchange of compounds through skin, in the case of higher organisms.

Conversely, in **bioaccumulation**, other sources of contamination such as food are also considered. This dietary uptake of contaminants supposes that bioconcentration has taken place at earlier stages. In a very general sense, bioaccumulation is also considered to increase with the age of the individual organism.

The entrance of contaminants into biological cycles occurs by adsorption of compounds which are initially dissolved in the water column and which possess a greater affinity to solid particles or lipid-rich tissues. This is characteristic of hydrophobic compounds, i.e., compounds that tend to be excluded from the aqueous phase. The influence of this general chemical characteristic of the hydrophobic nature of a compound on bioaccumulation will be considered later.

These are the initial steps in the accumulation of contaminants by living material. The terms **biomagnification** and **ecological magnification** are also currently used to refer to the increase in contamination from lower organisms to higher predators. This last process, which is an enrichment of contaminants in the food web during the transfer of food and energy, is a reiteration of similar processes which depend on the same chemical and biological factors as bioaccumulation.

2.2 Experimental Perception of Bioconcentration

Experimentally, bioaccumulation may be followed by measuring concentrations of contaminants in aquatic organisms during their exposure to contaminants. Fish, or any other aquatic organisms, are placed in aquaria filled with water containing a known concentration of a contaminant. Concentrations in fish increase until they reach an equilibrium, where they remain approximately constant. The bioconcentration factor (1) corresponds to this equilibrium concentration divided by the concentration in water. In many cases, depending on the characteristics of the compounds, the equilibrium concentration (plateau concentration) is only reached after long experiments, which may lead to difficulties in determining those concentrations and the bioconcentration factors obtained from these measurements. This has been called the 'steady state BCF'.

According to several authors (e.g., Davies and Dobbs, 1984), it is much easier to choose a kinetic approach to evaluate bioconcentration factors. Indeed, if concentrations are followed in biota and in water throughout the duration of the experiment, the concentration in organisms (C_B) may be described by simple first order laws that describe the uptake of contaminants from water and their elimination, or clearance:

$$dC_B/dt = K_1C_W - K_2C_B \quad (2)$$

where C_B and C_W are concentrations in the organism and in water, respectively. When equilibrium is reached, the concentration in biota remains constant and, accordingly, equation (2) becomes:

$$K_1/K_2 = C_B/C_W = BCF. \quad (3)$$

In conclusion, the bioconcentration factor is the ratio of rate constants K_1 (uptake) and K_2 (elimination); these constants can be determined at an early stage of accumulation and elimination in short-term experiments.

2.3 Importance of the Octanol–Water Partition Coefficient

Studies of bioconcentration have given rise to many determinations with many different compounds belonging to various chemical families, and using different target species. Correlations between BCF and physico-chemical characteristics were found a long time ago. Among those relationships, the octanol–water partition coefficient (K_{ow}) or the aqueous solubility (Sol.) of the compounds have been very useful to describe the bioconcentration potential.

One of the earliest examples was given by Ernst (1980), who established correlations between the bio-concentration of several organochlorine compounds by various molluscs and their aqueous solubility. It is important to note that, in this work, the best correlations have been observed for persistent organohalogen pesticides and for molluscs, which are the simplest species that possess a very reduced biotransformation capacity.

Later, Davies and Dobbs (1984) reviewed the different aspects of such correlations obtained for the bioconcentration of different organochlorine pesticides by various freshwater species. These relationships between the aqueous solubility (Sol.) and the bioconcentration potential have the general form:

$$\log BCF = b - a \log (Sol.) \quad (4)$$

with very significant regression coefficients. The same authors also reviewed similar correlations established using the octanol–water partition coefficient (K_{ow}). This coefficient has been very commonly, and successfully, used to describe the hydrophobic character of compounds. In most cases, for poorly soluble compounds K_{ow} is more easily determined than solubility in water. Several authors (Veith *et al.*, 1979; Bysshe, 1982; Spacie and Hamelink, 1984; Connell, 1992) have reviewed these correlations, which have the following general form:

$$\log BCF = a \log(K_{ow}) + b. \quad (5)$$

A few examples are given in Table A7.1. The best correlations are obtained for persistent compounds, mainly halogenated aromatic hydrocarbons, and for low trophic species. The constant a values are generally close to unity, whereas the constant b values correspond to the lipid contents expressed as a percentage of body weight.

These examples emphasize the importance of the octanol–water partition coefficients in describing the bioconcentration of organic compounds by aquatic organisms. In spite of some limitations, which will be discussed below, this physico-chemical characteristic appears to be the key parameter for explaining and predicting bioconcentration.

2.4 Thermodynamic Approach to Bioaccumulation

The empirical relationships between octanol–water partition coefficients and observed bioconcentration factors underline the similarity between the partitioning of chemicals in a water-immiscible solvent system (octanol) and their behaviour in biological systems. The octanol–water partition coefficient may be used to predict the bioconcentration of organic contaminants on the basis of those empirical relationships.

The distribution of a chemical substance between phases follows the laws of thermodynamics, i.e., equilibrium partitioning of a chemical between phases when the chemical potential of a compound in both phases is equal. By analogy, the distribution of substances in biological systems, i.e., bioconcentration, has been described by a similar principle which is basically the hypothesis of constant coefficient distribution in equilibrium conditions. It means that bioconcentration may be very simply described by equations (3) and (5).

Similar equilibrium equations have been used to describe other exchange processes that occur at the interfaces which act on the distribution of hydrophobic substances in the various compartments of the environment. They can also be expressed by partition coefficients. For example, the distribution of compounds, between water and sediment or suspended particulate matter, is defined by the adsorption coefficient K_D ($K_D = C_{Part}/C_W$), which is also correlated with the octanol–water partition coefficient and which depends on the characteristics of the solid particles such as granulometry and organic carbon content.

These exchanges and their relevant partition coefficients are the basis for the concept of fugacity and its use of environmental multimedia models (Mackay and Paterson, 1981). These models are useful for the assessment of the distribution of compounds in different compartments of the environment: water column, biota, air, sediment or soil. Using this approach, information on the distribution of chemical substances at equilibrium is obtained. Concerning bioaccumulation, concentrations in biota at equilibrium are calculated from the concentration in water and from the known or estimated octanol–water partition coefficients using the more appropriate evaluation of BCF from empirical relationships. With this thermodynamic approach, neither the rates of these exchanges which remain passive are considered nor the

Table A7.1. Relationships between log BCF and log K_{ow} of lipophilic compounds by aquatic organisms. Log BCF = a log(K_{ow}) + b (Connell, 1992).

Constants a	Constants b	N Values	r^2	Basis for BCF	Type of compound	Type of organism
0.7	–0.26	8	0.93	ww	pesticides	alga
0.68	0.16	41	0.81	ww	various organics	alga
0.46	2.36	8	0.83	ww	hydrocarbons and chlorohydrocarbons	alga
0.36	2.1	28	0.91	ww	mainly chlorohydrocarbons	alga
0.86	–0.81	16	0.96	ww	various organics	mussel
0.84	–1.23	34	0.83	ww	mainly chlorohydrocarbons	mollusc
0.54	0.12	8	0.95	ww	non-polar organics	fish
1.16	–0.75	9	0.98	ww	various organics	fish
0.85	–0.70	55	0.95	ww	various organics	fish
0.79	–0.40	122	0.93	ww	chlorohydrocarbons	fish
1.09	–0.87	11	0.99	ww	chlorohydrocarbons	fish
0.96	–0.56	16	0.98	ww	chlorohydrocarbons	fish
0.89	0.61	18	0.95	lw	chlorohydrocarbons	fish
0.96	0.25	18	0.96	lw	chlorohydrocarbons	fish
0.61	0.69	11	0.84	lw	chlorohydrocarbons	fish
0.95	–1.06	30	0.99	ww	chlorohydrocarbons and PAHs	fish
0.78	–0.35	22	0.95	ww	chlorohydrocarbons and PAHs	fish

ww = wet weight
lw = lipid weight

primary importance of several biological functions which for any animal act on the extent and kinetics of these exchanges.

2.5 Dynamic Approach to Bioaccumulation

Obviously, the rate and extent of bioaccumulation represent a dynamic process which depends on physico-chemical characteristics and, moreover, on the rates of exchange as well as the efficiency of biological functions which contribute to the enrichment of contaminants in biota. More sophisticated models have been developed which take into account the kinetic aspects of bioaccumulation. In such models, such as those of Norstrom *et al.* (1976), Thomann and Connolly (1984), and Connolly (1991), the basic equations of bioaccumulation express that the input of contaminants exceeds the output in any given organism.

A very general equation that summarizes those processes in a food chain might have the following form:

$$dC_i/dt = R_i \cdot a_{iw} \cdot C_w + \Sigma_{ij} F_i \cdot P_{ij} \cdot a_{ij} \cdot C_j - (E_i + G_i) C_i \qquad (6)$$

where C_w, C_i and C_j are the concentrations of the bioaccumulated contaminant in water, in the organism, and in its prey, respectively; R_i, F_i, E_i and G_i are the rates of respiration, feeding, elimination and growth of the organism i, respectively; P_{ij} represents the contribution of the various prey j to the diet of i, whereas a_{iw} and a_{ij} are the uptake efficiency terms for the contaminant by the organism i when it is absorbed from the water w or from the prey j, respectively.

This equation reflects the fact that intake of contaminants occurs from water by contact or more probably during respiration and from feeding on contaminated prey. Several processes take part in the reduction of contaminants in biota such as growth, which appears as a dilution, the excretion of compounds, their biotransformation, their redistribution within the organism, and their transfer to the next generation during reproduction.

In equation (6), the term that corresponds to the dietary uptake of contaminants may be quite complicated when several types of prey from different trophic levels are considered, that is, the case for biomagnification. If only one source of food is considered, which is sufficient to

discuss the biological aspects acting on bioaccumulation, the term becomes simpler:

$$dC_i/dt = R_i \cdot a_{iw} \cdot C_w + F \cdot a_{iF} \cdot C_F - (E_i + G_i)\, C_i \quad (7)$$

where F represents the food consumption rate without any precision concerning the type of food.

Equations (6) and (7), which are very similar to equation (2), clearly show that kinetic aspects of the processes are determined by the efficiency of physiological functions such as respiration rate, feeding rate, biotransformation capability, reproduction, etc. Then, in equations (6) and (7), if the input of contaminants is limited to the component due solely to water, which means that the contribution from food is zero, the resulting equation (8) resembles that of equation (2) describing bio-concentration.

$$dC_i/dt = R_i \cdot a_{iw} \cdot C_w - (E_i + G_i)\, C_i \quad (8)$$

In equilibrium conditions, the concentration in the organism remains constant; by comparing equation (8) with equation (2), uptake is related to respiration whereas the elimination rate depends on several different elimination processes including excretion, biotransformation, and reproduction.

The comparison of equations (2) and (8) points out that the same processes may be assessed by two different approaches, very crudely—the thermodynamic concept or the dynamic concept (some authors have called it trophodynamic). These two approaches may lead to two apparently different results about the relative importance of food and water as the main sources of bio-accumulation. In fact, these apparently conflicting results are related to the same processes which are not observed and described at the same level.

3 CHEMICAL FACTORS ACTING ON BIOACCUMULATION

3.1 General Characteristics

The bioaccumulation of organic compounds depends primarily on the nature of the compounds. Among the main characteristics acting on bioaccumulation by aquatic organisms, consideration must be given to their presence in the surrounding waters and their bioavailability, their hydrophobicity or affinity for lipid-rich tissues, and their persistence or their reduced capacity to be transformed.

Connell (1992) has summarized (see Table A7.2) the main chemical characteristics which govern bioaccumulation. These specifications affect the fate of the substances in the abiotic environment and, consequently, their availability as well as their capacity to be bioaccumulated.

Molecular weight and dimensions are directly related to the chemical structure of the compounds. These features determine the basic physico-chemical properties which, in turn, direct the fate of the compounds in the environment. The water solubility of the chemical substances or, inversely related, their octanol–water partition coefficient and their vapour pressure are useful parameters for describing the fate of the compounds.

Obviously, bioconcentration and, consequently, bio-accumulation may occur when compounds are present in the water column in a way that favours their uptake by aquatic organisms. Generally, fish and most aquatic species take up contaminants from water when chemicals are present in the truly dissolved phase. This means that bioconcentration occurs if the compounds are present in their dissolved form for long enough. Of course, volatile

Table A7.2. General characteristics of organic chemicals which exhibit bioaccumulation (Connell, 1992).

Characteristic	Features giving bioaccumulation
Chemical structure	High capacity: high proportion of C–C (aliphatic), C–H and C–halogen bonds. Limited capacity: low proportion of the bonds above with the presence of a variety of functional groups.
Molecular weight	>100 Da., giving a maximum capacity at about 350 Da., then declining to very low capacity at about 600 Da.
Molecular dimensions	Cross-section width <9.5 Å, molecular surface area between 208 and 460 $Å^2$, molecular volume between 260 and 760 $Å^3$.
Stability	Resistance to degradation reflected in soil persistence in the order of years.
Log K_{ow}	>2, giving a maximum capacity at about 6 and a decline to a very low capacity at about 10–12.
Water solubility (mole l^{-1})	<18, giving a maximum at about 0.002 with declining capacity at lower values.
Degree of ionization	Very low.

compounds, which possess a high vapour pressure, are rapidly removed from the water column by evaporation. Low molecular weight compounds, such as low chlorinated solvents or monoaromatic hydrocarbons, fall into this category. This group of contaminants is not subject to bioconcentration because they do not persist in the water phase.

At the other end of the volatility range, there are compounds which possess heavier molecular masses, larger dimensions, and a nonpolar character. These very stable compounds are nevertheless not bioconcentrated due to their adsorption to solids. In environmental conditions, compounds that possess high octanol–water partition coefficients are readily adsorbed onto suspended particulate material and, consequently, escape the dissolved phase. However, special mention must be made of the adsorption of contaminants to phytoplanktonic cells and other minute species, which although it limits the presence of compounds in the dissolved phase nonetheless contributes to the entry of the chemical substances into the biological cycle. This process will be discussed later on when dealing with the biological aspects of bioaccumulation (Section 4.2.1, below).

The size and, more generally, the stereochemistry of a molecule is another factor that may limit bioconcentration. In a group of related compounds, an increase in the number of substituents on the molecule also means a higher K_{ow} and, consequently, a higher bioconcentration potential. However, in spite of a higher octanol–water partition coefficient, such compounds are less bioconcentrated than expected due to the size of the molecule and to its steric hindrance which limits its mobility across biological membranes and its transport within organisms.

The presence of chemical substances in the water compartment is also related to their resistance to any degradation processes. The more chemically nonreactive the molecule is, the longer it will stay in solution and potentially be bioconcentrated. On the other hand, it is generally accepted that dissolved compounds are more easily degraded by chemical or biological processes. Here again, a lower solubility favours bioaccumulation, as it prevents rapid degradation. This means that the simplest molecules, which do not possess any reactive functional groups, are bioconcentrated compounds. In these groups, hydrocarbons and halogenated hydrocarbons are the most typical examples of bioconcentrated compounds.

The presence of a polar group in the molecule is another feature that limits bioconcentration. Apart from the fact that polar groups enhance the degradability of the compound, the presence of such functional groups on the molecule greatly reduces its affinity for lipid-rich tissues. For similar reasons, ionizable compounds are hardly, if at all, bioconcentrated. For these compounds, the equilibrium between the two forms, dissociated and non-dissociated, decreases the possibility of bioconcentration.

Once inside an organism, a compound may be biotransformed. Biotransformation is species-dependent and involves complex mechanisms during which compounds are ultimately transformed into more polar compounds that are more easily eliminated. A few well-known exceptions exist, showing that some metabolites are also bioaccumulated. This is the case for organochlorine pesticides such as DDT or chlordane. DDE is the main metabolite of DDT which is highly bioaccumulated in the food chain. Muir *et al.* (1988) have given other examples of bioaccumulated metabolites. These authors have followed the distribution of chlordane-related compounds in the food chain of the polar bear. In organisms from higher trophic levels, they have observed an increase in the concentration of oxychlordane, whereas this compound is not present in technical chlordane. Biotransformations are influenced by structural conditions such as the relative position of the substitutions in the molecule. Stereochemistry is an important feature that might affect enzymatic processes leading to the formation of derivatives. The coplanarity of a molecule seems to be an important factor. Recent studies have pointed out the enantioselective character of biotransformation reactions of certain contaminants, including alpha-hexachlorocyclohexane (α-HCH) and a few optically active PCB congeners (Hühnerfuss *et al.*, 1993; Hühnerfuss *et al.*, 1995).

In summary, bioconcentration and bioaccumulation depend on several chemical characteristics of the compound. A primary condition for bioconcentration is the presence of the contaminant in the proper form, usually in the dissolved phase, for a sufficient period of time. Accordingly, bioaccumulation of volatile compounds or very hydrophobic compounds which show a strong affinity to solid material is expected to be limited. Characteristics related to the structure of the molecule such as its size or the presence of functional groups are also believed to affect bioaccumulation.

3.2 Compounds that are Potentially Bioaccumulated

Due to their toxicity and their occurrence in various compartments of the environment, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and dibenzofurans (PCDFs) will be considered.

3.2.1 PAHs

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in the coastal marine environment. The major sources of PAHs are related to the use of fossil fuels and other combustion processes and the release of oil into the marine environment. In spite of their presence in

sediments and their elevated K_{ow}, PAHs have not been routinely found in fish but do occur in invertebrates especially near local sources. Several characteristics may partly limit the bioconcentration of PAHs. Probably the most important is that in some organisms, particularly the vertebrates, PAHs are rapidly biotransformed into more polar metabolites which favour their elimination: for example, hydroxylated metabolites of PAHs have been found in the bile and faeces of fish. However, in some invertebrates, especially those that have a poor capacity for metabolizing PAHs, bioconcentration does occur (Meador *et al.*, 1995). In coastal marine waters, PAHs become strongly associated with carbon-rich solid particles which may restrict their bioavailability. However, in the case of filter-feeding organisms such as mussels or sediment-dwelling invertebrates, ingestion of contaminated suspended particulate material will be a route of uptake. It is likely therefore that although PAHs can bioconcentrate in some invertebrates, they are probably not bioaccumulated in the food chain due to their limited availability and their rapid biotransformation at higher trophic levels. The presence of PAHs in the coastal marine environment is, therefore, a problem due to their toxicological properties, as several compounds possess mutagenic and carcinogenic activities.

3.2.2 PCBs

Polychlorinated biphenyls are one of the best known examples of contaminants that bioaccumulate. They are widely distributed in all compartments of the environment all over the world. In spite of their presence at very low levels in oceanic waters, typically at the pg dm^{-3} level for individual components (Schulz *et al.*, 1988), the highest concentrations have been determined in top predators. Concentrations at 1–100 mg kg^{-1} levels were determined in the fatty tissues of marine mammals (Abarnou and Loizeau, 1994; Boon *et al.*, 1997), which corresponds to an overall bioaccumulation factor of about 10^8. A very large quantity of information exists on PCB contamination in biota.

The interest in PCBs for bioaccumulation studies is related to the fact that they belong to the same group of 209 theoretically different congeners with varying physico-chemical properties, depending on the number and position of the chlorine atoms in the molecule. These features act directly on their octanol–water partition coefficient, their capacity to be biotransformed, and also their toxicity.

Systematic observations of PCB distribution in biological tissues provide useful information on these aspects. PCB distributions in organisms, or PCB patterns, are subject to important modifications according to the position of the animal in the food web (Muir *et al.*, 1988). Discrepancies from the expected linear relationship between BCF and K_{ow} correlations have been observed.

Fox *et al.* (1986) have followed the bioconcentration of 28 PCB congeners (from dichlorobiphenyl (CB9) to decachlorobiphenyl) in zebra fish (*Brachydanio rerio*). They concluded that BCF–K_{ow} relationships are best described by a second degree equation:

$$\log \text{BCF (wet weight)} = -0.397(\log K_{ow})^2 + 5.86(\log K_{ow}) - 15.8 \quad (9)$$

that shows an increase of the bioconcentration factor with K_{ow}, up to a maximum. According to these results, bioconcentration is optimal for penta-, hexa-, and heptachlorobiphenyls.

The uptake of PCBs from water follows a rapid kinetics independent of the characteristics of the compounds. Lower BCF values were observed for low-chlorinated CBs (< 4 Cl per molecule), which is explained by a more rapid elimination. On the other hand, the reduction of bioconcentration potential for highly chlorinated compounds is due to their higher hydrophobicity that decreases their bioavailability because of a more pronounced adsorption onto solid material. Moreover, the steric hindrance due to the increase in chlorine substitution, particularly in the *ortho* position, may partly reduce their passage through biological membranes and, consequently, limit the bioaccumulation of larger molecules.

In the PCB group, which is considered to be a very persistent group of contaminants, several components might have disappeared depending on the species of concern. Even compounds with very similar K_{ow} coefficients, such as CB101 and CB118 (log K_{ow} = 7.07 and 7.12, respectively; Rapaport and Eisenreich, 1984), are bioaccumulated differently. Biotransformation is an important process that limits bioaccumulation (Boon *et al.*, 1997; Sijm *et al.*, 1997). Biotransformation is part of the detoxifying mechanism that facilitates elimination of PCBs by the formation of more soluble hydroxylated or sulphonated metabolites that could, in theory, be more easily excreted. Certain structural requirements are needed to allow biotransformation of CBs. These conditions are related to the presence of two vicinal hydrogen atoms in the biphenyl molecule. This means that, depending on the number of unsubstituted pairs in *o-m* or in *m-p* positions, the compounds will be partially biotransformed (Kannan *et al.*, 1995; Niimi, 1996; Sijm *et al.*, 1992; Boon *et al.*, 1997). On the other hand, compounds which do not have these adjacent vicinal unsubstituted positions or, more simply, which possess 2,4,5- or 2,3,4,5-substitutions on either phenyl ring will be bioaccumulated. This is the case for major congeners in biota such as CB153, CB138, and CB180.

3.2.3 PCDDs and PCDFs

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are among the most toxic compounds present in the environment. Based on

their chemical properties, these compounds are potentially accumulated by aquatic organisms. However, limited information exists on the presence of these compounds in marine biota, mainly due to the high costs of analysis. Moreover, most of the existing data are restricted to the seventeen 2,3,7,8-substituted congeners that possess the most toxic properties.

Retention of PCDDs/PCDFs by aquatic organisms has been recently reviewed by Niimi (1996). It is clear that information on the bioaccumulation of these compounds is rather limited and studies involving marine biota are non-existent. Segstro *et al.* (1995) used Biota-Sediment Accumulation Factors (BSAFs) to characterize the bioaccumulation of a few PCDDs/PCDFs by benthic invertebrates. Their results show that bioaccumulation of these compounds was low, with an average BSAF below one. Similarly, Niimi (1996) reported low BSAFs for trout in Lake Ontario, below 3 for two 2,3,7,8-substituted congeners but for most well below one; this is an important contrast to the average BSAF value of 102 for 61 PCB congeners.

Opperhuizen and Sijm (1990) have proposed an overall explanation for the bioaccumulation characteristics of PCDDs/PCDFs by fish. They have suggested that three groups of congeners can be distinguished. The first group includes low-chlorinated 2,3,7,8-PCDDs, for which the behaviour can be predicted from correlations derived from octanol–water partition coefficients (K_{ow}). The PCDDs/PCDFs of the second group are taken up by fish with normal rate constants but rapidly eliminated, probably via biotransformation. Congeners of the third group are taken up with extremely low rate constants or efficiency by several fish species. This is probably due to inhibited membrane permeation.

4 BIOLOGICAL FACTORS ACTING ON BIOACCUMULATION

4.1 General Aspects

In a very general sense, the bioaccumulation of contaminants depends on biology. Many very common observations, such as the presence of contaminants in biota, the increase of concentrations with age or their increase within the food chain, implicitly establish a link between the accumulation of compounds and biological events. Indeed, biology interacts with bioaccumulation, adding an important part of the complexity and variability that characterize biological activity. Several main biological functions related to respiration, nutrition, and reproduction influence the uptake and/or elimination of contaminants. Table A7.3 contains a list of these main processes which fall into two major categories according to their effect on contamination: the increase or decrease of concentrations within the organism.

Unlike chemical factors, these various processes are very specific to each type of organism; they depend on the trophic level of the organism and are more or less subject to variation according to ecological and environmental conditions. The latter factors can influence food requirements as well as the availability of prey.

4.2 Uptake of Contaminants

4.2.1 Bioconcentration

Bioconcentration, the uptake of contaminants from water, represents the first and most important step in determining the behaviour of chemicals in biota. The entry of contaminants into the biological cycle is governed by the adsorption of contaminants onto solid inert particles or living material. This partition process is passive, rapid, and surface dependent. As such, it becomes very important for smaller particles and organisms which then constitute the food of higher organisms. Several authors (e.g., Brown *et al.*, 1982; Harding, 1986) have reviewed the dynamics of organic contaminants in planktonic species. The distribution of these contaminants between the water column and phytoplanktonic cells or even the smallest zooplanktonic species may be related to the octanol–water partition coefficient and the lipid content of the material. Brown *et al.* (1982) have proposed an experimental relation:

$$\log[PCB_{phyto}] = \log[PCB_{water}] + 0.46 \log K_{ow} + \log[lip] + 0.714 \quad (10)$$

that enables the estimation of PCB concentrations in planktonic cells from their levels in water and the lipid content of the cells. This relation resembles that used for the calculation of contaminants in suspended particulate matter. It seems very important to point out the rapidity of this partition process compared to most other biological mechanisms leading to bioaccumulation.

4.2.2 Respiration

For aquatic species, the uptake of contaminants occurs primarily during respiration. A large amount of water passes through the gills where exchanges of oxygen and contaminants can take place. Several studies have emphasized the role of respiration and, consequently, the importance of water as a major source of contamination. Bioconcentration is a rapid process which does not seem to be influenced by the structural characteristics of the compound. In most studies, the equilibrium is reached within a few weeks. In bioaccumulation models, the uptake of contaminants from water is currently related to the respiration rate and an assimilation coefficient. Temperature and dissolved oxygen that characterize the surrounding waters are environmental parameters that act indirectly on the extent of bioaccumulation.

Table A7.3. Main biological factors acting on bioaccumulation.

Factors that Increase Bioaccumulation		
Uptake from water		
Direct absorption	Passive process	
Diffusion	More important for small-sized species	
Adsorption	More important for low trophic level species	
Respiration	Active process O_2 consumption	Respiration rate Assimilation coefficient
Dietary uptake		
Food	Increasing importance for higher consumers	Feeding rate Diet composition
Sediment	Increasing importance for more hydrophobic compounds	Assimilation coefficient Lipid content
Factors that Decrease Bioaccumulation		
Excretion		
Via faeces	Faster for relatively soluble compounds	Excretion rate
Via urine		Assimilation coefficient
Biotransformation		
Liver	Presence of specific enzymes	Metabolism rate Metabolites[1] (Assimilation coefficient)
Growth		
Redistribution	Dilution within the body	Allometric relationships Lipid content
Reproduction[2]		
	Gonad maturation and spawning	GSI/RHI Seasonal variation
	Transfer *in utero* and via milk	Reproductive status

[1]Metabolites = persistent metabolites (DDE) bioaccumulated
[2]Reproduction = source of contamination for newborn marine mammals

4.2.3 Dietary uptake

The ingestion of contaminated food contributes to bioaccumulation. Whatever the type of food—living prey, suspended particulate material (SPM), or sediment—it is assumed that contamination has occurred by bioconcentration at a previous step according to the processes described above. Molluscs and other filter-feeding organisms absorb chemicals by ingestion of SPM which carries contaminants. Sediment represents an important source of contamination for benthic species. For these organisms, uptake occurs from the interstitial water or from particulate material. The PCB patterns in various benthic invertebrates show that feeding behaviour has a strong influence on the distribution of PCBs in these species. In molluscs, as filter-feeders, the low-chlorinated components are slightly more present than in other species, whereas in worms and crustaceans that feed on detritic material highly chlorinated compounds become more frequent.

The relative importance of both sources of contamination, either water or food, is still frequently discussed. Bioconcentration remains very important in the case of organisms from lower trophic levels; its influence on bioaccumulation decreases towards upper trophic levels. The more hydrophobic the compounds, the more dietary uptake dominates over other sources of contamination.

4.3 Processes Limiting Bioaccumulation

Once in the gastrointestinal tract, contaminants are subjected to several processes that contribute to a decrease in concentration. They may be eliminated directly, with or without biotransformation, redistributed within the body, remobilized during reproduction, and eventually transferred to the next generation.

4.3.1 Elimination and biotransformation

Direct elimination of compounds may occur without any modification of their structure. This direct excretion of foreign compounds occurs via faeces and urine. On the basis of bioconcentration experiments, the excretion of contaminants is included in the clearance process which is slower than uptake. In the case of PCBs, the depuration rate is slower than uptake and depends on the structure of the molecule, such that low-chlorinated compounds are eliminated more rapidly. In bioaccumulation models, the

term related to elimination is described by the assimilation coefficient which expresses the part of the contamination available in the prey remaining in the organism.

Biotransformation contributes to the reduction of contamination. This process is part of the detoxification mechanism that takes place in the liver whereby parent contaminants are transformed via enzymatically mediated reactions into more polar metabolites, which are more easily excreted. However, some metabolites have been found in biological tissues of higher organisms and are then subjected to bioaccumulation. Hydroxylated and methyl-sulphonated metabolites of PCBs have been detected in higher organisms. DDE is another well-known example of a bioaccumulated metabolite. Incidentally, it can be noted that the bioconcentration concept does not apply to these compounds which are, in theory, absent from the water compartment. The identification of the metabolites and, moreover, the kinetics of their formation is still a very difficult task as, in many cases, one parent compound may lead to several derivatives at much lower concentrations in biological tissues.

In the case of PCBs, the structural requirements for these biotransformation reactions have been briefly noted above. It was assumed that these processes were limited to the more developed organisms. However, observations of the PCB patterns in organisms from various trophic levels have revealed a capacity of transformation even for lower species. This capacity, which is very low or absent in molluscs, increases in higher organisms from fish and crustaceans to marine mammals such as dolphins, polar bears, and/or other top predators like seabirds.

4.3.2 Growth and tissue distribution

Unmetabolized contaminants, and any persistent metabolites, are redistributed throughout the body. The more lipid-rich the biological tissues, the more they are contaminated. The contaminants are stored in fatty reserves and may be remobilized according to energy requirements.

Growth is a function that obviously affects the concentration of contaminants in organisms. The variation of contamination with age seems to be similar to that of the growth curve with an apparently steady state reached at adulthood. In bioaccumulation models, growth is usually considered as dilution of the same amount of contaminant in a larger body.

4.3.3 Reproduction

At each developmental stage of an organism, the reproductive function has a very marked effect on the distribution of contaminants. This process, well known in mammals, has also been observed in lower species. In fish, sexual maturity creates new energy requirements which are satisfied by the remobilization of fatty reserves. Lipids and the associated hydrophobic contaminants are used for the formation of eggs. During spawning, a substantial part of the contamination may be rapidly eliminated from the body and delivered in millions of eggs.

For marine mammals, contaminants, which are stored mainly in blubber, are transferred to the foetus and, once born, to the young animal which feeds on its mother's milk. Obviously, for marine mammals, if reproduction—including gestation and particularly lactation—is an important factor that reduces the contamination in the mother's body, this same process also contributes to the contamination of the offspring until it can feed alone.

4.3.4 Importance of lipids

In most of the biological factors that act on bioaccumulation, lipid reserves seem to have a key function. Nutrition, mobilization of energy reserves, and reproduction are more or less related to the use of lipids by organisms. Bioconcentration and bioaccumulation of hydrophobic compounds are particularly influenced by lipid content and also by the lipid composition in the various compartments.

The importance of lipids has been recognized for a long time and several authors have highlighted the use of lipid content in the expression of results. The expression of concentrations on the basis of total lipid content (Bligh and Dyer method) might be a good option, provided that the analytical methodology is satisfactory. With these conditions, a part of the variability of the data due to biological factors may be reduced.

The composition of lipids in various parts of the body, the variation of this composition in relation to food availability, and biological or physiological status might also affect the distribution of contaminants in organisms by slightly modifying their relative distribution due to partition processes.

5 CONCLUSIONS

Bioaccumulation occurs when the intake of contaminants exceeds their elimination for any given organism. Obviously this mechanism depends on the characteristics of the compounds which are potentially bioaccumulated and on the organism which takes up the contaminant and stores it in bodily tissues.

The main chemical factors that govern the transfer of halogenated organic contaminants in the food chain are, firstly, related to the hydrophobicity of the compounds, defined by the octanol–water partition coefficient (K_{ow}) and, secondly, to their bio-persistence or resistance to biotransformation. These two main properties are determined by precise structural characteristics such as

size, substitution position, and stereochemistry. Halogenated aromatic hydrocarbons are potentially bioaccumulated and PCBs are one of the best-known examples.

The biological factors that act on bioconcentration depend on the usual main biological functions, such as respiration, nutrition, and reproduction. The rate and extent of bioaccumulation rely on these functions because they determine how fast and how much of the contaminant will be taken up, and whether these compounds will be bioaccumulated or not. Obviously, these features vary for each individual species, according to the position of this organism in the trophic web, its feeding habits and its capacity to metabolize chemicals. Environmental factors and local situations may also partly modify these general biological processes.

In a more global view, chemical and biological characteristics do not seem to play the same role in the processes leading to bioaccumulation. The chemical properties, mainly hydrophobicity, determine whether a compound may be bioaccumulated. Then, according to its resistance to the biotransformation processes, the compound will be bioaccumulated. On the other hand, biological processes act on the dynamics of bioaccumulation. Therefore, chemical properties are of prime importance for bioconcentration, whereas biological factors act more on bioaccumulation and biomagnification.

Because of this, the relative importance of water and food as the main sources of contamination for aquatic organisms is sometimes questioned. Earlier experiments on bioconcentration probably over-emphasized the importance of water, partly because, in many cases, the experimental concentrations in water used were greater by far than those found in natural conditions. On the contrary, field studies have shown a more pronounced contribution from dietary uptake. Bioconcentration is the first step by which foreign compounds enter biological cycles and remains very important for low trophic level species. For higher organisms, this contribution becomes negligible, particularly for poorly soluble contaminants.

Modelling can help to differentiate the relative importance of each source of contamination. Bioaccumulation models have recently become very useful for describing the complexity of these interrelated mechanisms and for predicting the behaviour of chemicals in biota. However, a very precise and reliable model would require substantial information on the presence of chemicals and trophic interrelationships. Such a model would, in the end, have a limited predictive capacity because of very specific conditions related to the choice of species, the local situation, and the environment. A better compromise, that would probably be more useful for a first assessment of bioaccumulation, might be found in a less sophisticated predictive tool based on simplified characteristics, provided that the limits of these assumptions are kept well in mind. For this purpose, the octanol–water partition coefficient is essential for characterizing the contaminant. Biological factors should focus on the more important processes that lead to an increase of contaminants in the most sensitive species rather than an exhaustive investigation of all intermediary species.

Lastly, it is important to recall that, from the perspective of better protection for the marine environment from human activities, the understanding of bioaccumulation itself cannot be considered as the main objective. The main objective is, of course, the assessment of the ecological hazard of contaminants. To take into account both the toxicity and the behaviour of compounds in biota, a multidisciplinary approach is required. In this context, chemists might still have a decisive contribution to make, such as obtaining high quality data on contamination levels, identifying 'new' bioaccumulated substances, chemically characterizing these compounds (K_{ow} determination), and investigating biotransformation processes.

6 DEFINITIONS

Definitions of terms are from Rand and Petrocelli (1984).

Bioaccumulation: General term describing a process by which chemicals are taken up by aquatic organisms from water directly or through consumption of food containing the chemicals.

Bioavailable: Term used for the fraction of the total chemical in the surrounding environment which is available for uptake by organisms. The environment may include water, sediment, suspended particles, and food items.

Bioconcentration: A process by which there is a net accumulation of a chemical directly from water into an aquatic organism resulting from simultaneous uptake (e.g., by gill or epithelial tissue) and elimination.

Note. This definition of bioconcentration is related to an experimental determination with a few requirements: long exposure to the contaminant, equilibrium conditions, low concentrations in water, constant concentration of the contaminant in water during the test (Lyman *et al.*, 1982).

Bioconcentration factor (BCF): A unitless value describing the degree to which a chemical can be concentrated in the tissues of an organism in the aquatic environment. At apparent equilibrium during the uptake phase of a bioconcentration test, the BCF is the concentration of a chemical in one or more tissues of the aquatic organism divided by the average exposure concentration in the test.

Biomagnification: Result of the process of bioconcentration and bioaccumulation by which tissue concentrations of bioaccumulated chemicals increase as the chemical passes up through two or more trophic levels. The term implies an efficient transfer of chemical from food to consumer, so that residue concentrations increase systematically from one trophic level to the next.

Depuration: Elimination of a chemical from an organism by desorption, diffusion, excretion, egestion, biotransformation or another route. The depuration phase of a test is the period during which previously exposed organisms are held in uncontaminated water.

Octanol–water partition coefficient (K_{ow}): The ratio of a chemical's solubility in *n*-octanol and water at equilibrium; also expressed as P. The logarithm of K_{ow} or P (i.e., log K_{ow} or log P) is used as an indication of a chemical's propensity for bioconcentration by aquatic organisms.

Note. The octanol–water partition coefficient has become a key parameter in studies on the environmental fate of organic chemicals, as it is related to water solubility, soil/sediment adsorption, and bioconcentration. K_{ow} represents the tendency of the chemical to partition itself between an organic phase (fatty tissue, organic-rich soil, etc.) and an aqueous phase. Compounds with low K_{ow} values (less than 10) are considered to be soluble (hydrophilic), whereas hydrophobic compounds possess high K_{ow} (greater than 10^4). (after Lyman *et al.*, 1982)

Partitioning: Distribution of a chemical between two immiscible solvents. The partition coefficient (P) is the ratio of the chemical concentrations at equilibrium. Partition coefficients are commonly measured between *n*-octanol and water (K_{ow}).

Steady-state or dynamic equilibrium: The state at which the competing rates of uptake and elimination of a chemical within an organism or tissue are equal. An apparent steady state is reached when the concentration of a chemical in tissue remains essentially constant during a continuous exposure. Bioconcentration factors are usually measured at steady state.

Uptake: Transfer of a chemical into or onto an aquatic organism. The uptake phase of an accumulation test period during which test organisms are exposed to the chemical.

7 ACKNOWLEDGEMENT

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ANNEX 8

MAPPING OF HARMFUL EVENTS RELATED TO PHYTOPLANKTON BLOOMS IN ICES MEMBER COUNTRIES

The purpose of this mapping exercise was to obtain a global, geographical overview of harmful events related to phytoplankton blooms in the ICES area for the ten-year period 1987–1996. The work was carried out through the ICES/IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD) under the chairmanship of Dr P. Gentien (IFREMER, Brest, France). Data, contributed by WGHABD participants, was processed by C. Belin (IFREMER, Nantes, France) and the final maps were generated by B. Raffin (IFREMER, Nantes, France) using ArcInfo© software.

The maps indicate the presence of toxins or observations of animal/plant mortality if detected, regardless of the level of toxicity. The maps also show regular monitoring sites and give an indication of the frequency of harmful bloom events during the ten-year period. Seven different types of events were considered: amnesic shellfish poisoning (ASP); ciguatera fish poisoning (CFP); diarrhetic shellfish poisoning (DSP); neurotoxic shellfish poisoning (NSP); paralytic shellfish poisoning (PSP); other toxic effects, such as cyanobacterial toxin poisoning; and animal/plant mortality. Each type of event is presented in a separate figure.

Figure A8.1. ICES Member Countries in Europe are indicated by gray shading. Regular monitoring of bloom events is carried out in areas represented with a heavy black line.

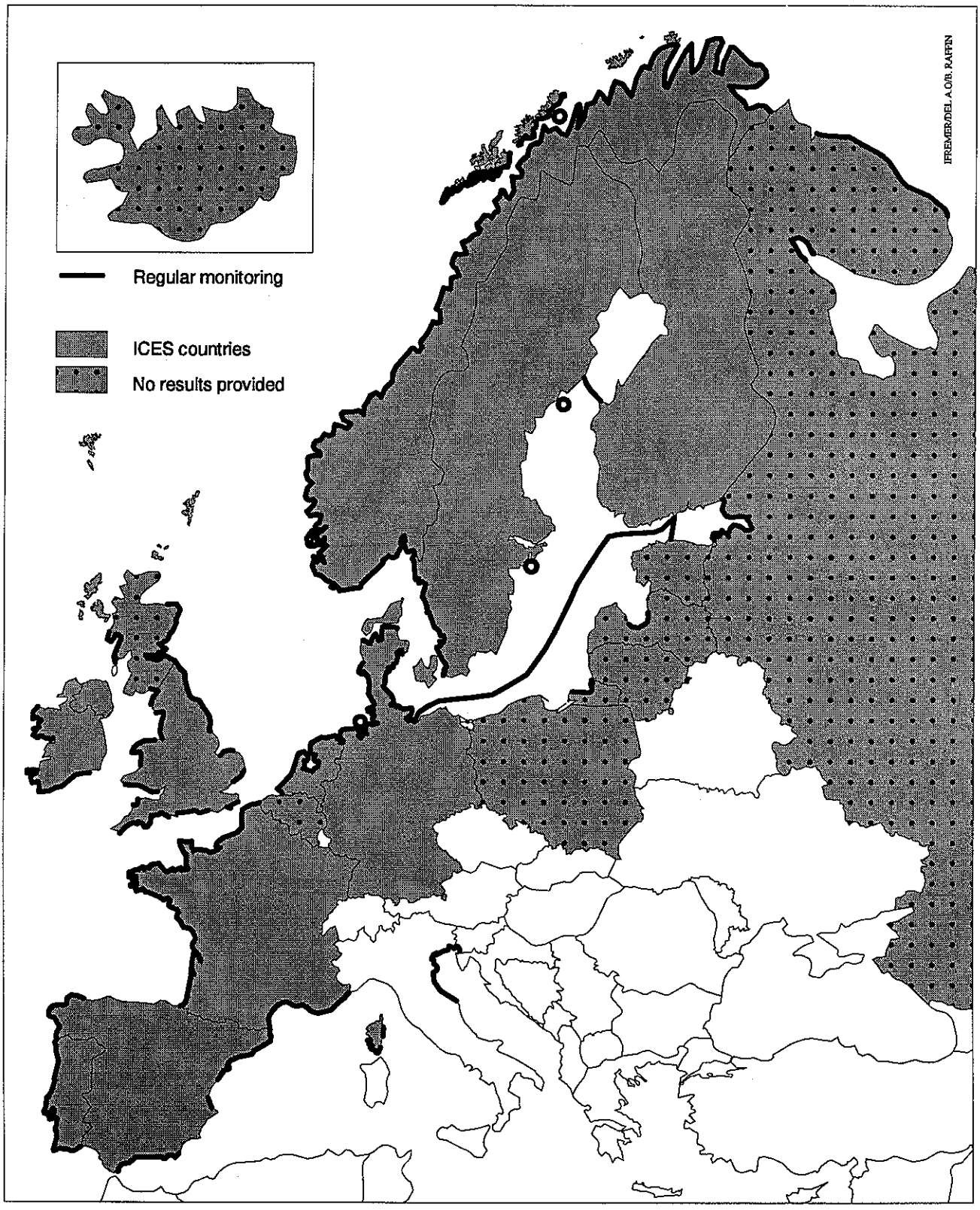


Figure A8.2. The occurrence and frequency of diarrhetic shellfish poisoning (DSP) events in Europe are indicated by the black circles.

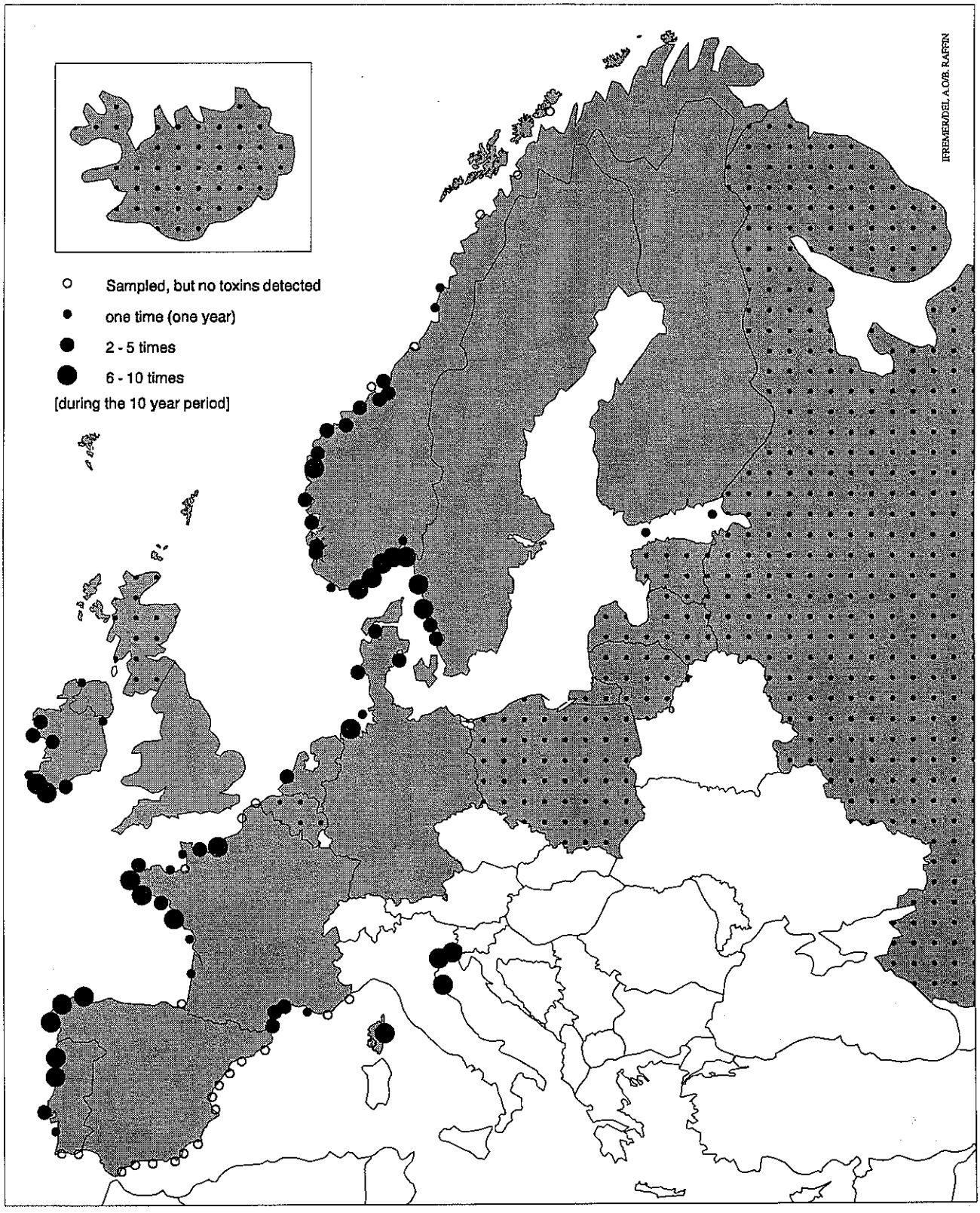


Figure A8.3. The occurrence and frequency of paralytic shellfish poisoning (PSP) events in Europe are indicated by the black circles.

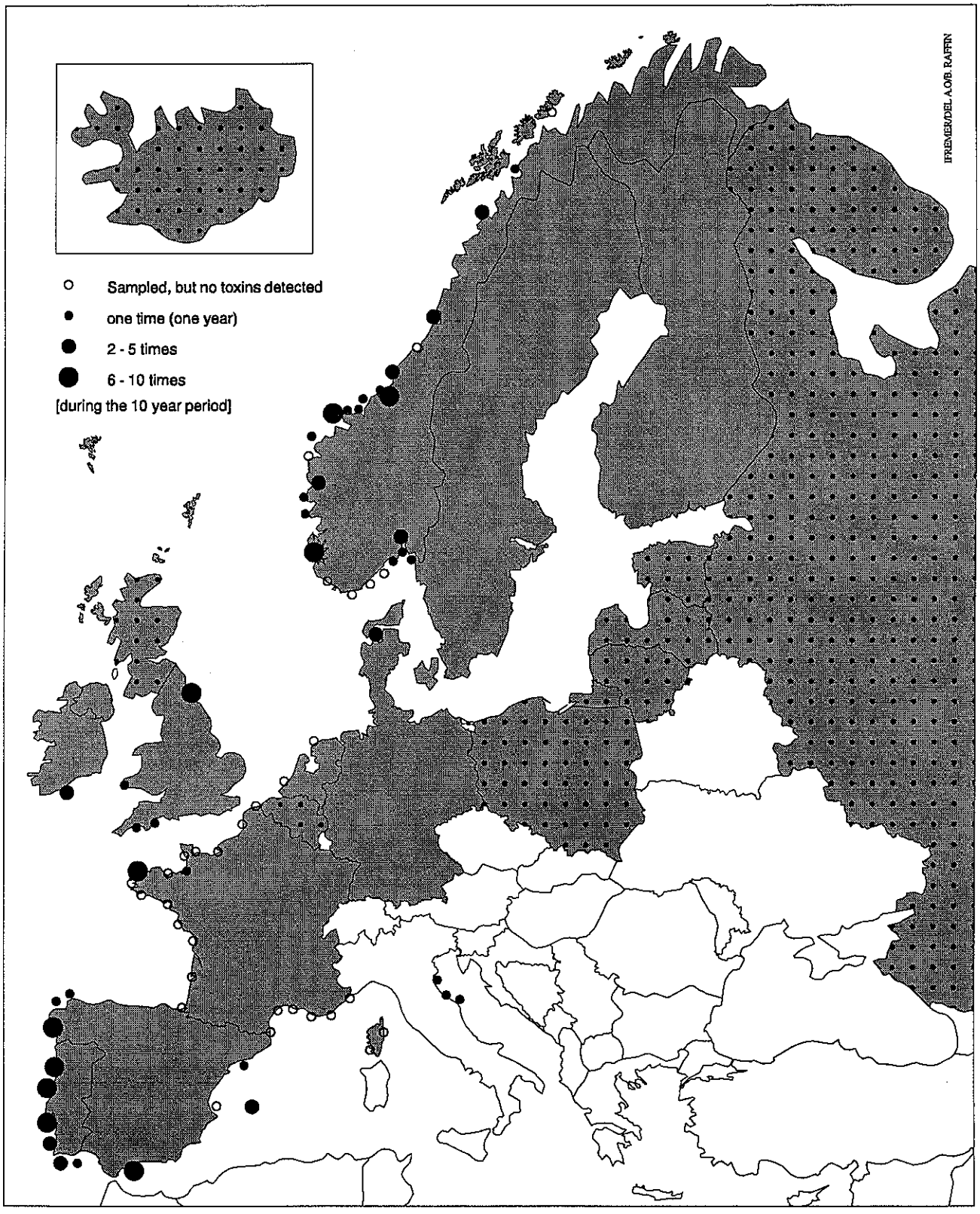


Figure A8.4. The occurrence and frequency of amnesic shellfish poisoning (ASP) events in Europe are indicated by the black circles.

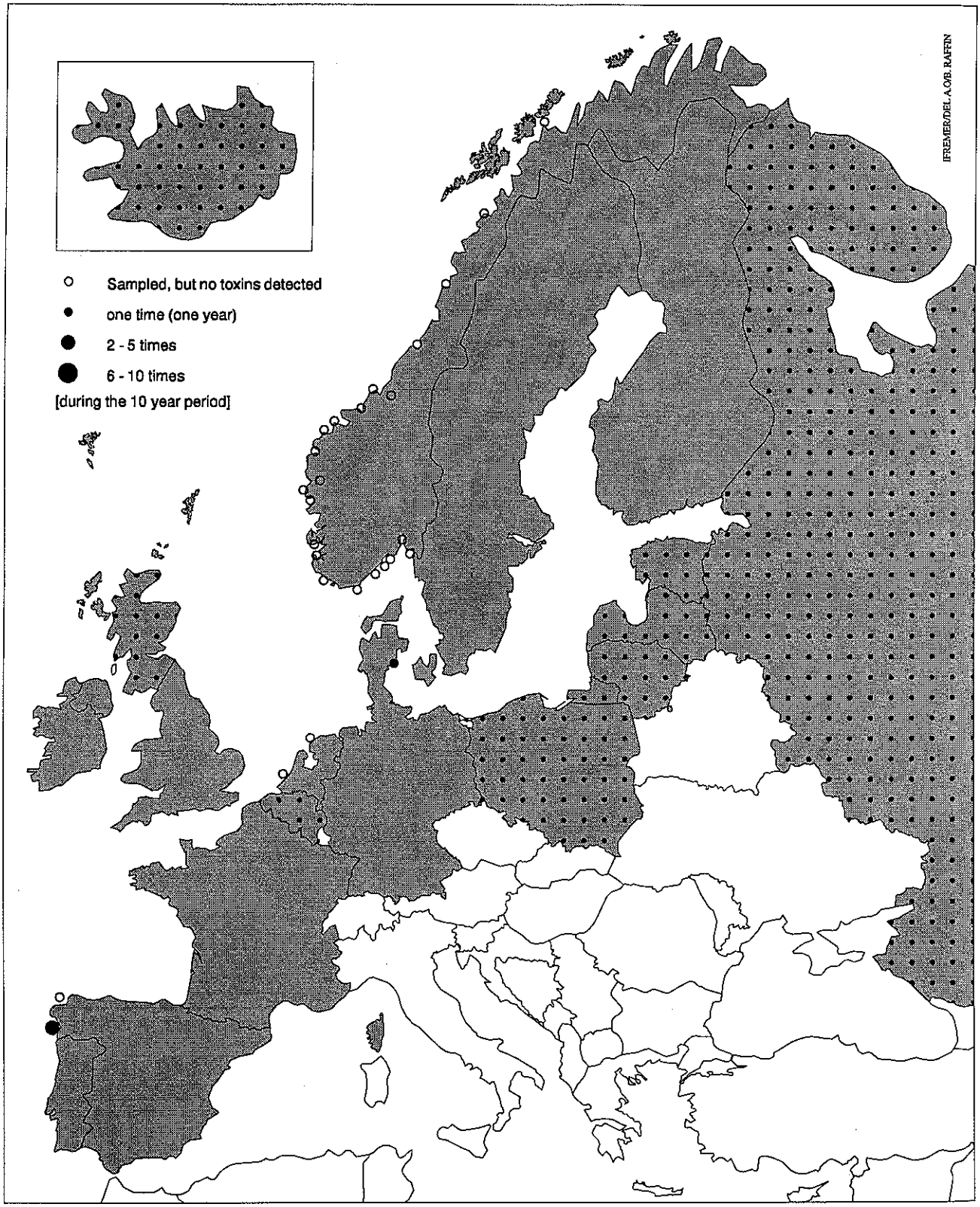


Figure A8.5. The occurrence and frequency of animal or plant mortality in Europe are indicated by the black circles.

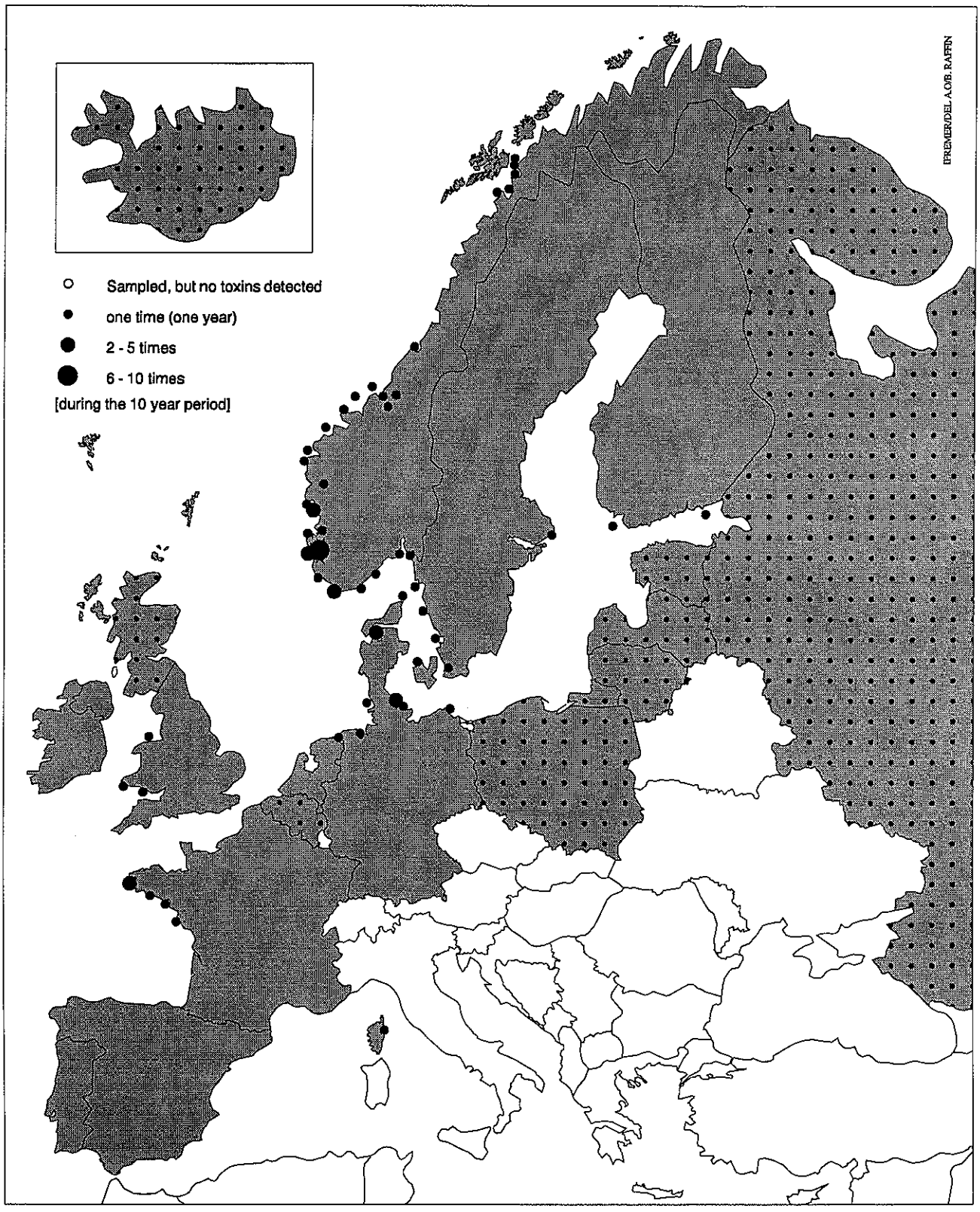


Figure A8.6. The occurrence and frequency of other toxic effects, such as cyanobacteria toxicity, in Europe are indicated by the black circles.

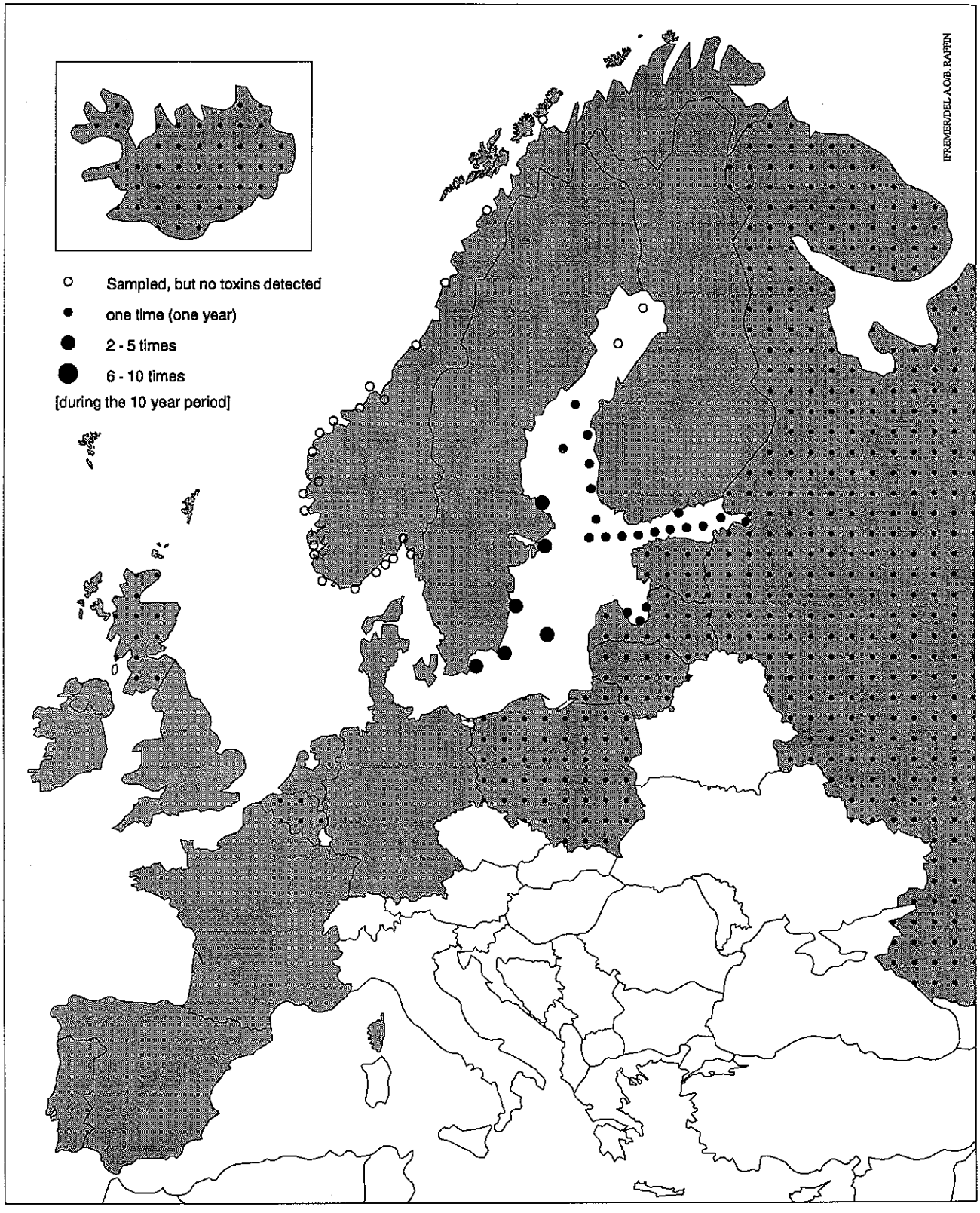


Figure A8.7. ICES Member Countries in North America are indicated by gray shading. Regular monitoring of bloom events is carried out in areas represented with a heavy black line.

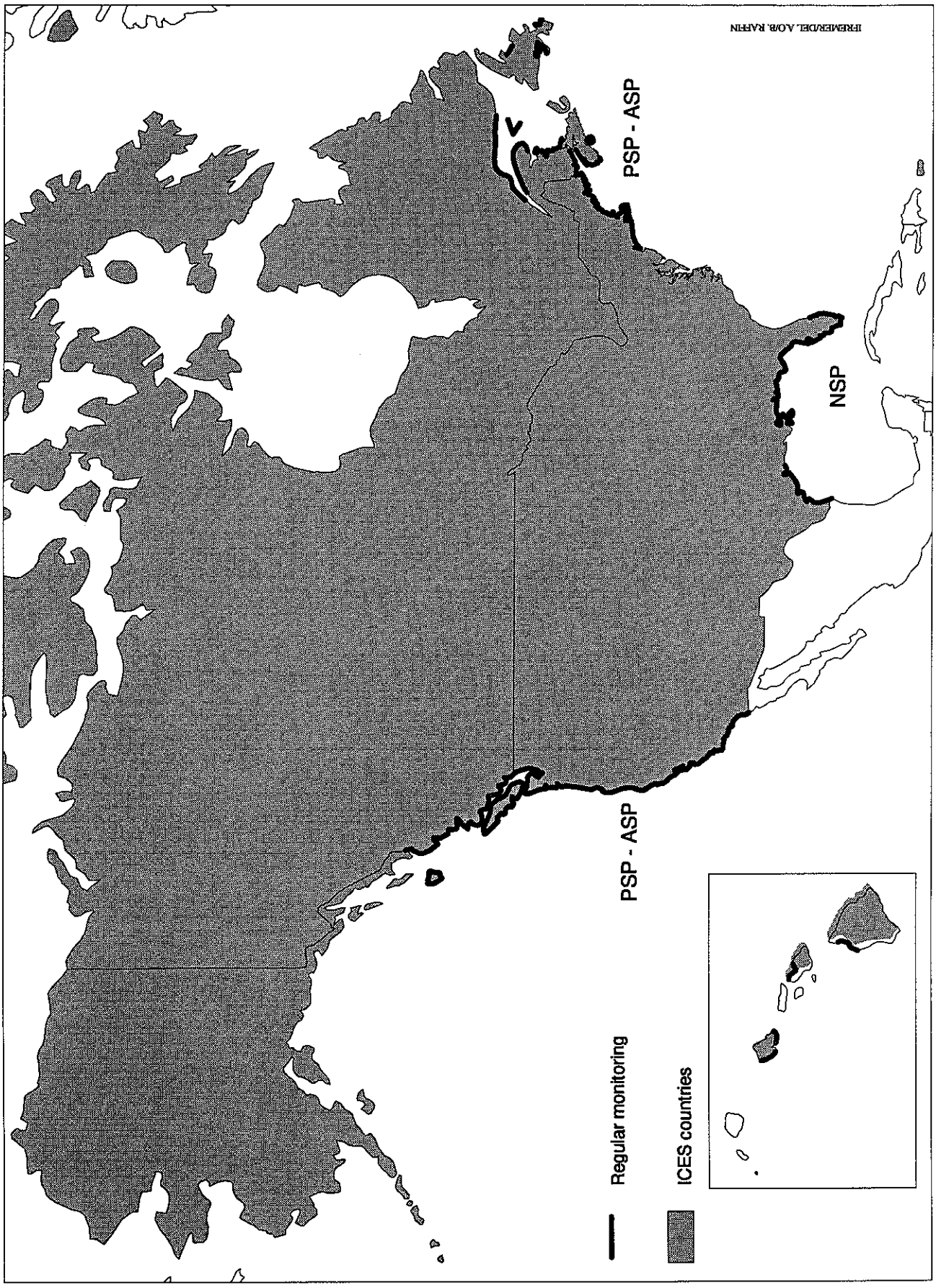


Figure A8.8. The occurrence and frequency of neurotoxic shellfish poisoning (NSP) events in North America are indicated by the black circles.

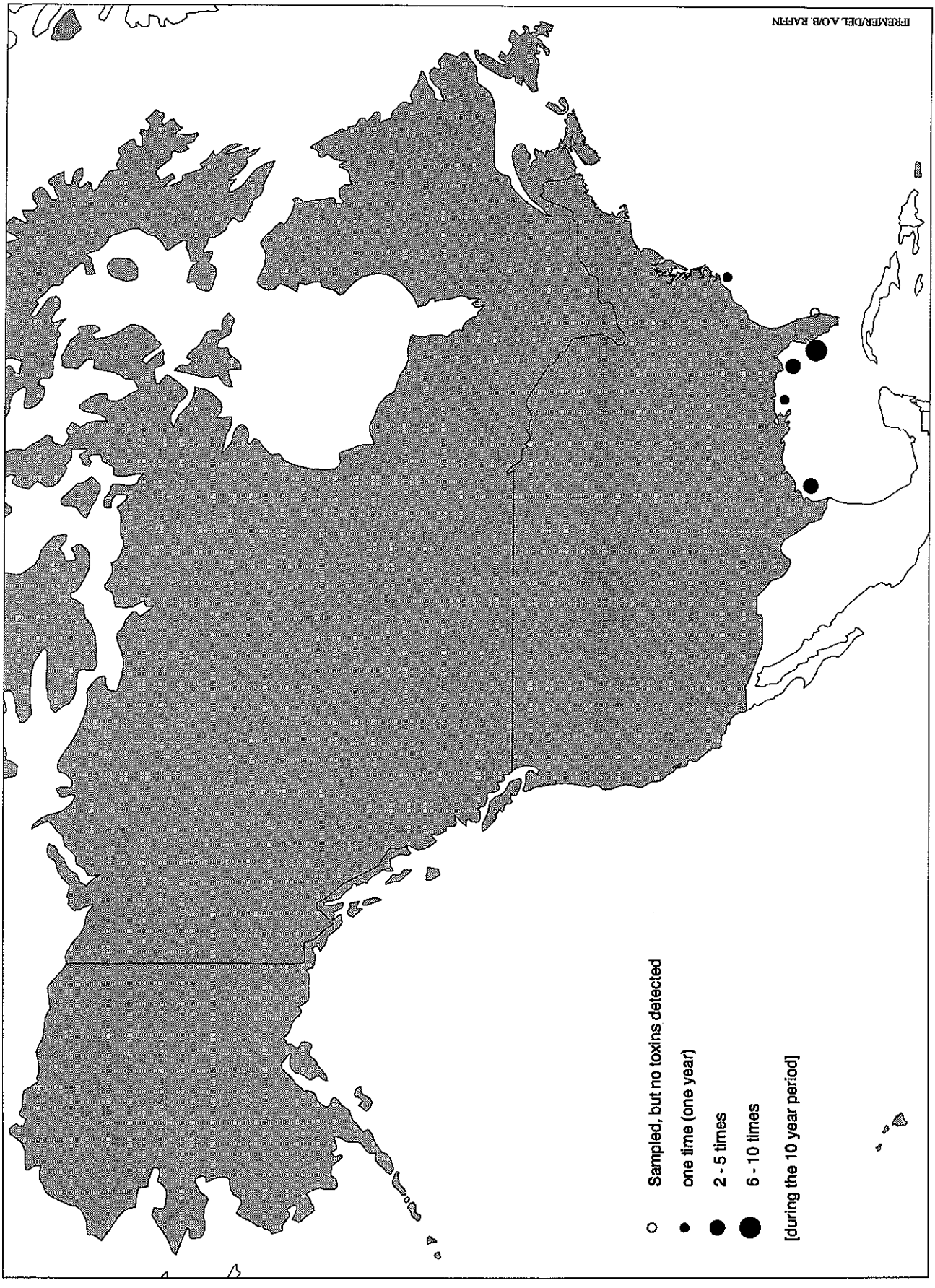


Figure A8.9. The occurrence and frequency of paralytic shellfish poisoning (PSP) events in North America are indicated by the black circles.

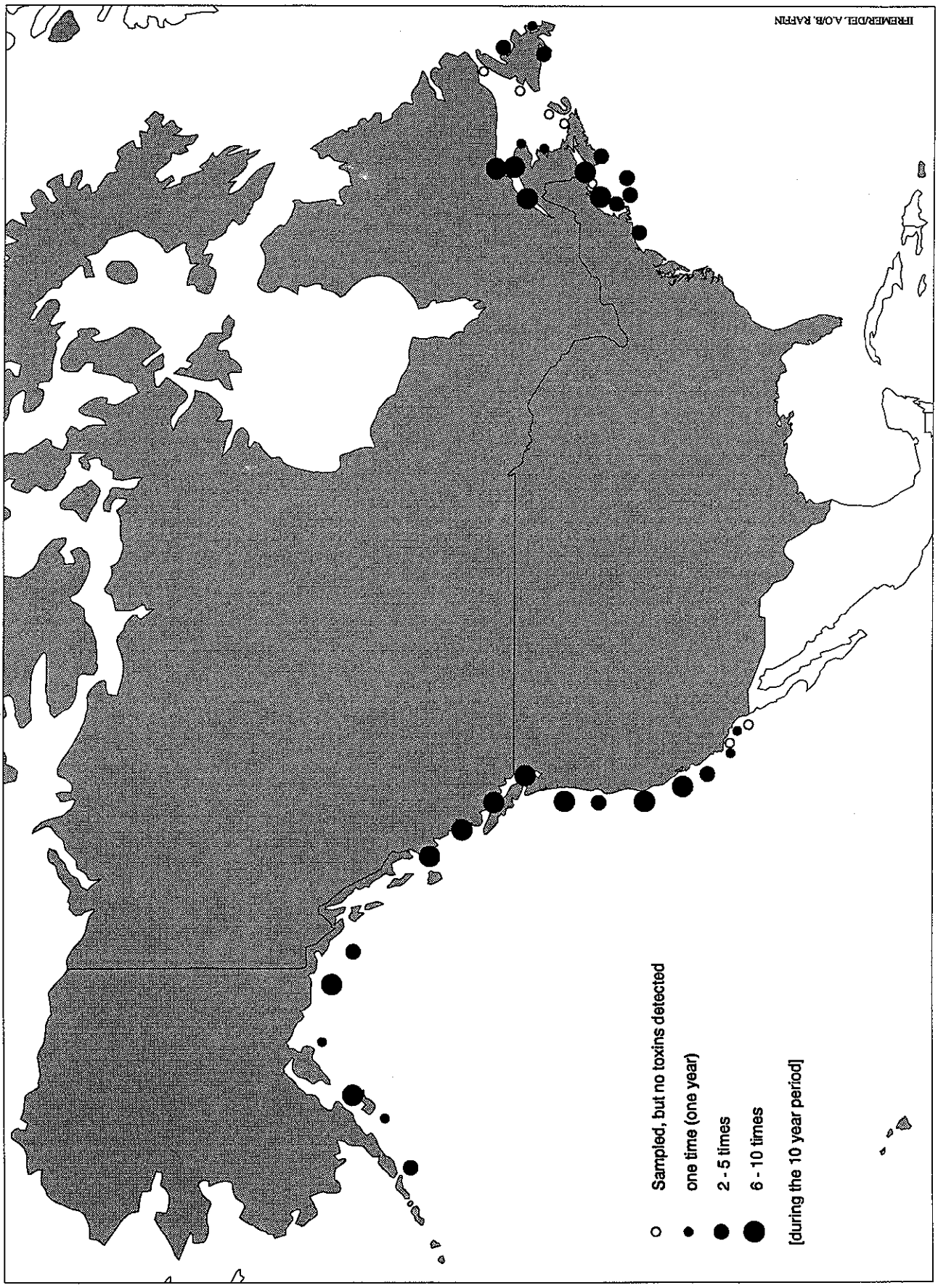


Figure A8.10. The occurrence and frequency of amnesic shellfish poisoning (ASP) events in North America are indicated by the black circles

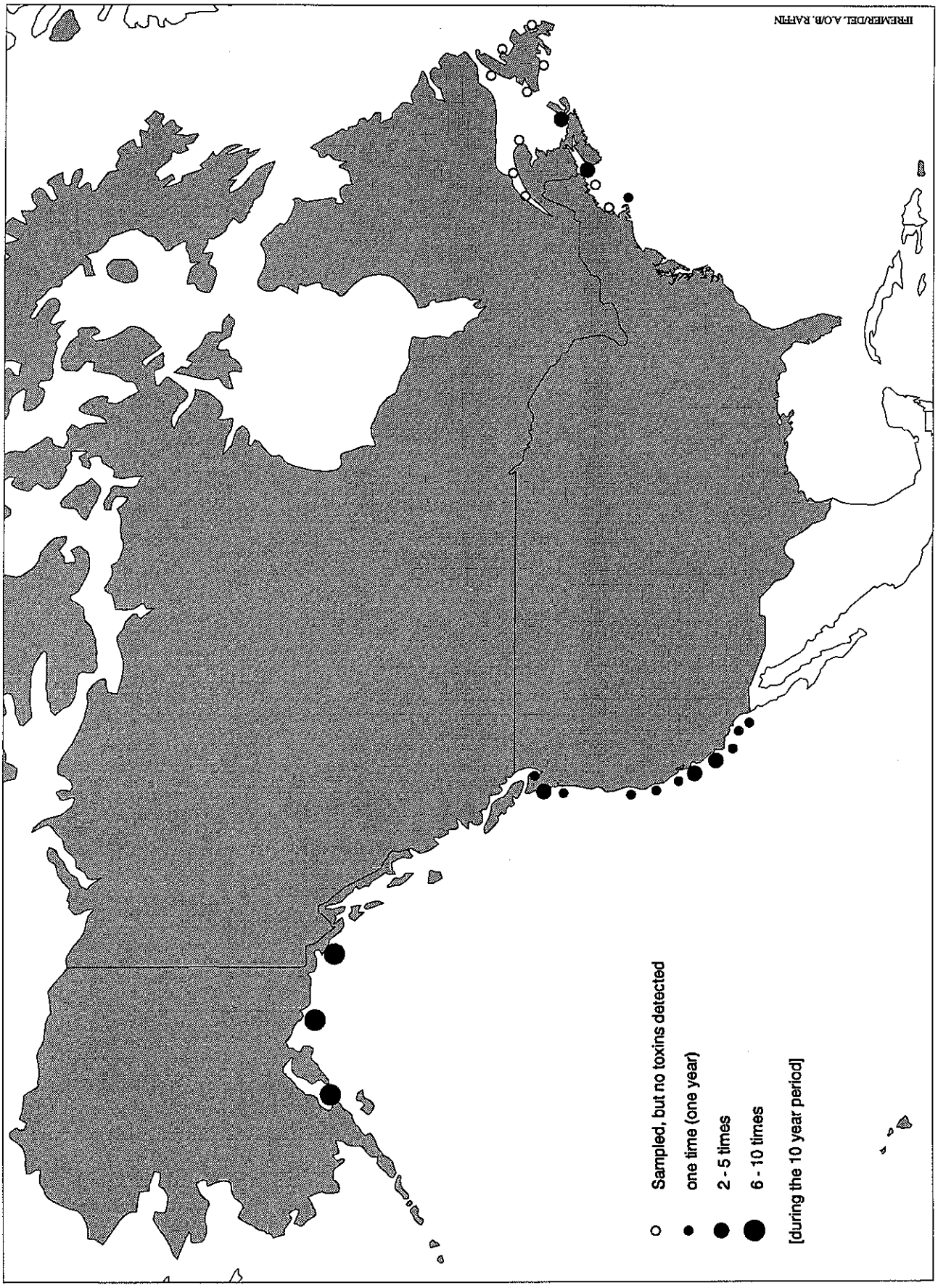


Figure A8.11. The occurrence and frequency of animal or plant mortality in North America are indicated by the black circles.

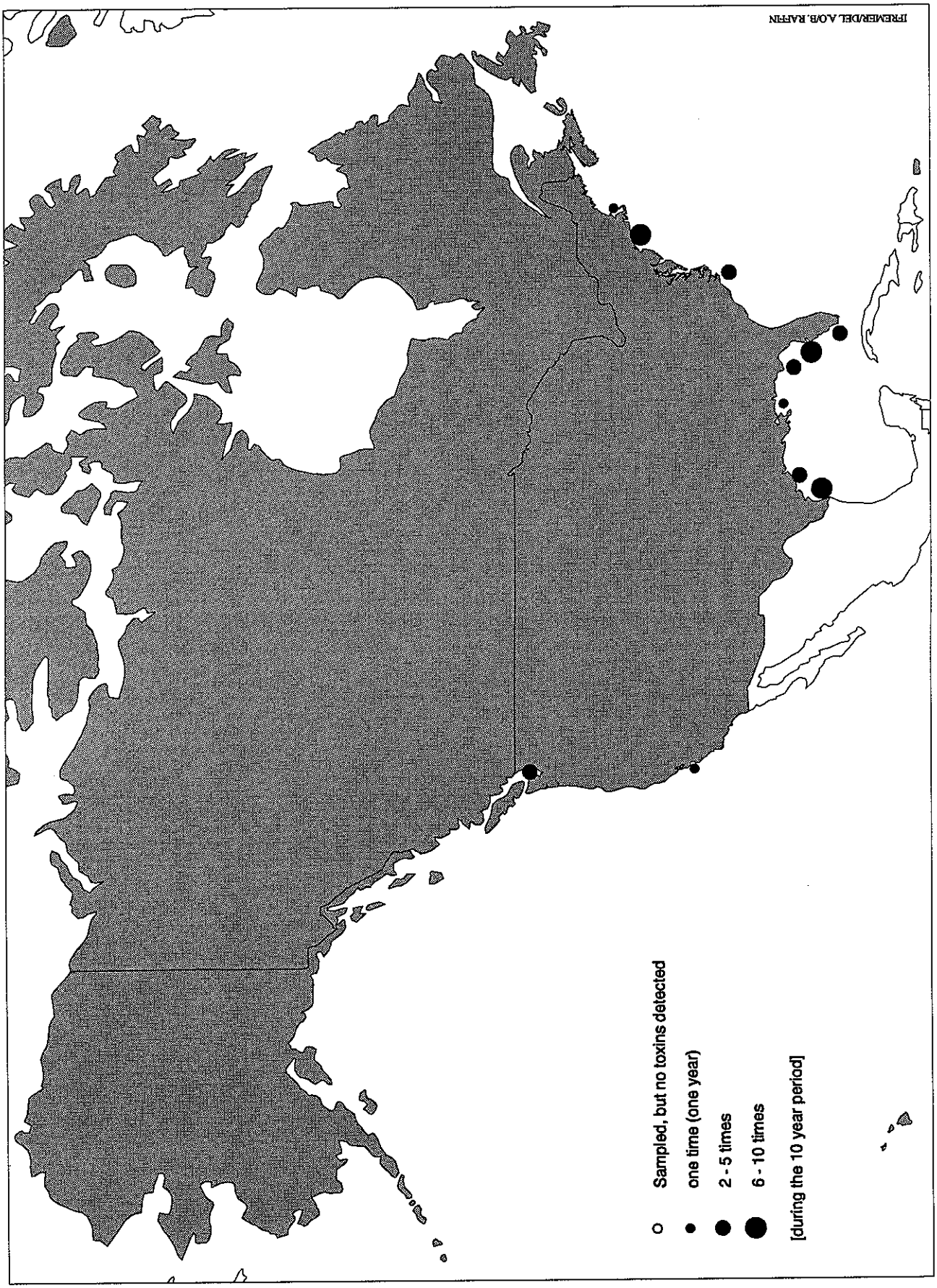
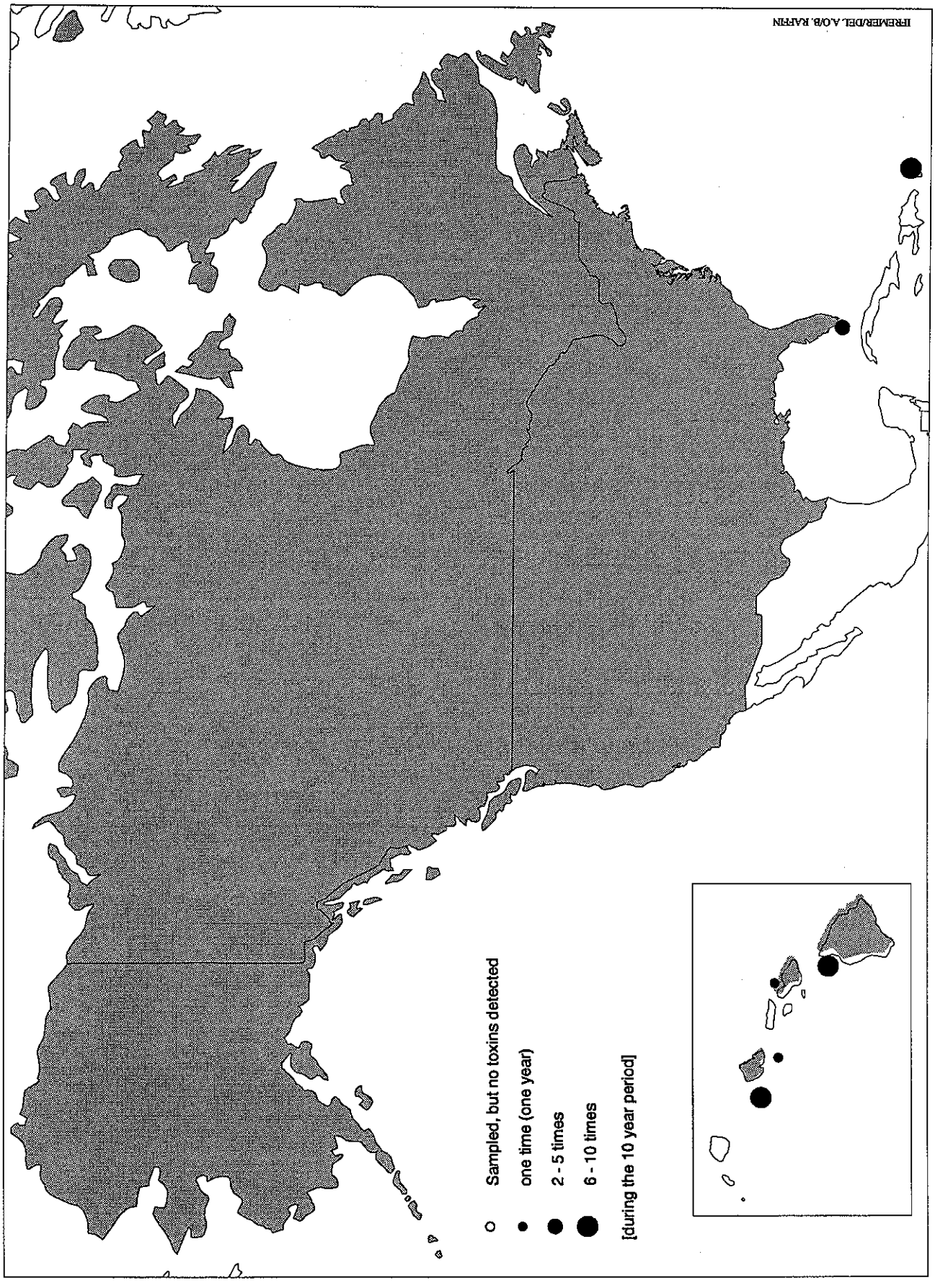


Figure A8.12. The occurrence and frequency of ciguatera fish poisoning (CFP) in North America are indicated by the black circles.



ANNEX 9

ICES SECRETARIAT COOPERATION WITH OSPAR ON EUTROPHICATION ISSUES

1 BACKGROUND

During the past 18 months, ICES has participated in various OSPAR meetings dealing with the development of a 'Common Procedure for Identification of the Eutrophication Status of the Maritime Area of the Oslo and Paris Conventions'. This development is now complete following its presentation to the April 1997 meeting of ASMO. However, work in connection with the quantitative assessment criteria for establishing eutrophication status is not yet complete. A continuing role of the ICES Secretariat may be expected as it is this aspect of the Common Procedure which has been the principle input by the Secretariat.

2 QUANTITATIVE CRITERIA

Progress in producing well-defined quantitative criteria has been slow, as much of the effort has been dominated by attempting to obtain more comprehensive data sets to provide the basis for common criteria across the whole OSPAR area. ICES has the most comprehensive nutrient data set compiled from conventional research activities as well as from data acquired during the Joint Monitoring Programme (JMP). However, a detailed review on behalf of OSPAR showed that in all but one of the OSPAR areas (Area II, North Sea) there are too few data in the ICES databanks to permit any kind of analysis. The situation is best for the classical nutrients nitrate+nitrite and phosphate, and very poor for other preferred parameters such as ammonia, total phosphorus, total nitrogen, and total carbon. In an attempt to address this problem, OSPAR made special pleas for additional data, but this yielded a disappointing response, especially for the OSPAR areas outside of the North Sea.

The quantitative assessment criteria which have been included in the Common Procedure depend on the classification of the existing eutrophication status (e.g., Problem, Non-Problem, and Potential Problem areas) and include:

a) nutrients, especially with respect to reference concentrations at defined salinity levels;

b) N:P ratio, relative to 16 (Redfield ratio);

c) chlorophyll *a*, relative to historical values—average from growth season;

d) oxygen relative to a threshold (in % saturation);

e) phytoplankton—various diatoms and dinoflagellates identified at nuisance and toxic concentrations.

Currently, only nutrient data are available in sufficient quantities to permit an attempt at elaborating criteria, and it is this aspect that has provided the main input from the ICES Oceanographic Data Centre. This work is now at the stage where efforts are being made to establish time series of average nutrient levels based on statistical analysis of the normalized relationship between salinity and nutrients. Because of natural environmental variability, analyses based on normalization provide the only chance for determining the status of nutrients in various areas. However, even this is of limited use because linear relationships between salinity and nutrients are not present across the entire OSPAR area. Such a relationship is, for example, absent in the northeastern North Sea and the Skagerrak/Kattegat.

2.1 Examples of Application of Criteria

During the course of the preparation for the various OSPAR meetings, many products were produced by the ICES Oceanographic Data Centre in an attempt to establish satisfactory ways for demonstrating quantitatively the extent of nutrient elevation and associated time trends. The most satisfactory of these were those analyses based on the nutrient/salinity relationships, a technique already frequently used by Dutch and Belgian groups in particular. Figures A9.1 and A9.2 illustrate the kind of information that can be teased out of the data as a result of normalization against salinity. These show time trends for phosphate (Figure A9.1) and nitrate + nitrite (Figure A9.2) calculated at a salinity of 0 for each winter period (January–March) from 1980–1996. The time series have been smoothed by taking three-year running averages.

These figures, which are based on all data for the three indicated areas of the North Sea, show trends which are very similar to those occurring in the principle rivers/estuaries in these areas (results not shown), confirming the robustness of this technique. However, the trends indicative of the east England area show very irregular behaviour, which points to a shortcoming in the data. This shortcoming is related to the lack of a regular monitoring scheme in this particular area.

3 CONCLUSIONS

It appears that the databases to satisfy the needs of those aspects of the Quantitative Assessment Criteria concerned with nutrient concentrations are currently inadequate in scope and scale across the OSPAR area, apart from the southern and eastern North Sea. This is in spite of the fact that the existing databases are relatively comprehensive and are of long duration. OSPAR is now

extending its efforts to the eutrophication effect parameters, possibly prompted by ICES advice on the need to monitor the consequences of nutrient fluxes rather than nutrient concentrations. It might be expected that similar effects of natural variability on the effect parameters, for which the databases are likely to be small for years to come, will lead to difficulties of interpretation unless measurement efforts beyond the scale presently under way for nutrient monitoring are implemented. This is clearly going to be impractical unless advanced methods of interpretation are developed, for example, improving data assimilation techniques into ecosystem models which in themselves also need to be improved.

Figure A9.1. Phosphate (µmol l^{-1}) at 0 salinity from 1980 to 1996.

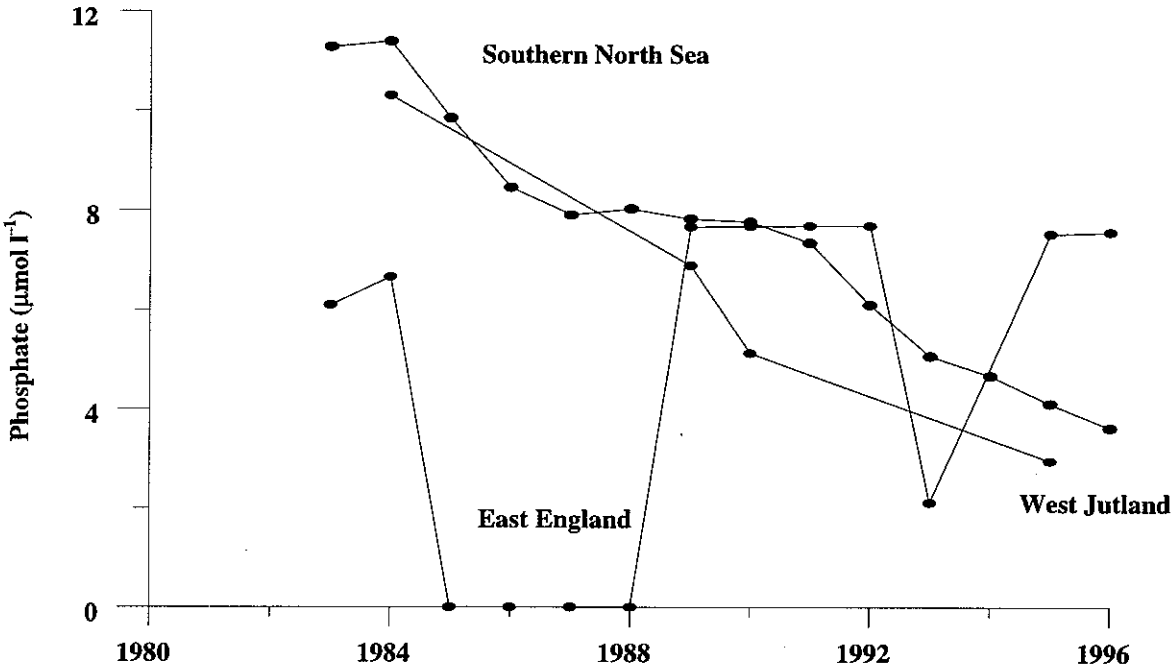
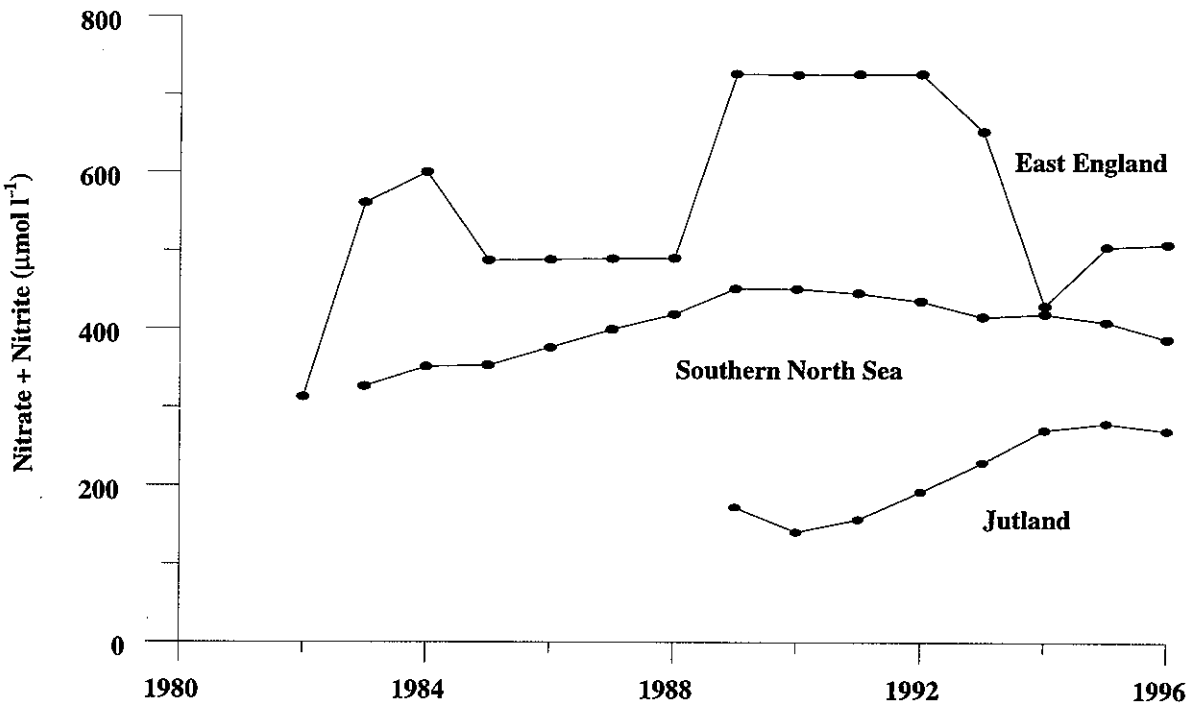


Figure A9.2. Nitrate + Nitrite (µmol l^{-1}) at 0 salinity from 1980 to 1996.



ANNEX 10

OVERVIEW OF INTERCALIBRATION/INTERCOMPARISON EXERCISES ON CHEMICAL ANALYSES COORDINATED BY ICES

TRACE METALS IN BIOTA

First ICES Intercalibration Exercise on Trace Metals in Biological Tissue
(1/TM/BT) 1972

Coordinator	:	G. Topping, United Kingdom.
Sample	:	Fish flour prepared from commercial fish meal.
Metals analysed	:	Hg, Cu, Zn, Cd and Pb.
Participation	:	8 laboratories from 7 countries around the North Sea.

Results published in *Cooperative Research Report* No. 80 (1978).

Second ICES Intercalibration Exercise on Trace Metals in Biological Tissue
(2/TM/BT) 1973

Coordinator	:	G. Topping, United Kingdom.
Samples	:	Fish flour prepared from unskinned muscle of inshore cod and acidified solution of metals.
Metals analysed	:	Hg, Cu, Zn, Cd and Pb.
Participation	:	15 laboratories in 11 countries around the North Sea and the Baltic Sea.

Results published for Baltic Sea laboratories in *Cooperative Research Report* No. 63 (1977) and for North Sea laboratories in *Cooperative Research Report* No. 80 (1978).

Third ICES Intercalibration Exercise on Trace Metals in Biological Tissue
(3/TM/BT) 1975

Coordinator	:	G. Topping, United Kingdom.
Samples	:	(a) Fish flour prepared from skinned muscle of distant water cod, and (b) individual reference standard solutions for each metal.
Metals analysed	:	Hg, Cu, Zn, Cd and Pb.
Participation	:	29 laboratories in 17 ICES Member Countries.

Results published for Baltic Sea laboratories in *Cooperative Research Report* No. 63 (1977) and for North Sea laboratories in *Cooperative Research Report* No. 80 (1978).

Fourth ICES Intercalibration Exercise on Trace Metals in Biological Tissue
(4/TM/BT) 1977

Coordinator	:	G. Topping, United Kingdom.
Samples	:	Same fish flour as in 3/TM/BT.
Metals analysed	:	Cd and Pb.
Participation	:	12 of the laboratories which had participated in 3/TM/BT.

Results published in *Cooperative Research Report* No. 108 (1981).

Fifth ICES Intercalibration Exercise on Trace Metals in Biological Tissue
(5/TM/BT) 1978

Coordinator	:	G. Topping, United Kingdom.
Samples	:	(a) Fish flour prepared from skinned muscle of distant water cod, and (b) the same fish flour extracted to produce a lower Hg concentration.
Metals analysed	:	Hg, Cu, Zn, Cd and Pb.
Participation	:	41 laboratories, including those associated with the Joint Monitoring Programme, from all 18 ICES Member Countries plus several laboratories in Australia.

Results published in *Cooperative Research Report* No. 108 (1981).

Sixth ICES Intercalibration Exercise on Trace Metals in Biological Tissue
(6/TM/BT) 1979

Coordinator	:	G. Topping, United Kingdom.
Samples	:	(a) White meat of edible crab freeze-dried and ground into powder, (b) commercial fish meal freeze-dried and ground into powder, and (c) digestive gland of Canadian lobster treated and ground into powder.
Metals analysed	:	Hg, Cu, Zn, Cd and Pb.
Participation	:	52 laboratories from 17 ICES Member Countries plus Australia.

Results published in *Cooperative Research Report* No. 111 (1981).

Seventh ICES Intercalibration Exercise on Trace Metals in Biological Tissue – Part 1
(7/TM/BT-1) 1983

Coordinators	:	S.S. Berman and V.J. Boyko, Canada.
Samples	:	(a) Lobster hepatopancreas homogenized, spray-dried and acetone extracted, (b) scallop adductor muscle freeze-dried and ground, and (c) plaice muscle freeze-dried and ground.
Metals analysed	:	Hg, Cu, Zn, Cd, As and Pb.
Participation	:	51 laboratories from 17 ICES Member Countries.

Results published in *Cooperative Research Report* No. 138 (1986).

Seventh ICES Intercalibration Exercise on Trace Metals in Biological Tissue – Part 2
(7/TM/BT-2) 1985

Coordinators	:	S.S. Berman and V.J. Boyko, Canada.
Samples	:	(a) Cod liver, acetone-extracted and freeze dried, (b) dogfish muscle, acetone-extracted and freeze dried, (c) dogfish liver, acetone-extracted and freeze dried, (d) whole dogfish, spray-dried, and (e) *Mytilus edulis* soft material, freeze dried.
Metals analysed	:	Hg, Cu, Zn, Cd, As and Pb.
Participation	:	49 laboratories from 16 ICES Member Countries.

Results published in *ICES Cooperative Research Report* No. 189 (1992).

TRACE METALS IN SEA WATER

First ICES Intercalibration Exercise for Trace Metals in Sea Water
(1/TM/SW) 1976

Coordinator	:	P.G.W. Jones, United Kingdom.
Samples	:	Two standard solutions of metals.
Metals analysed	:	Hg, Pb, Ni, Co, Fe, Cr, Cu, Cd, Zn and Mn.
Participation	:	41 laboratories from 14 ICES Member Countries.

Results published in *Cooperative Research Report* No. 125 (1983).

Second ICES Intercalibration Exercise for Trace Metals in Sea Water
(2/TM/SW) 1976

Coordinator	:	J. Olafsson, Iceland.
Samples	:	Two natural sea water samples and a mercury-spiked sea water sample, all acidified.
Metal analysed	:	Hg
Participation	:	14 laboratories from 10 ICES Member Countries.

Results published in *Cooperative Research Report* No. 125 (1983).

Third ICES Intercalibration Exercise for Trace Metals in Sea Water
(3/TM/SW) 1977

Coordinator	:	P.G.W. Jones, United Kingdom.
Samples	:	Two frozen samples of filtered sea water, one from open North Sea waters and one from coastal waters.
Metals analysed	:	Co, Fe, Ni, Pb, Cd, Cr, Cu, Mn, and Zn.
Participation	:	49 laboratories from 14 ICES Member Countries.

Results published in *Cooperative Research Report* No. 125 (1983).

Fourth ICES Intercalibration Exercise for Trace Metals in Sea Water
(4/TM/SW) 1978

Coordinators	:	J.M. Bewers, J. Dalziel, P.A. Yeats, and J.L. Barron, Canada.
Samples	:	Sets of six sea water samples consisting of four replicate sea water samples, one sample spiked with relevant metals and one dummy. Samples were frozen and acidified.
Metals analysed	:	Cd, Cu, Mn, Fe, Ni, Pb, and Zn.
Participation	:	43 laboratories from 13 ICES Member Countries plus Monaco.

Results published in *Cooperative Research Report* No. 105 (1981).

ICES/JMG Intercalibration for Mercury in Sea Water
(ICES/JMG/1/HG/SW) 1979

Coordinator	:	J. Olafsson, Iceland.
Samples	:	(a) Two samples of natural sea water, (b) sea water with a low Hg spike, and (c) sea water with a high Hg spike.
Participation	:	36 laboratories from all 13 Member Countries of the Oslo and Paris Commissions plus Canada, Japan, and the United States.

Results published in *Cooperative Research Report* No. 110 (1981).

ICES/JMG Intercalibration for Cadmium in Sea Water
(ICES/JMG/1/CD/SW) 1979

Coordinator	:	Y. Thibaud, France.
Samples	:	(a) Natural sea water, (b) sea water with a low Cd spike, and (c) sea water with a high Cd spike.
Participation	:	33 laboratories from all 13 Member Countries of the Oslo and Paris Commissions plus Canada and Monaco.

Results published in *Cooperative Research Report* No. 110 (1981).

Fifth ICES Intercalibration Exercise for Trace Metals in Sea Water
(5/TM/SW:3 and 5/TM/SW:4) 1982–1983

Coordinators	:	J.M. Bewers, P.A. Yeats, S.S. Berman, D. Cossa, Canada; C Alzieu, P. Courau, France.
Samples	:	(a) Sea water samples, filtered and acidified, for analysis of metals except Hg, and (b) sea water samples, natural and spiked, for analysis of Hg. In addition, 6 laboratories participated in an intercomparison of filtration procedures for coastal sea water samples.
Metals analysed	:	(a) Cd, Cu, Pb, Zn, Ni, Fe, Mn. (b) Hg.
Participation	:	59 laboratories from 15 ICES Member Countries plus Monaco.

Results published in *Cooperative Research Report* No. 136 (1986).

ICES Sixth Round Intercalibration for Trace Metals in Estuarine (Sea) Water
(ICES/JMG/6/TM/SW) 1986

Coordinators	:	S. Berman, Canada; A. Jensen, Denmark; W. Cofino, The Netherlands.
Samples	:	(a) Sea water samples, filtered and acidified, for analysis of metals except Hg (salinity ca. 12), (b) sea water samples, filtered and acidified, for analysis of metals except Hg (salinity ca. 28), and (c) sea water samples, filtered and acidified, for analysis of Hg (salinity ca. 12).
Elements analysed	:	Cd, Cu, Hg, Zn.
Participation	:	43 laboratories from 14 ICES Member Countries plus Italy.

Results published in *Cooperative Research Report* No. 152 (1988).

Seventh ICES Intercomparison Exercise on the Analysis of Trace Metals in Sea Water
(7/TM/SW) 1996

Coordinators	:	B. Pedersen, Denmark; S. Berman, Canada.
Samples	:	(a) Natural brackish water samples (salinity 8), (b) natural sea water samples (salinity 25).
Elements analysed	:	As, Cd, Co, Cr, Fe, Mn, Ni, Pb, Zn.
Participation	:	39 laboratories from 12 ICES Member Countries plus three other countries.

Results will be published in the *ICES Cooperative Research Report* series.

TRACE METALS IN MARINE SEDIMENTS

First ICES Intercalibration Exercise for Trace Metals in Marine Sediments
(1/TM/MS) 1984

Coordinator	:	D.H. Loring, Canada.
Samples	:	(a) Estuarine calcareous sandy mud sediment, (b) harbour sediment, and (c) Baltic mud sediment "MBSS" (from Baltic Sediment Intercalibration Exercise).
Metals analysed	:	Cd, Cr, Cu, Ni, Pb and Zn.

Optional metals : Ti, Fe, Mn and Al.
Participation : 40 laboratories from 11 ICES Member Countries.

Results published in *Cooperative Research Report* No. 143 (1987).

Baltic Sediment Intercalibration Exercise–Step 1
(Reference Samples ABSS and MBSS) 1983

Coordinators : L. Brügmann, German Democratic Republic, and L. Niemistö, Finland.
Samples : Two mud sediments ("ABSS" and "MBSS") from different locations, dried and homogenized.
Analytes : Cu, Pb, Zn, Cd, Mn, Fe, Cr, Ni, and organic C.
Optional : Hg, Co, Al, inorganic C, P and N.
Participation : 42 laboratories from 15 ICES Member Countries.

Additional Exercise on Hg and Cd, 1985.

Coordinator : A. Jensen, Denmark.
Samples : Six samples, some of which were pre-treated.
Metals analysed : Hg and Cd.
Participation : 8 (Hg) and 10 (Cd) laboratories from 6 countries around the Baltic Sea.

Baltic Sediment Intercalibration Exercise–Step 2
(Sliced Wet Cores) 1984

Coordinators : L. Brügmann, German Democratic Republic, L. Niemistö, Finland, and P. Pheiffer Madsen, Denmark.
Samples : 20 cm cores, sliced into 1-cm slices and deep frozen.
Main analytes : Cu, Cr, Zn, Pb, Mn, Cd, Fe, Ni, Al, Co, Hg, dry matter content, dating by Pb-210 technique.
Optional : Cs-137, organic C, N, P, clay minerals.
Participation : 11 laboratories from 6 countries around the Baltic Sea.

Results for the entire exercise published in *Cooperative Research Report* No. 147 (1987).

TRACE METALS IN SUSPENDED PARTICULATE MATTER

First ICES Intercomparison Exercise for Trace Metals in Suspended Particulate Matter
(1/TM/SM) 1984

Coordinators : P. Yeats and J.A. Dalziel, Canada.
Samples : Suspended particulate matter collected on pre-weighed 0.4 mm Nuclepore filters.
Analytes : Al, Fe, Mn, Zn, Cu, Pb, Ni, and Cd.
Participation : 8 selected laboratories from 7 countries.

Results published in *J. Cons. int. Explor. Mer*, 43: 272-278 (1987).

Second ICES Intercomparison Exercise for Trace Metals in Suspended Particulate Matter–Phase 1
(2/TM/SM-1) 1989

Coordinators : H. Hovind and J. Skei, Norway.
Samples : Standard reference materials from the National Research Council of Canada:
(a) PACS-1,
(b) MESS-1, and
(c) BCSS-1, from which participants should weigh out 1, 3, and 5 mg samples for analysis.
Analytes : Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn.
Participation : 19 laboratories from 11 countries.

Results published in *ICES Cooperative Research Report* No. 184 (1992).

Second ICES Intercomparison Exercise for Trace Metals in Suspended Particulate Matter–Phase 2
(2/TM/SM-2) 1993

Coordinator	:	C. Pohl, Germany.
Samples	:	Suspended particulate matter collected on pre-weighed 0.4 mm Nuclepore filters.
Analytes	:	Al, Cd, Co, Cu, Fe, Li, Mn, Ni, Pb, Zn.
Participation	:	15 laboratories from 10 countries.

Results published in *Accreditation and Quality Assurance*, 2: 2–10 (1997).

ORGANOCHLORINES IN BIOLOGICAL TISSUE

First ICES Intercalibration Exercise for Organochlorine Residues in Biological Tissue
(1/OC/BT) 1972

Coordinator	:	A.V. Holden, United Kingdom.
Samples	:	(a) Natural fish oil, and (b) same fish oil spiked with selected organochlorines.
Analytes	:	pp'-TDE, pp'-DDE, pp'-DDT, PCBs, dieldrin, γ-HCH
Participation	:	9 laboratories from 7 ICES Member Countries.

Results published in *Cooperative Research Report* No. 80 (1978).

Second ICES Intercalibration Exercise for Organochlorine Residues in Biological Tissue
(2/OC/BT) 1974

Coordinator	:	A.V. Holden, United Kingdom.
Samples	:	(a) unspiked maize oil, and (b) same maize oil spiked with selected organochlorines.
Analytes	:	pp'-TDE, pp'-DDE, pp'-DDT, PCBs, dieldrin, γ-HCH
Participation	:	30 laboratories from 13 ICES Member Countries.

Results published in *Cooperative Research Report* No. 80 (1978) and, for Baltic laboratories, in *Cooperative Research Report* No. 63 (1977).

Third ICES Intercalibration Exercise for Organochlorine Residues in Biological Tissue
(3/OC/BT) 1978

Coordinator	:	A.V. Holden, United Kingdom.
Sample	:	Fish oil (capelin).
Analytes	:	pp'-TDE, pp'-DDE, pp'-DDT, PCBs, dieldrin, α-HCH, γ-HCH.
Participation	:	30 laboratories from 16 ICES Member Countries.

Results published in *Cooperative Research Report* No. 108 (1978).

Fourth ICES Intercalibration Exercise for Organochlorine Residues in Biological Tissue
(4/OC/BT) 1979

Coordinators	:	J.F. Uthe and C.J. Musial, Canada.
Samples	:	(a) Fish oil prepared from herring muscle tissue and (b) same oil spiked with PCBs.
Analytes	:	PCBs
Participation	:	23 laboratories from 12 ICES Member Countries.

Results published in *Cooperative Research Report* No. 115 (1982).

Fifth ICES Intercalibration Exercise for Organochlorine Residues in Biological Tissue
(5/OC/BT) 1982

Coordinators	:	J.F. Uthe and C.J. Musial, Canada.
Samples	:	(a) Herring oil, and (b) same oil spiked with individual chlorobiphenyls (CBs).
Analytes	:	Individual CBs.
Participation	:	30 laboratories.

Results published in *Cooperative Research Report* No. 136 (1986).

Sixth ICES Intercalibration Exercise for Organochlorine Residues in Biological Tissue
(6/OC/BT) 1983

Coordinators	:	L. Reutergårdh and K. Litzén, Sweden.
Samples	:	(a) Standard solution of 12 pure CBs, (b) solution of an internal standard, and (c) herring oil.
Analytes	:	Individual CBs.
Participation	:	12 laboratories.

Results published in *ICES Cooperative Research Report* No. 183 (1992).

ICES/IOC/OSPARCOM Intercomparison Programme
on the Analysis of Chlorobiphenyls in Marine Media–Step 1
(7/OC/BT-1 and 1/OC/MS-1) 1989

Coordinators	:	J. de Boer, The Netherlands (for ICES), J.C. Duinker, Federal Republic of Germany (for IOC), J.A. Calder, United States (for JMG).
Samples	:	(a) Standard solution of 10 CBs in iso-octane, (b) solution of the 10 CBs in iso-octane at unknown concentration, (c) internal standard: octachloronaphthalene in iso-octane, and (d) blank: iso-octane.
Analytes	:	CB Nos. 28, 31, 52, 101, 105, 118, 138, 153, 180, 189.
Participation	:	57 laboratories from 17 countries.

Results published in *ICES Cooperative Research Report* No. 183 (1992).

ICES/IOC/OSPARCOM Intercomparison Programme
on the Analysis of Chlorobiphenyls in Marine Media–Step 2
(7/OC/BT-2 and 1/OC/MS-2) 1990

Coordinators	:	J. de Boer, The Netherlands (for ICES), J.C. Duinker, Federal Republic of Germany (for IOC), L. Reutergårdh, Sweden, and J.A. Calder, United States (for JMG).
Samples	:	(a) Standard solution (in iso-octane) of all CBs to be analysed; (b) seal blubber extract in iso-octane; (c) sediment extract in iso-octane; (d) internal standard solution in iso-octane; and (e) blank (iso-octane).
Analytes	:	CB Nos. 28, 31, 52, 101, 105, 118, 138, 153, 156, 180.
Participation	:	58 laboratories from 16 countries.

Results published in *ICES Cooperative Research Report* No. 207 (1995).

ICES/IOC/OSPARCOM Intercomparison Programme
on the Analysis of Chlorobiphenyls in Marine Media–Step 3a
(7/OC/BT-3a and 1/OC/MS-3a) 1991

Coordinator	:	J. de Boer, The Netherlands.
Sample	:	Certified Reference Material CRM 349 cod liver oil (from the Community Bureau of Reference (BCR) of the European Community).
Analytes	:	CB Nos. 52, 153, 156.
Participation	:	45 laboratories from 15 countries.

Results published in *ICES Cooperative Research Report* No. 223 (1998).

ICES/IOC/OSPARCOM Intercomparison Programme
on the Analysis of Chlorobiphenyls in Marine Media–Step 3b
(7/OC/BT-3b and 1/OC/MS-3b) 1992

Coordinators	:	J. de Boer and J. van der Meer, The Netherlands.
Samples	:	(a) A cleaned and an uncleaned sediment extract; (b) a cleaned and an uncleaned seal blubber extract; and (c) a standard solution.
Analytes	:	CB Nos. 28, 31, 52, 101, 105, 118, 138, 153, 156, 180.
Participation	:	46 laboratories from 15 countries.

Results published in *ICES Cooperative Research Report* No. 223 (1998).

ICES/IOC/OSPARCOM Intercomparison Programme
on the Analysis of Chlorobiphenyls in Marine Media–Step 4
(7/OC/BT-4 and 1/OC/MS-4) 1993

Coordinators	:	J. de Boer and J. van der Meer, The Netherlands.
Samples	:	(a) Seal oil, (b) sediment, (c) Atlantic cod muscle, (d) standard solution.
Analytes	:	CB Nos. 28, 31, 52, 101, 105, 118, 138, 153, 156, 180.
Participation	:	43 laboratories from 15 countries.

Results published in *ICES Cooperative Research Report* No. 223 (1998).

HYDROCARBONS IN MARINE SAMPLES

First ICES Intercomparison Exercise on Petroleum Hydrocarbons in Marine Samples
(1/HC/BT and 1/HC/MS) 1980

Coordinators	:	R.J. Law and J.E. Portmann, United Kingdom.
Samples	:	(a) Crude oil standard, (b) aliphatic fraction of crude oil standard, (c) marine sediment, and (d) mussel homogenate.
Analytes	:	Total hydrocarbons, aliphatic hydrocarbons (nC_7–nC_{33}), and several aromatic hydrocarbons.
Participation	:	36 laboratories from 12 ICES Member Countries and Bermuda.

Results published in *Cooperative Research Report* No. 117 (1982).

ICES/IOC Intercomparison Exercise on Petroleum Hydrocarbons in Biological Tissues
(2/HC/BT) 1984

Coordinators	:	J.W. Farrington, A.C. Davis, J.B. Livramento, C.H. Clifford, N.M. Frew, A. Knap, United States.
Samples	:	(a) Three samples of frozen, freeze-dried mussel homogenate, (b) reagent-grade chrysene, (c) methylene chloride solution of n-alkanes, (d) methylene chloride solution of aromatic hydrocarbons, and (e) Arabian Light Crude Oil standard.
Analytes	:	Aliphatic hydrocarbons (nC_{15}–nC_{32}) and selected aromatic hydrocarbons.
Participation	:	38 laboratories from 13 ICES Member Countries and 12 laboratories from 11 IOC Member Countries (most, if not all, ICES Member Countries are also members of IOC).

Results published in *Cooperative Research Report* No. 141 (1986).

Third ICES Intercomparison Exercise on Polycyclic Aromatic Hydrocarbons in Biological Tissue
(3/HC/BT) 1984

Coordinators	:	J.F. Uthe, C.J. Musial, and G.R. Sirota, Canada.
Samples	:	(a) Acetone powder of lobster digestive gland, and (b) the oil extracted during the preparation of this powder.
Analytes	:	21 selected polycyclic aromatic hydrocarbons.
Participation	:	11 laboratories from 7 ICES Member Countries.

Results published in *Cooperative Research Report* No. 141 (1986).

Fourth ICES Intercomparison Exercise on Polycyclic Aromatic Hydrocarbons in Marine Media–Stage 1
(4/HC/BT and 2/HC/MS) 1988–1990

Coordinator	:	R.J. Law, United Kingdom.
Samples	:	Solutions of 10 PAHs in acetonitrile (for HPLC analysis), or solutions of 10 PAHs in hexane (for GC analysis).
Analytes	:	Phenanthrene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*e*]pyrene benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*ghi*]perylene, and indeno[123-*cd*]pyrene.
Participation	:	17 laboratories from 9 countries.

Report on results published in *ICES Cooperative Research Report* No. 207 (1995).

NUTRIENTS IN SEA WATER

Fourth ICES Intercomparison Exercise for Nutrients in Sea Water
(4/NU/SW) 1989

Coordinators	:	D. Kirkwood, United Kingdom, A. Aminot, France, and M. Perttilä, Finland.
Samples	:	(a) Natural oceanic water, with no preservatives or pre-treatment, (b) natural shelf sea water, filtered, bottled in glass and autoclaved, and (c) sea water depleted in nitrate and phosphate, then filtered and bottled (blanks for nitrate and phosphate).
Analytes	:	Nitrate + nitrite, phosphate, silicate, nitrite, ammonia, total nitrogen and total phosphorus.
Participation	:	68 laboratories from all 18 ICES Member Countries.

Report on results published in *ICES Cooperative Research Report* No. 174 (1991).

Fifth ICES Intercomparison Exercise for Nutrients in Sea Water
(5/NU/SW) 1993

Coordinators	:	D. Kirkwood, United Kingdom, and A. Aminot, France.
Samples	:	Six samples of sea water (three for nitrate + nitrite determinations and three for ammonium and phosphate determinations).
Analytes	:	Nitrate + nitrite, ammonium, phosphate.
Participation	:	132 laboratories from 31 countries.

Report on results published in *ICES Cooperative Research Report* No. 213 (1995).

ANNEX 11

ACME/ACMP ADVICE BY TOPIC FOR THE YEARS 1986–1997

Numbers in the table refer to sections of the present report and of the ACMP or ACME reports from 1986 to 1997, in reverse chronological order.

*Signifies major advice on that topic.

Topic	Sub-topic	1997	1996	1995	1994	1993	1992	1991	1990	1989	1988	1987	1986
Monitoring	Strategy			5.1	*4; *Ann. 1	5	5.1				*4		
	Programme evaluation				4.2								
	Multi-purpose								6.2				
	Benthos		6.1.2; 11.1; *Ann. 8				8; *Ann .6	8.1	9	*Ann. 1	*7.1	8	
	NSTF/MMP					5.2			9.3	4			
	Sediments/guidelines	4.5; *Ann. 1	5.5; *Ann. 4		5.5	6.1; *Ann. 1				*14			15
	Sediment data normalization	4.5.2	5.5.1		5.5					*14.1	14.1	15.2	16
	Sediment sensitivity, variance factors			5.6							14.6; Ann. 2		
	Metals/sediments		9.5	5.6	5.5						12.6		
	Matrix tables • general (JMP)								*Ann. 1	*6.1	4		
	• organic								6.1				
	• NSTF								6.1				
	Contaminants that can be monitored												
	• organic		5.4	6.6	6.8								
	• inorganic	4.2											
	Use of seaweeds				5.1				6.8	6.3			
	Spatial monitoring	*4.7.2		5.3	5.1								
	JAMP/JMP guidelines	4.1	5.2;5.4	5.4			13.3						4.5
	BMP guidelines		5.1.2	5.4		5.3						*12	4.4
	AMAP		5.1.3		5.4								
	Effects of nutrient enrichment		9.1	5.8									

Topic	Sub-topic	1997	1996	1995	1994	1993	1992	1991	1990	1989	1988	1987	1986
Temporal trend monitoring	Strategy/objectives		*4; Ann. 1		*4; *Ann. 1								
	Guidelines		4.4		4;5.2						5.2	6.2;6.3	*4.1; Ann. 1
	Data analysis	15.2			5.2	6.2	5.2						
	Nutrients		5.7			6.3					11.1		
	Fish/JMP		5.6					5.1	6.2				Ann. 1
	Fish/CMP		5.6					5.1	6.5	*6.4	5.1	6.1	Ann. 1
	Biota/BMP			7.3									
	Mussels							5.1	*Ann. 3			6.3	
	Pooling								6.7; Ann. 5	6.4.3		6.2.1	
	Precision				5.2		*Ann. 1			6.4.4			
	Sediment storage										14.2		
	Sea water											6.5	4.2
	Sediments		4.3; 5.5.3								5.3; Ann. 2	6.5	4.2
	Statistical requirements			4.3									
Integration of biological/ chemical measurements	Sediment quality		5.2.2	4.2; Ann. 2	5.4	6.4; *Ann. 2							
Biological effects monitoring	Monitoring strategy		*5.3	4.1; *Ann. 1									
	Concepts									*7.1			
	Statistical design	4.31											
	Methods	4.3.2	5.3.2	Ann. 1			6.2	7.2					
	Molecular techniques			*5.2									
	Pathology	4.3.3	5.3.3	8.4	9.4								5.1
	Workshop results		5.3.2				6.1	7.1	8.1	7.2	6		5.2
	Fish egg bioassays												5.3
	Data analysis • general						6.3						
	• EROD						*Ann. 2						
	• oyster bioassay						*Ann. 2						

Topic	Sub-topic	1997	1996	1995	1994	1993	1992	1991	1990	1989	1988	1987	1986
Baseline studies	1985 Baseline fish											*4	4.3.1
	ICES Baseline TM/SW							6	*7	6.5	13	5	4.3.2
	Contaminants in • Baltic sediments	6.1	7.1	7.1	7.1	8.1	13.2	14.1	15.1			15.1	
	• North Sea sediments						13.1						
	HCH in sea water						14						
Regional assessments	Guidelines								5		*20.1		
	Preparation plans									5		21.4	
	Irish Sea											*21.1	24.2
	Skaggerrak/Kattegat											21.2	24.1
	North Sea QSR					4.1	4	4	5			21.3	23
	Baltic Sea		7.2	7.2	7.3					5			
	Baltic fish		7.3	7.2	7.3				17.2	17.3			
	Canadian waters							16					
	Nutrient trends–North Atlantic								13	12		16.1	
Quality assurance	Philosophy							13.6					
	Good laboratory practice											13.5	11.5
	Reference materials	4.2			*6.9	7.11				13.1	12.8		11.4
	Oxygen in sea water	*Ann. 3							14.5	13.6			
	Nutrients	5.7											
	Quality/comparability • organic contaminants			*6.6	*6.8								
	Hydrocarbons									13.7			
	Lipids			6.4	6.5								
	NSTF								14.7				
	Biological effects techniques	5.3	*6.2; *Ann. 5	6.2		7.1		7.3					
	Sediment quality criteria								15.2	22.2			
	QA of sampling	5.10					*12.8						
	QA info. in data bank	16.1.1			6.10								
	Chemical measurements– Baltic Sea	5.4	6.3	6.3	6.2	7.4							
	Biological measurements	5.1;5.2	6.1	6.1	6.1	7.3							
	Fish disease monitoring	5.3.2	*Ann. 6										

Topic	Sub-topic	1997	1996	1995	1994	1993	1992	1991	1990	1989	1988	1987	1986
Intercomparison exercises	Status	Ann. 10	Ann. 10	Ann. 7	Ann. 6	Ann. 5	Ann. 8	Ann. 3	Ann. 9	Ann. 2	Ann. 3		
	Nutrients/sea water		6.4	6.5	6.6	7.8	12.4		14.1	13.4	11.3	16.2	19
	Lindane (γ-HCH)												*13
	Specific hydrocarbons											13.3	11.1
	Hydrocarbons in • biota			6.7						13.7			
	• sediments									13.7			
	• sea water									13.7			*12
	PAHs/standards						12.2	13.1	14.2	13.2	12.1		
	PCBs/CBs in biota			6.6;6.7	6.3;6.4	7.5	12.1	13.2			12.3		
	Organochlorines in biota			6.6;6.7									
	CBs/standards								14.3	13.3	12.2		14
	CBs in sediments			6.6	6.3	7.5		13.2				14	14
	Metals in • sea water	5.5	6.5									13.1	
	• sediments										12.5		11.2.1; 11.2.2
	• biological tissue											13.2	
	• SPM				6.7	7.9	12.3	13.3	14.4	13.5	14.4; Ann. 1		18.2
	Dissolved oxygen in sea water						12.5	13.4	*14.5				
	Methyl Hg in biological tissue										12.4	13.4	
	Primary production										10.2		
	Oyster embryo bioassay						Ann. 4						
	EROD						12.6; *Ann. 3						
Methods	SPM in sea water												18.1
	Trace metals in SPM												18.2
	Total nitrogen												19.4
	Nutrients in sea water									13.5	*Ann. 4		19
	DO in sea water	*Ann. 3										13.6	
	Sediment normalization				5.5			14.2; 14.3				*14.1	14.1
	Analysis of total OCs												12.7

Topic	Sub-topic	1997	1996	1995	1994	1993	1992	1991	1990	1989	1988	1987	1986
Algal blooms	Primary production methods					6.5	11	11.1	12.1		10.2		
	Initiating factors							*11.3	12.2				8
	Dynamics	9.2			8	10							
	Exceptional blooms	Ann. 8						11.2					8
	Phycotoxins/ measurements							11.4	12.3		10.1		9
	C. polylepis bloom									*11.1	10.3		
Fish diseases and related issues	Relation to pollution	8.3	5.3.3	8.4	9.4			9.1		9.3			5.1
	Survey methods	7.2						9.2			8.2		
	Training guide								10				
	Baltic fish	7.2		8.1;8.2; 8.3	9.3								
	Survey results									9.1	8.1	9	6
	Data analysis	7.1	8.2		9.5	9.4	7						
	M-74 in Baltic salmon	6.2	7.4	7.4	9.1								
Mariculture	Interactions		15.1	14	13		9.1		*11	10	9	10	10
	Escape of fish—effects	14.1											
	Nutrient inputs/Baltic						*9.2		11.1	10.4			
	Use of chemicals	14.2							Ann. 6				
Introductions and Transfers	Code of Practice		14.2	13.1	14.1	12.1							
	Accidental transfers	13.2	14.4; *Ann. 9	13	14.2	12.3							
	Genetically modified organisms		14.5			12.2							
	On-going introductions	13.1	14.1										
	Baltic Sea		14.3										
Marine mammals	Contaminants/effects	11.4	5.4.2; 13.3; 13.4									*11.1	7.2
	Seal epidemic 1988								*18	*18.1			
	Baltic marine mammal stocks	11.1	13.1		10.2		*18					11.3	*7.1
	Populations/N. Atlantic				10.1	11.1	*18	18					
	Pathogens					11.2; Ann. 3							
	Impact of fisheries	11.2; 11.3	13.2										

Topic	Sub-topic	1997	1996	1995	1994	1993	1992	1991	1990	1989	1988	1987	1986
Overviews	Arsenic											*17.2	
	Mercury									*19.1			
	Hormone disruptors		Ann. 2										
	HCB								*20.1				
	Lindane (γ-HCH)								*20.1				
	Benzene/ alkylated benzenes			10.2 *Ann. 5									
	Chlorinated alkanes			10.1 Ann. 4									
	PCDDs and PCDFs									*19.2			
	PAHs												*20.2
	Tris(4-chlorophenyl) methanol/methane		10 *Ann. 7										
	Octachlorostyrene							20.1					
	Toxaphene	8.1; *Ann. 5			12.3								
	Atrazine				12.1								
	Irgarol 1051	8.1; *Ann. 4											
	PCDEs	8.1; *Ann. 6											
Classification/ assessment tools	Human health								19				
	Hazardous substances			12.3			*15						
	Background concentrations	15.1		12.1									
	Ecotoxicolgical reference values			12.2									
Sand/gravel extraction	Code of Practice								16				
	Effects	*6.3						15	16	15	15		
	Environmental impact assessment			*15	*15	13							

Topic	Sub-topic	1997	1996	1995	1994	1993	1992	1991	1990	1989	1988	1987	1986
Modelling	Radioactive contaminants/Baltic Sea						*17.1						
	Use in monitoring and assessment				16		17.2						
Data banks and management	Nutrients	17.2	16.1.2										19.5
	Contaminants	17.1	16.1.1; 16.3	17	2.2	2.2							
	NSTF						20	*21	22				
	ICES format		16.6										4.6
	ICES databases					14							
	Biological database	17.3; 17.4			11.2; Ann. 4								
	AMAP	17.1.1	16.2										
Special topics	Context of ACMP advice								Ann. 7	*21		22	25
	Patchiness in Baltic Sea									17.1	19.1	20.1	22.1
	Nutrient trends/eutrophication in OSPAR area	Ann. 9							13	12	11.1	16.1	
	Nutrients and eutrophication	9.1	9.1	5.8		6.3	10	*11.3			10.4		
	Sediments • Baltic	6.1	7.1	7.1	7.1			14.1	15.1	14.2	19.3	15.1	22.2
	• German Bight											Ann. 1	
	• Kattegat											Ann. 2	
	Sediments • bioavailability		9.3	4.2; Ann. 2	5.4	6.4; Ann. 2		7.4		7.3			17
	• release of contaminants									14.3			
	Bioaccumulation of contaminants	8.2; *Ann. 7											
	Acid rain studies/effects									20	17	19	
	Coastal zone fluxes					8.2							

Topic	Sub-topic	1997	1996	1995	1994	1993	1992	1991	1990	1989	1988	1987	1986
Special topics (cont.)	Riverine inputs • gross	*4.7.1									16.1	18.2	
	• net									16	16.2	18.3	21
	Inflow to Baltic					8.3							
	Atmospheric inputs	4.7.1									16.3	18.1; *Ann. 3	
	Effects of disturbance on benthos	9.3	11.2	9; Ann. 3	11.1		8.3	8.2					
	Ecosystem effects of fishing	*12	12		18		*19	19					
	Seabird/fish interactions	10			19								
	North Sea Benthos Survey			9			*Ann. 5						
	Organic carbon measurements	4.6; Ann. 2											
	GOOS	16											

ANNEX 12

TITLES OF RECENTLY PUBLISHED ICES COOPERATIVE RESEARCH REPORTS

No.	Title
200	Report of the Study Group on Ecosystem Effects of Fishing Activities
201	Patchiness in the Baltic Sea (Symposium proceedings, Mariehamn, 1991)
202	Chemicals Used in Mariculture
203	Joint Report of the ICES Advisory Committee on Fishery Management and the Advisory Committee on the Marine Environment
204	Report of the ICES Advisory Committee on Marine Environment, 1994
205	Spawning and Life History Information for North Atlantic Cod Stocks
206	Dynamics of Upwelling in the ICES Area
207	Report on the Results of the ICES/IOC/OSPARCOM Intercomparison Programme on the Analysis of Chlorobiphenyls in Marine Media—Step 2, and the Intercomparison Programme on the Analysis of PAHs in Marine Media—Stage 1
208	Results of the 1990/1991 Baseline Study of Contaminants in North Sea Sediments (Edited by S.M. Rowlatt and I.M. Davies)
209	Underwater Noise of Research Vessels: Review and Recommendations (Edited by R.B. Mitson)
210	Report of the ICES Advisory Committee on Fishery Management, 1994 (Part I and Part 2)
211	Intercalibration Exercise on the Qualitative and Quantitative Analysis of Fatty Acids used in *Artemia* and Marine Samples used in Mariculture
212	Report of the ICES Advisory Committee on the Marine Environment, 1995
213	Report on the Results of the Fifth Intercomparison Exercise for Nutrients in Sea Water
214	Report of the ICES Advisory Committee on Fishery Management, 1995 (Part 1 and Part 2)
215	Manual of Methods of Measuring the Selectivity of Towed Fishing Gears
216	Seabird/Fish Interactions, with Particular Reference to Seabirds in the North Sea
217	Report of the ICES Advisory Committee on the Marine Environment, 1996
218	Atlas of North Sea Benthic Infauna
219	Database Report of the Stomach Sampling Project, 1991
220	Guide to Identification of North Sea Fish Using Premaxillae and Vertebrae
221	Report of the ICES Advisory Committee on Fishery Management, 1996 (Part 1 and Part 2)

ACRONYMS

ACFM	Advisory Committee on Fishery Management
AChE	acetylcholinesterase
ACME	Advisory Committee on the Marine Environment
ACMP	Advisory Committee on Marine Pollution
ADI	acceptable daily intake
AGDATA	Ad Hoc Group of Databse/GIS Practitioners
AHF	altered hepato fico
AHH	aryl hydrocarbon hydroxylase
ALA-D	D-aminolevulinic acid dehydratase
AMAP	Arctic Monitoring and Assessment Programme
ASE	accelerated solvent extraction
ASMO	Environmental Assessment and Monitoring Committee (OSPAR)
ASP	amnesic shellfish poisoning
BCF	bioconcentration factor
BCPS	bis-*p*-chlorophenyl sulfone
BCR	European Commission Bureau of Community References
BEWG	Benthos Ecology Working Group
BMB	Baltic Marine Biologists
BMP	Baltic Monitoring Programme (HELCOM)
BSPAs	Baltic Sea Protected Areas
CBs	chlorobiphenyls
CDEs	chlorodiphenylethers
CD-ROM	compact disc: read-only memory
CEFAS	Centre for Environment, Fisheries and Aquaculture Science (UK)
CFP	ciguatera fish poisoning
CHBs	chlorinated bornanes
CIEM	Conseil International pour l'Exploration de la Mer
CMDGC	comprehensive multidimensional gas chromatography
CMP	Cooperative ICES Monitoring Studies Programme
COMBINE	Cooperative Monitoring in the Baltic Marine Environment (HELCOM)
CRIMP	Centre for Research on Introduced Marine Pests (Australia)
CRMs	certified reference materials
CTD	conductivity–temperature–depth
CUSUM	**Cu**mulative **Sum**
CV	coefficient of variation
DBT	dibutyltin
DCM	dichloromethane
DDE	dichlorodiphenylethylene
DDT	dichlorodiphenyltrichloroethane
ΣDDT	total DDT
DIFFCHEM	OSPAR Working Group on Diffuse Sources
DMA	dimethylarsenic
DNA	deoxyribonucleic acid
DO	dissolved oxygen
DOC	dissolved organic carbon
DSP	diarrhetic shellfish poisoning
DST	diarrhetic shellfish toxin
EC	European Commission
ECD	electron capture detection
EI	electron impact ionization
EIA	environmental impact assessment
EMD	evaporative mass detector
ENDS	Environmental Data Services (UK)
EOC	elemental organic carbon
EOCl	extractable organic chlorine
EPA	Environmental Protection Agency (USA)
EQG	environmental quality guidelines
EQS	environmental quality standard
EROD	ethoxyresorufin-*O*-deethylase
ESE	enhanced solvent extraction
EU	European Union
EUT	Ad Hoc Working Group on Eutrophication (OSPAR)
FDE	Fish Disease Data Entry Program
FID	flame ionization detection
GABA	gamma-aminobutyric acid
GC	gas chromatography

GC/ECD	gas chromatography/electron capture detection
GC/MS	gas chromatography/mass spectrometry
GESAMP	Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection
GPC	gel permeation chromatography
GLOBEC	Global Ocean Ecosystem Dynamics Programme
GOOS	Global Ocean Observing System
GSI	gonadosomatic index
GST	glutathion-*S*-transferase(s)
HAB	harmful algal bloom
HCB	hexachlorobenzene
HCH	hexachlorocyclohexane
H&E	haematoxylylin and eosin
HELCOM	Helsinki Commission (Baltic Marine Environment Protection Commission)
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HTCO	high temperature catalytic oxidation
IAEA	International Atomic Energy Agency
ICES	International Council for the Exploration of the Sea
ICS	International Chamber of Shipping
IEH	Institute for Environment and Health (UK)
IFREMER	Institut Français de Recherche pour l'Exploitation de la Mer
IJC	International Joint Commission (Canada and the USA)
IMO	International Maritime Organization
IMPACT	Working Group on Impacts on the Marine Environment (OSPAR)
IOC	Intergovernmental Oceanographic Commission
ISO	International Organization for Standardization
ITIS	Interagency Taxonomic Information System (USA)
IUCN	International Union for the Conservation of Nature and Natural Resources
IWC	International Whaling Commission
JAMP	OSPAR Joint Assessment and Monitoring Programme
JGOFS	Joint Global Ocean Flux Study (IGBP)
JMP	OSPAR Joint Monitoring Programme
JNCC	Joint Nature Conservation Committee (UK)
LD	lethal dose
LOI	loss-on-ignition
LRMs	laboratory reference materials
LRMS	low resolution mass spectrometry
MAST	Marine Science and Technology Programme (EC)
MBT	monobutyltin
MCWG	Marine Chemistry Working Group
MDGC	multidimensional gas chromatography
MDR	multidrug resistance
MEDPOL	Monitoring and Research Programme of the Mediterranean Action Plan
MFO	mixed-function oxidase
MMA	monomethylarsenic
MMHg	monomethylmercury
MMP	Monitoring Master Plan (NSTF)
MON	Ad Hoc Working Group on Monitoring (OSPAR)
MS	mass spectrometry
MSD	mass selective detector
mtDNA	mitochrondrial DNA
MXR	multixenobiotic resistance
NCI	negative chemical ionization
NIHS	National Institute of Health Science (Japan)
NIST	U.S. National Institute of Standards and Testing
NIVA	Norsk instittutt for vannforskning [Norwegian Institute for Water Research]
NMR	nuclear magnetic resonance
NOAA	National Oceanic and Atmospheric Administration (USA)
NOAEC	no observable adverse effects concentration
NOAEL	no observable adverse effects level
NODC	National Oceanographic Data Center (USA)
NOEL	no observable effects level
NORWECOM	**Nor**wegian **Eco**logical **M**odel

NOWESP	North-West European Shelf Programme (EU MAST Project)
NRC	National Research Council (Canada)
NSP	neurotoxic shellfish poisoning
NSTF	North Sea Task Force
OCs	organochlorines
OIE	Office International des Epizooites
OM	oxidizable matter
OPs	organophosphates
OSPAR	Oslo and Paris Commissions
PAHs	polycyclic aromatic hydrocarbons
PAR	photosynthetic available radiation
PBDEs	polybrominated diphenylethers
PBTs	persistent, bioaccumulative, toxic compounds
PCA	principal component analysis
PCBs	polychlorinated biphenyls
PCDDs	polychlorinated dibenzo-*p*-dioxins
PCDEs	polychlorinated diphenylethers
PCDFs	polychlorinated dibenzofurans
PCNA	proliferating cell nuclear antigen
PCNs	polychlorinated naphthalenes
PCP	pentachlorophenol
PCTs	polychlorinated terphenyls
PEEK	polyetheretherketone
PFC	plaque-forming cell
PICT	pollution-induced community tolerance
POC	particulate organic carbon
POPs	persistent organic pollutants
PROD	pentoxyresorufin-*O*-deethylase
PSP	paralytic shellfish poisoning
PSU	practical salinity unit
PTFE	polytetrafluorethene
QA	quality assurance
QC	quality control
QSARs	quantitative structure–activity relationships
QSR	quality status report
QUASIMEME	Quality Assurance of Information for Marine Environmental Monitoring in Europe
QUASH	Quality Assurance of Sampling and Sample Handling (EC)
RIA	radioimmunoassay
RIKZ	Rijksinstituut voor Kust en Zee [National Institute for Coastal and Marine Management]
RMs	reference materials
RUBIN	Rutin för Biologiska Inventeringar
SFE	supercritical fluid extraction
SFG	scope for growth
SG	Study Group
SGBSC	Steering Group for the Coordination of the Basline Study of Contaminants in Baltic Sea Sediments
SGBWS	ICES/IOC/IMO Study Group on Ballast Water and Sediments
SGFDDS	Study Group on Statistical Analysis of Fish Disease Data in Marine Fish Stocks
SGMBIS	Study Group on Marine Biocontrol of Invasive Species
SGMPCS	Study Group on Monitoring Programmes for Contaminants in Sediments
SGQAE	ICES/OSPAR Steering Group on Quality Assurance of Biological Measurements related to Eutrophication Effects
SGQAB	ICES/HELCOM Steering Group on Quality Assurance of Biological Measurements in the Baltic Sea
SGQAC	ICES/HELCOM Steering Group on Quality Assurance of Chemical Measurements in the Baltic Sea
SGSEAL	Study Group on Seals and Small Cetaceans in European Seas
SGSFI	Study Group on Seabird/Fish Interactions
SIM	selected ion monitoring
SIME	Working Group on Concentrations, Trends and Effects of Substances in the Marine Environment (OSPAR)
SMLIPA	ICES Special Meeting on the Use of Liver Pathology in Flatfish for Monitoring Biological Effects of Contaminants
SOAEFD	Scottish Office Agriculture, Environment and Fisheries Department
SOPs	Standard Operating Procedures
SPM	suspended particulate material
SRMs	standard reference materials

TALs	total annual loads
TBA	tetrabutylammonium
TBPS	tertiary butylbicyclophosphorothionate
TBT	tributyltin
TCDD	tetrachlorodibenzo-*p*-dioxin
TEF	toxic equivalency factor
TIE	toxicity identification evaluation
TIMES	*ICES Techniques in Marine Environmental Sciences*
TIP	training and intercalibration programme
TMA	trimethylarsenic
TOC	total organic carbon
TPT	triphenyltin
UK	United Kingdom
UNEP	United Nations Environment Programme
Unesco	United Nations Educational, Scientific, and Cultural Organization
U.S.	United States
USA	United States of America
USEPA	United States Environmental Protection Agency
UV	ultraviolet
VIC	Voluntary International Contaminant Monitoring in Temporal Trends
VTG	vitellogenin
WGAGFM	Working Group on Application of Genetics in Fisheries and Mariculture
WGBAST	Baltic Salmon and Trout Assessment Working Group
WGBEC	Working Group on Biological Effects of Contaminants
WGBFAS	Baltic Fisheries Assessment Working Group
WGBME	Working Group on the Baltic Marine Environment
WGEAMS	Working Group on Environmental Assessment and Monitoring Strategies
WGECO	Working Group on Ecosystem Effects of Fishing Activities
WGEIM	Working Group on Environmental Interactions of Mariculture
WGEXT	Working Group on the Effects of Extraction of Marine Sediments on the Marine Environment
WGHABD	ICES/IOC Working Group on Harmful Algal Bloom Dynamics
WGITMO	Working Group on Introductions and Transfers of Marine Organisms
WGMDM	Working Group on Marine Data Management
WGMS	Working Group on Marine Sediments in Relation to Pollution
WGNAS	Working Group on North Atlantic Salmon
WGPDMO	Working Group on Pathology and Diseases of Marine Organisms
WGPE	Working Group on Phytoplankton Ecology
WGSAEM	Working Group on the Statistical Aspects of Environmental Monitoring
WGSE	Working Group on Seabird Ecology
WGSEAL	Working Group on Seals and Small Cetaceans in European Seas
WGSSO	Working Group on Shelf Seas Oceanography
WGZE	Working Group on Zooplankton Ecology
WKSLAS	Workshop on the Interactions between Salmon Lice and Salmonids
WKSMTD	ICES/OSPAR Workshop on the Identification of Statistical Methods for Trend Detection
WOCE	World Ocean Circulation Experiment
WWW	world wide web