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REPORT OF THE WORKING GROUP ON HARMFUL EFFECTS OF ALGAL BLOOMS ON MARICULTURE AND MARINE FISHERIES

Lisbon, Portugal
11 - 13 April 1988

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REPORT OF THE WORKING GROUP ON THE HARMFUL EFFECTS OF ALGAL BLOOMS ON MARICULTURE AND MARINE FISHERIES

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1. OPENING OF THE MEETING

1.1. The meeting was opened at 10.00 hrs. on 11 April 1988 and was hosted by Dr. M.A. Sampayo, INSTITUTO NACIONAL DE INVESTIGACO DAS PESCAS (INEP), Portugal. Prof. L. Saldanha, Director, welcomed participants. The Chairman of the Working Group, Miss J. Doyle thanked Prof. L. Saldanha and introduced new members of the Group.

1.2 The agenda was adopted and is attached at Annex I.

1.3 A list of participants is given in Annex II.

1.4 Dr. R. Gowen was appointed as Rapporteur.

1.5 The Chairman advised the Working Group of Council Resolution 2:39 which revised the title of the Group and established the following terms of reference:

a) provide information useful for toxin monitoring programmes protecting public health, seafood and recreational industries;

b) evaluate analytical techniques for routine measurements of the toxins found in bivalves, finfish, dinoflagellates, and other possible causative organisms;

c) recommend research programmes on management techniques designed to alleviate or minimise the effects of harmful blooms;

d) liaise with the Study Group on the Ecology of Algal Blooms, and keep abreast of possible causes of the apparent spread of toxic species and increase in toxic blooms;

e) evaluate the feasibility of predicting toxic episodes in time and space scales relevant to fisheries and mariculture;

f) prepare a report on the currently known causes of and species involved in Algal Blooms with Harmful Effects on Fisheries and Mariculture, with a view to publication.

1.6 The Chairman also drew attention to Council Resolution 2:37 concerning the Working Group on Primary Production and Council Resolution 2:38 concerning a proposed Study Group on the Ecology of Algal Blooms with which the Group had been asked to liaise. Concern was expressed at the extent of overlap between the terms of reference given to separate groups and the potential for duplication which could arise. It was also noted that many countries have limited travel funds and this would limit participation by some members and thus hinder all these groups in carrying out the tasks assigned to them by the Council. The Chairman had been advised by two previous members of the Working Group on Exceptional Algal Blooms (now Harmful Effects.....) of their non attendance for this reason.

It was agreed that Drs. Edler and Lassus act as observers for the Working Group on "Harmful Effects of Algal Blooms on Mariculture and Marine Fisheries" at the

forthcoming meeting of the Study Group on the Ecology of Algal Blooms which is to meet in L'Houmeau, France on May 10-13 1988.

1.7 As requested the Working Group reviewed the report by the GESAMP working group on Nutrients and Eutrophication in the Marine Environment. It was accepted as a general overview of the problems. A number of comments and suggested amendments were forwarded to Dr. J. Portmann by the chairman of the Working Group.

1.8 The Working Group considered the Glossary of Aquaculture Terminology (CM 1986/F:34) which had been circulated in advance of the meeting. It was decided that in view of the time available to the Working Group that contributions to the Glossary which might also be required for the Co-operative Research Report should be prepared during the intersessional period and submitted to Dr. Gowen (see Item 5 Action List).

2. NATIONAL REPORTS AND MONITORING PROGRAMMES.

National reports on the occurrence and effects of harmful algae from ICES member countries were tabled and discussed. The reports are appended as Annex III (will be tabled at the Statuatory Meeting as well as circulated to members shortly). The following points arose from the discussion:

1. Harmful algae caused problems in coastal waters of some ICES countries, where there had been no reports prior to 1987. PSP was recorded for the first time on the Mediteranian coast of Spain. In Canada, Prince Edward Island, *Gonyaulax tamarensis* was recorded for the first time, however 1987 was also the first time that monitoring had been undertaken in that region. In France *Dinophysis sacculus* was found in a new area. In France there were more algal blooms compared to previous years. However, in general it is difficult to assess whether the frequency of occurrence and spread of harmful algae is real or an artifact resulting from increased awareness of harmful algae and more extensive monitoring. The Working Group agreed that careful analysis of time series data derived from monitoring programmes might provide some insight to the question of the spread of harmful algae.

2. The extent of the problem caused by PSP and DSP varied in different countries, as did the extent of the problem in some countries when compared to previous years. For example in Ireland, DSP was more persistent and higher levels of toxicity were recorded in 1987 compared to previous years.

3. It was noted that some shellfish farmers considered that deep frozen storage of bivalves might be used as a method of destroying toxins. It was agreed that this was not true. For example Dr. Hageltorn informed the Working Group that samples of mussels collected in 1985 and stored frozen since then were still toxic.

4. The Working Group considered reports from Japan that low cell numbers (60 cells l^{-1}) induced DSP toxicity. Dr. Kat reported that toxicity of shellfish could be maintained by only 30 cells l^{-1}. However it was agreed that at the present time it is not possible to determine whether such low cell numbers can induce toxicity or that an initial high cell density is required.

Details of existing monitoring programmes were provided. A number of working documents prepared by individual members of the Working Group were briefly discussed. Titles are given in Annex IV.

3. EXCHANGE OF INFORMATION

In earlier reports of the Working Group (Doc, C.M. 1985/F:58) the following recommendation was made:

- that member countries designate an appropriate national co-ordinating centre for information exchange on exceptional blooms, to facilitate national co-ordination of

action and control andinternational information exchange.

This arises because at a national level many different organizations have an interest in the occurrence of and effects of harmful algae. With respect to mariculture and shellfisheries, these include:

Local Public Health Control Authorities
Research Institutes
Universities
Centers of taxonomic expertise.

There can be problems of reporting within countries arising from such diverse interests.

The National Co-ordinating centres were intended to receive and disseminate information to relevant agencies (including industry, scientists and the public) and to co-ordinate international information exchange at a formal level. This need arises when:

1 a country wishes to warn a neighbour of the occurrence of harmful species in its national waters that might subsequently affect it, or

2 when harmful algae have caused toxicity in fish and shellfish products which might be exported to other countries.

An example of the former is the onset of *Gyrodinium aureolum* blooms in the North Sea/Skagerrak area which is usually the first occurrence in Danish coastal waters and is subsequently transported to Norwegian and Swedish coastal waters.

In both cases formal contact (ideally through the National Co-ordinating Centres) is to protect fishery interests. It is very important that only carefully balanced factual information is given, as the fish and shellfish trades are easily damaged by distorted "scare stories". In addition individual countries will need to demonstrate that they can protect their producers and consumers particularly where an export trade is concerned.

In addition to the formal contact, exchange of information occurres between scientists usually to gain information and help when incidents occur, so the first requirement for communication is a classified directory of experts.

The Working Group agreed that members would provide information to build up a directory of algal bloom expertise using the revised format agreed at the 1987 meeting. This information should be updated regularly.

Dr. Edler (Sweden) has kindly offered to prepare the final draft and it will be finalized and circulated to members with the report of the Working Group Meeting in Lisbon.

4. DATA FILING AND INFORMATION

ICES agreed in 1982 to an annual record of incidents in the archival journal "Annales Biologiques" under the editorship of Dr. J.P. Mommaerts.

The Working Group regarded this form of record as valuable and expected that it would form the basis of an archived data base. However, it is now noted that this publication has been discontinued by decision of the Council.

There is an urgent need for an international data filing and information exchange centre. Ideally the information supplied to ICES should be transmitted through the national centre in a standard format, and the reported data should be freely available from ICES in the same way as material on national fisheries statistics. The Working Group will seek the advice of the Marine Data Management Working Group on the present format of reporting and, to this end, the Chairman will forward the National Reports for 1987 presented at this meeting through the Secretariat for advice on future format for archive purposes.

5. PREPARATION OF DRAFT CO-OPERATIVE RESEARCH REPORT

The Working Group then convened in Subgroups to prepare draft chapters for a Co-operative Research Report on the following topics:

1 Detection and Determination of Algal Toxins - Chairman: P. Krogh

2 Monitoring for the Occurrence of Harmful Algae - Chairman: J. Martin

3 Management Strategies for reducing the Effects of Harmful Algae on Mariculture and Marine Fisheries - Chairman: E. Black

4 Predicting the occurrence of Harmful Phytoplankton - Co-Chairman: S. Fraga, O. Lindahl

5 Site selection - Chairman: P. Lassus and R. Gowen

The draft reports of each sub-group were later discussed at a plenary session of the Working Group. These drafts are attached at Appendix V-IX. Amendments and bibliographies will be prepared for the final draft prior to publication.

6. RESEARCH ACTIVITIES

Having considered the reports of the sub-groups the Working Group identified the following areas of research which are considered to be of major importance in extending the understanding of the problems associated with harmful effects on fin-fish and shellfish caused by phytoplankton.

I EFFECTS

* Studies on the effects of harmful phytoplankton (present in low concentrations, and blooms) on marine organisms. These should include histopathological studies, and toxic effects.

II MITIGATION TECHNIQUES

* Methods for protecting shellfish and fin-fish from harmful phytoplankton.
* Effects of wastes from fish and shellfish farms on phytoplankton growth.

III PREDICTABILITY

* Compilation and evaluation for predictive purposes of case studies of occurrences of harmful phytoplankton species and associated losses.

IV TOXICOLOGY AND TOXIN ANALYSIS

* Further development of biological and chemical procedures for monitoring of marine algal toxins.
* Development of rapid field tests for detection of marine algal toxins.
* Evaluation by WHO and national health authorities of the human health risks by exposure to newly discovered marine algal toxins, so that adequate action l levels can be determined.
* Toxicity including toxin profiles of toxic species.
* Studies on factors controlling the persistence of PSP and DSP toxicity when no t toxic species are present.
* Laboratory and field experiments to evaluate methods of detoxification.

V BIOLOGY AND ECOPHYSIOLOGY OF HARMFUL PHYTOPLANKTON SPECIES

* Identification and culture of harmful phytoplankton species.
* Ecology of harmful phytoplankton which includes studies in the open sea and in large enclosed and controlled ecosystems and laboratory based ecophysiological growth experiments.
* Distribution and ecophysiology of cysts.
* Determination of minimum cell numbers which induce toxicity.

VI MULTIDISCIPLINARY STUDIES

* Studies of biological, physical and chemical interactions influencing bloom development in off-shore areas, particularly shelf sea fronts.
* Physical processes leading to the transport of blooms between frontal areas and coastal mariculture sites including the relationship between oceanic and coastal phenomena.
* The effects of eutrophication on the frequency and intensity of harmful phytoplankton occurrences should be further investigated.

7. ACTION LIST FOR MEMBERS OF THE WORKING GROUP

1. Dr. Edler to finalize the Draft Directory of National Expertise.

2. The Chairman to contact the Marine Data Management Group for advice on long term archiving of toxic bloom event data.

3. The Chairman to convey the comments of the Working Group on the Report of the GESAMP Working Group on Nutrients and Eutrophication in the Marine Environment to the IOC Secretariat and Chairman Dr. J. Portmann.

4. Drs. Lassus and Edler to liase with the Study Group on the Ecology of algal Blooms which is meeting under the chairmanship of Dr. S. Maestrini at L'Houmeau, France from May 10-13 1988.

5. All members to prepare a list of terms and definitions for the Working Group's specialised area, for inclusion in the Glossary of Aquaculture Terminology prepared by the Mariculture Committee of ICES. Members to work by correspondence (Dr. Gowen to act as co-ordinator of this task).

6. All members to compile information on the of occurrence of blooms and/or harmful events to establish possible trends in frequency. (Mr. Dahl to act as co-ordinator of this task).

7. All members to prepare a list of currently known harmful species together with factors responsible for their occurrence, to be included in the proposed publication in accordance with term of reference f (C.Res. 1987/2:39). This material must be with the Chairman by November 1988 to allow synthesis and circulation in time for the next meeting of the Working Group.

8. RECOMMENDATIONS

1. The Working Group on the Harmful Effects of Algal Blooms on Mariculture and Marine Fisheries will meet in Nantes, France 11 - 14 April 1989 to undertake the following tasks:

   a. to finalize draft chapters on site selection, monitoring protocols, predictability, research priorities and toxin detection methodology for inclusion in a proposed Co-operative Research Report on "Management of Effects of Harmful Algal on Mariculture and Marine Fisheries".

   b. To complete a report on the currently known causes of and species involved in algal blooms with harmful effects on fisheries and mariculture with a view to publication.

   c. To discuss the report on possible trends in the occurrence of algal blooms and/or harmful events.

2. In order to facilitate rapid exchange of information on potential problems to and from neighbouring countries in accordance with C. Res. 1985/4 : 22 recommends that the ICES Secretariat reminds national delegates of the terms of this resolution.

ANNEX I

ICES WORKING ON THE HARMFUL EFFECTS OF EXCEPTIONAL ALGAL BLOOMS ON MARICULTURE ALGAL BLOOMS AND MARINE FISHERIES

AGENDA

Monday, April 11

1.	10.00 hours:		Opening of the meeting
2.			Adoption of the agenda
3.			Appointment of Rapporteur
4.		a	Consideration of revised terms of reference and liaison with other ICES working groups
		b	Review of the Report of Gesamp Working Group on nutrients and eutrophication in the marine environment
5.		a	Consideration of national reports on Bloom events in 1987
		b	National monitoring programmes
		c	Preparation of a report on analytical techniques for toxin detection
		d	Research programmes on management techniques
		e	Progress in the prediction of toxic episodes
		f	Complete advice on site selection
6.			Glossary of terminology
7.			Any other matters
8.			Adoption of the report and recommendations

ANNEX II

List of Participants

Mr.	E. Black	Canada
Dr.	A. Cembella	Canada
Mr.	E. Dahl	Norway
Ms.	J. Doyle (Chairman)	Ireland
Dr.	L. Edler	Sweden
Dr.	A. Fiksdahl	Norway
Dr.	S. Fraga	Spain
Ms.	S. Franca	Portugal
Dr.	R. Gowen	United Kingdom
Dr.	M. Hageltorn	Sweden
Ms.	M. Kat	The Netherlands
Dr.	P. Krogh	Denmark
Dr.	P. Lassus	France
Ms.	C. Lima	Portugal
Dr.	O. Lindahl	Sweden
Dr.	J. Martin	Canada
Ms.	T. Moita	Portugal
Dr.	J. Rodriguez-Vazquez	Spain
Ms.	M. Sampayo	Portugal
Mr.	K. Vagn Hansen	Denmark
Dr.	J. Worms	Canada

ANNEX IV

DOCUMENTS* CONSIDERED BY THE WORKING GROUP

Dahl, E.	Monitoring of Toxic Phytoplankton causing fish Mortality and Mussel toxcicity in Norwegian Waters
Fraga, S.	Prediction of *Gymnodinium catenatum*-blooms in Spain
Hageltorn, M	Methods for detecting and quantifying DSP and PSP
Jones, K.J.	Research strategies and techniques for use in scientific investigations into bloom events
Lassus, P. and Erhard, Le D. E.	Draft Report on Criteria for site selection of shell fish farms for the avoidance of toxic blooms

NATIONAL MONITORING PROGRAMMES

Anderson, D.	Monitoring Programs for Toxic Shellfish in the United States
Black, E.	Plankton Monitoring on the Canadian Pacific Coast
Dahl, E.	Monitoring of Toxic Phytoplankton in Norwegian Waters
Doyle, J.	Ireland - National Monitoring Programme for Phytoplankton and Investigations of Toxic Effects on Fish and Shellfish
Edler, L.	Monitoring Programs for Toxic Shellfish in Sweden
Fraga, S.	Monitoring Programmes in Spain
Franca, S.	Toxicity in Dinoflagellates and Resulting bivalves toxicity - state of the Analytical Techniques in Portugal
Sampayo, M.	Monitoring Program in Portugal

* Lodged with ICES Secretariat for consultation on request

ANNEX V

DETECTION AND DETERMINATION OF ALGAL TOXINS

1 INTRODUCTION

Any recommended method for the analysis of algal toxins in shellfish and finfish must meet five basic criteria: a) reliability, b) acceptable cost, c) precision, d) sensitivity, and e) specificity. A further requirement, particularly for the application of a given method in a monitoring programme, is for the technique to gain general acceptance through extensive homologation and inter-laboratory comparisons. Specifically there is a need for internationally recommended methods in order to safeguard international trade in shellfish and finfish. This may be achieved by conducting collaborative studies of methods through international organizations, such as International Union of Pure and Applied Chemistry (IUPAC) and Association of Official Analytical Chemistry (AOAC).

In many cases, there exists a distinction between the optimal method for purely analytical purposes and that which may be most successfully adopted by a regulatory agency. The level of precision and sensitivity yielded by a method required by a monitoring programme may be substantially less than that potentially achievable using advanced technology, yet still prove adequate in a regulatory role. In this context, we have directed most attention to methods which have proven to be workable and in widespread use.

2 PRESENT STATUS

2.1 Paralytic Shellfish Poisoning (PSP)

2.1.1 Biological Methods

a) Mouse Bioassay

The proceedure requires an intraperitoneal injection of mice with an acidified tissue extract, and the determination of the resulting death time (AOAC, 1984; Adams and Miescier, 1980). By standardizing the bioassay conditions (mouse weight, pH of extract, salt concentration, determination of the mouse strain sensitivity, using a saxitoxin standard) a fairly reliable routine procedure has been established. When the assay was tested collaboratively, the reproducibility coefficient of variation was about 20% (Adams and Furfari, 1984). The lowest detectible PSP level is about 40 ug/100 g wet weight of tissue, however the exact level depends on the sensitivity of the mouse strain employed.

b) House Fly Bioassay

The procedure involves injection of acidified tissue extract into the thorax of house flies (*Musca domestica*) and subsequent observation of decreased mobility. The flies are kept immobilized during injection by maintaining them on a chilled petri dish (Ross *et al.*, 1985).

2.1.2 Fluorometric Methods

Chemical techniques for the analysis of PSP toxins are generally based upon alkaline oxidation of toxins to fluorescent derivatives. The original manual method (Bates and Rapoport, 1975) and later modifications (Bates *et al.*, 1975; Shoptough *et al.*, 1981), can lead to rather severe discrepancies in toxicity levels, although such techniques remain in use in a few regulatory laboratories. Part of this discrepancy arises from the use of saxitoxin alone as the calibration standard, whereas in many toxic shellfish, this toxin may be only a minor component. The variation in specific fluorescence/toxicity ratio among the gonyautoxins can yield large under-estimates of total toxicity if N-1 hydroxy toxins are dominant. Conversely, if high fluorescent derivatives (e.g. GTX2 and GTX3) are abundant toxicity is over-estimated by this technique.

Attempts to automate sample processing using an autoanalyser system with a fluorescence detector equipped with a flow cell (Jonas-Davis *et al.*, 1984; Sullivan *et al.*, 1985) has shown promise for use in PSP regulatory programmes. For shellfish from a limited geographical area, results generally show a reasonable correlation with the AOAC bioassay. The method offers rapid sample processing and high sensitivity.

The HPLC methods (Sullivan *et al.*, 1985; Sullivan and Wekell, 1984; Nagashima *et al.*, 1987; Oshima *et al.*, 1984) in current use for the analysis of PSP toxins are all based upon the post-column oxidation of toxins to fluorescent products. Of the HPLC techniques available the Sullivan method probably offers

the greatest potential in a high volume PSP regulatory programme, for the following reasons: 1) the column was specifically selected for longevity and tolerance to relatively impure shellfish samples; 2) the separation of significant shellfish toxins is effected in a single injection; 3) the method is optimized for the separation of the specific toxins most commonly present in shellfish rather than in the dinflagellates; 4) on a worldwide basis, more HPLC facilities have been configured for this method than the alternatives; this will aid in international laboratory calibrations; and 5) the mouse bioassay/HPLC comparisons have been extensively carried out by Sullivan and co-workers.

The HPLC method offers some unique advantages in a PSP regulatory programme. It can meet the basic criteria for a monitoring tool: a) reasonable operating cost($ 2-3 U.S. per sample); b) precision (+/-3% at optimum); c) reliability (generally good once the system is fully operational); d) specificity (the most significant PSP toxins can be resolved and quantified); e) sensitivity (depending on the specific toxin, 4-400 times more sensitive than the mouse bioassay, average sensitivity less than or equal to 1 ug STXeq/100 g).

2.2 Diarrheic Shellfish Poisoning (DSP)

According to Japanese reports (Murata *et al.*, 1982; Yasumoto *et al.*, 1985; Murata *et al.*, 1986, 1987), the known toxins involved in the DSP complex are, the polyether fatty acid okadaic acid (OA), dinophysistoxins 1 and 3 (DTX 1, 3), the polyether lactone pectenotoxins 1, 2, 3 and 6 (PTX 1,2,3,6) and the recently described polyether sulphate yessotoxin (YTX): However diarrheic effects have only been proved for OA; DTX 1 and 3 (Hamano *et al.*, 1985)

2.2.1 Biological Methods

a) Techniques for the determination of total DSP toxicity

Mouse bioassays are performed on extracts of digestive glands or total soft tissue. Homogenates are extracted with acetone (Ministry of Health and Welfare, Japan 1981; Yasumoto *et al.*, 1987) and transfered to diethylether. Reactions and eventual death are recorded following intraperitoneal injection into mice.

b) Techniques for the detection and quantification of diarrheic effects.

Extracts prepared as described above can be administered by gavage into four to five day old mice and the effects measured as a fluid accumulation ratio (FAR) which is expressed as a ratio of weight of intestines to that of the body (Hamano *et al.*, 1985).

Digestive glands without any pre-treatment can be offered to experimental animals, such rats. Food avoidance and faecal consistency are indicative of the presence of a gastro-intestinal toxin (diarrheic agent)(Kat *et al.*, 1982).

2.2.2 Chemical Methods

A quantitative chemical method for the detection of OA and DTX 1 has been developed based on an HPLC technique (Lee *et al.*, 1987).

2.3 Domoic Acid

The recent addition of domoic acid to the list of natural marine toxins with potential human health impact has prompted significant efforts to be targeted at developing screening and detection methods which will be specific enough to be used by regulatory agencies. While still in a developmental stage, three approaches are currently used, based upon the data accumulated during the peak of the eastern Prince Edward Island (Canada) episode.

2.3.1 Biological Methods

Mouse Bioassay

Extraction Method: The standard AOAC water-acid extraction method is used on the total soft tissue. Its relative efficiency for domoic acid extraction is about 85%. Although more efficient extraction procedures have been found, namely aqueous/methanol (about 95%), the standard AOAC method is a good compromise between practicability and efficiency for regulatory purposes.

Bioassay: The test as enforced now uses CF1 or CD1 females, weighing 18 to 23 g. One ml of extract is

intraperitoneally injected into three mice. The mice are observed continuously for four hours and kept in cages for an additional fourteen hours, for a total test duration of eighteen hours. Symptoms are carefully recorded as well as the eventual time of death.

Symptoms typical of domoic acid intoxication are as follows:

- hunched-huddled position on injection,
- scratching of the ear region, alternatively on both sides (most typical symptom),
- barrel movements,
- aggressive behaviour with fighting,
- shallow breathing,
- convulsions,
- eventual death,

The test is considered positive when two or three mice die within eighteen hours. Mouse sensitivity to domoic acid is not yet fully clarified, mainly due to inadequate supplies of pure domoic acid. Correlation between time of death and domoic acid concentration still requires further investigation.

2.3.2 Chemical Methods

a) Analytical method (National Research Council/Atlantic Research Laboratory, Halifax, Canada).
An HPLC technique has been developed which employs a very basic system consisting of an isocratic pump, standard UV detector, etc (details of the procedure will be made available later, including sensitivity). The maximum UV absorption peak of domoic acid is around 240 nm.

b) Rapid detection method (R. Pocklington, Department of Fisheries and Oceans, Halifax, Canada)
The need appeared quickly for a field "spot test" which would at least indicate the presence of domoic acid in a given sample. A method is being developed, based on the colour reaction of ninhydrin with the secondary amine group of domoic acid. Much remains to be done to refine this test in terms of its sensitivity and specificity.

3 CURRENT PROBLEMS AND FUTURE RESEARCH REQUIREMENTS

3.1 Sample Handling

Accurate determinations of toxin levels must be based on a representative sample of sufficient weight or volume, to account for toxin heterogeneity among individuals and sampling sites. The proper storage of collected samples is a critical factor in yielding reproducible and accurate toxin measurements, particularly for DSP. Freshly collected samples must be kept refrigerated to minimize toxin degredation, and frozen as rapidly as possible. For DSP determinations, samples should be boiled prio to freezing to minimise the interference by free fatty acids. It is recognised that some toxin will be inevitably lost through the extraction procedure. This can be controlled for by introducing a "spike" of reference standard in known uncontaminated samples.

3.2 Bioassays

Apart from the mouse bioassay for PSP (AOAC, 1984) the bioassays listed in the previous section are only semi-quantitative at best. In addition, the toxin specific symptoms are poorly defined for most bioassays, with the exception of the characteristic signs observed in the PSP mouse bioassay, and the diarrhea which develops in the rat and suckling mouse DSP bioassays.

It should be emphasized that only highly skilled and experienced technicans can operate bioassay units, and observe and describe symptoms with a precision sufficient to detect effects of all toxins present. A further factor limiting the application of bioassay methods is the restriction on the use of experimental animals which has already been introduced or is presently being considered in a number of countries. Apart from the scientific desirability of introducing quantitative chemical methods as alternative tools, it is possible that the use of bioassays employing experimental animals, especially mammals, will be banned completely in many countries within a 5 to 10 year period.

3.3 Chemical Methods

The major drawbacks with the autoanalyser approach to measure total toxicity by fluorescence are

related to the lack of control for possible non-toxic fluorescent artefacts in the samples, and the relative lack of knowledge regarding its application to shellfish exhibiting widely different toxin spectra. Recently, a more sophisticated automated flow-injection fluorescence toxin analyser (Cembella and Therriault, 1988) has been adopted for rapid sample analyses (less than 2 minutes per sample). Correction for fluorescent artefacts, the predetermination of the specific toxin profiles from batch shellfish samples by HPLC, and the use of an appropriate mixture of gonyautoxins for calibration, has served to alleviate some of the problems associated with earlier fluorescence-based methods for total toxicity.

A particular constraint on the more widespread application of the HPLC method has been the limited availability of PSP standard toxins for calibration. For the analysis of PSP toxins in shellfish, the spectrum of gonyautoxins, most particularly GTX1, GTX2, GTX3 GTX5, NeoSTX and STX, is required by monitoring laboratories. This is a problem which will be alleviated in the near future, as a result of current efforts to produce PSP toxin standards of certified purity and distributed according to the demand by the Atlantic Research Laboratory (National Research Council, Halifax, Canada).

The analysis of DSP by HPLC requires okadaic acid and DTX1, specifically for identification purposes. Since currently available standards for DSP are of variable purity, it is recommended that an internal standard be employed for quantitative determinations. Such an internal standard would be an organic compound with chemical and physical properties very similar to the DSP toxins, but which is readiily obtainable in highly purified form. The internal standard must be carefully chosen for high stability, both before and after fluorescent derivation and its chromatographic behaviour must be reproducible. By adding a known quantity of the internal standard to the extracted sample, the recovery of the analagous DSP toxins can be followed. After chromatographic separation, the intergrated peak area of the reference internal standard can be compared with that of the sample for quantitative toxin determination.

Several attempts to apply the Sullivan HPLC method for routine shellfish toxin analysis have proven frustrating, and have even been abandoned in some cases. The hardware requirements for a functional system, including a gradient HPLC pump, sensitive fluorescence detector with dual monochromator and xenon lamp, a suitable post-column reaction system and, preferably, an autosampler and automated data integrator, necessitates a capital outlay beyond the capacity of many regulatory laboratories. The methodological difficulties arising from the use of post-column reaction systems (large "dead volume", peak broadening, the requirement for stable, pulseless flow rates, critical reaction temperatures, clogging and crystallization in the mixing "Tees" and reaction coil, the formation of unidentified oxidation products, etc.) are essentially similar among the available alternative HPLC techniques. Successful operation of these systems requires the care and attention of a highly trained technician. Efficient sample processing is most readily achieved if the HPLC is operated continuously. Once configured for PSP toxin analysis it is best to dedicate the system to this specific task and to avoid multiple usage. Since sample analysis is sequential, an analytical output of about 40 samples per day is the maximum that can be achieved.

The intercalibration between the mouse bioassay and the Sullivan HPLC method for PSP has been well established, but further research is required to compare the HPLC analysis for DSP and its respective mouse bioassay. If the fly bioassay gains wider acceptance as a possible replacement for the PSP mouse bioassay, such a systematic comparison with HPLC should be undertaken.

Further development of HPLC methods for the quantitative determination of yessotoxin and pectenotoxins will be needed when toxicological studies are completed and if they indicate significant hazard to human health.

At present there are only a few HPLC facilities dedicated to the analysis of either PSP or DSP toxins. There are even fewer laboratories engaged in the routine analysis of these toxins for regulatory purposes. If these methods are to be brought from the purely analytical stage to function effectively in a monitoring capacity, extensive inter-laboratory calibration, using standardized reference material and samples prepared at a single source is required. Research and analytical laboratories which can supply reference material must be identified, as well as individuals who can serve as contacts for the distribution of standards.

Given the above limitations of the HPLC methods, it is unlikely that they will be adopted as regulatory tools for routine screening of large numbers of samples. However, they can provide a valuable quality control to complement the traditional bioassay methods, and can yield confirmatory

identification of specific toxins when such detailed information is required.

3.4 Immunological Methods

The immunological approach to the monitoring of algal toxins offers perhaps the most promising avenue of future research. Immunological methods are advantageous since, in general, they can be adapted to yield extremely high sensitivity and high toxin specificity, while requireing little, if any, capital expenditure. When properly designed, such methods could be applied by field technicians and even shellfish producers themselves, at least as a preliminary screening procedure. The sample extraction and preparation required for an immunological assay should be relatively rudimentary, since interference by other metabolites should be minimal.

At present, there are no immunological test kits in general usage in regulatory programmes, although several have reached the stage of prototype, advanced development, or limited field research and marketing. An immunological kit for DSP based upon an ELISA method (UBE Industries, Tokyo, Japan) is commercially available, but requires further comparison with conventional methods before acceptance is assured. A similar immunological technique for PSP is in the latter stage of development at the Armand-Frappier Institute (Montreal, Canada). Current research on this PSP kit is directed towards confirming the degree of cross-reactivity with the complete spectrum of gonyautoxins.

4 INTERNATIONALLY RECOMMENDED METHODS

The availability of internationally recommended methods is mandatory in order to safeguard international trade in shellfish and finfish. Two international organisations, the International Union of Pure and Applied Chemistry (IUPAC), Commission of Food Chemistry, and the Association of Official Analytical Chemists (AOAC), are presently involved in the evaluation and collaborative testing of methods for algal toxins. The results of these studies will form the statutory basis for recommendation of methods. Specifically, work is in progress to study collaboratively the Sullivan HPLC procedure for PSP and the method for DSP (Okadaic acid, DTX1 and DTX3) (Lee *et al.*, 1987).

5 EVALUATION OF HEALTH RISKS OF ALGAL TOXINS TO HUMANS

Within the last 5 to 10 years a number of novel algal toxins have been identified in shellfish. These toxins include okadaic acid, DTX1 and 3, pectenotoxins, yessotoxin, brevetoxins, and domoic acid. The toxic properties of these chemicals have been elucidated to varying degrees. As there is undoubledly a human exposure to these toxins, the Working Group recommends that ICES directly call the attention of the World Health Organization to the matter, so that health evaluation can be carried out.

6 CONCLUSIONS

Reliable HPLC methods that can detect and quantify individual toxin components, are now available for paralytic shellfish poisons (PSP) and for the commonly occurring components of diarrheic shellfish poisons (DSP). Further reference material for the commonly occurring PSP and DSP components are likely to become commercially available within 6 to 12 months. Hardware requirements in HPLC are, however, capital demanding, and HPLC procedures are therefore unlikely to be generally used for monitoring and regulatory purposes, but may well serve as backup confirmatory tools. Thus bioassays will most likely continue to be the general methodological principle in monitoring and regulatory analytical operations. However, the routine use of experimental animals, especially mammals in bioassays is likely to be drastically reduced by law enforcement in a number of countries within the next five to ten years. This dilemma may be solved by the introduction of immunoassays. Thus ELISA procedures for PSP and DSP (okadaic acid) have been developed and are at present being evaluated for reliability and specificity. If these methods prove reliable this type of test would be the method of choice in monitoring and regulatory purposes, because the tests are easy to perform and large numbers of samples can be analysed at low cost.

Newly discovered algal toxins in shellfish and finfish constitute a special problem in terms of management and regulatory operations. These toxins, discovered within the last 5 to 10 years, have been characterized toxicologically, but evaluation of the health risks for humans has not yet been performed, and consequently action levels for the toxins in seafood cannot be defined. The Working Group calls the attention of the World Health Organization to this matter.

ANNEX VI

MONITORING FOR THE OCCURRENCE OF HARMFUL ALGAE

1 GENERAL INTRODUCTION

In addition to causing public health problems harmful phytoplankton can cost mariculture industries and traditional fisheries loss of stock and markets. There have been two approaches to these problems, monitoring for product toxicity and monitoring for the occurrence of harmful phytoplankton species. It is essential that monitoring programmes are correctly designed to ensure that: 1) adequate warning of the occurrence of harmful species is given, to protect public health and in the case of fish farming enable farmers to initiate management strategies to minimize losses. 2) to ensure that the cost of monitoring is related to the value of the industry.

As will be discussed, monitoring programmes rely, in part, on an understanding of the hydrography and phytoplankton ecology of the coastal area to be monitored. In some locations a lack of baseline information may hamper the development of adequate monitoring programmes. In such cases there is no alternative but to initiate extensive monitoring which may be reduced as information becomes available. In this respect research into basic hydrography and phytoplankton ecology would aid the development of suitable monitoring programmes.

2 PHYTOPLANKTON MONITORING

2.1 Introduction

For PSP and DSP phytoplankton monitoring can be used as a pre-screening technique to determine when the species responsible for PSP and DSP are present and hence when to initiate toxin detection in the shellfish. However for finfish farming monitoring the phytoplankton is often the only method of providing a warning of the presence of harmful algae. There are both government and industry based monitoring programmes. However, where the problem covers a large geographical area industry based programmes may be more cost effective.

2.2 Establishment of key sampling stations

To establish key sampling stations a number of factors should be considered:

1. The position of hydrographic boundaries, for example shelf sea fronts.

Shelf sea fronts provide conditions suitable for the growth of dinoflagellates including harmful species (references in here **). The presence of a front offshore from a mariculture area might therefore act as a source of harmful species. For example in Ireland the front off the south west coast is held to be the origin of *Gyrodinium aureolum* which has caused mortalities of fish farmed in more inshore waters. Monitoring the phytoplankton composition at the front could therefore provide an early warning of the presence of a harmful species. In some estuaries there is often a convergence between outflowing surface brackish water and inflowing sea water. Being motile and therefore buoyant dinoflagellates would accumulate at such a convergence. Where the convergence is a persistent feature sampling at such a location might provide an early warning of the presence of a high biomass of a harmful species. Sampling at hydrographic boundaries will often be difficult because of the distance that the boundary might be offshore. In such cases satellite imagery could be used to detect changes in the position of the front or in phytoplankton biomass therein. In areas where there are a number of mariculture operations, to optimise sampling efficiency and reduce cost a co-operative approach to monitoring is recommended. However with respect to the latter this would not provide information on the composition of the phytoplankton at the boundary. Furthermore since a low cell density of some harmful species (for example *Dinophysis* spp. and *Chaetoceros convolutus*) can cause problems it would be necessary to collect discrete samples for species analysis .

2. Water Currents.

Where there is a known transport mechanism, for example from an offshore front or a long-shore current, monitoring stations should be sited "up stream " of the mariculture operation.

3. Flushing time.

For some semi enclosed water bodies it may be necessary to monitor at the head of the inlet (where the residence time of the water might be sufficient to allow a high algal biomass to develop) and at the entrance to the inlet (to monitor phytoplankton being advected into the inlet).

4. Historical records.

Where historical records on the occurrence of harmful algae or problems associated with harmful algae exist, such records might be of value in determining the location of monitoring stations.

2.3 Sampling protocols

2.3.1 Sample collection

It is important to ensure that sample collection takes into account the distribution of the organisms to be harvested. This might appear obvious, however in the case of *Prorocentrum lima*, an epiphytic dinoflagellate, for example, collection of water samples during the day time would be inappropriate since this species is predominantly epiphytic during the day.

2.3.2 Phytoplankton biomass

A general method for quantifying phytoplankton biomass would be chlorophyll estimation (pigment anaysis). However, this method does not distinguish individual species. Furthermore since some harmful species can cause problems when present in low cell densities, the use of chlorophyll estimation is therefore, only likely to be of use in detecting the development of a phytoplankton bloom.

2.3.3 Phytoplankton species composition

Semi-quantitative methods, for example net hauls or shell fish gut content can be used to determine the presence of harmful species. In the case of shellfish gut content this could not be used to determine the presence of those species which do not have a cell wall or theca, for example naked dinoflagellates and microflagellates. Quantitative water sampling (using vertically integrated or discrete water samples collected from selected depths) are necessary to provide accurate estimation of cell densities. In general it is advisable to collect integrated samples, collected over a depth range corresponding to the depth of the euphotic zone. This will ensure that a harmful species which is present but confined to a narrow depth range will be detected. Furthermore since low cell densities have been shown to cause harm, it is necessary to ensure that a large volume of water is collected. The phytoplankton in such a large sample can be concentrated by methods such as filtration, centrifugation and settlement.

2.3.4 Samplimg frequency

It is important to relate the frequency of sampling to the growth rate and/or rate of accumulation of the species being monitored. For example, in the case of rapidly growing species it may be necessary to sample at 3 to 4 day interval. For slower growing species an interval of 7 to 10 days may be sufficient.

2.4 Monitoring during the occurrence of a harmful species

When increasing cell densities are encountered, the monitoring protocol should be modified to include additional sampling. This is necessary in order to initiate mitigation techniques. The aquisition of additional data may allow the development of a predictive capability. Depending on the availability of resources regular monitoring of all or some of following are recommended: vertical profiles of salinity and temperature, abnormal behaviour of stock, dissolved oxygen, and meterological conditions. Extra measurement might include chlorophyll and pheopigment estimation, pH, light attenuation, adenosine triphosphate (ATP) and nutrient (inorganic nitrogen, phosphate and silicate). A common approach to monitoring the occurrence of harmful algal species was prepared by Dr. Tett in 1985 and a further refinement was presented by Dr. Lindahl in 1986 and is being evaluated. The method to be recommended will be included in this section of the proposed co-operative research report.

3 TOXICITY MONITORING

In general, because of their filter feeding and capacity to accumulate toxins bivalves are the usual organism monitored. However, in some instances it may be necessary to monitor other locally consumed and/or of commercial value organisms (e.g. ascidians and grazing moluscs).

The criteria for locating key sampling stations are similar to those described for phytoplankton monitoring. Furthermore, with the exception of offshore sampling station they should be located as close as possible to stations used for phytoplankton monitoring. However, in the case of shellfish cultivation on suspended long lines it is necessary to consider the position on the long line from which the bivalves are collected. For example in the case of the toxic algae being confined to a narrow subsurface water layer bivalves growing near the surface might not be toxic and this could lead to the wrong conclusion being drawn. The Working Group recommends that mussel sample be collected from the top, middle and bottom of the suspended culture. Once samples are collected appropriate testing should be undertaken (see section XX).

ANNEX VII

MANAGEMENT STRATEGIES FOR REDUCING THE EFFECTS OF HARMFUL ALGAE ON MARICULTURE AND NATURAL FISHERIES

1 GENERAL INTRODUCTION

For both mariculture and natural fisheries there are a number of management strategies which can be adopted to ensure the protection of public health in the case of toxic algae ,and farmed stock in the case of both toxic algae and those species which might inflict physical harm. With respect to public health this involves careful monitoring of the natural stock and/or the farmed stock and this is discussed in section*. This section presents an assessment of different management strategies which could be employed by mariculturists to avoid or reduce the effect of harmful algae. The section is divided into two parts, the first deals with site selection prior to the establishment of a mariculture operation and the second discusses strategies for established operation.

2 PRE SITING MANAGEMENT (SITE SELECTION)

2.1 INTRODUCTION

Marine fin fish cultivation generally relies on feeding the fish with a manufactured diet. As such the farming of fish is independent of the natural food which might be available. It is clear therefore that one method of avoiding or reducing the problems associated with harmful algae it in the selection of sites where harmful species do not occur or where they only occur infrequently. However, Shellfish farming depends on natural phytoplankton production. It is therefore necessary to site farms in areas with sufficient productivity. Natural shellfish beds will also be located in productive areas. This presents a problem since such areas might also be where harmful algal blooms occur frequently. For PSP and DSP monitoring techniques can safeguard public health. However, some harmful blooms have been reported to kill larvae, reduce the growth of post larval stages and possibly affect reproduction. With respect to finfish farming the effects range from stress to mortality. Both toxin producing species, for example *Gymnodinium catenatum, Protogonyaulax tamarensis ,Dinophysis* spp., Flagellate X (*Heterosigma akashiwo*) and *Gyrodinium aureolum* , together with species which cause physical damage to finfish (for example *Chaetoceros convolutus*) have been involved in direct and indirect loss of production at fin and shell fish farms.

For fin and shell fish farming, where the occurrence of harmful species could seriously reduce production, site selection prior to the establishment of the farm provides a management strategy for reducing this problem.

All mariculture and shellfish fisheries are located in what can be regarded as inshore waters, with many operations located in estuaries, rias, fjords, and embayments. However, exchange of water takes place between inshore embayments and coastal water, as a result of movements of the halocline, tidal pumping and freshwater and/or wind-driven circulation. This can result in exchange of phytoplankton between offshore coastal water and inshore regions. The risk to a farm, in inshore waters, from harmful species is thus threefold:

1 The development of a harmful population *in situ* (within the water body where the farm or natural fishery is located) resulting from the growth of a species present in that water body

2 Transport of an inoculum to the site area and subsequent growth to a population size which is harmful

3 The development of a harmful population at an external site and subsequent transport to the sitearea

These are important distinctions which make it essential to consider not only conditions influencing phytoplankton growth in the immediate vicinity of the farm or natural fishery site but also that of adjacent coastal regions. The following criteria for assessing the probable occurrence of harmful algae are based on an assessment of the main factors which influence phytoplankton growth, species succession and the accumulation of phytoplankton in inshore and offshore coastal waters.

2.1.1 Nutrients and Lights

With the exception of turbid and deep, vertically mixed waters the growth of phytoplankton, during

the spring and summer, is limited by the availability of dissolved inorganic nitrogen. Thus coastal waters and semi enclosed water bodies with high, natural levels of nitrogen or which receive nitrogen from anthropogenic sources (for example domestic sewage, industrial waste and run-off from agricultural land) have the potential to support a higher level of primary production than nutrient poor waters. High densities of harmful algae are therefore more likely to occur in waters enriched with nitrogen.

In turbid and deep, vertically mixed waters the availability of light rather than nutrients is likely to control phytoplankton growth. In locations where light is controlling phytoplankton growth blooms are unlikely to develop.

2.1.2 Shelf sea fronts

Ecological studies of coastal phytoplankton have shown that shelf sea fronts are regions of enhanced primary production (see for example Pingree et al., 1978). These fronts which are a summer feature, range from simple boundaries between inshore tidally mixed water and offshore thermally stratified water (for example the English Channel fronts) to more complex thermohaline fronts (for example fronts off the west coast of Scotland). Once established thermal shelf sea fronts generally persist for several months during the summer. The physical and chemical conditions found at fronts result in an enhancement of primary production and the duration of fronts is sufficiently long to allow species succesion from diatoms to dinoflagellates to take place. Consequently during summer the phytoplankton found at these fronts might be dominated by dinoflagellates (Simpson et al., 1979) and large populations can develop (Holligan, 1979). Some frontal boundaries could therefore act as a source of a harmful bloom or provide an 'inoculum' of a harmful species. For example, red tides of *Gyrodinium aureolum* which have caused mortalities of farmed fish in Scotland and Ireland were thought to have originated at offshore fronts (Jones et al., 1982; Doyle et al., 1984). In addition, *G. aureolum* blooms which caused mortalities of Scallop larvae reared in some French coastal embayments probably originated at the Ushant front (Lecorre *et al*, 1986).

2.1.3 Flushing time of estuaries

The dilution rate of an estuary will influence the accumulation of biomass as a result of growth within the estuary. With respect to Scottish, west coast sea lochs, Gowen *et al.* (1983) suggest that calculating a flushing time is a means of ranking sea lochs as environments for phytoplankton growth. Thus when the timescale of flushing is less than or equal to the timescale of phytoplankton growth high algal standing crop is unlikely to develop. Only in those sea lochs which have a flushing time in excess of the timescale of phytoplankton growth, is growth of phytoplankton likely to result in a high algal standing crop. Thus in rapidly flushed sea-lochs the probability of blooms developing is low. It might be possible to assess other estuaries using the same approach.

2.1.4 Water column stability

During the summer, stratification of the water column isolates nutrient depleted surface waters from deeper water with higher concentrations of nutrients. In some estuaries stratification is weak and there are periodic mixing events. Such mixing can introduce nutrients in deep water into the euphotic zone and stimulate phytoplankton growth. This growth could result in the development of a harmful population in estuaries in which stagnating nutrient rich bottom water is mixed into the euphotic zone.

2.1.5 Transport mechanisms

For a harmful bloom or an 'inoculum', which develops at a shelf sea front, to cause mortalities of farmed fish or shellfish in an estuary or a bay requires a mechanism to transport phytoplankton from the front into these coastal areas. Transport mechanisms have been suggested for specific locations (see for example Jones et al., 1982) but in general the processes which result in the transport of phytoplankton from offshore fronts into estuaries or bays are poorly understood. Furthermore a distinction should be made between localized transport mechanisms which might operate over distances of several kilometres (see Jones et al., 1982) and large scale mechanisms which might operate over distances of several hundred kilometres (Lindahl, 1983).

2.1.6 Site specific concentration mechanisms

The density at which a bloom becomes toxic might be reached by a combination of growth and concentration of the algae. The concentration of phytoplankton might be due to a hydrographic feature

such as a convergence between brackish water and saline water or a meteorological feature such as an onshore wind or merely to the pumping system used in a shellfish larval production pond.

2.1.7 Farm Operation

Fish farms release large quantities of ammonium (excretory waste) into the marine environment (Gowen and Bradbury, 1987) and shell fish farms can enhance the cycling of nitrogen through the food chain. Under certain conditions both types of operation might stimulate phytoplankton growth. It has been shown that soluble organic waste from fish farms can stimulate the growth of dinoflagellates (Nishimura, 1982). Furthermore the toxicity of *Gyrodinium aureolum* might be under environmental control (Turner et al., 1984) and could be influenced by waste from fish farms.

3 POST-SITING MANAGEMENT

3.1 INTRODUCTION

For a chosen site and existing mariculture operations there are a number of management strategies which can be initiated to reduce the effect of harmful species. Management to reduce the effects of harmful algae can be divided into three cathegories; 1 farm design,
2 monitoring for the presence of harmful species and/or the development of a bloom, 3 management during the presence of harmful species (mitigation techniques)

3.2 Farm design

If consideration is not given to the design of farm structures, certain management techniques are precluded from use when a population of harmful algae reaches a sufficient size to cause problems. One option which should be considered is the possibility of moving the culture structures away from the affected area. For example, in Ireland fish cages were moved away from a localised bloom of Flagellate X and mortalities of farmed stock avoided (Doyle, unpubl. data). However it should be pointed out that this strategy will only work where the occurrence is localised. With respect to moving cages three factors should be considered:

1. The size of the water body in which the farm is located.
   This should be sufficient to allow the movement of the farm structures. In Scotland some fish farms are located in sea lochs which are too small to allow effective movement of the cages.
2. Moorings.
   The use of easily detatchable moorings would make the operation of moving the structures easier and faster. It may also be necessary to have permenant moorings at an alternative site for use when cages are moved.
3. Structure design.
   Farm structures should be designed to move well through the water, minimize constriction of suspended net pens, long lines should not tangle and the structure should move safely with reasonable speed. In the case of fin fish farms consideration should be givento cages with greater depth which would allow the fish to distribute themselves vertically to avoid a harmful population which might be confined to a narrow depth range. For raft culture of bivalves a harmful algal population, restricted to a narrow depth range could be avoided by raising or lowering the bivalve culture. However, lowering bivalve culture into deeperwater with a low phytoplankton biomass for an extended period of time is likely to reduce bivalve growth. For mariculture operations which rely on pumped water there is the potential to monitor the intake water and to take action if the presence of harmful species is suspected. Detection could be carried out by periodic discrete sampling followed by estimation of phytoplankton biomass and species composition. Alternatively it is possible to monitor phytoplankton biomass in the intake water continuously. This could be achieved indirectly by measuring turbidity or directly by estimating chlorophyll. Since turbidity is an indirect method the latter is recommended. However, chlorophyll only provides an estimate of microalgal biomass therefore, it is recommended that discrete samples for species analysis should be taken periodically, particularly if the concentration of chlorophyll increases. Land and sea based farms which rely on pumped water should be designed with the capabilty of altering the depth of the intake port(s) . An example is a land-based farm in Scotland which has a duel intake system has successfully reduced mortalities during near surface blooms of harmful algae by switching from a shallow intake (at adepth of 5 m) to a deep intake (at a depth of 20 m).

3.3 Monitoring for development of a bloom

Unless forwarned of the occurrence of harmful algae certain effective measures to reduce the impact of the algae may not be feasible. It is therefore essential to monitor for the presence of harmful species. This requires monitoring which is discussed in section *.

3.4 Management (mitigation) during the presence of a harmful population

Some of the techniques described below require the use of specific items of equipment. Given the speed with which many algal blooms develop there is seldom time to make or purchase this equipment. Thus for effective measures to be implimented rapidly, such equipment should be on site.

Some of the techniques outlined below involve movement of the stock or the introduction of water free of the harmful algae into the culture structure. In both cases it is important to have information on the horizontal and vertical distribution of the harmful species and the quality of the water used to replace water containing the algae. It must be emphasised that without such information problems could be encountered which are as costly as not taking any action. For example the pumping of deep, anoxic water into fish cages to disperse or dilute a bloom would result in mortalities of the stock by asphixiation.

For many of the techniques outlined below maximum benefit will only be achieved if action is initiated before the algal population reached a harmful size. However even if implemented when harmful effects are manifest some benefit may be derived.With respect to all of these techniques there is the factor of cost effectiveness. If a prediction of the level of toxicity or potential loss of stock could be made, this will allow an assessment of the cost effectiveness of these methods. Potential management options are outlined as follows:

1. Pre-emptive harvesting. Where a seasonal occurrence of a harmful species has been defined, this together with phytoplankton monitoring and negative bioassays would allow early harvesting to minimize economic loss. For farmed fish harvesting prior to the occurrence of a harmful species could also avoid complete loss of stock and market.

2. Movement of cultured stock to water free of the harmful species. This may involve the deeper cages or longer culture lines. As discussed earlier this presupposes an ability to move the structures and information on the distribution of the harmful species.

3. *In situ* shielding of farmed fish. Farm stock could be isolated from harmful algae in a number of ways. First, by using a non-porous barrier (for example polythene sheets), which may completely enclose the fish or be placed around the sides of the cages. Second, a bubble curtain may also isolate the stock although there is some doubt of the effectiveness of this. Third, the injection of water, free of the harmful species and of good quality (for example free of particulate material and with sufficient dissolved oxygen) into fish cages. This might dilute the algal population to a size which does not cause harm to the stock or act as a barrier, preventing the fish from being exposed to the algae. The pumping of deep water is likely to be most successful when employed with a physical barrier. Additional support in the form of oxygenation and current generators may be required. The cost and benefits of this type of approach should be carefully examined.

With fish culture the reduction of the animals oxygen requirements might be important in its survival. Cessation of feeding, provision of shade and reduced human activity in the culture area lessen the rate of oxygen uptake by the fish. Because the reduction of respiration rate requires time these measures must be initiated in advance of the onset of the harmful effects.

After a harmful algal population has developed, in fish culture a range of options remain. Two options are, dilution of the bloom by articifial currents or pumped water and, providing the fish with access to water free of harmful algae by lowering the cage structures or increasing the depth of the net cage. However this type of mitigation presupposes that the farmer has an understanding of the hydrographic conditions around his site. For example it would be deleterious to dilute the bloom with anoxic water.

In areas of short daylight duration illumination of fish culture waters at night appears to releave distress associated with some algal events. The mode of action is not understood and should be determined. Another approach is to reduce stress levels and consequent oxygen requirements of the fish. In bivalve culture, once a harmful algal population has deveolped (i.e. the bivalves are toxic) the only known option is detoxification. However, the cost effectiveness of this approach is in question.

ANNEX VIII

PREDICTING THE OCCURRENCE OF HARMFUL PHYTOPLANKTON

1 GENERAL INTRODUCTION

The aims of predicting the occurrence of harmful phytoplankton in low cell densities and blooms are two fold. First, to provide mariculturists and shellfish harvesters with sufficient time to initiate management strategies. Second, to better focus monitoring programmes and optimise resources.

It is necessary to distinguish between prediction in time and prediction in space. Further useful distinctions are those made between small-scale (or local) and large-scale (or regional) prediction, and between prediction in a short-term (a few days or a week) and in the long term (seasons and years). The distinction between the short-term local and the long-term regional prediction is like the distinction between weather and climate. It may be fruitful to keep in mind the different problems encountered in weather forecasting and climatic description and apply this by analogy to the problem of predicting harmful marine blooms. Another useful distinction is that between the empirical, statistical and the theoretical approaches to prediction. It should be pointed out that the requirements for prediction differ somewhat between fin-fish and shellfish cultivation. Finally since harmful effects can result from the presence of a species in low cell densities as well as a bloom the use of the word "occurrence" implies both low cell number and blooms.

In predicting the occurrence of harmful algal species it is important to identify the source of the species: *a,* growth of the species at the site as a result of local conditions; and *b,* the advection of the species from an external seed-area, such as an offshore front, and intrinsic growth of the population at the site. In addition to growth, physical and biological factors might concentrate the algal cells such that cell densities reach harmful proportions at the site. It is also important to distinguish between an occurrence resulting from excystment of benthic cysts, which may have persisted for several years in the sediment and those developing from a planktonic inoculum.

It must be emphasised that existing methods of predicting the occurrence of harmful species do not provide a prediction of effect. Clearly an ability to predict the level of toxicity in shell fish or the potential losses of fin fish would assist in management decisions to deal with the event.

2 PREDICTION OF LOCAL OCCURRENCES

2.1 Prediction based on time series data

Data derived from monitoring of a site or sites can provide short term prediction of the occurrence of harmful species and this can provide fish and shellfish farmers with sufficient warning to initiate strategies to minimise the effect. Where there are good time series of data derived from regular monitoring effective long-term predictions can be made. This applies to blooms and those toxic species occurring at low cell densities (for example *Dinophysis* sp., *Protogonyaulax* sp., *Gymnodiniun catenatum*). This method is successfully employed for a range of species in France, Spain, Sweden, Canada and the United States. For example in the Netherlands phytoplankton data collected since 1973 provide evidence for an annual September increase in *Dinophysis acuminata* populations (Kat, in press). However, this does not provide a prediction of whether the *Dinophysis* -populations induce toxicity in mussels.

2.2 Empirical models

Some geographical areas which have well-defined seasonal weather patterns might be more amenable to prediction than areas which have more variable weather patterns. Such models are a valuable addition to monitoring programmes. *The following example is taken from work by* Fraga *et al.,* (in press). Blooms are summer and autumn phenomena in the Rias of Galicia (NW Spain), but *Gymnodinium catenatum* blooms have only occurred during the autumn.

The hydrography of the West coast of the Iberian peninsula is characterized by a seasonal upwelling. During summer, the anticyclone over the Azores is the cause of the dominant North wind that causes the upwelling. In the autumn, the high pressure moves to the South and is substituted by low pressure that

change the north winds to south winds not favourable for upwelling. Although upwelling systems are usually associated with a lack of thermal stratification, inside the rias, during summer the upwelling is generally the cause of thermal stratification due to the cooling of bottom water of the ria while the summer sun heats the surface waters.

In this area the non motile phytoplankton (diatoms) are taken out of the euphotic zone by the downwelling. Motile phytoplankters (flagellates) remain in the surface waters by upward swiming and are concentrated horizontallly by convergent water movements. If downwelling is strong, slow swimming species are also taken out of euphotic zone. As a result, faster swimming species, for example the chain forming dinoflagellates *Protogonyaulax affinis* and *Gymnodinium catenatum*, could dominate and might reach bloom proportions.

3 PREDICTING THE OCCURRENCE OF HARMFUL ALGAE ON A REGIONAL SCALE

Where there are known persistent advective mechanisms which result in transfer of phytoplankton along the coast or from offshore frontal boundaries it might be possible to provide regional prediction. In such cases there is the potential to give considerable advance warning to the final target areas by regular monitoring of the region of bloom initiation. Monitoring could be achieved by looking at phytoplankton species composition, biomass, or of toxin in natural shellfish beds or especially installed mussel rafts. Remote sensing of sea surface temperature and chlorophyll might also be a means of monitoring the occurrence and changes in phytoplankton biomass.

4 LONG TERM PREDICTABILITY

Long-term prediction can be addressed from two perspectives. The first would be based on the detection and statistical validation of trends in historical toxicity data. Examples would be a cyclical recurrence of toxicity at regular intervals or a sustained increase or decrease in toxicity with time. In Canada continuous shellfish toxicity records since 1940 exist fron the Bay of Fundy. There appears to be a significant correlation between high toxicities of the mid 1940's, early 1960's and late 1970's and the 18.6 year lunar tidal modulation cycle (White, 1984).

The second approach towards long-term prediction seeks to identify statistical relationships between blooms and one or more measured environmental variables that in themselves vary in a predictable manner. An example would be if temperature or some other parameter critical to bloom development could be shown to vary in a statistically consistent manner. This would require resolution of trends superimposed on the normal seasonal variation.

The above discussion relates to prediction based on monitoring or empirical models. At present, models exist which allow the prediction of phytoplankton biomass (see for example Tett, 1981 and references cited therein). However, to date none of these models have the capability of predicting the occurrence of specific phytoplankton species.

5 BASIC RESEARCH TO IMPROVE PREDICTABILITY

To improve predictive capability the following areas of research are considered to be to essential;

Analyses of harmful phytoplankton occurrences, meterological and hydrographic data should be carried out to discover whether such occurrences are correlated with specific conditions and may provide long term prediction.

Development of models which provide an assessment of the degree of toxicity in shellfish and the level of mortality in finfish. The formulation of such models requires a better understanding of the ecophysiology of harmful species in relation to hydrodynamic processes.

The manner in which envionmental factors influence physiology and behaviour and hence the responce of the target organism (cultured and harvested) to the effects of harmful algae.

Progress in these areas would allow management strategies to be optimised.