

Intergovernmental Oceanographic Commission
Training Course Reports

36

MAST-IOC
Advanced Phytoplankton Course
on Taxonomy and Systematics

Marine Botany Laboratory
Stazione Zoologica 'A. Dohrn' di Napoli

Casamicciola Terme (Island of Ischia), Naples, Italy
24 September - 14 October 1995

TABLE OF CONTENTS

	Page
ABSTRACT	iii
1. BACKGROUND, ORGANIZATION AND GOALS	1
1.1 BACKGROUND	1
1.2 ORGANIZATION	1
1.3 GOALS	2
2. CONTENT	2
2.1 OPENING AND INTRODUCTION	3
2.2 MANUALS	3
2.3 THEORETICAL SESSIONS	3
2.4 PRACTICAL SESSIONS	3
2.4.1. Species observation	3
2.4.2. Techniques	4
2.4.3. Exercises	4
2.5 FIELD TRIP AND OBSERVATION OF LIVE NATURAL SAMPLES	4
2.6 SCANNING (SEM) AND TRANSMISSION ELECTRON MICROSCOPE (TEM) DEMONSTRATIONS	5
2.7 WORKSHOPS	5
2.8 REGIONAL REPORTS ON HARMFUL ALGAL BLOOMS	5
2.9 SEMINARS	5
3. QUESTIONNAIRE AND CONCLUDING REMARKS	6
3.1 QUESTIONNAIRE	6
3.1 CONCLUDING REMARKS	6

ANNEXES

I	Programme
II	Faculty
III	Participants to the five previous Courses
IV	Organizing Committee
V	List of participants
VI	Financial statement
VII	Workshops
VIII	Regional reports
IX	Questionnaire
X	List of Acronyms

ABSTRACT

This was the sixth in a series of Courses which started in 1976 upon the recommendation of the SCOR-Working Group of Phytoplankton Methods (WG 33). The first three Courses (1976, 1980 and 1983) were organized in Norway by the Marine Botany Section of the University of Oslo (Norway). The fourth, fifth and present Courses were organized by the Marine Botany Laboratory of the Stazione Zoologica "A. Dohrn" (SZN), Naples (Italy). The 1995 faculty included some of the former teachers of the Course since 1976 (G. R. Hasle and J. Throndsen) and 1983 (K. A. Steidinger), the staff of the Marine Botany Laboratory of the SZN (D. Marine, M. Montresor and A. Zingone) and other teachers and lecturers. Most of these lecturers had participated as students in previous Courses (M.-J. Chretiennot-Dinet, J.D. Dodge C.B. Lange, J. Larsen and C. R. Tomas).

The main goal of the Course was to train and upgrade qualified students in the identification of phytoplankton species using light microscope. Students were chosen among already experienced scientists working in different fields of phytoplankton research and/or monitoring.

The Course consisted of theoretical sessions, including lectures on general morphology and taxonomy of the different marine phytoplankton groups, followed by practical sessions, with examination of fixed, cultured and slide material. Each student had the chance to study samples using microscope, but samples were also shown by a video camera connected to a microscope. Relevant techniques aimed at collecting and culturing phytoplankton specimens, as well as for displaying taxonomic characters essential for identification, were presented. Several exercise sessions were organized based on mixed material provided by both the faculty and the participants. The manual on phytoplankton identification was used as a text book. A very comprehensive collection of about 3000 reprints and identification literature was available for consultation during the sessions.

Special workshops on harmful or nuisance species and seminars covering several aspects of phytoplankton research were organized.

1. BACKGROUND, ORGANIZATION AND GOALS

1.1 BACKGROUND

Phytoplankton taxonomy and systematic is an open and actively developing field of research due to the extremely high and still increasing number of identified microalgal species. The correct identification of phytoplankton species relies on characters which can only be detected through different special techniques. Moreover, the knowledge of species from the taxonomic point of view is a tool that cannot be renounced for any ecological or ecophysiological work on marine phytoplankton. Due to its small scale response to environmental changes, phytoplankton community composition represents a synthetic parameter to interpret the dynamics of the pelagic ecosystem. In particular during the last years the importance of taxonomic work has been stressed by the international scientific community in the frame of the increasing interest in the assessment of natural community biodiversity. This is directly related to recommendations adopted by UNCED in Rio de Janeiro and by documents produced by the European Community in the field of research and technological development.

The need for advanced courses on phytoplankton identification was first identified by Professor Trygve Braarud (1903-1985) of the University of Oslo, who considered taxonomy to be fundamental to the knowledge of ecology and physiology of marine phytoplankton. The SCOR-Working Group of Phytoplankton Methods (WG 33), established in 1969, suggested a tentative plan for a "Phytoplankton Course for Experienced Participants", along with a list of contents for a manual including methods and literature for the identification of marine phytoplankton. The Marine Botany Section of the University of Oslo was chosen to be responsible for the teaching programme of the Course, which was held for the first time in 1976 in Oslo, with 17 participants from 13 different countries. The Phytoplankton Manual, (A. Sournia ed.), focused on non-chemical methods for the quantitative study of phytoplankton, was published two years later in the UNESCO Monographs on Oceanographic Methodology. A first part of a manual (C. R. Tomas ed.) for taxonomy and identification was published in 1992 and a second part is presently at the second proof stage.

Since the first Course, two other advanced Courses were organized by the Marine Botany Section of the University of Oslo and held at the Biological Station in Drøbak, in 1980 and 1983. Over the years, the teaching faculty basically included the same teachers (G. R. Hasle, J. Throndsen, K. Tangen, B. Heimdal and, from 1983, K.A. Steidinger). In 1985 and 1990, the Course, still under the direction of G.R. Hasle, was organized by the Marine Botany Laboratory of the Stazione Zoologica "A. Dohrn" of Naples (SZN). Since then, some new teachers (almost all past-students) have in turn joined the faculty and some teachers have left, but basically the same teaching programme, reinforced and adjusted by previous experiences, has been used (Annex I).

A total number of 101 participants, representing 37 countries have so far attended the six Advanced Phytoplankton Courses. They form a rather special community, including several well known scientists engaged, at times in leading positions, in the field of phytoplankton research (Annex H) and sharing, together with the more than 500 people who have so far applied for the Course, the original idea of Trygve Braarud on the importance of taxonomy in phytoplankton research.

1.2 ORGANIZATION

The organization of the present Course (Annex III) started at an informal meeting which was held in Newport, USA, in October 1990. It was first announced in October 1993 at the second session of the IOC-FAO Intergovernmental Panel on Harmful Algal Blooms, during which the organization of training Courses on phytoplankton taxonomy at different levels of specialization was recommended. The announcement was mailed to about 400 research institutions around the world and appeared at the beginning of 1995 in the most important scientific journals including marine phytoplankton as their main topics. A total number of 132 applications from 38 different countries reached the organizers, and the selection of 20 participants and 2 auditors (Annex IV) was performed based on the curriculum, the positions held, the need and usefulness for training of the applicants.

Due to severe space limitations at the SZN, the Course was held in a small hotel (Hotel Gran Paradise) located in Casamicciola Terme (island of Ischia), which also hosted the faculty and the students. The classroom and the laboratory were both located in the main meeting hall of the hotel. The classroom held the students' benches, each equipped with a light microscope, and the desk with two microscopes connected to a video camera, and slide and transparency projectors. On a side, a library was prepared holding a collection of about 3000

IOC Training Course Report No. 36

page 2

reprints belonging to the Marine Botany Laboratory, as well as taxonomy textbooks lent by the SZN library. The laboratory was equipped with a small growth chamber for cultures, a centrifuge, a filtration apparatus and a number of laboratory devices and disposable for cultures maintenance and sample preparations and reprints belonging to the Marine Botany Laboratory as well as taxonomy textbooks lent by the SZN library. A course office was prepared in a separate room, which was equipped with computer, telephone, e-mail and Xerox-copy machine.

Financial support for the Course was provided by the EC-MAST program, which covered honoraria, travel and living expenses for teachers, living expenses for the 20 participants, rent of the classroom and consumable expenses. SZN provided funds for the organization and secretariat, as well as living expenses and salary of the staff of the Institute involved in the Course. IOC contributed travel expenses for some of the participants from developing countries. Carl Zeiss S.p.A. kindly lent 22 microscopes for the practical sessions.

1.3 GOALS

The Course was meant for already experienced researchers actively working in fields of research such as phytoplankton ecology and physiology that require species identification, and for experts in taxonomy and systematic who need to upgrade their expertise.

The objectives of the Course were:

- (i) to provide an updated theoretical background for the morphology, taxonomy and classification of the most important taxonomic groups;
- (ii) to teach, in practical classrooms, methods and criteria for correct identification of species, with special emphasis on light microscopy techniques;
- (iii) to allow students to acquire updated information on specialized literature;
- (iv) to distribute information and awareness on toxic and potentially toxic species;
- (v) to create a forum for discussion of general and specific aspects of systematic, ecology and geographic distribution of phytoplankton species.

2. CONTENT

The Course included the following activities:

- (i) theoretical classrooms;
- (ii) practical classrooms;
- (iii) field trip and observation of the collected material;
- (iv) scanning (SEM) and Transmission Electron Microscope (TEM) demonstrations
- (v) workshops;
- (vi) regional reports on harmful algal blooms;
- (vii) seminars.

2.1 OPENING AND INTRODUCTION

The twenty-two participants, the members of the faculty and the Organizing Committee were welcomed by G. R. Hasle, director of the Course and by Donato Marine, head of the Organizing Committee of the Course. G. R. Hasle briefly presented the historical background of the Course. She showed the list of participants in the previous Phytoplankton Courses and stressed the leading positions within the scientific community attained by a large number of them. G. R. Hasle concluded her welcome speech by presenting the financial budget and expressing on behalf, of the faculty, her thanks to the Institutions that generously supported the Course (Annex VI).

D. Marino illustrated the programme (Annex 1) and gave practical information on the use of the laboratory facilities, the collection of reprints and identification literature, He also presented the manuals on the different phytoplankton groups that were distributed to the participants and expressed his thanks to the editor. C. R. Tomas and to the authors of the different sections, G.R. Hasle, B. Heimdal, K. Steidinger. E. Syvertsen K. Tangen and J. Throndsen.

2.2 MANUALS

The manuals "Marine Phytoplankton - A guide of naked flagellates and Coccolithophorids" (ISBN 0-12-693010-4) by J. Throndsen and B. Heimdal (C. R. Tomas ed.) and "Identifying Marine Phytoplankton - Diatoms and Dinoflagellates" by G. R. Hasle & E. Syvertsen, K. Steidinger & K. Tangen (C. R. Tomas ed.) were used as textbooks during the Course, As mentioned in chapter 1.1, the publication of such manuals was recommended by SCOR-WG33 in 1976. The books developed from the hand-outs usually distributed during the previous courses, which were thoroughly re-elaborated and completed to include a detailed description of numerous planktonic genera and species, along with taxonomic keys, drawings and references, were also distributed.

2.3 THEORETICAL CLASSROOMS

General features of diatoms, dinoflagellates, coccolithophorids and other flagellates were illustrated during the different sessions. Morphological characters of taxonomic groups and species as seen in light and electron microscopy were presented and their distinctive features were stressed using visual aids (transparencies and slides), The use of identification keys was introduced when possible. In some cases, permanent slides of selected species were shown from the video camera connected to a microscope. For coccolithophorids and dinoflagellates having alternate stage life cycles, different life stage morphologies were illustrated.

The teachers gave detailed information on the use of the different sections of the manuals drawing attention to schemes and illustrations useful for species identification. They also provided updated literature lists and gave the historical background of literature and nomenclature concerning "problematic" species.

2.4 PRACTICAL CLASSROOMS

2.4.1 Species Observation

Permanent slides and living or fixed clonal cultures of selected phytoplankton species were distributed to all the participants to be observed and identified in the light microscope. Special attention was given to bloom species and harmful species belonging to the different groups.

Fixed natural samples were also distributed in order to identify species belonging to the different groups. This material was mainly provided by the faculty, but special sessions were also devoted to the examination of natural samples provided by the students themselves.

2.4.2. Techniques

Different techniques for collecting, culturing and handling phytoplankton were illustrated and their possible applications and limitations were highlighted. Practical demonstrations were provided and the opportunity was given to the participants to practice.

The following methods were included:

- (i) collection of phytoplankton samples with different tools: bucket samples, Niskin bottles, plankton nets - these methods were illustrated during the field trip (see paragraph 2.4);
- (ii) concentration of phytoplankton samples: sedimentation, filtration, Tangential Flow Filtration (TFF);
- (iii) serial dilution cultures established from natural bottle samples for flagellate enumeration and identification. Series prepared at the beginning of the Course were observed during the exercise sessions:

- (iv) single cell isolation by micropipetting and cultivation techniques. Different recipes for preparation of culture media were distributed to the participants and discussed;
- (v) frustule cleaning with different acid mixtures and permanent slides preparation for diatoms;
- (vi) permanent jelly mounts for coccolithophorids and dinoflagellates;
- (vii) squashing and thecal plate staining for thecate dinoflagellates;
- (viii) dinoflagellate resting cyst collection and treatment of cyst samples for observation;
- (ix) preparation of TEM grids and SEM stubs using both culture material and mixed samples;

2.4.3 Exercises

Exercise sessions, held during the last days of the Course, consisted in observation of natural samples from different geographical areas, comparisons of similar species, serial dilution cultures and permanent mounts of diatoms and dinoflagellates. Lists of species identified were compiled by the participants and checked by the teachers.

2.5 FIELD TRIP AND OBSERVATION OF LIVE NATURAL SAMPLES

A field trip took place on the research vessel 'Vettorica' of the SZN. Several stations were visited and net and bottle samples were collected to be observed in the afternoon of the same day. Large volume samples were also collected by a submerged pump to be concentrated by the TFF apparatus. The use of different equipment was demonstrated, e.g. CTD probe and rosette sampler.

2.6 SCANNING (SEM) AND TRANSMISSION ELECTRON MICROSCOPE (TEM) DEMONSTRATIONS

The Course was meant to focus on the identification of phytoplankton species in the light microscope. However, during the lessons students were made aware of the impossibility of identifying certain species or even genera without the aid of the electron microscope. Ultrastructural features underlying classification in several genera were illustrated by slides and transparencies during the lessons.

An electron microscopy session was held during the Course at the SZN. Students divided into small groups had the chance to observe material at the SEM and TEM under the guide of some of the teachers. Samples prepared during the previous days, as well as other demonstrative samples, were showed. and morphological characters of interest were illustrated for some species.

2.7 WORKSHOPS

The following workshops were organized (Annex VII):

- (i) Toxic Dinoflagellates;
- (ii) Diatoms: toxic and nuisance species;
- (iii) Naked Flagellates with Emphasis on Nuisance Species;
- (iv) Interactive Taxonomy.

2.8 REGIONAL REPORTS ON HARMFUL ALGAL BLOOMS

A short synopsis of problems related to toxic and nuisance phytoplankton in their respective countries was presented by the participants (Annex VIII). The countries presented were: Argentina, Brazil, Canada, Chile, China, Germany, Ireland, Italy, Korea, Mexico, Norway, Russia, Spain, United Kingdom, and United States.

2.9 SEMINARS

The following seminars, given by students and members of the faculty, were organized on both taxonomy and other aspects relevant to marine phytoplankton:

Flagellates from the Norwegian coasts - W. Eikrem;
Molecular biology techniques applied to dinoflagellate phylogeny - G. D'Onofrio, SZN;
Parasitic dinoflagellates - J. H. Landsberg;
Biogeography of phytoplankton species - J. D. Dodge;
Immunofluorescence techniques in the identification of phytoplankton species- D. Sarno;
Processing phytoplankton data - Q. Dortch.

3. QUESTIONNAIRE AND CONCLUDING REMARKS

3.1 QUESTIONNAIRE

During the last days each participant was requested to give comments on the Course with a questionnaire (Annex IX) which included questions on both organizational and scientific aspects.

All the participants expressed their enthusiastic appreciation for the organization and the scientific content of the Course. Suggestions for improvements included the following comments:

- (i) some phytoplankton groups, that is naked dinoflagellates (3 participants) coccolithophorids (2 participants) and pennate diatoms (2 participants) should have been dealt with in greater detail and others, that is cyanobacteria (5 participants), added to the programme of the Course;
- (ii) participants should have been given more time to try out some of the techniques included in the Course. that is TEM preparations (4 participants), staining and preservation of samples (3 participants). counting methods (5 participants) and others, e.g. epifluorescence (10 participants);
- (iii) time devoted to electron microscopy observation should have been increased (3 participants);
- (iv) the length of the Course should be extended to 4 weeks (2 participants).

3.2 CONCLUDING REMARKS

G. R. Hasle, director of the Course, and D. Marino, on behalf of the Organizing Committee, presented the concluding remarks. They stressed the very positive outcome of the Course. All the participants took the opportunity to highly increase their ability in species identification and application of different techniques, as well as to go through the most updated taxonomic literature. G. R. Hasle and D. Marino expressed their appreciation for the pleasant collaborative atmosphere established during the sessions, which promoted the exchange of knowledge and experience not only vertically, from the faculty to the students, but also among the students. They also referred on the results of the questionnaire and committed themselves to organize another Course in three years,

ANNEX I

PROGRAMME

Sunday, 24 September 1995

Arrival of participants, ice breaking, with welcome party in the evening,

Monday, 25 September

- 0900-0930: Opening of the Course, general recommendations (how to use microscopes, literature. etc.) Hasle, Marino.
- 0930-1300: Methods - Demonstration of procedure steps for preparation of diatom slides, Starting serial dilution cultures. Lange, Throndsen, Marino, Zingone, Forlani.
- 1500-1900: Diatoms - Morphology, terminology, general systematic. Hasle. Observation of Thalassiosiraceae. Hasle, Lange.
- 2100-2200: Diatoms - Demonstration of Melosiraceae and Leptocylindraceae. Lange, Hasle.

Tuesday, 26 September

- 0900-1300: Diatoms - Observation of Coscinodiscaceae. Stellarimaceae. Demonstration of Hemidiscaceae, Asterolampraceae and Heliopeltaceae. Lange, Hasle. Observation of Rhizosoleniaceae (1st part). Hasle, Lange.
- 1500-1900 Diatoms - Observation of Rhizosoleniaceae (2nd part), Hemiaulaceae and Cymatosiraceae. Hasle, Lange. Demonstration of Lithodesmiaceae and Eupodiscaceae. Hasle, Lange.
- 2100-2200: Diatoms - Observation of Chaetocerotaccae. Lange, Hasle.

Wednesday, 27 September

- 0900-1300: Diatoms - Demonstration of Fragilariaceae and Rhaphoneidaceae. Lange, Hasle. Observation of Thalassionemataceae. Hasle, Lange. Demonstration of Naviculaceae. Lange. Hasle. Observation of Bacillariaceae. Hasle, Lange.
- 1500-1900 : Coccolithophorids - Morphology and terminology. Heterococcolithophorids. Chrétiennot-Dinet. Observation of living material in culture (*Emiliana* and *Gephyrocapsa*). Chrétiennot-Dinet, Zingone.
- 2100-2200: Diatoms - Rc-observation of selected samples. Hasle, Lange, Marino.

Thursday, 28 September

- 0900-1300: Coccolithophorids - Holococcolithophorids. Collection and preservation techniques, preparation of permanent slides for light microscopy, preparation of stubs for SEM. Chrétiennot-Dinet. Observation of living material in cultures (*Pleurochrysis*, *Cruciplacolithus*, *Ochrosphaera*, *Hymenomonas*) Chrétiennot-Dinet, Zingone, Throndsen.

IOC Training Course Report No. 36
Annex I - page 2

1500-1900: Cocolithophorids - Reproduction and life cycles, synonyms and recent taxonomic changes. Chrétiennot-Dinet. Observation of selected samples. Chrétiennot-Dinet, Zingone, Throndsen.

Friday, 29 September

0900-1300: Diatoms - Re-observation of *Pseudo-nitzschia* species. Hasle, Lange.

1500-1900: Flagellates - Morphology, terminology, general systematic. Throndsen. Raphidophyceae, Chrysophyceae. Observation of selected species. Throndsen, Zingone, Chrétiennot-Dinet.

2100-2200: Flagellates - Prymnesiophyceae. Observation of selected species. Throndsen. Zingone, Chrétiennot-Dinet.

Saturday, 30 September

0900-1300: Flagellates - Cryptophyceae. Observation of selected species. Throndsen, Zingone. Techniques: TEM preparations of flagellates and cocolithophorids. Throndsen, Zingone, Chrétiennot-Dinet.

1500-1700: Social events - Manzù sculpture exhibition at the Ischia Castle.

1700-1900: Social events - Time free for shopping.

1930-2100: Social events - Organ concert by Maestro Livia Mazzanti.

2100: Social events - Pizza party.

Sunday, 1 October 1995

1030-1600: Excursion by boat around Ischia and Procida islands.

Monday, 2 October

0900-1300: Flagellates - Euglenophyceae and Chlorophyceae. Observation of selected species. Throndsen, Zingone.

1500-1900: Flagellates - Prasinophyceae. Observation of selected species. Throndsen, Zingone.

2100-2200: Seminar: "Flagellates from the Norwegian coasts" W. Eikrem

Tuesday, 3 October

0900-1300: Dinoflagellates - Morphology, terminology, general systematic. Steidinger. Demonstration and observation of *Prorocentrum*, *Dinophysis* and *Ornithocercus* species. Steidinger, Montresor.

1500-1900: Dinoflagellates - Techniques (permanent slides, squashing, plate staining, etc.). Steidinger, Montresor. Demonstration and observation of *Protoperidinium*, *Scrippsiella* and *Ensiculifera* species. Steidinger, Montresor.

2100-2200: Seminar ‘-Molecular biology techniques applied to dinoflagellate phylogeny’. G. DOnofrio (Marine Botany Laboratory, SZN),

Wednesday, 4 October

0900- 1300: Dinoflagellates - Demonstration and observation of *Alexandrium*, *Goniodoma*, *Pyrodinium*, *Gambierdiscus*, *Coolia*, *Ostreopsis* species, Steidinger, Montresor.

1500-1900: Dinoflagellates - Demonstration and observation of *Gonyaulax* and *Ceratium* species. and other armored dinoflagellates, Steidinger, Montresor,

Thursday, 5 October

0900-1300: Dinoflagellates - Demonstration and observation of naked species. Larsen

1500-1900: Dinoflagellate resting cysts - Introduction, demonstration and observation of organic and calcareous cysts. Montresor, Larsen.

2100-2200: Seminar: “Parasitic dinoflagellates” J. H. Landsberg.

Friday, 6 October

0960-1100: Group 1: Field trip. Tomas, Montresor
Group 2: Examination of samples from the Gulf of Naples. All faculty.
Demonstration of TFF technique. Zingone, Forlani.

1100-1300: Group 2: Field trip, Tomas, Montresor
Group 1: Examination of samples from the Gulf of Naples. All faculty.
Demonstration of TFF technique. Zingone, Forlani.

1500-1900: Examination of samples from the Gulf of Naples. All faculty.

Saturday, 7 October

0900-1300: Techniques for SEM preparations of dinoflagellates and flagellates and TEM preparations of dinoflagellates. Montresor, Throndsen, Larsen,

1500-1900: Cultivation. Tomas, Montresor, Throndsen, Larsen.

Sunday, 8 October

Free

Monday, 9 October

All day at the SZN - SEM and TEM demonstrations (the participants will be divided into 4 groups) and lecture on the history of the SZN. All faculty, Forlani, Esposito and the technicians of the electron microscopy laboratory (Dafnis, Iamunno, Gragnaniello).

Tuesday, 10 October

0900-1100: Exercises - Examination of mixed samples from different areas. Hasle, Lange, Steidinger, Throndsen, Zingone.

IOC Training Course Report No. 36
Annex I - page 4

1130-1200: Examination of serial dilution cultures. Throndsen, Zingone.

1500-1900: Workshop on "Toxic dinoflagellates". Tomas, Dodge, Larsen, Steidinger, Zingone.

Wednesday, 11 October

0900-1100: Lecture - Biogeography of phytoplankton species. Dodge

1130-1300: Exercises - Examination of mixed samples from different areas. Hasle, Lange, Steidinger, Throndsen, Zingone.

1500-1900: Workshop on "Diatoms; toxic and nuisance bloom species ". Hasle, Lange.

2100-2200: Exercises - Re-observation of serial dilution cultures. Throndsen, Zingone.

Thursday, 12 October

0900-1100: Exercises - Examination of mixed samples from different areas. Lange, Steidinger, Throndsen, Zingone.

1130-1150: Lecture - Immunofluorescence techniques applied to phytoplankton identification. Sarno .

1150-1300: Exercises - Examination of mixed samples from different areas. Lange, Steidinger, Throndsen, Zingone.

1500-1900: Workshop on "Naked flagellates with emphasis on nuisance species". Throndsen, Tomas, Zingone.

2100-2200: Seminar: "Processing phytoplankton data". Q. Dortch.

Friday, 13 October

0900-1100: Exercises - Comparison of selected species. Throndsen, Zingone.

1130-1300: Examination of mixed dinoflagellate and diatom samples from different areas. Dodge, Lange, Hasle, Zingone.

1500-1900: Workshop on "Interactive taxonomy". Dodge, Steidinger, Zingone, Sarno.

20.00: Gala Dinner

Saturday, 14 October

0900-1100: General discussion. All faculty.

11.30: Summary and conclusions. Hasle, Marino

Afternoon free

Sunday, 15 October

Departure

IOC Training Course Report No. 36
Annex 11

ANNEX II

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IOC Training Course Report No. 36
Annex II - page 2

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IOC Training Course Report No. 36
Annex III

ANNEX III

PARTICIPANTS TO THE FIVE PREVIOUS COURSES

Judy C. Acreman Canada. 1976	Marta M. Estrada Spain. 1976	Derek S. Harbour United Kingdom. 1976
Emelia R. Anang Ghana, 1976	Maria A. Faust USA, 1983	David R. A.' Hill Australia, 1990
David Hernandez-Becerril Mexico, 1985	Martha E. Ferrario Argentina, 1985	Michael A. Hoban USA, 1985
Lyse Bérard-Therriault Canada, 1983	Santiago Fraga Spain, 1983	Giorgio Honsell Italy, 1985
Pensri Boonruang Thailand. 1983	Jaqueline Fresnel France. 1990	Nobuhito Hosaka Japan, 1985
Susan A. Brady USA. 1985	Greta A. Fryxell USA. 1976	Maja Huttunen Finland. 1983
Ian Bryceson Tanzania, 1983	Ken Furuya Japan, 1980	Lydia Ignatiades Greece, 1985
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Marie-J. Chrétiennot-Dinet France. 1983	David L. Garrison USA. 1983	Ian R. Jenkinson Ireland, 1976
Einar Dahl Norway, 1980	Gisele Gaumer Algerie, 1980	Roberto S. Jiménez Equador, 1976
Elvira de Reyes Venezuela, 1980	Ana Maria Gayoso Argentina, 1990	Lakshmanan Kannan India, 1983
Malte Elbrächter Germany, 1976	Giovanni E. Giuffré Italy, 1990	Marie Kat Nederland, 1976
Svein Rune Ergs Norway, 1985	Gustaaf M. Hallegraeff Australia. 1983	Tufan Koray Turkey, 1990
	Regina Hansen Germany. 1990	Miryam Kutner Brasil, 1980

I0C Training Course Report No. 36
Annex III- page 2

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Pauline Lawrence Canada, 1980	Elisabeth Sahlsten Sweden, 1980	Ladda Wongrat Thailand, 1976
Jin-Hwan Lee South Korea, 1983	Leila Samson Algerie, 1980	Adriana Zingone Italy, 1985
Georgina S. Lembeye Chile, 1990	Francisco Sanchez Spain, 1980	
Sérgio Licea-Durán Mexico, 1976	Victor Smetacek Germany, 1976	
A. Noemi Ochoa Lopez Peru, 1985	Giorgio Socal Italy, 1985	
Ivona Marasovic Yugoslavia, 1983	Filipina B. Sotto Philippines, 1983	
Donato Marino Italy, 1980	Alain Sournia France, 1980	
Linda Medlin USA, 1980	Bo Sundström Sweden, 1990	
Øjvind Moestrup Denmark. 1983	Helene M. Sørensen Denmark, 1990	
Maria Teresa Moita Garnel Portugal, 1985	Jens Petter Taasen Norway, 1976	
Caterina Nuccio Italy, 1990	Thórunn Thórdardóttir Iceland, 1990	
Clarisse Odebrecht Brasil, 1990	Lena C, Tinnberg Sweden, 1976	
Frederick I. Opute Nigeria, 1990	Carmelo R. Tomas USA, 1983	
Trevor Platt Canada, 1976	Elizabeth L. Venrick USA, 1976	
Akshinthala Prasad India. 1990	Damir Vilicic Yugoslavia. 1985	

IOC Training Course Report No. 36
Annex IV

ANNEX IV

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IOC Training Course Report No. 36
Annex V

ANNEX V

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IOC Training Course Report No. 36
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IOC Training Course Report No, 36
Annex VI

ANNEX VI

FINANCIAL STATEMENT

Total Income:

MAST CEC grant (up to 56,605 ECU) ⁽¹⁾	LIT	120,681,860
UNESCO grant (5000 US\$) ⁽²⁾	LIT	8,000,000
Funds provided by Stazione Zoologica to the Course	LIT	7,662,308
Funds provided by Stazione Zoologica to the Marine Botany Laboratory		10,191,520
Registration fee (LIT 400,000x20)	LIT	8,000,000
ZEISS s. r.l free renting of 22 light microscopes		

TOTAL LIT 154,535,688

Total costs:

Travel refunds for invited lecturers	LIT	15,349,150
Travel refunds for selected students	LIT	8,015,000
Subsistence costs for invited lecturers	LIT	13,625,000
Subsistence costs for lecturers from SZN	LIT	8,644,520
Subsistence costs for participating students	LIT	42,000,000
Honoraria for lecturers	LIT	9,505,200
Other costs	LIT	57,396,818

TOTAL LIT 154,535,688

(1) Ratio ECU/LIT was calculated as 1/2132

(2) Ratio US \$/LIT was calculated as 1/1600

ANNEX VII

WORKSHOPS

Toxic Dinoflagellates

The workshop was begun by an introductory presentation by C. R. Tomas on the apparent global spreading of toxic events involving dinoflagellates. Differences in regional distribution of toxic species and episodes was stressed.

K. A. Steidinger, presented a synopsis of the toxic species in the Gulf of Mexico and the hydrodynamics governing them. *Gymnodinium breve* is the major pelagic toxic species causing extensive losses in fin and shell fish as well as severe impacts on humans at beaches causing danger to human health and tremendous losses to the tourism industry. *Alexandrium monilatum*, a PSP producing species, is responsible for blooms in the Gulf but has yet to be associated with a toxic event there. Benthic toxic dinoflagellate species are also present and abundant. These included *Gambierdiscus toxicus* (Ciguatera), *Prorocentrum lima* (OA, DSP), *Coolia monotis*, *Ostreopsis siamensis*, *O. lenticularis*, *O. ovata* (DSP and possibly Ciguatera) and *Dinophysis* species such as *D. fortii* and *D. wipex* (DSP). *Pseudo-nitzschia seriata* (DA) is also present in the Gulf although no cases of ASP have been reported. Mechanisms of bloom formation and transport were discussed.

J. Larsen gave a short presentation of species from Danish waters emphasizing the DSP producing dinoflagellates as the major problem. These included several species of *Dinophysis* and the importance of the percent these species represent of the total population was stressed. Low abundances which may represent a larger proportion of the total phytoplankton population can render bivalves more toxic than when the *Dinophysis* is in higher concentration but in lower proportion..

A. Zingone introduced the IOC- Harmful Algal Bloom Programme and illustrated the outline of the Programme, with emphasis on implemented, ongoing and planned activities.

Following the discussions, living (cultures) and preserved specimens of toxin producing dinoflagellates, including species of the genera *Prorocentrum*, *Alexandrium*, *Gymnodinium*, *Dinophysis*, were observed and studied. Mixed preserved samples brought to the Course by a number of participants were examined for identifying toxic species.

Diatoms: toxic and nuisance species

G. R. Hasle introduced the problem of harmful diatoms and gave account of the different diatom species ever associated with nuisance event. The different effects of nuisance species were stressed:

- (i) Anoxic conditions: *Ceratium pelagicum* (death of benthic bony fish and shellfish)
Mucilage production: several potential species responsible ("mare sporco" (dirty sea) in the Adriatic Sea - Mediterranean Sea)
- (ii) Mechanical damages: *Coscinodiscus centralis* and *C. concinnus* (bird mortality caused by oil on the feathers); *Thalassiosira mala* and other mucus-producing *Thalassiosira* (bivalve gill clogging); *Coscinodiscus wailesii*: (fishermen's net clogging); *Chaetoceros* spp. (clog the gills of farmed fish or setae may penetrate gill tissue).
- (iii) Toxicity: *Pseudo-nitzschia* species (DA production, causing ASP)
C. B. Lange presented different aspects of ASP events and production of domoic acid by species in the genus *Pseudo-nitzschia*. The distribution of the different species in the genus *Pseudo-nitzschia* was discussed.

A list of relevant papers on toxic and nuisance species - mainly based on the list included in the chapter Taxonomy of Diatoms, by G.R. Hasle and G.A. Fryxell, In : Hallegraeff et al. (eds) 1996 - UNESCO monograph on Harmful Marine Microalgae - was provided and discussed.

Diatoms involved in harmful events were examined in the light microscope. Taxonomic characters to be used in the identification of species in the genera *Pseudo-nitzschia*, *Thalassiosira*, *Coscinodiscus* and *Chuetoceros* were highlighted,

Naked flagellates with emphasis on nuisance species

J. Throndsen described the development of the prymnesiophycean blooms in Scandinavian waters (*Chrysochromulina* in 1988, *Prymnesium* in 1989, 1990 and 1991, and *Chrysochromulina* in 1991), including their effects on caged fish and on natural fauna and flora, as well as their economic impact. It was pointed out that blooms like those reported would hardly been noticed if it had not been for the fish farms which acted as large scale biosensors for toxicity events,

C. R. Tomas illustrated different physiological and autoecological aspects of *Heterosigma akashiwo* which could be related to its capacity of produce massive blooms in coastal waters of several sites.

A. Zingone reviewed the existing literature on the potential effects of viruses as regulators of nuisance species blooms. The cases of *Heterosigma akashiwo* and *Aureococcus anophagefferens* were presented, with emphasis on the mechanisms underlying virus-algae dynamics,

The distinguishing features of flagellate species associated with toxic/harmful effects were reviewed and possibilities for confusion with similar species were elucidated. Literature necessary for the identification was listed and commented upon. Re-observation of culture material of some of the species followed. Species in the following genera were taken into consideration: *Chrysochromulina*, *Prymnesium*, *Phaeocystis* spp. (Prymnesiophyceae), *Heterosigma*, *Chattonella*, *Fibrocapsa* (Raphidophyceae), *Aureococcus* (Chrysophyceae or Pelagophyceae), *Dictyocha* (Dictyochophyceae).

Interactive taxonomy

J. D. Dodge gave an introduction to the principles of taxonomy, classification and nomenclature. He explained some of the difficulties of taxonomic identification and gave examples to show why it is important to be aware of the codes of nomenclature. A discussion of different taxonomic approaches followed, including the use of pictures, the use of taxonomic keys and new approaches using computer-based methods.

K. A. Steidinger illustrated some applications and perspectives of computer-based image analysis identification systems, and the development of neural networks.

J. D. Dodge introduced various types of computer based taxonomic systems for identification, such as the "on-line" system, a character set system, and the ETI Linnaeus system, which combines traditional taxonomic keys and data sets with a simple character-based approach. Students split into groups for demonstrations of the Linnaeus system. A computer-based sample reading and database building system for phytoplankton analysis was also demonstrated.

ANNEX VIII

NATIONAL/REGIONAL REPORTS

ARGENTINA and the situation in URUGUAY (Virginia Villafane)

Argentina has a long coast-line and a great potential for economic exploitation of maritime resources. In spite of that, the economy has mostly relied on land resources and comparatively little activity is found in the marine environment. Although there is a long history of marine science research in Argentina, most of the phytoplankton work has been oriented towards systematic studies of different organisms and there has been almost no activity in the field of toxic phytoplankton species up to 1980.

In the spring of 1980, the death of two fishermen was attributed to the ingestion of toxic mussels taken from the coast off Peninsula Valdés, Chubut. The symptoms indicated that deaths were due to PSP. The toxic phytoplankton species responsible was identified as *Alexandrium tamarense*. In 1985 several fatalities also occurred in the proximity of the Chubut River estuary, Chubut, due to the same species.

Since then, the Argentinean government has established monitoring programs for toxicity due to phytoplankton species along the Atlantic coast, and all efforts are directed toward the detection of toxicity and education at all levels of the population about the importance of a toxic event. This required the implementation of routine sampling of molluscs in all provinces that could be affected by this phenomenon (Buenos Aires, Río Negro, Chubut, Santa Cruz and Tierra del Fuego) and the setting up of laboratories that could measure toxin levels in bivalves and relay this information to the public. In the case of a toxic event a quarantine is established and no consumption or commercial uses of the toxic molluscs are allowed.

INSTITUTIONS INVOLVED

In Argentina, monitoring of toxicity is organized at both national and provincial levels. The Servicio Nacional de Sanidad Animal (SENASA, National Service of Animal Health) is the governmental office in charge of final decisions on quarantine, and also each province is in charge of monitoring along their coasts.

Buenos Aires Province: The Instituto Nacional de Investigación y Desarrollo Pesquero (Institute of Research and Fisheries Development, INIDEP) in Mar del Plata, Province of Buenos Aires is in charge of the management of renewable resources in the Argentinean Sea and carries out monitoring of toxicity levels along the Buenos Aires coast. Usually, once a year they do a cruise along the entire Argentinean coast where they measure biological, chemical and physical parameters. Both the Universidad de Buenos Aires (UBA) and Universidad de Mar del Plata (UMDP) have carried out research activities related to toxic events in cooperation with INIDEP personnel.

Rio Negro Province: The Instituto de Biología Marina y Pesquera (IBMP, Institute of Marine Biology and Fisheries), is in charge of collecting data on toxicity levels along the coast. This Institute, which also has laboratories for scientific research, belongs to the Provincial Government.

Chubut Province: The Dirección de Medio Ambiente (Environmental Office) is in charge of monitoring toxic events, also in collaboration with local offices which sample along the beaches. In Chubut there is also the Regional Center of "Red Tides", which compiles the information on toxicity in bivalves from Tierra del Fuego, Santa Cruz and Chubut. As in all provinces, many of the samples are provided by fishing vessels, which send the samples to laboratories for analysis before commercial use. In this province, scientific research is carried out in both the Centro Nacional Patagónico (CENPAT, National Patagonic Center), an Institution which belongs to the national Government and the Universidad Nacional de la Patagonia (UNP). This University is the only one in the Patagonian region and has five locations in different provinces.

Santa Cruz Province: Monitoring is carried out by the Dirección de Bromatología, which is an office of the Provincial Government. No information is available on present scientific activities carried out in this province regarding toxic phytoplankton.

Tierra del Fuego Province: Monitoring is done by the Dirección de Pesca (Office of Fisheries). In this province the Centro Austral de Investigaciones Científicas (CADIC) is located, This is an institution belonging [o the national Government which is dedicated to research in several fields of marine biology.

STATUS AND NEEDS

Economic conditions in Argentina do not permit a rigorous program of monitoring of toxic episodes, but the basics are carried out in order to set safe limits for the public. Toxicity analysis are carried out following a standard mouse bioassay. At present, HPLC techniques are not applied for toxin analysis.

In general, research institutions, universities and government laboratories are not well equipped for science, and often there is a lack of essential materials such as microscopes, computer supplies and chemicals. In regard [o electron microscope facilities, they are available in several places in Buenos Aires province. There is one SEM available in the Chubut Province, which apparently is not operable full time at present. The closest one available for the Patagonian region is in southern Buenos Aires.

Argentina had pointed out the necessity of training in determination of toxins, as well as the need to carry out interdisciplinary studies, including the physical, chemical and biological aspects during a toxic episode.

One of the main problems is the lack of information, particularly of specialized journals. There are no well implemented libraries (especially in the Patagonian region) and no access to computerized searching systems.

Most of the research activities on toxic phytoplankton along the Argentinean coast have been directed towards the identification of organisms and description of toxic phenomena. In the taxonomy field, the most extensive work in both toxic and non-toxic dinoflagellate species has been done by Prof. Balech (who has provided a very extensive literature). There are also several descriptive works on "red tides" along the Argentinean coast, produced mostly by *Alexandrium* species, most of them having been carried out by personnel of INIDEP. Some physiological studies with toxic dinoflagellates species have also been done. Finally, there is some literature on the medical aspects of mollusk poisoning.

At present, there is a project at the Universidad Nacional de La Plata (Buenos Aires) to study toxic diatoms along the Buenos Aires coast. They plan to include the study of toxic diatoms along the Patagonian coast. Their focus is on *Pseudo-nitzschia* species, and they plan to relate their results to studies carried out with bivalves, especially the yellow clam *Mesodesma mactroides*. They plan to focus mostly on the taxonomy of the species, but environmental parameters will also be considered.

NOTE ON THE SITUATION IN URUGUAY

Toxic episodes have been observed since 1980 in Uruguay also. The species cited as responsible of toxicity in bivalves belong to the genera *Gymnodinium* and *Alexandrium*, which cause economic losses in the exploitation of mussels and clams (no human deaths have been registered due to mollusc poisoning). There is a monitoring program for toxicity along the coasts, and toxicity levels are referred to mouse units. Sampling is carried out every 10-15 days at five fixed stations, and includes the sampling of phytoplankton and mussels. At the same time, environmental parameter measurements are also taken.

The Instituto Naconal de Pesca (INAPE, National Institute of Fisheries) is the office responsible of monitoring. It informs the Government about toxicity levels, which in turn is responsible to establish a quarantine if necessary. There is also a cooperation between Uruguay and Argentina (through the Comisión Técnica Mixta del Frente Marítimo), which is reflected in workshops and planning of cruises with scientific personnel of both countries to study toxic phytoplankton blooms along the Atlantic coast.. Although Uruguay has HPLC equipment to carry out

toxin analysis, it lacks the appropriate expertise. This country has also pointed out the necessity of training in physical oceanography and modelling.

BRAZIL (Sylvia M. Susini Ribeiro)

Despite the length of the Brazilian coast, information about economic and/or health impacts caused by harmful algal bloom events is scarce. A review of these blooms is summarized in the Table 1.

The Intergovernmental Panel on Harmful Algal Blooms (IPHAB II) stimulated an increasing awareness regarding HAB aspects in Brazil. There are now some projects in development in southern Brazil, where recurrent massive shellfish mortality caused problems mainly in the coastal area. The establishment of an aquiculture programme since 1989 resulted in a rapid increase in shellfish production, mainly of the mytilid *Perna perna*, in Santa Catarina State. The annual production of this shellfish increased from 150 to 1,800 tons between 1989 and 1994. During periods when human intoxication events have been registered in this State, shellfish commercialization was interrupted. However, this decisions had no scientific support. Now there is a proposal for a HAB pilot programme including the monitoring of microalgae and shellfish toxins by HPLC. This is an important initiative, and the experience will help to expand this activity to other regions in the near future.

At a broader regional level, a joint activity between Brazil and Uruguay was undertaken during the last massive shellfish mortality at the boundary between the two countries in December 1994. This activity functioned as a calibration exercise for harmful phytoplankton cells counting between scientific groups of both countries.

In addition to what listed above, there are some problems with species identification, mainly due to an inappropriate, and sometimes delayed, phytoplankton sampling and transport. It is important to stress that at this level there is the necessity to support training activities for Brazilian scientists in order to increase the number of persons involved in HAB studies in this country not only at the Universities but also at Governmental Foundations.

CANADA - Lower Estuary and Northeastern Gulf of the St. Lawrence (Connie Vincent Lovejoy)

The Quebec and Gulf Region are part of long term phytoplankton projects at: Ste-Flavie, New London Bay, Cardigan, Brudenell, Murray and Miramichi Rivers. These projects are funded by the Canadian Department of Fisheries and Oceans (DFO), DFO) Inspection Services Branch operates a phytoplankton watch project at 30 stations in these areas. This presentation will concentrate on the lower estuary and Northeastern to Northern part of the Gulf, which is referred as the Québec Region. The area to the south near Prince Edward Island (PEI), New Brunswick and Northern Nova Scotia is considered to be the Gulf Region and is the responsibility of other laboratories, primarily, the Fisheries and Oceans Department of the Gulf Fisheries Centre in Moncton N. B.

The Quebec Region contains a suite of toxic and potentially toxic species of algae, which will be mentioned in relation to the toxic effects.

1. PSP

Historically there have been toxic algal blooms and shellfish contamination every summer in the northeast Gulf and Gaspé regions. The problem has been principally associated with *Alexandrium tamarense*, (also classified as *A. fundyense*, and *A. excavatum*). The form *A. fundyense* has no ventral pore between plates 1' and 4', as in *A. tamarense*, although it has recently been found that there is no justification for separating these into species and that the form is not a good predictor of toxicity.

The Canadian Department of Fisheries and Oceans has had an ongoing monitoring program in this region for many years and collects samples from sites around the lower estuary and northern gulf in collaboration with the Inspection Services branch as part of the Canadian phytoplankton monitoring program. The Gulf has an early-warning system for the presence of harmful algae as component of a phycotoxin program.

Phytoplankton samples are collected from 10 stations in the Quebec Region, including a long term monitoring at site at Ste-Flavie (MLI), where nutrients are also collected weekly, There are also 2-3 station in Magdalen Islands and a station in the Baie-des-Chaleures,

Blooms of *Alexandrium* spp. are largely confined to the plumes of the Manicouagan and Aux-Outardes Rivers in the lower St. Lawrence Estuary promoted by the stabilizing input of fresh water, In the Gulf, annual outbreaks occur in the Gaspé Current, these have been correlated with transient stratification towards the end of August. However this year, which was one of the warmest on record, no blooms had occurred in [his area up to the end of August.

2. DSP

There is always potential for DSP to become a problem in the Gulf since the species normally associated with DSP such as *Dinophysis* spp. (*D. acuminata*, *D. norvegica* and *D. rotundata*) and *Prorocentrum lima* are frequently present. No toxin producing clones of these have been isolated. No notable outbreaks of DSP have occurred.

3. ASP

Since the 1987 Prince Edward Island outbreak of ASP there have been organized inspections by the DFO inspection service. This outbreak was attributed to *Pseudo-nitzschia multiseries* (= *Pseudo-nitzschia pungens* f. *multiseries*). While *Pseudo-nitzschia* spp. are occasionally seen in our area there is no record of bloom proportions. *Pseudo-nitzschia seriata*, which can also produce domoic acid, is frequently encountered in low numbers throughout the Gulf, it has never been linked to any major bloom or ASP outbreaks.

4. EFFECTS ON FINFISH AQUACULTURE, AND WILD FISH

Many species associated with farmed fish mortality are found in the Gulf (*Chaetoceros* spp., especially *Chaetoceros concavicornis* and *Chrysochromulina* spp. as yet not identified). However, there have been no reports of problems. There was a bloom of *Gyrodinium aureolum* in the Gaspé current near Mont-Louis in September 1993. This organism has also been encountered in other parts of the Gulf. Although this represents a threat to fish and other marine organisms, no effects have been documented.

In the wild fishery, the association of *Chrysochromulina* spp. with a condition known as blackberry feed in Atlantic Cod (*Gadus morhua*) caught along the Labrador and Newfoundland coasts has recently been investigated. This condition is characterized by a strong sulfur smell in the fish. For several weeks from mid-July to early August these fish are known to feed almost exclusively on the pteropod, *Limacina helicina*, which in turn has grazed on high concentrations of *Chrysochromulina* spp. which produces DMSP (dimethyl-sulfoniopropionate, the precursor to DMS, dimethylsulfide).

In summary there are many potentially toxic algal species present in the Quebec region. The overriding problem has been with *Alexandrium tamarense* and associated forms which are highly toxic in this area. Bacterial contamination has also been a problem during summer in this area with the result that close monitoring of shellfish is necessary and this fishery is frequently closed during extended periods every year. In Canada, there is a protocol which has been established using HPLC and mouse bioassay by DFO and Health Canada to ensure public safety.

CHINA (Douding Lu)

Red tides have mainly occurred in the coastal regions of China, especially in the convergence zone. In August 1982, a *Noctiluca* red tide occurred in the Yangtze river estuary encompassing a region of 10 km². In 1986, a red tide of *Rhodomonas* sp. and *Mesodinium rubrum* covered 300 km² while in August 1988, a *Noctiluca* bloom covered 6100 km². In May-June 1995, a *Prorocentrum minimum* bloom covered massive area from Yangtze estuary to the coast of Zhejiang. In 1990, several blooms of *Noctiluca* in Dapong Bay occurred within a region of only 10 km², but in recent years, red tides of *Noctiluca* spread along almost the entire coast of Dapong Bay. Toxic algal blooms were also reported in the last decade. On December 1, 1986, a toxic algal bloom was reported in Ciyao

village, Dongsang county, Fujian province, One hundred thirty six persons became sick and one person died. as a result of eating the bivalve *Ruditapes philippinensis*. The causative species was identified as *Gymnodinium* sp.

In 1989, a large bloom occurred in Bohai Bay from August to October. Many fish and shellfish were killed, devastating a million yuan shellfish resource according to incomplete statistics. In April 1989, 1000 tons of shrimp were lost due to blooms of *Noctiluca* in Hebei province. An estimated one million fish (2 million yuan) were lost when sea water containing the red tide organisms were pumped into the cultivating ponds. During May-August 1993, a large blooms of *Gyrodinium* sp. and *Noctiluca* occurred in Zhejiang coast, which caused mortality of cultivated shrimp (*Penaeus orientalis*). 300 million yuan were lost.

Up to now, nearly 100 species of causative red tide organisms have been reported. The harmful, toxic or potentially toxic species include: *Noctiluca scintillans*, *Prorocentrum minimum*, *P. micans*, *P. sigmoides*, *Alexandrium tamarense*, *A. catenella*, *Gymnodinium nagasakiense*, *G. catenatum*, *G. aureolum*, *Cochlodinium* sp., *Pyrodinium bahamense*, *Chattonella marina*, *Heterosigma akashiwo*, *Rhizosolenia styliformis*, *Proboscis alata* f. *gracillima*, *Pseudo-nitzschia pungens*, *Skeletonema costatum*, *Mesodinium rubrum*, *Trichodesmium hildebrandtii*, *T. thiebautii* and *T. erythraeum*. Several investigations have discovered dormant cysts along the Chinese coasts. Some of the vegetative cells produced by these cysts were identified in Dapeng Bay, in the South China Sea.

The most important research projects relative to harmful algal blooms in China are summarised in the following list. Some projects were sponsored by high level governmental bodies, such as the National Natural Science Foundation and the National Committee of Science and Technology. Research goals of these projects were relatively wide to cover more areas and concentrate on more basic aspects. Other projects focused on regional incidence and were supported by different governmental administrations, research institutions or local governments. Agencies involved in HAB studies are the SOA (Research Institutes, Subbranch of SOA), Academy of Science, some fishery Institutions and Universities.

MOST IMPORTANT RESEARCH PROJECTS IN CHINA

- 1978-80 Eutrophication and red tide problems in Bohai Bay
- 1984-85 Organic pollution and red tide outbreaks in Haihe River estuary
- 1985-87 Observation of red tides and their ecological features in Dalian Bay
- 1986-88 Patterns of outbreak and variation of red tides in South China Sea
- 1987-88 Comprehensive survey on red tides in the west area of Xiamen Harbour.
- 1987-89 Causes of red tides in Yangtze River Estuary
- 1988-89 Foundation studies on red tides control in inner bays.
- 1990-93 Chemical environmental study on red tides along Zhejiang coast
- 1991-93 Causes of red tide outbreak along Shangdong coast.
- 1991-94 Red tide biology study along Zhejiang coast.
- 1992-95 Prediction and prevention of red tides in coastal waters
- 1995-97 Short-term forecasting of red tides in marine aquiculture areas.

CHILE (Juan Carlos Uribe Paredes)

Chilean coasts extend from ca. 18 to 56° S, with a coast relatively uniform as far as 420 S. The southernmost portion is characterized by many fjords and channels and this is the area where HAB events occur. The following is a brief account based on the published information.

In 1972 the first episode of PSP outbreak was recorded, involving the central part of the Strait of Magellan. the Beagle Channel and surroundings waters. In 1981 a new event took place, in northern areas partly overlapping those of the former episode. A third event was recorded in 1989, although in a very restricted area. The first two events caused five deaths and economical losses over one US million dollars each. Since 1991, the situation has changed dramatically; with continuous episodes taking place and the affected area extending considerably to the North. At present, most of the coast remained closed for shellfish harvesting. In all cases, *Alexandrium catenella* has been considered the causative organism.

DSP outbreaks were first reported in 1970 in the northernmost fjord areas, and successively in 1979 and 1986. In 1980 and 1984 DSP outbreaks were detected 250 km South. Since 1991, these events have been detected more regularly. *Dinophysis acuta* has been identified as the causative organism. The number of human affections has risen to many hundreds and shellfish harvesting has been banned for most part of the last period.

Since 1984, fish farming (salmon) has been a growing activity in Chile, especially in the northern part of the fjords. In 1989 an extensive bloom of *Heterosigma akashiwo* caused a massive fish kill, with economical losses over 7 million US dollars.

MONITORING AND RESEARCH

In the Magellan region, the southernmost part of the fjord area, a monitoring program has been supported by regional government since 1982, although it is being carried out irregularly. Since 1994, National Authorities support a bigger program aimed at detecting PSP, DSP, and toxic phytoplankton. Regional health services are in charge of the toxicological control over any product marketed.

Most part of the research has been related with the description of toxic events. At present, the major part of the research is carried out by a multi-disciplinary and multi-institutional team dedicated to investigate the toxicology of PSP and DSP,

Besides the above mentioned species, the monitoring program has put into evidence the extensive distribution, in both space and time, of the genera *Pseudo-nitzschia* and *Dinophysis* all along the fjords. Species such as *Dinophysis acuminata* and *D. (= Phalacroma) rotundata*, mentioned as toxic elsewhere, have been identified. Fish-killing diatoms, such as *Chaetoceros convolutes*, are also present.

National resources related to HAB in many areas are deficient, phytoplankton taxonomy being one of the weaker ones. There are regular publications only related to diatoms. Groups such as phytoflagellates have never been studied in Chile. Most of the bigger equipment necessary to carry out research is concentrated in universities located in central Chile. The progressively increasing use of coastal waters for aquiculture activities stresses the need of personnel prepared to work in phytoplankton taxonomy.

GERMANY (Jeanette Gobel)

Some of the goals of the Algenfrüherkennungssystem (ALGFES) = Early Algal Detection System are to identify toxic or potentially toxic species, to follow the phytoplankton fluctuation, especially mass developments and their possible harmful effects on flora, fauna and on swimmers. New species previously not detected were discovered in our sampling area. Species composition, direction of spreading blooms and toxic events associated with "new" species are some of the objectives achieved by this project.

The main and most intensive sampling period extends from May to the end of August. Two sampling areas are located within the small province Schleswig-Holstein, the first on the North Sea coast and the other on the Baltic Sea coast. Sampling programmes for these two areas are not the same because of their very different hydrographic conditions.

North Sea: The sampling is carried out monthly or fortnightly in April, May, September, October, fortnightly and some times weekly from June to the beginning of September. Fifteen stations, most of which located a bit offshore, are sampled by helicopter. The following parameters are included: phytoplankton abundance, chlorophyll, nutrients, temperature, salinity, pH.

Baltic Sea: In April and May, 15 stations located mainly inside fjords and bights along the coast are sampled fortnightly by helicopter. Parameters include temperature, pH, salinity, phytoplankton species, abundance. From June to the beginning of September, up to 23 sampling points are sampled by coast guard ships, with measurement of temperature and estimates of phytoplankton abundance.

Public information: The dominant and critical phytoplankton species should be identified 3-4 days after sampling. Afterwards two reports have to be written. In the "Algenreport", some kinds of general information about phytoplankton and the current situation are briefly described. If possible, a short-term prognosis should be given. Drawings of the predominant species are illustrated in the text. The "Algenreport" will be sent off to the Ministry of Nature and Environment, health officials, German and foreign marine biology institutes, tourist information offices and environmental groups as well.

In addition, a second report is written with more scientific information like cell numbers, salinity, etc. This report gives additional and more detailed information. This report is sent to health officials and marine biology institutes, but not to touristic officials.

OTHER MONITORING PROGRAMS

Other different phytoplankton monitoring systems with different goals exist in Germany.

1. HELCOM - monitoring in the Baltic Sea, which will end by 1995,

With the beginning of 1996, a new monitoring programme will be established on the North Sea side and on the Baltic Sea as well.

2. Two additional but separate phytoplankton-monitoring systems exist in Schleswig-Holstein and in Niedersachsen, which are the two provinces in North and North-West of Germany. In the near future, these two systems will be a part of the coming new monitoring program. Both have the same goals:

- (i) early algae detection system
- (ii) to follow the phytoplankton fluctuations, especially mass developments
- (iii) species composition, especially for toxic and potentially toxic species
- (iv) "new species" which have not been detected before in the sampling area.

3. Scientists from Helgoland and List/Sylt Institute Biologic Arstalt Helgoland examine the species composition weekly.

Almost every year, DSP toxins were detected in mussels either from Schleswig-Holstein and/or from Niedersachsen. Though *Dinophysis* species can be very abundant in the late summer, no evidence exists that this genus is the reason for the detected DSP. Outbreaks of foam on the beaches (e.g. North Sea: *Phaeocystis*), intensively reddish or brown coloured waters (e.g. North Sea: *Phaeocystis*, Baltic Sea: *Prorocentrum minimum*, *Heterocapsa triquetra*), and waters which smell bad (e.g. North Sea: *Noctiluca*, *Phaeocystis*) are also bloom events noted for this region. Although PSP-producing species and/or their cysts occur in the areas (*Gonyaulax* sp., *Alexandrium* sp., cysts of *Gymnodinium catenatum*), no PSP has been detected.

In Germany, no regular phytoplankton courses exist. A kind of workshop lasting 2-3 days was carried out in the last few years.

IRELAND (Jacqueline H. T. O'Mahony)

Since 1976, there have been both noticeable algal blooms and occurrences of harmful algae in the Republic of Ireland. Between 1976 and 1981, *Gyrodinium* cf. *aureolum* was the dominant dinoflagellate species recorded and implicated in mortalities of invertebrates and farmed rainbow trout (*Salmo gairdneri*) in the south west. Mortalities of finfish occurred also in 1982 on the west coast, associated with "Flagellate X", subsequently considered to be *Heterosigma* cf. *akashii*. Since 1984, because of the association of *Dinophysis* species with Diarrhetic Shellfish Poisoning, a monitoring programme for phytoplankton and toxins was established by the Fisheries Research Centre (FRC), at the Department of the Marine, in Dublin.

The FRC is the government laboratory responsible for monitoring both phytoplankton and toxins, reporting these to the Department of Health (responsible for regulating domestic consumption of shellfish) and also to the Fish

Quality Officers of the Department of the Marine (responsible for certification of exports), No human cases of DSP have been reported to date. Detection for DSP in Ireland is principally by a combination of phytoplankton sample examination, mouse bioassay and HPLC. Since 1987, there have been many closures of shellfish growing areas due to the presence of DSP toxins, some for up to six months, *Dinophysis* species recorded in Ireland include *D. acuminata*, *D. acuta*, *D. caudata*, *D. norvegica*, *D. hastata*, *D. dens* and the related *Phalacroma rotundata*. *D. acuminata* and *D. acuta* are the most commonly recorded species of *Dinophysis*. Analysis for PSP toxins is also carried out, using the mouse bioassay, if *Alexandrium* species are detected in routine phytoplankton samples.

Results of a baseline qualitative survey of dinoflagellate cysts present in coastal sediments during 1993/1994, revealed the presence of cysts of *Alexandrium* spp. such as *A. cf. minutum* and *A. cf. tamarense*, but these cyst occurrences were rare, Blooms of *Alexandrium* species were recorded during the 1980's in Cork Harbour (*Alexandrium* sp., *Alexandrium ibericum*), but these appeared to be non-toxic.

Mortalities of lobsters on the south coast in 1981 appeared to be associated with diatom blooms of *Nitzschia* spp. (*Pseudo-nitzschia* spp.), which clogged lobster pots and fishing nets, In 1994, human skin irritation was associated with high numbers of diatoms again including *Nitzschia* spp. (?*Pseudo-nitzschia* spp.) on the west coast.

Most work on marine phytoplankton in Ireland is concerned with monitoring at aquaculture sites (FRC, Dublin; independent consultancies). Some research is carried out on phytoplankton in areas of upwelling (Depts. of Oceanography & Microbiology, University College, Galway; FRC, Dublin), and identifying marine toxins (FRC, Dublin; Regional Technical Colleges, Cork & Athlone).

Funding of basic research in the marine sciences in general and marine phytoplankton in particular, in Ireland is a problem.

ITALY - Adriatic Sea (Marina Carbrini)

The requisites for coastal and brackish water quality, in which mussels naturally grow or are cultivated in rope culture, have been established by Italian law in order to reduce the risk of DSP. The Italian law (D. M. 1/8/90, n. 256 and D.L. 1/9/90, n. 131) indicates methods to determine and count phytoplankton in the water and algal biotoxins in the molluscs. The method to detect DSP toxins is the mouse bioassay according to the Italian law,

The DSP producers such as *Dinophysis* and *Prorocentrum* species are monitored by the use of the Utermöhl method. These toxic species are usually scarce and their densities reach about 10-10³ cells/liter. At present, fifteen potentially toxic species have been found in the North Adriatic Sea, where *D. fortii* and *D. acuminata* are the most abundant with a different seasonal pattern: *D. fortii* is preferably an autumnal species while *D. acuminata* is more frequent in spring. In Sicilian coastal waters also, some *Dinophysis* species were detected including *D. sacculus* as the dominant species.

Species of the genus *Alexandrium*, potentially PSP-producers, have been reported since 1982 in the Adriatic Sea, but are less abundant than *Dinophysis* species. Up to now, five species have been identified and no PSP outbreak was recorded till May 1994, when concentrations up to 75,000 cells/liter of *Alexandrium minutum* were recorded in coastal waters near the Po River estuary. On this occasion, toxin level in mussels exceeded the tolerance level for the Italian regulation, reaching 80 µg/100g. These results were obtained by mouse bioassay and confirmed by HPLC analyses.

REPUBLIC OF KOREA (Joon-Back Lee)

The Korean Peninsula has many bays, in which shallow water is prevalent. It is divided into three parts, comprising southern, western, eastern waters. Among these, the southern coastal area has been known to be an important spawning and nursing ground for fish and shellfish for many years. However it has recently become notorious for its eutrophication and frequent red-tides due to the establishment of industrial complexes around the bay since the 1970s. Red-tides sometimes cause serious damages to cultured shellfish and other living organisms therein. Especially in the summer of 1995, there were serious damages throughout the southern part due to red-tide caused by *Prorocentrum* and *Cochlodinium* species. It followed a big oil tanker

crash accident, which spilled a huge volume of oil and spread though the whole southern coastal area. At the moment it is not possible to say which factor affected red-tide occurrence, but perhaps some chemicals poured into sea water for oil removal might have triggered phytoplankton blooms. The red-tide affected many fish farms and shellfish aquaculture areas causing the death of these organisms. However, the real cause of this death is not clear.

Since the 1970s, when a first investigation of phytoplankton blooms was reported in Korean waters, blooms have been caused by diatoms or dinoflagellates or other flagellates, changing year by year. So far a total of 67 species have been described as blooming organism from Korean coastal waters, including 5 Cyanophyceae, Cryptophyceae, 34 Dinophyceae, 21 Bacillariophyceae, 2 Raphidophyceae, 2 Chrysophyceae, 1 Euglenophyceae. Among these, dinoflagellates and diatoms have been important in the southern area, However some phytoflagellates and a few ciliate species have occurred sometimes as part of dominant species in Korean waters,

In Korea, the red-tide study is one of the important projects in the ocean and fishery sciences. There are two national authorities, Korea Ocean Research and Development Institute (KORDI), and National Fisheries Research and Development Agency, which organize main monitoring programs for red-tide. KORDI has responsibilities on basic studies concerning red-tide, while National Fisheries Agency pursuits real applications including monitoring, prediction, protection of red-tide and training fishery guiders.

Additionally there are about 50 specialist belonging to different universities and institutes taking part in phytoplankton and harmful algal blooms projects.

MEXICO (Ma. Esther Meave Del Castillo)

As in other countries around the world, in Mexico the high growth of human population, touristic expansion and multiplication of mariculture have contribute to increase eutrophication in coastal areas and provide conditions for the development of red tides. Many permanent programmes for red tide monitoring have been established. Only in two localities on the Pacific coast, long term series of data are available (Guaymas and Mazatlan, with a 24 and 16 years time-series, respectively).

A recent review was presented at the 7th International Conference on Toxic Phytoplankton (Sendai, Japan), taking into account the scarce information on this topic since 1957. It shows that phytoplankton blooms and red tides are common events in Mexico, with toxic ones produced by several species, mainly dinoflagellates, with densities ranging from 0,5 to 36×10^6 cells/litre. Nearly 70% of the recorded events have been registered in the last 5 years. Species involved are *Gymnodinium catenatum* and *Gonyaulax polyedra* in the Gulf of California, *Pyrodinium bahamense* var. *compressum* on the Pacific coast (as a recurrent event since 1989), and *Gymnodinium breve* in the Gulf of Mexico.

Cases of PSP by bivalve mollusc consumption have been recorded in several locations on the Pacific coast. such as Mazatlan (Jalisco), Acapulco (Guerrero), Huatulco and Salina Cruz (Oaxala), and Puerto Madero (Chiapas), mainly due to *Pyrodinium bahamense* var. *compressum*, but nine different potentially toxic species have been found in the bay of Mazatlan.

Fatal cases have also been documented, although they could be more than those reported due to misidentification of symptoms. Toxicity caused by blooms of *Dinophysis caudata* has been found in *Agropecten circularis* and *Pinna rugosa* at Bahia de Concepcion, Gulf of California. Cases in the Gulf of Mexico include mass mortality of fish due to *Gymnodinium breve*.

The Mexican Caribbean Sea is a virtually unstudied region, but isolated cases of fish consumption (*Sphyrna* sp.) have led to intoxications with no deaths, possibly related to a ciguatera species, *Gambierdiscus toxicus*. These events, as well as recently findings of *Pseudo-nitzschia australis* in the Gulf of California, induce to urgently establish monitoring stations in these locations.

NORWAY (Wenche Eikrem & Cecile Hellum von Quillfeldt)

Over the past years, several harmful algal blooms have occurred in Norway. Species known to be responsible for such events are presented below.

In May 1988 *Chrysochromulina polylepis* was the cause of an extensive toxic bloom in the Skagerrak killing wild fauna, flora and caged fish. The toxin produced have adverse effects on cell membranes. The first bloom of *Gymnodinium mikimotoi* (= *Gyrodinium aureolum*) was observed in the fall 1966 when brown water killed fish. This species produces recurrent blooms in Southern Norway and there is one report from Northern Norway. *Prymnesium parvum* and *P. patelliferum* bloom regularly in Ryfylkefjordane, on the west coast, These species seem to have limited effects on the wild fauna and flora, but kill caged fish. Traditionally this has been an area for fish farming and the fish farmers have suffered big losses over the years. *Chrysochromulina leadbeateri* is believed to be responsible of a toxic bloom in the Lofoten area in Northern Norway. The bloom killed caged fish, but no damage was observed on the wild fauna. Also this bloom caused great losses to the fish farming industry,

As for shellfish toxicity caused by algae, the PSP producing alga *Alexandrium tamarense* is common in Norwegian coastal waters and has caused local blooms on some occasions, Two human fatalities have been registered from ingestion of blue mussels since 1901. The DSP producing algae *Dinophysis norvegica*, *D. acuta*, *D. acuminata*, *D. rotundata* occur regularly. Due to DSP producing algae, the shellfish industry is poorly developed in Norway, Diatoms producing domoic acid that cause ASP are common in Norwegian coastal waters (e.g. *Pseudo-nitzschia multiseries*, *P. pseudodelicatissima*, *P. seriata*), but so far they have not been reported to have caused problems, Heavily silicified setae from certain *Chaeroceros* species may physically harm fish gills.

Other algae reported to be harmful that occur and occasionally bloom, but which have not been described to have adverse effects in Norwegian waters are: *Heterosigma akashiwo*, *Prorocentrum minimum*, *P. lima*, *Alexandrium ostenfeldii* and *A. minutum*. At present there is no organized surveillance programme, but water samples are collected on a regular basis to screen for presence of toxin-producing algae.

RUSSIAN FEDERATION (Yuri Borisovich Okolodkov)

BLOOMS

Studies on harmful algae on a regular basis have not been conducted in the Russian Federation. Also, there are no reliable medical statistics on algal toxin poisoning. The only well documented harmful algal bloom was caused by a *Chattonella* sp. in Amursky Bay, Sea of Japan, in 1987. The peak concentration reached 15×10^6 cells/l. High concentrations, about 10^3 - 10^4 cells/l, of toxic *Dinophysis* spp. occur every summer in coastal waters of the Sea of Japan. There are some reports on algal blooms in the Baitic, White and Black seas, the Sea of Okhotsk and the Sea of Japan. The nature of the blooms is poorly investigated.

Red tides in the Far East (Avachinsky and Olyutorsky bays) were recorded in June-August 1984, 1986 and 1987 (temperature 10 to 15[°] C) and were produced by *Alexandrium excavatum*, *A. tamarense* and *A. acatenella*. Bloom of the latter, with concentration up to 4×10^5 cells/l, was accompanied with increasing toxicity of mussels. Human poisoning by toxic shellfish occurred in Pavla Bay, the Bering Sea, in 1945, and in Avachinsky Bay, in 1973, when saxitoxin was found in mussels. Red tide caused by the ciliate *Mesodinium rubrum* was observed in Avachinsky Bay, in 1983. *Gymnodinium* cf. *mikimotoi* caused red tides in Amursky Bay, in September-October 1989 and 1990. The highest concentration of *Dinophysis acuminata*, about 5×10^5 cells/l, was observed in August-October 1988, at 10- 12[°] C. A raphidophycean, *Heterosigma akashiwo*, previously not known from the Russian Far Eastern seas, was found in 1988 in Kronotsky and Avachinsky bays at a density of up to 7×10^4 , at 10 - 15[°]C. Konovalova (1993) presented a list of 14 species known as producers of toxic red tides or hazardous species from the Russian Far Eastern seas (1 *Prorocentrum*, 5 *Dinophysis*, 4 *Gymnodinium*, 1 *Gyrodinium* and 3 *Alexandrium* species).

EXPERTISE

There are a few experts on harmful algae and, in particular, on marine dinoflagellates in Russia: Dr G. V. Konovalova of the Kamchatka Department of the Pacific Institute of Oceanology, Russian Acad. Sci.,

Petropavlovsk-Kamchatsky, who regularly participates in the international symposia on toxic/harmful algae, and has published a number of works on the morphology of *Alexandrium* spp.; Dr Y. B. Okolodkov of the Komarov Botanical Institute, Russian Acad. Sci., St. Petersburg, studies the systematic, taxonomy and biogeography of marine dinoflagellates mainly in the Eurasian Arctic.

Results on dinoflagellate taxonomy, ecology and biogeography obtained by Russian authors are published in the Botanical Journal, Russian Acad. Sci., in the materials of the international symposia on toxic/harmful algae, and in some international and national (Polish and Norwegian) periodicals.

SPAIN - Catalan Area (Maximino Delgado)

Phytoplankton monitoring in the Catalan coast was established in 1988 as a contract between Direcció General de Pesca Marítima (Generalitat de Catalunya) and Instituto de Ciencias del Mar (CSIC) to check any possible toxicity or toxic organisms in relation with bivalves, following the European recommendations. Within this programme, water and shellfish samples are collected weekly at fixed stations in the bays of Ebro Delta and with different time intervals in other areas of the Catalan coast. Environmental information is also recorded. In the Ebro Delta area, important aquiculture and fisheries activities are located. Mussels are cultured in the bays (raft cultures), some fish farms exist in the margins of the bays (ponds) and also fish is cultured in cages outside the bays.

This year the surveillance of commercial harbours was included, along with zones exposed to high nutrient discharges where dense phytoplankton blooms occur. Phytoplankton samples are regularly collected in these semi-enclosed areas on a weekly interval in the warm season (April-October) and every other week during the cold season (November-March).

POTENTIALLY TOXIC SPECIES

Potentially toxic organisms present in Catalan waters include PSP and DSP producers, whereas other toxic species such as NSP- and ASP-producing species have not been reported. *Alexandrium minutum* is considered the most important toxic dinoflagellate in the area. It produced a red tide in San Carlos de la Rápita harbour on May 1989 and also was present in the neighboring bay of Alfacs, with toxicity levels exceeding the allowed level in mussels. Shellfish harvesting was stopped in the region and no human illnesses occurred. After this incident, *A. minutum* has been recorded every year in Alfacs Bay during the winter months but never reached high concentrations, nor PSP toxicity was detected. *Alexandrium tamarense* was also found on different occasions, but never caused toxic events.

Several DSP-producing dinoflagellates have been recorded in this area in both open and coastal waters. although positive results of DSP toxicity by the mouse test have never been obtained in Catalonia. *Dinophysis* spp. are present usually in the cold seasons in net samples, but usually with very low concentrations (below 100 cells/l). Among the most frequently recorded species are: *D. acuta*, *D. caudata*, *D. fortii*, *D. hastata*, *D. ovum*, *D. rotundata*, *D. sacculus* and *D. tripes*. *D. sacculus* is the most frequent and abundant morphotype in the coastal area (concentrations reaching 2,000-3,000 cells/l, and over 10,000 exceptionally). Other DSP-producers dinoflagellates in the area are *Prorocentrum lima*, *Prorocentrum mexicanum* and *Ostreopsis siamensis*. The benthic species *P. lima* (epiphytic, resuspended from the sediments) has been observed frequently, but always in low concentrations. This year *P. mexicanum* was also observed in concentrations up to 9,000 cells/l. Also the benthic species *Ostreopsis siamensis* was recorded this year in the Catalan coast. This is not the first report of *Ostreopsis* in Mediterranean waters because the occurrence of *O. ovata* in Tyrrhenian and in Libanese waters has been recently reported.

WATER DISCOLORATIONS

Most of the water discolorations recorded along the Catalan coast are very reduced in space and time (local phenomenon in semi-confined environments as harbours and ponds usually lasting 1-3 weeks). Organisms causing water discoloration in Catalonia are: *Noctiluca scintillans*, *Peridinium quinquecorne*, *Kryptoperidinium foliaceum*, *Gyrodinium impudicum*, *Heterocapsa triquetra*, *Prorocentrum triestinum*, *Prorocentrum minimum*, *Chattonella marina*, and *Eutreptiella gymnastica*. Red tides of *Noctiluca scintillans* are detected periodically along the Catalan coast, every 2-3 years. It is an extensive phenomenon (more than 100 Km). Such blooms are first produced in open

IOC Training Course Report No. 36
Annex VIII - page 12

waters and transported later towards [he coast. A particular case is the occurrence of *Alexandrium* cf. *taylori*, which produced a dense green patch in La Fosca beach (Costa Brava) on summer of 1994 and 1995 (and there are references of its occurrence every year), lasting 2 months. Although water discolorations due to dinoflagellate blooms were produced on most occasions during the warm season, winter dinoflagellate blooms have also been observed (i.e. *Gyrodinium corsicum*).

OTHER HARMFUL EVENTS

Fish mortality (*Sparus aurata*) has been recorded from December 1994 to March 1995 in culture ponds located at the margin of Alfacs Bay, coinciding with water discolouration caused by the dinoflagellate *Gyrodinium corsicum*. Mortalities of mussels (*Mitylus galloprovincialis*) were also recorded in the Alfacs bay and dead wild fauna was observed during this incidents. This was the first recorded case of fish mortalities associated to a dinoflagellate bloom in Catalonia. There is only one reference of fish mortality attributable to phytoplankton, which occurred in a brackish coastal lagoon in the Ebro Delta in 1977, caused by *Prymnesium parvum*.

SWEDEN (Lars Edler)

East and south of Sweden, in the brackish Baltic Sea, blooms of cyanobacteria area common feature in the summer. This has been going on for a long time and has been scientifically reported since the middle of the last century. It is sometimes claimed that eutrophication during the last decades has caused an increase of the blooms, but the evidence is not convincing. The number of toxic events due to these blooms, however, have increased which of course may be related with an increased awareness.

The blooms which are dominated by *Nodularia spumigena* and *Aphanizomenon flos-aquae* ÓbalticaÓ float on the surface as the vacuoles are filled with gas near the end of the bloom development. The blooms are thus easily seen on satellite images. The mats of bluegreen algae are driven by the wind to the shores of the Baltic Sea. Since the beginning of the '80s there have been several toxic cases when domestic animals, mainly cattle, dogs and birds, have been killed after drinking the brackish water with high concentrations of bluegreen algae.

On the west coast of Sweden, which includes the Kattegat and the Skagerrak, the salinity is higher and problems of harmful phytoplankton are more similar to other marine areas. Since the beginning of the '80s oxygen deficiency has been a considerable problem in the Kattegat. One reason for this are dinoflagellates blooms, especially of *Ceratium* spp. During the first half of the 80-ties these blooms were very large, but have since decreased in magnitude, whereas the annual September oxygen deficiency remains and actually seems to spread of larger areas of the Kattegat.

Since 1984 DSP has been a considerable problem along the Swedish Skagerrak coast. Investigations in the area showed that *Dinophysis acuta* was the toxin producing species. Neither *Dinophysis norvegica*, nor *D. acuminata* can, however, be ruled out as responsible for the toxin production. All three *Dinophysis* species are abundant in the summer, but toxicity is generally not found until the beginning of October. Some years the mussels have remained toxic for very long time (more than 6 months) although *Dinophysis* species have been present in the water in very low abundance. Other toxic dinoflagellates, such as *Alexandrium tamarense*, is a regular component of the plankton flora in the Kattegat and Skagerrak. However, there has not been any documented PSP outbreak in Sweden.

In 1988 the very large *Chrysochromulina polylepis* bloom developed in the Kattegat and Skagerrak. Cell densities of up to 100 million cells/l were registered. The effects were drastic. All sorts of marine organisms were affected. Since this big bloom there has been an increased awareness of the presence of *Chrysochromulina* and even if blooms with cell densities of 5-10 million cells/l have been observed, there have been no signs of toxic effects since 1988.

Partly due to the *Chrysochromulina* bloom, a new national monitoring programme of phytoplankton (as well as chemical and physical oceanography) has developed. Nowadays there are four Óintensive stationsÓ, one in the Skagerrak, one in the Kattegat, one in the Baltic and one in the Bothnian Sea, sampled about 25 times per year. A net of offshore stations, from the outer part of the Skagerrak to the innermost part of the Bothnian Bay, are sampled more or less monthly.

REGIONAL PROGRAMMES CLOSE TO THE COAST

After the *Chrysochromulina* bloom, some Information Centres were setup - one for the Kattegat, Skagerrak area, one for the Baltic and one for the Bothnian Sea. Results from all the different programmes are reported to these centres where they are compiled and discussed. An overview of the situation, concerning toxic phytoplankton, phytoplankton blooms, mussel toxicity, oxygen deficiency, nutrient situation etc. is then released to authorities, communities, health inspection, universities, media and anybody else who subscribes.

At present the most severe problem we face in this work in Sweden is the lack of taxonomic expertise. We have to rely on our neighbors in Oslo and Copenhagen.

UNITED KINGDOM (Jane Mary Lewis)

NUISANCE SPECIES

Nuisance blooms of microalgae have been known around the British Isles since phytoplankton investigations began around the turn of the century. A recent analysis of the literature shows that about 16 species have been recorded as forming blooms. However, it seems that the appearance of many of these species in bloom proportions are singular occurrences. A survey of data held by the Ministry of Agriculture Fisheries and Food (MAFF) and the National Rivers Authority (NRA) revealed the six taxa listed below as being the most common cause of nuisance or toxicity in the last few years:

- Alexandrium tamarense* (PSP toxins)
- Chaetoceros* and *Attheya* spp (farmed fish deaths, and foam on beaches)
- Dinophysis* spp (DSP toxins)
- Gyrodinium aureolum* (fish and invertebrate deaths, red water)
- Noctiluca scintillans* (red water)
- Phaeocystis* sp (foam on beaches and other related problems)

MONITORING

Monitoring is carried out by the agencies listed below. Monitoring may fall to two different agencies in each of the countries concerned. Taking England and Wales as an example, the MAFF is responsible for compliance with EC directive 91/742 concerning the health conditions for the production and marketing of live bivalve molluscs. It regularly samples in shellfish production areas and samples are counted with respect to the main nuisance microalgal species. The NRA is responsible for compliance with EC directive 76/160 concerned with the quality of bathing waters. Whilst carrying out this remit they monitor coastal areas for visually obvious algal problems and would investigate any events in bathing areas. They are also considered to be "guardians of the water environment" and as such the public contact them with any aquatic "cause for concern". This also leads to the NRA investigating algal events in marine areas. Additionally (particularly in Scotland) some fish farming concerns carry out microalgal monitoring at their farms.

RESEARCH AND COURSES

There is a continuing tradition of phytoplankton research in both Universities and Government Laboratories in the UK. The Natural Environment Research Council (NERC) funds research into phytoplankton ecology and taxonomy and the MAFF funds research into toxic blooms and related subjects. Within the UK there is expertise on most of the major microalgal groups. Courses have been run by myself and other researchers for government, and NRA scientists and fish farmers involved in microalgal monitoring.

MONITORING AGENCIES

England and Wales:

Ministry of Agriculture Fisheries and Food (MAFF)
National Rivers Authority (NRA)

Scotland:

Scottish Office, Agriculture and Fisheries Department (SOAFD)
River Purification Boards

Northern Ireland:

Department of Agriculture Northern Ireland
Department Environment for Northern Ireland

U.S.A. - N.W. Atlantic, Gulf of Maine (Maureen Keller)

The Gulf of Maine is a large, semi-enclosed shelf sea located on the east coast of the United States. Three U.S. states and two Canadian provinces share its coastline, yet the Gulf remains relatively pristine. Its enormous primary production, which supports a variety of fisheries, and its "clean" state, are a function of extensive and strong tidal and frontal mixing. Many noxious or toxic species are present in its coastal waters, but the conditions that result in problems, e.g. eutrophication, etc., do not occur so far, except in a few, isolated occurrences. The exception is the dinoflagellate, *Alexandrium tamarense*. Since 1958, the state of Maine has had a monitoring program for PSP, after an outbreak in the Canadian provinces in 1957. Until the early 1970's, the toxic area was confined to the N.E. part of the Gulf, but in 1972, the bloom extended along the entire Maine coast and resulted in large closures. By the late 1970's, it had extended into southern Gulf of Maine coastal waters and toxic events have recently been recorded on Georges Bank, a prime off-shore fishing ground. Closures now occur in many areas every year. The causative organism in all cases is the dinoflagellate, *Alexandrium tamarense*. The spreading of the bloom has been attributed to the establishment of cyst beds in different areas and advective transfer. There are some areas that are permanently closed because of extensive cyst beds. Typically, the bloom period extends from April to November, with most extensive/intensive periods in June and September. The distribution of other species of *Alexandrium* (e.g. *A. fundyense*) in the Gulf of Maine is unclear.

In September, 1988, an isolated bloom of the dinoflagellate, *Gymnodinium mikimotoi* (*Gyrodinium aureolum*?), resulted in massive shellfish mortalities in a small coastal bay in the S.W. portion of the Gulf. This species had not been previously recorded, although *Gyrodinium aureolum* was described from this area by Hulburt, and it has not caused problems since this single incident.

Many other species, identified in other areas as problem algae, are regular components of the Gulf of Maine flora. The prymnesiophyte *Phaeocystis pouchetii* is a regular and sometimes dominant component of the spring bloom, but it does not reach the densities recorded in NE. Atlantic coastal waters and thus foam banks on beaches, etc. have not been observed. Many species of *Dinophysis* are common throughout the spring and summer, including those implicated in DSP toxicity. Dinoflagellates of the genera *Prorocentrum* and *Katodinium* form extensive, dense blooms in late summer and early fall, and in late winter and early spring, respectively, resulting in typical "red tides," but no problems have been associated with these events. For example, no anoxia has been attributed to the occurrence and degradation of any such high biomass blooms, analogous to the massive kills associated with a *Ceratium* bloom in N.Y. waters in 1976.

Recently, using immunofluorescent probes, the "brown tide" organism *Aureococcus anophagefferens* (Chrysophyceae or Pelagophyceae) has been identified in several areas of the Gulf of Maine. This species has caused extensive mortalities of shellfish in coastal waters of Long Island Sound, N.Y. and Narragansett Bay, R. I., to the south of the Gulf of Maine. This organism first appeared in Rhode Island and Long Island in 1985 and has since occurred every year, but not with the catastrophic results of 1985. This year (1995), mortalities were recorded

again. Examination of material collected before 1985 revealed the presence of this "hidden flora," but as a minor component of the nanoplankton. No problems with *Aureococcus* have been recorded in the Gulf of Maine. The raphidophyte, *Heterosigma akashiwo*, associated with fish mortalities, has been forming blooms in Narragansett Bay since at least the 1960's. It has not been reported in the Gulf of Maine.

The first reports of domoic acid poisoning, caused by *Pseudo-nitzschia* sp., were from waters immediately to the north of the Gulf of Maine, but to date, ASP has not been reported in the Gulf of Maine.

U.S.A. -Northern Gulf of Mexico (Quay Dortch)

The low salinity estuaries and coastal zone of the northern Gulf of Mexico are highly eutrophic and, in general, characterized by extremely high phytoplankton biomass. A major problem for this entire region is the lack of systematically gathered data about the distributions of potentially toxic species or about toxins in fish and shellfish. It is generally believed that the low salinities prevent the occurrence of blooms of toxic phytoplankton and that no monitoring is necessary in this area, despite the economic importance of the shellfish and fishing industries. However, high concentrations of many species occur, some of which are usually considered either noxious or potentially toxic. Abundances of other "normal" species, such as *Skeletonema costatum*, are so high that they may be considered noxious because their sinking, either directly or in fecal pellets, results in a large area of hypoxic bottom water on the shelf.

So far there have been no known human health problems associated with toxic phytoplankton in this region. However, *Pseudo-nitzschia* spp., some of which cause Amnesic Shellfish Poisoning, reach concentrations exceeding a million cells/liter, especially in spring when the flow of the Mississippi River is highest. Toxic, non-toxic, and sometimes-toxic species have been identified in the area, but their spatial and temporal distribution is unknown. Fortunately, *Pseudo-nitzschia* spp. occur less frequently and concentrations seem to be lower in the estuaries where most shellfish are harvested. Another potential problem is Diarrhetic Shellfish Poisoning. Okadaic acid in oysters in Mobile Bay has been measured. None of the dinoflagellates known definitively to produce okadaic acid in other regions are observed in this area, but a large variety of *Dinophysis* and *Prorocentrum* species are present, sometimes in high numbers.

The occurrence of fish kills associated with algal blooms and water discoloration events are well documented for this area. Particularly frequent are blooms of *Alexandrium monilatum* and *Gymnodinium sanguineum*, which can be associated with fish and shellfish mortality and may produce toxins. In addition, there are fish kills with no apparent cause (neither obvious algal blooms, low oxygen, fish by-catch nor lost net sets), which may be due to the predatory dinoflagellate genus, *Pfeisteria*. Less frequent are water discoloration events due to *Noctiluca* sp., *Prorocentrum minimum*, *Lingulodinium polyedra*, *Heterosigma* cf. *akashiwo*, and *Katodinium rotundum*. Recently, in the very low salinity estuaries there have been massive blooms of filamentous cyanobacteria, probably *Anabaena*.

U.S.A. and CANADA - Northwest Pacific (Jean-Marie Adamson)

Alexandrium catenella, *A. acatenella*, *A. tamarense* (PSP)

An outbreak of PSP in 1942 caused the deaths of 3 people and resulted in the annual closure of a stretch of the Washington open coast to the harvest of bivalve molluscs. One section of the northern Oregon coast was closed to razor clam harvesting for three continual years, due to alternating problems with PSP and DA. PSP was not a problem in the more inland waters of Puget Sound until 1978, but closures there are now common. Organisms which have been found to pose a problem through accumulation of saxitoxin include mussels, oysters, rock scallops, razor clams and cockles.

Two other potential saxitoxin producers, *A. ostenfeldii* and *A. hiranoi*, have recently been identified in British Columbia, Canada. Thus far, these species have not been associated with any toxic blooms in the area.

The resources to accomplish identification and monitoring appear to be adequate, at least in Washington and British Columbia. Dr. Rita Homer (U. Washington, Seattle) provides training in phytoplankton identification. There is a program funded by the U.S. Food and Drug Administration in Washington, D. C. that helps fund the

purchase of nets and field microscopes to aid in field monitoring. In Oregon, only sporadic sampling of potentially toxic phytoplankton is done due to financial restrictions and to a recent relocation of responsibility for shellfish safety from the state Dept. of Health to the Dept. of Agriculture. Routine monitoring of sentinel organisms (mussels) takes place during the warm summer months to provide early detection of biotoxin problems. The source of the blooms, whether advective or *in situ*, is generally unknown.

Pseudo-nitzschia australis, *P. multiseriata*, *P. pseudodelicatissima* (DA, or ASP)

Domoic acid (DA) poisoning was first found in the Pacific northwest in October, 1991 when razor clams were found to contain significant levels of the toxin, resulting in closure of both commercial and recreational harvests. Oysters and mussels were tested at that time and did not contain DA, although Dungeness crabs tested positive. In October 1994 a weekly phytoplankton monitoring program, including 20 coastal and inland sites, was begun by the Washington State Department of Health in conjunction with the U.S. Food and Drug Administration to determine the distribution of problem species. In November 1994, DA was first found in mussels in inland waters, indicating that the problem is increasing both in geographic extent and in the number of species affected in this region. The causative agent of ASP has not been identified with certainty but it has been assumed that the *Pseudo-nitzschia* species named above are responsible. Identification of these species is difficult, usually requiring at least scanning electron microscopy. Instrumentation and taxonomic expertise are adequate in this region, and routine monitoring continues in Puget Sound.

In Oregon, mussels are tentatively being used as the sentinel organisms for DA as well as PSP, although their appropriateness in this role is questionable. The conditions giving rise to the problem, as well as the causative organism, are not understood.

Chaetoceros convolutus, *C. concavicornis*, and perhaps *C. danicus* (finfish mortality)

Blooms of these species seem to cause problems because the barbs on the setae lodge within fish gills, resulting in excess mucus production and severe tissue damage. The problem has existed since at least 1961, primarily among fish reared in net pens. Fairly low concentrations ($<10^4$ cells l^{-1}) can be lethal.

Heterosigma akashiwo (finfish mortality)

This has been an annual problem since 1960 in British Columbia, less frequently in Washington, for both cultured and wild fish. Larger fish are usually killed before smaller ones, probably due to organ damage by superoxide radicals and hydrogen peroxide. *H. akashiwo* (Hada) Hada, which is synonym of *H. carterae* and at times has been erroneously confused with *Olisthodiscus luteus* Carter, is a raphidophyte (chloromonad) flagellate. *Ceratium fusus* and *Gymnodinium sanguineum* (shellfish mortality)

These dinoflagellates have been associated with mortality of oyster larvae and adults and spot prawns. The mode of action is not known, although either a toxin or mechanical damage is suspected.

Table 1

A summary of toxic and noxious algal blooms and their effects

DATE	AUTHORS	LOCAL	ORGANISMS	OBSERVATIONS
06/1913	Faria (1914)	Guanabara Bay Rio de Janeiro State	<i>Scrippsiella trochoidea</i> (= <i>Glenodinium trochoideum</i>)	Fish mortality
12/1920	Dias (1992)	Cananéia São Paulo State	unknown	Fish mortality
08/1946	Oliveira (1947)	Guanabara Bay Rio de Janeiro State	<i>Scrippsiella trochoidea</i> (= <i>Glenodinium trochoideum</i>)	Fish mortality
04/1948	Oliveira (1950)	Guanabara Bay Rio de Janeiro State	<i>Scrippsiella trochoidea</i> (= <i>Glenodinium Trochoideum</i>)	Fish mortality
06-07/1949	Oliveira (1950)	Guanabara Bay Rio de Janeiro State	<i>Prorocentrum micans</i>	Rusty water no fish mortality
02-03/1961 10/1963	Satô (1963/4)	Recife Pernambuco State	<i>Oscillatoria erythraea</i> (= <i>Trichodesmium erythraeum</i>)	"Tamandaré fever" (like a cold) No fish mortality was proved
02-03/1967	Barth (1967)	Water from Brazil Current Rio de Janeiro State	<i>Oscillatoria erythraea</i> (= <i>Trichodesmium erythraeum</i>)	No fish mortality
07-08/1971	Aguiar & Corte-Real (1973)	Rio Grande do Sul State	<i>Asterionellopsis glacialis</i> (<i>Asterionella japonica</i>)	Discolored water No fish mortality
04/1978	Machado (1978) Rosa (1978)	Tramandai, Mostardas and Hermenegildo villages Rio Grande do Sul State	<i>Gyrodinium aureolum</i> (= <i>Gynmodinium sp.</i>)	Respiratory irritation No fish mortality Shellfish mortality
04/1978	Zavala-Camin & Yamanaka (1980)	from Itanhaém to Peruib	<i>Asterionellopsis glacialis</i> (<i>Asterionella japonica</i>)	Fish mortality (32 species of fishes)
05-06-07/1978	Sevrin-Reyssac <i>et al.</i> (1978)	Guanabara Bay Rio de Janeiro State	Chloromonads and numerous dinoflagellates	No fish mortality (07-4.8 x 10 ⁷ cells/l) (Chl. =31.7 -47.7 mg/m ³)
01/1979	Tommasi & Navas-Pereira (1983)	Santos Bay São Paulo State	<i>Skeletonema costatum</i>	No fish mortality (2.2 x 10 ⁷ cells/l)
09/1990	Tommasi & Navas-Pereira (1983)	Santos Bay São Paulo State	<i>Asterionellopsis glacialis</i> (<i>Asterionella japonica</i>)	No fish mortality (2.5 x 10 ⁶ cells/l)
05-07/1981	Rosa & Buselato (1981)	Hermenegildo and Barra do Chui villages Rio Grande do Sul State	<i>Gyrodinium aureolum</i>	Respiratory irritation Invertebrates mortality Food intoxication
02/1981	Freitas & Lunetta (1982)	Rio de Janeiro State	<i>Gonyaulax</i> sp.	Discolored water
08/1983	CETESB (1983)	São Paulo State	<i>Gymnodinium</i> sp.	Fish mortality
01/1984	Roberto & Navas-Pereira (1984)	Ubatuba São Paulo State	<i>Mesodinium rubrum</i>	Water stratification (High PO ₄ values)
03/1990	Owen <i>et al.</i> (1992)	Ubatuba continental shelf São Paulo State	<i>Mesodinium rubrum</i>	Subsurface discoloured water (>4000 cells/ml) (Chl. = 79 mg/m ³)
11/1990	Gianesella-Galvão <i>et al.</i> (in press)	Northern coast of São Paulo State	<i>Oscillatoria erythraea</i>	30 km patchiness (Chl. = 400 mg/m ³)
03-04/1993	Odebrecht <i>et al.</i> (1995)	Cassino Beach Rio Grande do Sul State	<i>Asterionellopsis glacialis</i> (10 ⁶ - 10 ⁷ cells/l) <i>Prorocentrum balticum</i> (10 ⁶ cells/l) <i>Gymnodinium</i> cf. <i>aureolum</i> (10 ⁵ cells/l) <i>Noctiluca scintillans</i> (10 ⁵ cells/l) <i>Dinophysis acuminata</i> (10 ² cells/l)	Massive shellfish mortality (<i>Mesodesma mactroides</i> , <i>Donax hanleyanus</i>) and crustacean (<i>Emerita brasiliensis</i>)

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Known or potentially toxic algal species in the Gulf of Mexico and western North Atlantic (Jan H. Landsberg)

SPECIES	HABITAT	AREA	TOXIC	IMPACT	COMMENTS
DINOFLAGELLATES					
<i>Alexandrium monilatum</i>	Estuarine/coastal/ planktonic	Gulf-wide, NW Atlantic	+	Animal kills	Seasonal in estuaries, appears sporadically. Has caused marine animal mortalities in Florida and Texas, but not implicated in shellfish poisonings e.g. PSP. Currently in September 1995, blooms have been recorded off of Florida, Mississippi, Louisiana and Texas at the same time.
<i>Coolia monotis</i>	Benthic	Gulf-wide, NW Atlantic	+	?	Toxic in lab tests, ciguatera?
<i>Dinophysis acuminata</i>	Estuarine/coastal/planktonic	Gulf-wide	+	?DSP	Toxic in lab studies
<i>Dinophysis caudata</i>	Estuarine/coastal/planktonic	Gulf-wide, NW Atlantic	+	?DSP	Toxic in lab studies
<i>Dinophysis fortii</i>	Estuarine/coastal/planktonic	Gulf-wide	+	?DSP	Toxic in lab studies
<i>Phalacroma mitra</i>	Estuarine/coastal/planktonic	Gulf-wide	+	?DSP	Toxic in lab studies
<i>Phalacroma rotundatum</i>	Estuarine/coastal/planktonic	Gulf-wide, NW Atlantic	+	?DSP	Toxic in lab studies
<i>Gambierdiscus toxicus</i>	Benthic	South Florida	+	Ciguatera Human illness Fish kills/disease?	Possible reported cases from Texas, southeast Florida
<i>Gymnodinium breve</i>	Oceanic/coastal/estuarine/ planktonic	Gulf-wide, NW Atlantic	+	NSP, fish (animal?) kills, respiratory irritation	Occurred 57 times in the last 150 years. Persistent in Florida coastal waters over the last 45 years. In 1994-1995 it occurred in Florida from Dry Tortugas to Panama City and co-occurred with <i>A. monilatum</i> in northwest Florida
<i>Gymnodinium mikimotoi</i>	Offshore/coastal/planktonic	Florida	+	?	Known to be toxic in other areas
<i>Gymnodinium sanguineum</i>	Estuarine/coastal/ planktonic	Gulf-wide, NW Atlantic	+?	Fish kills, low d.o.	One lab study indicates toxicity
<i>Ostreopsis lenticularis</i>	Benthic	South Florida	+	?	Implicated in ciguatera
<i>Ostreopsis siamensis</i>	Benthic	South Florida	+	?	Implicated in ciguatera
<i>Pfiesteria piscicida</i>	Estuarine/coastal/planktonic	?Gulf-wide/WN Atlantic	+	Fish kills	May be responsible for fish kills in estuarine areas typically associated with low d.o.
<i>Pfiesteria</i> sp.	?Estuarine/coastal/planktonic	?	?+	?Fish kills	Known from aquarium studies, potentially toxic to fish
<i>Prorocentrum heliceanum</i>	Benthic	South Florida	+	?Ciguatera	Toxicity in lab studies

<i>Prorocentrum hoffmanianum</i>	Benthic	South Florida	+	?DSP/Ciguatera	Toxicity in lab studies
<i>Prorocentrum lima</i>	Benthic	Gulf-wide, NW Atlantic	+	?DSP/Ciguatera	Toxicity in lab studies
<i>Prorocentrum marimum</i>	Benthic	Gulf-wide, NW Atlantic	+	?DSP/Ciguatera	Toxicity in lab studies
<i>Prorocentrum mexicanum</i>	Benthic	Gulf-wide	+	?DSP/Ciguatera	Toxicity in lab studies
<i>Prorocentrum minimum</i>	Coastal/estuarine/planktonic	Gulf-wide, NW Atlantic	+	?Fish kills	Toxicity in lab studies
DIATOMS					
<i>Pseudo-nitzschia multiseries</i>	Benthic/tychoplanktonic	Gulf-wide	+	ASP	No human shellfish toxicity cases in this area, but shellfish have tested positive for ASP
<i>Pseudo-nitzschia pseudodelicatissima</i>	Benthic/tychoplanktonic	Gulf-wide	+	ASP	No human shellfish toxicity cases in this area, but shellfish have tested positive for ASP
<i>Pseudo-nitzschia delicatissima</i>	Benthic/tychoplanktonic	Gulf-wide	+	ASP	No human shellfish toxicity cases in this area, but shellfish have tested positive for ASP
RAPHIIDOPHYCEAE					
<i>Chattonella antiqua</i>	Coastal/planktonic	Florida, Louisiana, Texas	+	?Fish kills	Appear in eutrophic inshore areas
<i>Chattonella marina</i>	Coastal/planktonic	Florida, Louisiana, Texas	+	NSP, ? Fish kills	
<i>Heterosigma akashiwo</i>	Coastal/planktonic	Florida, Louisiana	+	?Fish kills	
PRYMNESIOPHYTA/HAPTOPHYTA					
<i>Prymnesium parvum</i>	Coastal/estuarine/planktonic	Gulf-wide	+	?Fish kills	
<i>Chrysochromulina</i> spp.	Coastal/planktonic	Gulf-wide	+	?Fish kills	
CYANOBACTERIA					
<i>Schizothrix calcicola</i>	Estuarine/planktonic	Tampa Bay, Florida	?	?Benthic marine animal kills, low d.o.	Toxic in lab studies
<i>Synechococcus elongatus</i>	Coastal/estuarine/planktonic	Florida Bay	?	Sponge mortalities	Mechanical damage or toxicity to sponges
<i>Trichodesmium erythraeum</i>	Offshore/coastal/planktonic	Gulf-wide, NW Atlantic	+	?Fish kills/ciguatera	Implicated in fish kills?, ?coral mortality

ANNEX IX

QUESTIONNAIRE

1. Considering where you are in your professional development, do you feel that this Course was an important experience to have? Could you have received the same training elsewhere?
2. Course content: with a limited period of time (three weeks) do you feel each group of phytoplankton species was covered adequately? If not, what would you suggest be included in future offerings?
3. Techniques: are there other techniques you would have liked to have seen or of the ones presented to have greater detail?
4. What general suggestions would you make to improve the Course?
5. Do you feel that each student was given equal instruction?
6. Were laboratory facilities adequate?
7. Were housing and meal arrangements adequate?
8. When you return to your home institution, do you believe you will be given sufficient time and facilities to do more detailed taxonomic work?
9. Please feel free to make additional comments you may wish regarding the Course experience.

ANNEX X

LIST OF ACRONYMS

ASP	Amnesyc Shellfish Poisoning
DA	Domoic Acid
DSP	Dyarrhetic Shellfish Poisoning
EC	European Committee
ETI	Expert Center for Taxonomic Identification
HPLC	High Performance Liquid Chromatography
IOC	Intergovernmental Oceanographic Commission
HAB	Harmful Algal Blooms
MAST	Marine Science and Technology
OA	Okadaic Acid
NSP	Neurotoxic Shellfish Poisoning
PSP	Paralytic (or, better, Paralyzing) Shellfish Poisoning
SDC	Serial Dilution Cultures
SEM	Scanning Electron Microscopy
SZN	Stazione Zoologica “A. Dohrn” of Naples
TEM	Transmission Electron Microscopy
TFF	Tangential Flow Filtration