# Intergovernmental Oceanographic Commission

Workshop Report No. 57



# IOC Workshop on International Co-operation in the Study of Red Tides and Ocean Blooms

Takamatsu, Japan, 16-17 November 1987



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6	17-22 February 1975. Report of the CCOP/SOPAC- IOC IDOE International Workshop	IOC, Unesco Place de Fontenoy	English	20	Second CCUP-IOC Workshop on IDOE Studies of East Asia Tectonics and Resources, Bandung, Indonesia, 17-21 October 1978.	IOC, Unesco Piace de Fontenoy 75700 Paris, France	English
	on Geology, Mineral Resources and Geophysics of the South Pacific, Suva, Fiji, 1-6 September 1975.	75700 Paris, France		21	Second IDOE Symposium on Turbulence in the Ocean, Liège, Belgium, 7-18 May 1979.	IOC, Unesco Place de Fontenoy 75700 Paris, France	English French Spanish
7	Report of the Scientific Workshop to Initiate Planning for a Co- operative Investigation in the North and Central Western Indian Ocean, organized within the IDOE	IOC, Unesco Place de Fontenoy 75700 Paris, France	English French Spanish Russian	22	Third IOC/WMO Workshop on Marine Pollution Monitoring, New Delhi, 11-15 February 1980.	IOC, Unesco Place de Fontenoy 75700 Paris, France	Russian English French Spanish
	under the sponsorship of IOC/FAO (IOFC)/Unesco/EAC, Nairobi, Kenya, 25 March-2 April 1976.			23	WESTPAC Workshop on the Marine Geology and Geophysics of the	IOC, Unesco Place de Fontenoy	English Russian
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11	Adjacent Regions, Port of Spain Trinidad, 13-17 December 1976. Collected contributions of invited	IOC Linesco	English	27	CCOP/SOPAC-IOC Second International Workshop on Geology, Mineral Resources and Geophysics of	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
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12	and Adjacent Regions, Port of Spain, Trinidad, 13-17 December 1976.		Faction		environmental variation on the survival of larval pelagic fishes Lima, 20 April-5 May 1980.	Place de Fontenoy 75700 Paris, France	
	plinary Workshop on Scientific Programmes in Support of Fisheries Projects, Fort-de-France, Martinique	Place de Fontenoy 75700 Paris, France	French Spanish	29	WESTPAC Workshop on Marine biological methodology Tokyo, 9-14 February 1981.	IOC, Unesco Place de Fontenoy 75700 París, France	English
13	28 November-2 December 1977. Report of the IOCARIBE Workshop on Environmental Geology of the	IOC, Unesco Place de Fontenoy	English Spanish	30	International Workshop on Marine Pollution in the South-West Atlantic Montevideo, 10-14 November 1980.	IOC, Unesco Place de Fontenoy, 75700 Paris, France	English (out of stoc Spanish
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	Workshop on Marine Pollution in the Gulf of Guinea and Adjacent Areas, Abidjan, Ivory Coast,	Place de Fontenoy 75700 Paris, France	English French	32	UNU/IOC/Unesco Workshop on International Co-operation in the Development of Marine Science and	IOC, Unesco Place de Fontenoy 75700 Paris, France	English French Spanish
15	2-9 may 1978. CPPS/FAO/IOC/UNEP international Workshop on Marine Pollution in the South-East Pacific. Santiago	IOC, Unesco Place de Fontenoy 75700 Paris, France	English (out of stock)		the Transfer of Technology in the context of the New Ocean Regime Paris, 27 September - 1 October 1982		
	de Chile, 6-10 November 1978.	, er of a land, i terroe		CONT	D ON INSIDE OF BACK COVER		

Intergovernmental Oceanographic Commission

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SC-89/WS-27

#### 1. OPENING

The Chairman of the Local Organizing Committee for the Workshop, Prof. T. Okaichi, called the Session to order at 09.00 hours on 16 November 1987. He welcomed the participants to University of Kagawa and invited Prof. T. Nemoto, Chairman of WESTPAC, and Dr. P. Bjornsen, IOC representative, to address the Workshop.

Professor Nemoto emphasized the economic and environmental importance of red tides and ocean blooms, and the timeliness of holding a workshop on international co-operation in this field. He then recalled the aims of the Workshop as specified in the invitation letter to the participants:

- (i) to provide an overview of relevant research in selected regions, partly on the basis of presentations and discussions during the International Symposium preceeding the Workshop;
- (ii) to formulate research strategies and propose methodologies for a global Sub-programme on Ocean Blooms and Red Tides, under the Ocean Science on Living Resources (OSLR) Programme; and
- (iii) to suggest major research topics within the framework of OSLR and GIPME, e.g. on eutrophication, changes in the ecosystem structure and population distributions (natural and manmade), associated changes in recruitment and chemical conditions (e.g. oxygen levels), and mass mortalities of marine organisms.

The IOC representative welcomed the participants on behalf of IOC and conveyed a greeting for a successful Workshop from the Secretary IOC to the local organizing Committee and the participants. The IOC representative further introduced the background for the Workshop, the scope of which exemplifies the increasing need for interdisciplinary approaches to ocean science, including physical and chemical oceanography as well as ecology, physiology and toxicology. The apparantly rapid expansion of bloom phenomena in a number of regions stresses the demand for international  $_{\rm CO}$ -operation and for close liaison between the scientific community and the international organizations. He outlined the possible role of IOC in supporting a regional implementation of a Sub-programme on Ccean Blooms and reference materials, organizing training and intercalibrations and facilitating exchange of data, and he expressed the hope that this Workshop could provide a technical basis for the initialization of such a Sub-programme.

The IOC Representative thanked the Local Organizing Committee for its great efforts made in arranging the Workshop in conjunction with the International Symposium on Red Tides held in Takamatsu, 10-14 November 1987. The tying of the Workshop to an international scientific symposium had proven very beneficial, as it had ensured the availability of top scientists covering different aspects of the field and with a broad geographical representation; furthermore the participants had had the possibility of exchanging views and information before the workshop.

#### 2. ADMINISTRATIVE MATTERS

The Chairman of the Local Organizing Committee, Prof. T. Okaichi, proposed Prof. T. Nemoto to chair the Workshop, and the proposal was approved by the participants.

The Agenda given in Annex I was introduced by the Chairman. With reference to the aims of the Workshop as listed above, the Chairman suggested that the research review aspect largely could be covered by the solicited written contributions and the presentations and discussions at the International Symposium preceeding the Workshop, thus allowing the Workshop to dedicate the available time mainly to discussing needs and possibilities for future international collaboration.

The Chairman proposed to arrange part of the Session in three Subgroups dealing with ecological, taxonomical and toxicological aspects, respectively. He then invited Drs. T. Smavda, K.V. Hansen and D.M. Anderson to chair the Sub-groups, and Drs. P.M. Holligan, K. Steidinger and I. Jenkinson to act as rapporteurs for the sub-group discussions.

The proposed administrative arrangements were approved by the participants. The division of the participants into the sub-groups is indicated in Annex III.

#### 3. REGIONAL EXPERIENCES

A number of brief reports on regional occurrences of phytoplankton blooms and red tides, solicited from the participants to the Workshop, are given in Annex II. Together with the information presented at the International Symposium on Red Tides preceeding the Workshop, these reports clearly illustrate that phytoplankton blooms and red tides phenomena are global in distribution and appear to be spreading within and between regions and to be becoming increasingly frequent and extensive as well. Some of the more alarming reports can be summarized as follows:

Red tides of <u>Gvrodinium aureolum</u> have caused massive kills of farmed and wild fish (and other marine animals) in northwestern Europe since the mid 1960s. Remarkably, this dinoflagellate is not recorded in the plankton records for the Northeast Atlantic before this time. Now <u>G</u>. <u>aureolum</u> is a common and often dominant component of the phytoplankton community in this region. The organism has also caused mass mortalities of caged fish in Japan (where it is referred to as <u>Gymnodinium nacasakiense</u>).

Red tides of <u>Chattonella anticua</u> have caused devastating kills of caged fish (mostly yellowtail) in the Seto Inland Sea in Japan on many occasions since about 1970. The annual damage from <u>C. anticua</u> red tides on fish farming in Japan often reaches billions of yen.

It is now well-documented that certain dinoflagellates can cause severe gastrointestinal disturbance in humans, including accute diarrhoea, nausea, vomiting, and abdominal pain (DSP). Although no mortalities caused directly by toxicity from DSP have been reported, the affliction is highly debilitating for 2-3 days. The responsible dinoflagellates can contaminate shellfish even when cell concentrations in the water are very low. Starting as a virtually unknown phenomena 10 years ago, DSP is now considered to be the most important shellfish toxicity problem in many countries throughout the world, including Japan and western Europe.

Another important and disturbing series of shellfish toxicity emisodes are associated with the dinoflagellate <u>Phyrodinium bahamense</u>. Occurrences in 1976 in Sabah and Erunei in the western Pacific region appear to be related to the spreading of this species from Papua New Guinea where PSP toxicity was a common occurrence. A sudden and unexpected outbreak of the species in the central Philippines in 1983 closed the mussel fishery for 8 months in that region, again presumably related to the spreading of <u>P</u>. <u>bahamense</u> from other areas. The existence of a dormant resting cyst of this species explains its persistance in these regions after the initial introduction or inoculations. An equally alarming scenario can be imagined for Central America now that a PSP outbreak has occurred in western Guatemala for the first time, in August 1987. The causative dinoflagellate was <u>P</u>. bahamense, suggesting that the toxic variety of this species may have incossed the Pacific and now might spread within Central and South America in the same manner as it has in Southeast Asia.

Blooms and red tides of several species of <u>Protoconvaulax</u> have caused contamination of shellfish stocks with paralyzing toxinies in many countries around the world, resulting in numerous cases of human illness and death (PSP). Although <u>Protoconvaulax</u> blooms generally have negligible effects on shellfish, they have tremendous impacts on the economics of the shellfish industry around the world because of the harvesting and marketing restrictions necessary to avoid the danger to public health. Blooms of <u>Proconvaulax</u> are apparantly spreading around the world in recent years. Elooms of this organism also kill fish and have been associated with mass nortalities of herring in eastern Canada and caged salmon and trout in the Farce Islands.

For many years toxic algal blocms and red tides have had severe economic impact on shellfish and finfish resources, public health, and the aquatic environment throughout coastal regions of the world. It is now evident that the magnitude, frequency and geographic extent of these occurrences have increased significantly during the last two decades. This phenomenon affects developed countries with established natural fisheries and mariculture resources as well as developing countries that rely heavily ion coastal fisheries for their food supply and economics.

Algal blooms can take many forms, some of which have serious moverse effects due to the sheer abundance of organisms or to the production of toxins by those organisms. As described in many of the solicited regional reports, there are both economic and public health impacts from these occurrences.

Certain plankton blooms can result in human mortalities, most commonly during PSP outbreaks. Other events such as DSP outbreaks can cause scute illnesses, but evidence is now appearing that chronic problems occur as well, due to the apparent tumor promoting activity of the DSP toxins. Ciguatera poisoning has both acute and chronic effects as well. Massive red tides in the Gulf of Mexico not only kill fish but also cause severe respiratory irritation among coastal inhabitants due to the toxin that becomes airborne in the surf zone.

Shellfish toxicity such as paralytic shellfish poisoning (PSP), ciarrheic shellfish poisoning (DSP) and neurotoxic shellfish poisoning (NSP) cause major disruptions of harvesting and marketing operations

throughout the world and often necessitate expensive monitoring and surveillance programmes. In addition to the direct losses to established fisheries, the presence of plankton-related shellfish toxicity in a region can constrain aquaculture development. This is occurring, for example, in Tasmania, Australia, where recent outbreaks of PSP have led local authorities to recommend that shellfish farmers in one region either grow different species or move their operations to a different region of the country.

Mortalities of cultured fish, such as those that frequently occur in the Inland Sea of Japan, or the massive kills of wild fish in the Gulf of Mexico have major economic costs, estimated to be \$19,500,000 in Japan in 1983 alone. Plankton blooms can also clog fishermen's nets or alter fish behaviour, both of which can dramatically reduce catch sizes. Blooms can even impact the tourist industry in certain regions when dead fish wash up on shore, when surf activity creates thick layers of smelly foam on beaches or when local sea food is suspected inedible.

#### 4. THE BLOOM CONCEPT

As a startpoint for the discussions, the three subgroups were charged with the task of defining plankton blooms in the context of this Workshop. The definitions which came up reflected the different approaches of the three sub-groups, as indicated in the following three chapters.

It was generally agreed that phytoplankton blooms are discrete events in space and time, constituted by elevated concentrations of unicellular phytoplankton organisms. The causative mechanism of concentration may be either physical or biological, as when the growth rate of phytoplankton biomass exceeds the rate of losses due to sinking, lysis and grazing. Potentially adverse effects of phytoplankton blooms comprise discoloration and foam production, toxicity, changes in ecosystem structure, diversity and cycling of matter, and increased sedimentation load on the benthic communities.

From a toxicological point of view it is interesting to note that only a rather limited number of phytoplankton species seem to produce toxins, though the list of potentially toxic species is still being extended. These species are not permanently toxic, rather the toxicity appears to be expressed under certain environmental and physiologicl conditions, yet incompletely understood. Although toxicity is normally connected with mass occurrences (i.e. blooms) of the concerned species, some phytoplankton species prove toxic even at low cell densities. Apart from the phytoplankton, some heterotrophic planktonic organisms (e.g. Ciquatera dinoflagellates) display similar toxic effects and shoud therefore be considered in this context.

The taxonomy sub-group reviewed a list of potentially bloomforming phytoplankton species. The list is shown in Annex IV. It is remarkable that species of all the major phytogenetic groups of marine phytoplankton are known to form blooms in perturbed and non-perturbed coastal waters as well as in the open oceans. From an ecological point of view this observation is important, because it lifts the focus from the autecology of selected species to the level of synecology, specifically aiming at elucidating the physical, chemical and biological factors controlling the outbreak, climax and degradation of phytoplankton blooms. In coastal areas, phytoplankton blocms are often connected to elevated

concentrations of nutrients, particularly the combined inorganic forms of nitrogen and phosphorus, and phytoplankton blooms have therefore often been regarded as an effect of eutrophication. The occurrence of phytoplankton bloom in the open ocean, however, suggest that other mechanisms might as well be operative in triggering and maintaining phytoplankton blooms.

#### 5. ECOLOGICAL STUDIES

#### 5.1 INTRODUCTION

The Ecology Subgroup initiated its discussions by further elaborating the definition of plankton bloom as spatial and temporal discrete elevations of phytoplankton biomass relative to a baseline situation, as outlined in Fig.1, which illustrates the stages of a typical bloom event: the initiation and growth phase, the climax or maintenance phase and the decline or degradation phase. Being restricted phenomena in time and space, relative to a baseline which itself is fluctuating and rarely well established, blooms probably often escape observation. Furthermore, blooms defined as elevated concentrations of phytoplankton can be natural phenomena, such as the spring blooms normally occurring in temperate coastal waters.



Fig. 1 The stages of a bloom: (i) initiation, (ii) exponential growth, (iii) maintenance, (iv) decline. The bloom is constituted by the elevation in biomass (illustrated by the hatched area) above the baseline (v).

In order to conceptually distinguish exceptional blooms from natural blooms, it is necessary to include a quantitative aspect in the bloom definition. Phytoplankton blooms can be considered excessive in terms of:

- (i) amplitude, i.e. the biomass reached during the maintenance phase;
- (ii) spatial extension, i.e. the geographical area affected;
- (iii) duration of the maintenance phase; and
- (iv) frequency of successive blooms.

Viewed on a global or regional scale, the monitoring of blocm occurrences is normally too scanty to facilitate an identification of exceptional blooms on these quantitative criteria, and the Sub-group therefore considered the acquisition of long-term routine data on phytoplankton biomass to be a matter of immediate concern.

#### 5.2 BLOOM DEVELOPMENT

Even a detailed observational programme on bloom occurrences will, however, not improve the understanding of bloom development, unless it includes the physical, chemical and biological factors that control bloom formation. At present, the general understanding of bloom dynamics is limited, and the sub-group considered the development of consistent and testable hypotheses within this field to be a prime research need. The Subgroup discussed a number of physical, chemical and biological factors, that might be operative in controlling the different stages of bloom formation (Table 1).

#### Table 1.

#### FACTORS CONTROLLING BLOOM DYNAMICS

<u>Bloom</u> Stages	Physical	rolling factors Chemical	 Biological
Initiation	Vertical mixing (Upwelling)	Excess nutrients	Seed population
Exponential . growth	Temperature, light		Species composition Lack of predation
<u>Maintenance</u>	Water mass stability	Limiting nutrients	Luxury uptake Remineralization
Decline	Horizontal mixing	Nutrient exhaustion Toxic substances	Sedimentation Grazing Lysis

It is generally believed that <u>biomass</u> of marine phytoplankton in the baseline situation (cf. Fig. 1) is controlled by the availability of inorganic nutrient in the surface layer, particularly of the combined inorganic forms of nitrogen and phosphorus, while phytoplankton <u>production</u> in the baseline situation is limited by the mobilization of nutrients from remineralisation processes within the surface layer. The production will be

balanced by losses due to grazing by zooplankton, sedimentation and excretion of dissolved organic matter. The sedimentation will represent a loss of nutrients from the productive surface layer, which will be replaced by a steady diffusion of dissolved nutrients from deep layers.

A bloom might be initiated by an increase in availability of inorganic nutrients, provided the presence of a sufficient seed population of potentially bloom forming phytoplankton species (cf. Annex IV). Nutrient enrichment can be caused by input from river run off or land-based sources or by upwelling of nutrient-rich bottom water. Although nutrient enrichment probably represents the major causative factor initiating blooms, other mechanisms of physical, chemical or biological nature could also be operative as triggering factors. Studies of phytoplankton blooms in the open sea may contribute to elucidate the importance of such mechanisms.

After the initiation stage, in which the phytoplankton adapt to the changed environmental conditions, a build up of phytoplankton biomass will occur, reaching a maximum biomass determined by the availability of the most limiting nutrient, i.e. the nutrient with the lowest concentration compared to the phytoplankton demand (cf. Liebig's law of the Minimum). The duration of this maintenance stage is influenced by the physical stability of the water mass, the regeneration of nutrient by heterotrophic processes within the surface layer and the ability of the phytoplankters to reallocate intracellular nutrient pools (luxury uptake). Finally, degradation of a bloom can be achieved through physical mixing and by increasing loss rates due to sedimentation, lysis and grazing. IQC Workshop Report No. 57



Fig. 2 Physical, chemical and biological parameters constituting an integrated monitoring of ocean blooms. Parameters within the circle represent core measurement, while additional parameters are listed outside the circle. Sea surface temperature, chlorophyll and light can be monitored by satellite imagery.

An adequate understanding of blocm development can only be acquired through a well integrated programme of physical, chemical and biological measurements (Fig. 2). At a basic level, information on environmental factors such as wind, temperature, salinity and light attenuation is required, together with analyses of dissolved nitrogen, phosphorus and oxygen, chlorophyll determinations and counts of phytoplankton, bacteria and cysts for water and sediment samples.

Furthermore, if resources permit, there is a strong need to identify experimentally the factors that control the timing, magnitude and frequency of phytoplankton blocms, and to determine the effects of human activities in the coastal environment (coastline engineering, mineral exploitation, river discharges and dumping, aquaculture developments, etc.) on growth conditions for phytoplankton.

# 5.3 ECOLOGICAL EFFECTS OF BLOCMS

(a) Upper Water Column. As most blocms are not detected until late in growth phase (see Fig. 1), associated changes in the distributions of other planktonic organisms (e.g. bacteria, protozoa, copepods, gelatinous organisms, fish larvae and juveniles) are generally poorly documented. More detailed information on the structure of the planktonic food chain during bloom development as well as under non-blocm conditions is needed to assess the ecological impact of the phytoplankton blocms. Special attention should be given to making reliable measurements of algal growth, sinking and grazing rates. Also, observations relating climatic trends and hypernutrification to blocm frequency require careful evaluation, especially with respect to the problem of predicting blocm occurrences.

(b) Lower Water Column and Sediments. The declining phase of blooms often involves large fluxes of organic matter to bottom water and sediments. The effects on the feeding and survival of benthic organisms and on the activity of micro-organisms leading to oxygen depletion are often severe and need careful monitoring. Studies on the distribution of cysts and specific biochemical markers in sediment cores to provide historical information on blocm occurrences should be encouraged. Information on cyst type abundance in recent sediments is required to assess the potential for toxicity accumulation by benthic animals and for the development of blocms in succeeding years.

(c) Global Environmental Changes. There is increasing evidence that phytoplankton blocms strongly influence the global cycles of elements such as carbon, nitrogen and sulphur. Important problems for future research should include the effects of blocms on the air-sea exchange of carbon dicxide and organic sulphur compounds, on fluxes of nitrogen and phosphorus across the water-sediment interface, and on the exchange of particulate and dissolved organic material between coastal and oceanic waters.

#### 5.4 MONITORING AND PREDICTION

(a) Remote sensing. Infra-red and visual waveband sensors on satellites, aircraft and balloons provide the only means of obtaining largescale, synoptic information on the sea surface distributions of water temperature and phytoplankton abundance. Sequences of images also represent a powerful method for assessing the dynamic properties of surface waters. Improved resources for the processing and dissemination of remotely sensed data are required, and further research into the interpretation of colour signatures for coastal (optical case II) waters is strongly encouraged.

(b) Ships and Buoys. Continuously recording instruments for basic physical, chemical and biological measurements are needed to make effective use of ship time for ecological studies. Similar instruments should also be developed for automatic recording on buoys, in particular for measurements of temperature and dissolved nutrient levels and for the collection and preservation of water samples.

(c) Modelling. One, two and three dimensional models to simulate the basic physical and biological processes underlying the formation of phytoplankton blooms must be developed in parallel to observational programmes. These models, together with simple heuristics models to investigate particular processes, should have the aim of predicting phytoplankton blooms. Computer-based information systems would also be essential for diagnostic analysis, consultation and communication services.

#### 5.5 RESEARCH STRATEGIES

(a) Data Acquisition. Seasonal and longer term observations are needed to understand bloom development and to enable the detection of interannual changes of bloom distributions. Strategies for observational and experimental work should be designed for basic or advanced level work so that the best use is made of the available personnel and technological resources. The full co-operation of research institutions, national maritime agencies, fishermen co-operatives and appropriate commercial organizations should be sought.

(b) Co-operative Efforts. Within national programmes, a balanced interdisciplinary approach to studies on phytoplankton blocms must be

maintained. When this is not possible, appropriate external assistance should be sought. The rapid exchange of information between countries on the planning and results of studies on phytoplankton blooms will promote awareness of new problems as they arise.

(c) Development of Predictive Methods. For conceptual and diagnostic models to have a real predictive value, they should be based as far as possible on objective ecological principles and be capable of accommodating new information on particular processes.

#### 6. TAXONOMICAL STUDIES

#### 6.1 GENERAL PROBLEMS IN TAXONOMY

The available taxonomical literature is scattered and does not contain a cohesive guide to plankton blocm species that incorporate new findings based on conservative, easily recognized and consistent characters. In many countries this makes identification of plankton bloom species impossible during monitoring programmes or specific bloom events. In addition, new approaches need to be pursued at specialized research facilities to systematically characterize species through generic and biochemical studies. At our current level of expertise and knowledge, we must, by necessity, rely on morphology of species as studied by light microscopy in order to have effective identification by scientists and technicians minimally trained in the identification of plankton blocm organisms.

#### Table 2.

# PROBLEMATIC PLANKTON BLOOM TAXA.

Category I

Priority 1. Problem taxa requiring immediate attention.

- Protogonyaulax / Alexandrium / Gessnerium / Pyrodinium / Gonyau
- <u>Gymnodinium</u> / <u>Gyrodinium</u> / <u>Ptychodiscus</u>
- <u>Chattonella</u>
  - Priority 2.
- Prorocentrum / Exuviaella
- Ultraplankton and Picoplankton
- <u>Cochlodinium</u>
- <u>Ostreopsis</u> / <u>Coolia</u>
- <u>Mesodinium</u>
- Oscillatoria / Trichodesmium
- <u>Scrippsiella</u> / <u>Ensiculifera</u>

#### Category II

- Dinophysis / Phalacroma
- Heterosicma / Olisthodiscus
- <u>Goniodoma</u> / <u>Heteraulacus</u> / <u>Triadinium</u>
- Katodinium / Massartia

The plankton bloom genera listed in Table 2 present taxonomical uncertainties because of synonymies, possible hononymies or lack of meaningful morphological characters. They are presented in two categories: 1) synonymies and new species problems arising from reliable morphological characters and 2) nomenclatural and synonymy problems.

Although ideal classification would be at the genetic level of species identification, at present we are limited to morphospecies at the phenetic level. Therefore, we need to refine our morphological observations by 1) determining morphological variation (large scale and small scale) within species, 2) establishing and verifying conservative and meaningful characters for each speciel species complex, and 3) conducting life cycle studies to verify cyst morphological characters and possible polymorphism within a population. We need to know all life cycle stages in order to map distribution of mobile vegetative cells and bottom cysts, or hypnozydotes The scientific community working with plankton blocm species needs to establish identification criteria for all taxonomic ranks from species to order, or even to higher taxa.

## 5.2 GLOBAL RESEARCH NEEDS AND DIRECTION

Precise identification of unicellular organisms causing blocms is a prerequisite for ecological and toxicological studies/measurements, and for adequate warning and preventive measures to minimize effects on aquaculture, fisheries, and public health. The following steps are considered useful in this context.

(a) A list of experts in taxonomy, by country, should be compiled, based on expertise relevant to the different taxa. Experts should be invited to form working groups in order to reach common agreement on

synonymies and homonymies and to advise on content and format, and contribute to an updated identification manual.

(b) Based upon existing identification sheets and manuals compiled for use in monitoring programmes (e.g., Japan) and taxonomic courses (a follow-up of SCOR WG 33 recommendations), these should be revised for publication and expanded by guidelines on sampling and culturing. These publications could be used in traing workshops (3 weeks) in regions requiring instruction and guidance, and taught by invited instructors.

(c) Culture banks of living mobile cells and cysts should be established for specific outbreaks/events so that they will serve as archive material for future taxonomic studies. These banks can be established at individual laboratories or regional centres. The establishment of major regional centres that have analytical capabilities would accelerate knowledge on biology and toxins. The need for basic equipment, such as inexpensive field microscopes for less than 800 U.S. dollars, is just as important to local laboratories that need equipment for monitoring and docimenting specific blocm events.

(d) Establishment of an International Plankton Bloom Information Centre to handle distribution of identification manuals, colour photographic slides, permanent mount glass slides, preserved field material, lists of references, directories of experts and other informational and educational aids. The network of national experts could be used to screen submitted material prior to availability for distribution.

Plankton bloom species should be studied from a systematic approach concurrent with clarification or morphological criteria, nomenclature and life cycles. This would involve genetic analyses to characterize genomes, immunological analyses for species identification and differentiation and biochemical analyses to characterize other markers such as toxins, pigments sterols, fatty acids, etc.

# 7. <u>TOXICOLOGICAL STUDIES</u>

#### 7.1 INTRODUCTION

The toxicology sub-group considered it relevant to include the following problems and effects in its discussion:

PSP is caused by a variable (according to species and incident) range of related neurotoxins of which the best studied is saxitoxin. These toxins are concentrated by marine bivalves which remain unaffected by them.

Human consumption of the bivalves results in intoxication, with respiratory paralysis and death in the most severe cases. About 11 species from three genera (<u>Gonvaulax</u>, <u>Pvrodinium</u> and <u>Gvmnodinium</u>) are known to produce PSP. PSP producing species are naturally endemic to some areas, notably the Pacific coast of Alaska, Canada and the U.S.A. and strict controls are maintained on bivalve harvesting.

DSP is associated with species from the two genera <u>Dinaphysis</u> and <u>Prorocentrum</u>. This is a recently described phenomenon recorded in Europe, South America and Japan. Toxins are concentrated by marine bivalves and cause diarrhoea in humans consuming them. There is, as yet, little information on the ecology of the causative dinoflagellates.

NSP is normally associated with the dinoflagellate <u>Gvmnodinium</u> <u>breve</u> (= <u>Ptvchodiscus brevis</u>), common along the coast of Florida and in the Gulf of Mexico. NSP includes potent icthyotoxins which cause spectacular fish kills. Though direct human fatalities have not been reported, on-shore winds sometimes carry toxic cells to coastal settlements causing widespread human respiratory distress and skin irritation requiring hospitalization in the worst cases.

A thorough review of the effects on human health which can arise as a consequence of direct or indirect contact with certain types of phytoplankton blocms is contained in a WHO Environmental Health Criteria Document (WHO, 1985).

There are presently several regional groups concerned with adverse effects of plankton blocms (e.g. WESTPAC, ICES) and the experiences from these regions could be useful elsewhere. However; many regions are experiencing problems with toxic blocms for the first time, and need assistance. Programmes are needed in Central and South America, Africa, the Indian Subcontinent, the Mediterranean and Black Seas and the Caribbean. Particular concern at present focusses on spreading by <u>Pvrodinium bahamense</u> in tropical regions.

7.2 MONITORING

In those situations where the identity of a toxic organism is not known, it may be worthwhile to collect plankton samples for toxin analysis. If this is done in different environmental conditions, one can ascertain the variability in toxin production in any particular region.

The Sub-group recommended, that in areas:

- (i) where PSP, DSP or NSP has occurred in the past; or
- (ii) where plankton or cyst surveys suggest a potential problem; or
- (iii) of planned or ongoing mariculture;

countries should select a few key stations to be monitored <u>regularly</u>, generally once per month, but once every two months outside the period when blocms typically occur. Key stations, shown to be susceptible to early increases in toxicity, should be selected from a preliminary-based monitoring study. When toxicity is detected, the frequency of monitoring should be increased to twice per week. This frequency may be relaxed during closure of a fishery due to high toxin levels, but sampling frequency should again be increased during the declining phase of toxicity so that the fishery may be re-opened as soon as possible. The fishery should not be opened, however, until toxin levels in three successive samples fall below quarantine limits. A well co-ordinated approach to sample collection and analysis is necessary, and the number of institutions involved should be kept at a minimum.

At this time the Sub-group did not recommend that ciguatera monitoring programmes be investigated because of the dynamic nature of fish movement, dinoflagellate patchiness and inadequate assay methodologies. The Sub-group, however, did recommend epidemiological surveys to document the magnitude and timing of ciguatera. As soon as an approved assay methodology is available, countries with ciguatera problems should begin monitoring fish at the market level.

Since there has often been a correlation between reef destruction and outbreaks of ciguatera, the Sub-group recommended that large-scale construction or dredging activities in affected countries be followed carefully by monitoring local coral reefs for toxins.

#### 7.3 CHEMICAL TECHNIQUES FOR TOXIN ASSESSMENT

7.3.1 PSP

Mouse bioassays according to the Association of Official Analytical Chemists (AOAC) method, standardized using saxitoxin from U.S. Food and Drug Administration (FDA).

High-performance liquid chromatography (HPLC) or fly bioassay may be suitable, but at the time of writing, these methods do not yet have official ACAC approval.

7.3.2 DSP

In developing countries, the mouse bicassay is recommended, but the Sub-group recognized difficulties in interpretation due to false positive results. Thus a few samples should be sent to an established laboratory for confirmation using HPLC. Advanced laboratories may use HPLC or enzyme immunoassay when approved by ACAC, but at present only the mouse bicassay is officially sanctioned. Details on the method may be obtained from Dr. Yasumoto (Yasumoto et al 1980).

7.3.3 VSP

Mouse bioassay. The method may be obtained from the Japanese Ministry of Health and Welfare or Dr. Yasumoto.

#### 7.3.4 Mass mortalities of fish and other organisms

When mortalities of marine animals have occurred, port-mortem analyses should be conducted on fresh specimens. Fish should also be frozen immediately for subsequent analysis. Intensive plankton sampling and counts should be immediately carried out, along with preservation of sample organisms by freezing, and determinations of oxygen concentration using the Winkler method or a calibrated electrode. Care should be taken to make these measurements in the late part of the night.

#### 7.3.5. <u>Ciguatera</u> toxicity

Mouse bioassay, as recommended by WHO. Standard toxins may be obtained from Dr. Yasumoto.

#### 7.4 RESEARCH NEEDS

The Sub-group identified the following list of problems which need further study:

- (i) Toxigenesis (e.g. intracellular bacteria, toxin variabilities in culture, toxin function);
- (ii) Toxins in fishkilling species;
- (iii) "Field" assay kits;
- (iv) Antibodies;
- (v) DSP assay methodology (alternatives to animal bioassays);
- (vi) PSP assay methodology with HPLC; alternative to mouse bicassays;
- (vii) Ciguatera assays methodology (alternative to mouse bloassay);
- (viii) VSP assay methodology;
  - (ix) Toxin conversion and detoxification in shellfish ;
  - (x) Toxin effects on larvae of marine organisms.

#### 7.5 REQUIREMENTS FOR REGIONAL PROGRAMMES

The Sub-group recommended that a directory of scientists, foctors, public health officials and fisheries managers, familiar with symptoms and treatment of PSP, DSP, NSP, VSP, Ciguatera and fish mortalities be created and distributed.

The Sub-group recommended that fact sheets on symptoms and treatment of marine phycotoxins be prepared and distributed to public health officials in coastal areas.

7.5.1. Training in chemical analysis of toxins

Postgraduate fellowships or exchange programmes between countries should be set up, particularly in toxicology.

Workshops should be conducted, with correct selection of participants (with emphasis on technicians rather than administrators, for example) being extremely important. One week's duration is considered sufficient, and "hands-on" experience with appropriate equipment is essential.

Training visits are also considered to be a vital part of the transfer of expertise, and are preferred to workshops. Specifically, visits of technicians to expert laboratories are useful, but visits of experts to

the countries needing the training is preferred.

Although a programme on red tides and phytoplankton blocms must focus on marine blocms, similar problems do exist in freshwater lakes, and the countries so affected could benefit from the techniques and expertise of the marine scientific community.

In conjunction with training in use and interpretation of the output data, specialized equipment is necessary, and should be supplied to laboratories to developing countries. Examples are HPLC equipment, evaporators, standard laboratory mice, technical literature, etc.

Many countries cannot afford up-to-date information on plankton blooms and their adverse effects. The Sub-group therefore strongly recommended that funds be allocated to purchase books and reference materials to be either lent or given to appropriate laboratories.

Intercalibration of assay methodologies should be conducted and co-ordinated periodically. This is a very important and necessary procedure.

### 7.5.2. Co-ordinated monitoring

The clear correlation between coastal pollution and red tide events and the apparent increase in the frequency and magnitude of shellfish toxicity episodes on a global level, suggests that monitoring of red tides should be co-ordinated with the existing monitoring of marine pollution. On a global level, the International Mussel Watch Programme has been lauched by UNEP and IOC to assess the levels of chlorinated hydrocarbon pesticides in bivalves collected from coastal marine waters around the world. Bivalves are useful indicator organisms due to their worldwide distribution and general ability to bioconcentrate pollutants. An expansion of the existing Mussel Watch Programme to include phycotoxins, which are bioconcentrated by bivalves, could reveal useful information, not only on the global distribution of toxic phytoplankton blooms but, inasmuch, their relationship to marine pollution.

# 8. EXCHANGE OF INFORMATION AND DATA

Due to lack of time this agenda item was only briefly discussed. It was generally agreed, however, that the global nature of the red tide problem and its apparently increasing frequency and geographical spreading, makes information exchange a prime interest for international co-operation in this field. Four initiatives which could advance this situation were identified:

- (i) a directory of institutions and individual experts engaged in research and monitoring of plankton blooms. A format proposal from ICES was presented (shown in Annex V of this report);
- (ii) a questionnaire to the institutions and experts mentioned above on ongoing research and monitoring (e.g. number and location of stations, sampling frequency, measured parameters);
- (iii) a newsletter reporting on outbreaks of red tides and exceptional phytoplankton blooms, their effects of fishery, mariculture and ecosystem function and on ongoing activities, contact points for

further information, etc.; and

(iv) regional data banks receiving submitted data on phytoplankton blooms according to a standardized data-logging format.

#### 9. <u>SUMMARY</u>

The IOC Workshop on International Co-operation in the Study of Red Tides and Ocean Blooms was held at the University of Kagawa, Takamatsu, Japan, on 16-17 November 1987, following an International Scientific Symposium of Red Tides, 10-14 November 1987. Out of the 240 scientists attending the Symposium, 30 participants formed a wide geographical representation and a coverage of different aspects of the problem.

The aim of the Workshop was to:

- (i) review the global occurrences of, and studies of, plankton blooms; and
- (ii) to propose components for a global programme on ocean blooms and red tides.

In the context of the Workshop, phytoplankton blooms are defined is discrete events in space and time, comprising elevated concentrations of inicellular algae, above the baseline concentrations normally recorded in a particular area. The mechanisms of concentration might be either physical in biological, as when reproduction exceeds loss processes (grazing, sedimentation, lysis).

Blocm phenomena call for concern for several reasons. Some blocmforming phytoplankton species produce toxins which can reach human beings through different pathways and eventually cause severe health problems. Some of these phycotoxins also cause massive fish kills, particularly in mariculture systems, leading to substantial economic losses. Beside the directly toxic effects, coastal phytoplankton blocms can affect amenities by discolouration of the water and by production of foam, which accumulates on beaches. The decay of sedimented blooms might increase benthic oxygen consumption above the rate of supply, possible casuing anoxia, which can affect survival of the benthic community including fish stocks. On a larger scale, oceanic blocms might affect global fluxes of chemical elements.

Based on the solicited contributions and on presentations during the Symposium, it was clear that coastal blocms and red tides are worldwide phenomena, showing increasing frequency and geographical extension.

In several areas, this expansion of coastal blocms is evidently linked to increased anthropogenic inputs of nutrients to the marine environment, though other mechanisms are also operative in triggering of blocms. Blocm phenomena have been recorded also in open oceans, but the available data do not yet allow conclusions on trends or causes to be drawn concerning these areas. Although the scientific approaches taken in studies of coastal and oceanic blocms have differed, it appears reasonable to suggest that both are regulated by the same basic mechanism. This is further supported by the observation that species representing all major phylogentic groups of phytoplankton are known to form blocms in coastal seas as well as in the open ocean. On this basis, it seems scientifically sound that a Sub-programme on Blocms and Red Tides may consider the phenomena in general and their potential impacts on the marine environment and man's interest in the oceans and their resources.

The Workshop concluded that international co-operation in the study of blocms needs improvement in the fields of biological oceanography (understanding of mechanisms of blocm dynamics and effects on the ecosystem), taxonomy (identification of potential blocm forming or toxin producing species) and toxicology (direct assessment of toxins in biota).

Within the field of biological oceanography, the main concerns are the lack of data to elucidate the extent of blocm phenomena on a regional basis and the lack of hypotheses to consistently explain the linkage of blocms to hydrographical and ecological conditions and to imputs of nutrients. In order to better understand the basic mechanisms of blocm formation, maintenance and degradation, studies integrating physical, chemical and biological techniques in an ecological and oceanographic framework should be carried out in areas of observed or expected blocms, leading to long-term series of data based on standardized methods. Concurrent studies on natural and exceptional blocm events are required to develop hypotheses to explain the dynamics of blocms, to identify the environmental factors controlling their formation, and eventually to be able to predict their occurrence. In many regions, there is an immediate need for training and equipment to carry out basic measurements. On a large time-scale, remote sensing and budys can be more widely applied to acquire data series <sup>in</sup> space and time, and modelling could find increased use as a communication means between disciplines in data interpretation.

Within the field of taxonomy, the Workshop identified global needs for:

- (i) clarification of nomenclature within a number of specified systematic groups;
- (ii) updating of identification manuals covering bloom-forming algae;
- (iii) a network of taxonomical specialists to assist in specific identification problems; and
- (iv) banks of preserved reference samples and living cultures of blocmforming algae.

Regionally, there is an immediate need for training in sampling, identification and culturing, and for basic equipment. As a simple and inexpensive startpoint, plankton samples should be collected on a regular basis and preserved for later analysis. Much valuable information on blocm dynamics is lost, due to lack of stored samples.

Within the field of toxicology, the actual source of phycotoxins, and the biological conditions inducing their production are topics of future research. The major immediate concern is routine assessment of toxins in marine biota in order to prevent human intoxications. Analytical methods are now available for the measurement of the different types of toxins produced by certain blocm-forming algae. These methods range from relatively simple bio-assays to advanced biochemical analyses. In order to improve a worldwide application of these methods, the Workshop suggested that regional centres be established with the required instrumentation and qualifications, and with access to standard reference materials. As a parallel to the global "Mussel Watch" programme on chlorinated hydrocarbons, the ability of bivalves to bioconcentrate toxins could be utilized in a global monitoring programme of toxic blooms. On a local level, there is a general need for information on symptoms and treatments of poisoning from marine phycotoxins, as well as training in simple methods for controlling concentrations of toxins in fish and shellfish.

Co-operative international research on Ocean Blooms and Red Tides must draw upon expertise from a wide spectrum of disciplines, including oceanography (physical, chemical, biological), remote sensing, ecology, taxonomy, physiology, toxicology and analytical chemistry; such activities would naturally fall as a sub-programme within the Ocean Science Living Resources (OSLR) programme. The apparent increase of coastal bloom occurrences due to eutrophication by antropogenic nutrient inputs to the sea, and the potential impact of blooms on ecosystem functions, implies a natural linkage to GIPME and, in particular, its Group of Experts on Effects of Pollutants (GEEP). Also, the MARPOLMON System of GIPME can contribute to the observational data base required for, such a sub-programme. On the other hand, the toxic effects of some blooms on living resources are a subject of interest to FAO which is already a co-sponsor with IOC of the Guiding Group of Experts on the CSLR. In due course, collaboration with WHO might be sought on human health aspects.

A possible step to initiate implementation of the proposed sub-programme would be the review of the conclusion and recommendations of the Workshop by the IOC/FAO Guiding Group of Experts on OSLR, with a view to:

- advising on on-going regional activities and stimulating new ones; and
- (ii) advising the most appropriate arrangements to deal with the sub-programme, possibly through the establishment of a small specialised joint sub-group of experts, covering the needed disciplines, between the IOC/FAO GGE (OSLR) and GIPME through GEEP.

#### ANNEX I

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- 3. REGIONAL EXPERIENCES
- 4. THE BLOOM CONCEPT

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# ANNEX II

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#### P.S.P. CONDITIONS IN INDONESIAN WATERS

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Knowledge on red tides in Indonesia is still far from complete, and until now, no research programme on red tides has been carried out. There are, however, several reports of PSP in Indonesia, some of which are summarized below.

In November 1983, PSP occurred in Flores strait, East Indonesia waters. More than 200 persons got sick, and 4 persons died after having consumed clupeid fish. This was suspected to be caused by <u>Pyrodinium</u> <u>bahamense</u> var. compressa, which has occurred in South East Asian waters (Philippines, Brunei, Malaysia, Indonesia and Papua New Guinea).

PSP has also occurred around Sulawesi Island, middle part of Indonesia. Fish die seasonally north of Sulawesi Island, and in August 1987 four persons died after having consumed oysters from south of Sulawesi Island.

In July 1986 PSP occurred and fish died in Jakarta Bay along the coast of North Jakarta, probably due to high concentrations of ammonia caused by eutrophication of that area, while <u>Noctiluca</u> was also blooming.

<u>Protogonyaulax cohorticula</u> which is known to be a red tide organism, was found in Aceh North Sumatera, West Indonesia, 1987, and identified by Dr. F. Fukuyo. In March, 1988 some shrimp cultures were damaged in this area.

In January 1988 at Balansiku-Setabu-Bulungan, East Kalimantan, more than 60 persons got sick and 2 persons (a woman and a boy) died after having consumed oysters - hard clam (<u>Meritrix meritrix</u>). The test for trial was done by the staff of the local hospital as follows: an oyster was boiled, then given to a dog, which died after about 5 minutes. The occurrence of the bloom lasted several days. The possible causative organism was <u>Pyrodinium bahamense</u> var compressa.

Red tides and PSP occurrences are still a problem in Indonesia. Delay in transportation from the site of incidence (often remote islands) to the laboratory is a problem which must be solved. This will give us better opportunity to identify most of the red tide occurrences. IOC Workshop Report No. 57 Annex II - page 2

RED TIDES AND TOXIC ALGAL BLOOMS IN THE SOUTH WESTERN ATLANTIC

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Among the organisms responsible of discolorations in coastal and neritic waters of the South Western Atlantic, diatoms, dinoflagellates, <u>Phaecocystis</u>, the cyanophycean <u>Trichodesmium</u> sp. and the ciliate <u>Mesodinium</u> <u>rubrum</u> have been reported (Fig. 1).

It is interesting to point out that most of the mentioned blooms have been produced in frontal systems. Blooms of <u>Alexandrium excavatum</u>, <u>Prorocentrum micans</u> and <u>Phaeocystis</u> have been observed in the frontal system of Peninsula Valdes. Several discolorations and a toxicity outbreak have been reported in the Rio de la Plata frontal system. <u>Mesodinium rubrum</u> red tide has been related to the shelf break front generated by the turbulent flux of nutrient rich waters of the Malvinas current.

The occurrence of toxicity in shellfish in Uruguay was first noted in February 1980 when several people reported illness symptomatic of Paralytic Shellfish Poisoning (PSP).

The first toxic outbreak of PSP was recorded in the Argentine Sea during spring of 1980. The toxic species was identified as <u>Alexandrium</u> <u>excavatum</u>. It is difficult to explain if this species has been recently introduced in the area. However, it seems clear that since the 1980's bloom, the distribution of the species widely spread. Our data indicate that <u>A. excavatum</u> is at present a common, minor component of the spring bloom observed in most of the neritic Argentine ecosystem, associated to the subantarctic shelf waters.

Unispecies blooms of this species have been observed only in frontal systems. However, this condition seems to be necessary but not sufficient. Although the determining factors of blooms are difficult to explain, their consequences cause serious problems in this region (Fig. 2). Besides the species above mentioned, <u>Gymnodinium catenatum</u>, PSP producer, and <u>Dinophysis fortii</u> and <u>Dinophysis acuminata</u>, DSP producers, have also been found in the Argentine Sea.

The problem of red tides and toxic algal blooms in Argentina and Uruguay are really serious and deserve to be studied in detail in an international framework programme of training, communication and collaborative research.



Fig. 1. - Red Tides and toxic algal blooms reported in the South Western Atlantic.

# RED TIDE EXPERIENCES IN THE PHILIPPINES

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Probably the earliest record of a "red tide" in the Philippines has been that of a reddish rusty discoloration and bioluminiscence in Manila Bay in 1908. Similar phenomena have been noted in the Tawi-tawi area in 1976 and in Maribago, Cebu in 1983. Human fatalities, widespread report of PSP cases and a loss of about 10 M from a government-initiated mussel mariculture project from a bloom of Pyrodinium bahamense var compressa in Maqueda Bay and adjacent waters in Samar in 1983 alarmed the government. A monitoring programme has been initiated, data from which have shown seasonal and spatial occurrence of the species in the area from 1983 to 1987. Another bloom of this organism occurred in June 1987, this time outside of Maqueda Bay and in Zambales area. From May 26 to August 7, 1987, there were 211 reported PSP cases, of which 6 were fatal. Periodic blooms of the species have also been recorded in the Indo-Pacific region. Aside from routine monitoring of the physico-chemical parameters in the affected areas, most of the work so far made is mainly the identification and determination of the concentrations of the suspected and associated organisms. Toxicological work and testing of local antidotes have been done where verifications or further works are needed. There has been no attempt to undertake more in-depth study on the local representatives of P. bahamense var compressa.

As in the case of other less developed countries, the following are the needs/problems to be addressed in order to improve the understanding of red tides and to act accordingly before, during and after red tides:

- 1) lack of technical expertise and equipment;
- lack of reference materials for the identification and other studies on the causative organisms;
- lack of rapid warning system and public awareness campaign; and
- lack of multidisciplinary research approaches on the phenomenon.

At regional and/or national levels, the following could be done:

 development/training of a pool of local expertise to undertake research and supervise/train local manpower (technical assistants, fishermen, etc.) who could be utilized for regular monitoring, surveillance and other related works;

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- implementation of long-term collaborative multidisciplinary research on the biology, economy and toxicity of the problematic species <u>P, bahamense var compressa;</u>
- development of manuals for identification and culturing of causative organisms;
- 4) development of rapid warning systems as in the one found useful in the management of "Nori" culture in Japan, and
- 5) public information campaign to minimize if not prevent negative impact on human lives during outbreaks of red tides.

#### OCCURRENCE OF RED TIDES IN THE SEAS AROUND INDIA

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As regards the status of occurrence of red tides in the waters India, Trichodesmium erythraeum is the most important red tide around forming organism. Practically every year massive red tides occur especially in the Arabian Sea during the premonsoon season (February - May). High concentrations of inorganic PO4-P, NH4-N and NO3-N have been observed associated with these red tides. Other phytoplankton organisms were abundant and some diatoms appeared in profusion in association with the red Chlorophyll a and 14C uptake values were considerably high during tides. The ability of Trichodesmium to fix atmospheric nitrogen and the period. subsequently to release inorganic nutrients during their decay enriches the environment and leads to proliferation and succession of other planktonic organisms. Future plans of work are:

- evaluation of N regimes which might initiate <u>Trichodesmium</u> red tides;
- nitrogen fixation rate by red tides and computation of the import of this N into the marine ecosystem;
- decay of <u>Trichodesmium</u> and the rate of release of different N compounds like DON, NH4, etc;
- 4) utilization of the released N by other plankton and succession.

Recently we have observed outbreaks of toxic red tides due to dinoflagellates causing PSP and DSP problems and human death. During 1984 a green tide caused by <u>Pedinomonas noctilucae</u> (Subr.) occurred in Sweeney harbouring. <u>Noctiluca Scintillans</u> was responsible for severe setback to fisheries on the west coast of India.

Important steps envisaged for future studies are:

- regular monitoring of regions which are proned to red tide outbreaks;
- 2) more stress to studies of micronutrients and Vitamin B12;
- 3) more concentration on cyst studies;
- application of remote sensing techniques for buoyant red tide forming organisms.

#### TOXIN ANALYSIS - TOXICITY IN DINOFLAGELLATES AND RESULTING BIVALVES TOXICITY

#### S. Franca

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The first report on toxicity in bivalves along the Portuguese coast related to a <u>Gonyaulax tamarensis</u> bloom was made by Silva (1962) whose contributions to the study of dinoflagellates led to a better knowledge on cultures and on characterization of several important details on life cycle, ecology, morphology, ultrastructure and association with endocellular bacteria and toxicity of <u>Dinophyceae</u>.

The scientific understanding of the problem related to the dinoflagellates, namely their toxicity and resulting bivalves toxicity was the scientific basis for the management programme now established.

Since April 1986, and in order to satisfy the impositions for commercial export, the work on toxin analysis in molluscs bivalves has been developed within a design leading to a surveillance on harvesting areas for PSP and DSP toxins. The prime objectives are related to <u>public health</u> and to <u>coastal economy</u>. Since the programme was initiated, toxicity has been found.

The control of the sanitary conditions of the bivalves from the Portuguese coast in relation to dinoflagellate toxins has been accomplished and the close integration with researchers interested in the study of dinoflagellate toxicity led to the collecting of data and to a description of the relationship between toxicity and causative organisms. Also it has been possible to preserve both organisms (isolated from natural populations and maintained for cytological and toxicological studies) and the toxic shellfish extracts for further research studies.

PSP and DSP toxins were monitored by mouse bioassays. Until now no attempts were made to purify the crude extracts. The extraction of PSP toxins was made according to the AOAC (Association of Official Analytical Chemists) method (1985). The toxin level is expressed in ug of saxitoxin equivalents by 100 g of shellfish meat. Table 1 summarizes our knowledge on the actual occurrence of PSP toxin along the Portuguese coast from April 1986 to October 1987.

The extraction of DSP toxins was made according to Yasumoto (1980). Table 2 briefly presents the preliminary results obtained in bivalves along the Portuguese coast from May 1987 to October 1987.

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#### <u>Table 1</u>

Occurrence of PSP toxins in the bivalves of the Portuguese coast

Results on PSP analysis and brief charact. of mouse bioassay		No.	۶	causative organism	
PSP	present	>80 ug - like STX stand sol.	64	8.1	<u>G. catenatum</u>
PSP	n	<80 ug - " " " "	50	6.4	**
PSP	"	survivors, recovered from mild STX sympt.	86	11.0	"
PSP	absent		49	6.2	
PSP	?	death time <60 min., unlike STX	467	59.7	unknown *
PSP	?	" " (60min24h), " "	65	8.3	**

Total = 781 shellfish-samples analyzed

\* - These results are commonly found in analysis of oysters. The causative organism was not identified. These toxic extracts need a detailed chemical study and a post-mortem examination of mice may be necessary. Mice undergo a hypoactive behaviour after the i.p. injection of 1 ml extract.

## <u>Table 2</u>

Preliminary results on the occurrence of DSP in bivalves of Port. coast

			No.	causative organism
DSP	present, death time	< <30 min, severe symptoms	23	<u>D. saculus, D.acuta</u> *
DSP	absent, no death 24	hours	21	

\* - (pers. comm. M.A. Sampaio);

It is obvious that there are important gaps in our knowledge.

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Besides the need for maintaining our research programme in course on "Dinoflagellates Toxicity" and the "Study of the Relationships Between Dinoflagellate and Bacteria", we think that the findings in the surveillance programme suggest even more questions.

The most important research topics in this area are:

- culturing, which is essential to the development of studies leading to the detailed characterization of organisms and for production of toxins;
- ii) characterization of structure, ecology, nutrition, and growth rates under experimental conditions, periodical events of lifecycle, biosynthesis of toxin production, relationship between dinoflagellates and endocellular bacteria;
- iii) production of cell extracts and shellfish extracts;
- iv) purification and chemical and biological characterization of toxins; and
- v) comparative studies on toxins obtained from different origins: shellfish, dinoflagellates (field collection and/or culture collection), bacteria (isolated from dinoflagellate cells).
## TAXONOMY OF RED TIDE PHYTOPLANKTON

#### Tasuwo Fukuyo

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More than 100 species belonging to eight taxonomical classes can be listed as red tide causing and/or toxic organisms. Among them 29 species of <u>Dinophyceae</u>, 5 of <u>Raphidophyceae</u> and 2 of <u>Haptophyceae</u> listed below are known to be hazardous.

Dinophyceae: Prorocentrum lima, P. concavum, P. mexicanum, Dinophysis acuminata, D. acuta, D. fortii, D. mitra, D. norvegica, D. tripos, Amphidinium carterae, A. klebsii, Cochlodinium polykrikoides, Gymnodinium breve, G. catenatum, G. nagasakiense, G. veneficum, Gyrodinium aureolum, Gonyaulax polyedra, Pyrodinium bahamense f. compressum, Alexandrium monilatum, Protogonyaulax acatenella, P. catenella, P. cohorticula, P. fratercula, P. tamarensis, Ostreopsis ovata, O. siamensis, Coolia monotis, Gambierdiscus toxicus, Peridinium polonicum.

<u>Raphidophyceae</u>: <u>Chattonella antigua</u>, <u>C. marina</u>, <u>C. subsalsa</u>, <u>Fibrocapsa japonica</u>, <u>Heterosigma akashiwo</u>.

<u>Haptophyceae</u>: <u>Phaeocystis pouchetii</u>, <u>Prymnesium parvum</u>

As taxonomical criteria of some genera and species vary among taxonomists, alternative species names are proposed and recognized as synonyms. For example, Dr. Balech uses genus name <u>Alexandrium</u> instead of <u>Protogonyaulax</u>. Consequently <u>A. acatenellum</u> is used as a senior synonym of <u>Protogonyaulax acatenella</u>, although their diagnoses vary from each other because of difference of species criteria. <u>Gymnodinium breve</u> is another example. Dr. Steidinger uses <u>Ptychodiscus brevis</u>, because she found that the morphological characteristics of the species were enough for separation of the species from the genus <u>Gymnodinium</u>.

The case of <u>Gymnodinium nagasakiense</u> and <u>Gyrodinium aureolum</u> is a little more complicated. The latter species appearing in European waters has different characteristics from the original description of specimens from Woods Hole, Atlantic coast of North America, but the same ones as the former species occurring in Japanese coastal waters. At the same time Dr. Tangen considers the former is conspecific with <u>Gymnodinium mikimotoi</u>. Therefore, if we follow his opinion, European <u>G. aureolum</u> and Japanese <u>G.</u> <u>nagasakiense</u> are both junior synonym of <u>Gym. mikimotoi</u>, and we must use <u>Gym.</u> <u>mikimotoi</u>.

Same species names have been adopted to different but very similar

morphotypes. <u>Dinophysis acuminata</u> in European waters is probably a mixture of <u>D. acuminata</u> and <u>D. sacculus</u>. Dr. Steidinger reported two Japanese <u>Gymnodinium breve</u> clones differing from each other, and moreover differing from <u>Ptychodiscus brevis</u>. It means that there is no Florida type <u>G. breve</u> in Japan. In <u>Raphidophyceae</u> <u>Olisthodiscus luteus</u> is often used instead of <u>Heterosigma akashiwo</u>.

These taxonomical disagreements impede a smooth and proper development of biological, ecological and toxicological studies of red tide and/or toxic species. However, it is very difficult to get complete agreement of position and criteria (definition) of all species among all taxonomists within a few years.

Therefore, any practical standard authorized among taxonomists for identification is very important. For practical use guide books introducing techniques for plankton collection and observation and taxonomical system is helpful. A guide book containing description of species, i.e. morphology, life history, distribution, synonyms, must be prepared. For supplemental use a model showing a profile of species and photo-slides are useful.

Regular training workshops to create experts in identification and collections of plankton cultures and reference publications should also be considered.

## TOXIC DINOFLAGELLATE BLOOMS IN AUSTRALIAN WATERS

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On a global scale, both the number and intensity of toxic plankton blooms seem to be on the rise and their geographic extent seems to be spreading. Toxic dinoflagellates that can cause paralytic shellfish poisoning (PSP) in humans are well-known from temperate waters of Europe, North America and Japan, but until recently no PSP problems were known from Australian waters.

The toxic dinoflagellate Gymnodinium catenatum was first identified from Southern Tasmanian waters in April 1985. Its production of paralytic shellfish toxins was confirmed by performing mouse bioassys on natural plankton samples, and appropriate government officials were then notified of potential public health risks. A subsequent screening of historic plankton samples indicated that this dinoflagellate had been present in Tasmanian waters at least since 1980, and a screening of local hospital records revealed that 2 unambiguous human PSP poisonings had occurred previously in this region in 1980. Until then only a very limited number of PSP tests on commercial shellfish had been carried out and these had all proved negative. In February 1986, 2 further human poisonings were reported from Southern Tasmania from an area not previously included in the monitoring programme. Unacceptably high toxin concentrations (up to 8000 mg /100 g shellfish meat) were detected in commercial mussels, oysters and scallops from the area. In March, 1986, up to 15 shellfish farms thus had to be closed for periods of up to 6 months. Renewed shellfish farms closures (only 5 farms affected) had to be imposed in March 1987. By then, a smooth running monitoring programme was in operation to test for dinoflagellates in net samples and, whenever these are present, to test for toxins in shellfish (both mouse bioassay and HPLC assay). This monitoring programme has met the approval of the United States Food and Drug Administration and a Memorandum of Understanding has been signed which allows Tasmania to export its shellfish products to the American market.

# SAMPLING METHOD FOR NAKED FLAGELLATE RED TIDE STUDIES

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In situ sampling for studies of naked-flagellate red tides involve several problems.

- i) The naked flagellates have feeble cell membranes, making fixation impossible. Therefore, laboratory work must be done shortly after sampling.
- Cell division often takes place during night time, thus cell counting must be completed on the sampling day by early nighttime.
- iii) Diurnal vertical migration can concentrate the population at the surface at mid-daytime. In order to accurately measure population density, it is necessary that an integrated water sampling method is used no matter when and where sampling may be done.
  - iv) Uneven horizontal distribution (patchiness) requires several samples to be taken.

Accordingly, it is desirable to carry out integrated watersamplings at as many points as possible in the field and to pool the samples for laboratory work.

We employed a "boring" method to actually carry out red tide sampling in the field and to study the growth process of the surface population (0.5 m) of <u>Gymnodinium nagasakiense</u> in Omura Bay. In order to estimate correctly the variable cell densities in terms of time and space, we collected integrated water samples at as many points as possible with a silicon hose, which is 15 m long and 18 mm and 25 mm in inner and outer diameters, with two simple devices for separating a 15 m water column into three layers: 0.5 m, 5.10 m and 10.15 m. A total of 36 integrated samples were collected at 12 points distributed in a studied area of 6 square miles. All water samples were gathered in three large vessels by layer. Then, a part of the vessel water was brought back to the laboratory in only three sample bottles and the remaining water of the vessel was allocated to <u>in</u> <u>situ</u> enclosure experiments for determining the daily growth rate of each layer population.

# NUMERICAL EXPERIMENTS ON OUTBREAKS OF RED TIDES

## Saburo Ikeda

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A research programme on "Integrated Models of Red Tide Phytoplankton Blooms in Eutrophicated Coastal Waters" is being carried out with T. Okaichi as principal investigator. The components of the research programme are summarized below. The basic processes involved in the modelling of red tides are shown in Fig. 1. Different steps towards the development of an integrated simulation model of red tide outbreakes are listed below.

## Meteorological Conditions

# Oceanographic Structure (currents, front, mixing, temperature layers, etc.)

# <u>High Growth</u>

# **Agglomeration**

# <u>Dissipation</u>

- physical

<u>Cysts</u> Formation of optimum condition  Nutrients (metals, chemicals)
de-inhibitory materials
predation

physical factors (wind, upwelling (thermocline) ory biological factors (vertical migration competition, etc)

- biological (?)

(?)

## Human Activities

- nutrients loading (urban runoff, etc)

- fishery & culture

- marine development (traffic, reclamation)

<u>Fig. 1</u>

- (i) One dimensional models of basic processes (vertical profile).
  - A germination model of cysts.
  - A growth model of phytoplankton with prey-predator and competition relationship. (Michaellis-Menten, cell quota, nutrient ratio (N/P), light inhibition, grazing ratio and size effects, etc.)
  - A growth model with vertical migration of "red tide" plankton.
  - A decay (dissipation) model.
- (ii) Two dimensional models of basic processes (horizontal profile).
  - A growth model under upwelling or supply of nutrients from bottom water.
  - A growth model of the multi-layer temperature zone (thermocline).
  - A growth model of wind-driven currents.
- (iii) Three dimensional models of integrated hydraulic and biological processes.
  - A model of two layer current field.
  - A model of three layer current field.
  - Spatial dimension (mesh size, boundary conditions, etc.).
  - Time dimension (days, weeks, months, etc.).
  - Data on nutrient loading.
  - Stability of numerical computation scheme.
  - Introduction of spatial temperature profile and other physical oceanographical elements.
  - (iv) Policy-oriented models of "forecasting" or "controlling" outbreaks of red-tides.
    - A real-time forecasting model of red tides (peak time, spatial distribution: scale and location of plankton patch).
    - A combination of numerical simulation models with statistical forecasting or other heuristic methods.
    - An integration into coastal resource management models such as fishery, farming, and recreational use (monitoring, detection, prevention activities).

## IMPORTANCE OF BACTERIA IN RED TIDES

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In natural environments there are various kinds of biological processes involved in intimate association and transfer of substances between algae and bacteria. Bacteria often affect the growth and phototaxis of red tide algae. I have taken up the typical cases in which the bacterial cells of bacterial metabolites are essentially utilized by red tide algae.

- (i) Freshwater Chrysophyceae <u>Uroglena american</u>, which usually forms a freshwater red tide in lake Biwa, is unable to grow in the absence of bacteria, and able to grow by engulfing common bacterial cells in the presence of light (photophagotrophy). "The bacterial factor" is phospholipids such as phosphatidyl-ethanolamine which is the normal component of bacterial cell membrane (B. Kimura & Y. Ishida, 1985).
- (ii) <u>Asterionella glacialis</u>, a red tide algae in Maizuru Bay, is unable to grow in axenic culture, but can be grown satisfactorily when some particular bacteria such as Pseudomonas sp.022 are present. Some <u>Vibrio</u> are not effective. The amino acids fraction in the culture filtrate of <u>Pseudomonas</u> sp.022 seems to be essential for the growth of <u>A</u>. <u>glacialis</u> (C. Requelme & Y. Ishida, in preparation).
- (iii) In the case of <u>Skeletonema costatum</u>, a red tide algae in Dokai Bay, the axenic culture loses the ability to grow after transferring 10-30 times in PES medium, though the unaxenic culture constantly grows. The cause is not yet apparent. (M. Yamada, personal communication).
  - (iv) In <u>Chattonella antiqua</u> cultivated in ASP7 medium, the accompanying bacteria strongly stimulated algal growth and phototaxis (Yoshida & Kawaguchi, 1983).

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# A NEW APPROACH TO THE IDENTIFICATION OF GENUS CHATTONELLA - METHODOLOGY FOR TAXONOMY

# Yuzaburo Ishida

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<u>Chattonella antiqua</u> and <u>C. marina</u> are the major organisms causing red tides in the coastal Sea of Japan. As the identification of these species based on morphological characteristics has been ambiguous, some exact criteria for their identification have been desired, which would avoid the necessity of relying solely on their sizes and shapes for identification. A more reliable criterion such as monoclonal antibody is necessary to identify them in routine works.

We propose a new criterion for the identification of the interspecies and intra-species of genus <u>Chattonella</u> by using monoclonal antibodies. Four kinds of monoclonal antibodies were prepared from 2 strains of <u>C. marina</u> and of <u>C. antiqua</u> at each. Reactivities of those monoclonal antibodies to 15 strains of genus <u>Chatonella</u> isolated at different dates and from different areas were examined by indirect fluorescence assay.

The results indicate that two of the prepared antibodies (AT-86 and MR-18) distinguish between <u>C</u>. <u>antiqua</u> and <u>C</u>. <u>marina</u>, respectively. The third antibody (MR-21) was reactive with all strains of <u>C</u>. <u>marina</u>, and a part of <u>C</u>. <u>antiqua</u>, and the forth antibody (AT-83) was reactive with all strains of <u>C</u>. <u>antiqua</u> and a part of <u>C</u>. <u>marina</u>.

By the reactivities of these four monoclonal antibodies it became possible to classify the strains of each species into two respective groups. In the near future we will confirm these results by the isozyme method.

# RED TIDES IN THE MEDITERRANEAN AND BLACK SEA

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After a brief and incomplete review of the literature, five geographical areas have been identified in which mortalities of wild fish have occurred. In other areas, extensive bloom-associated mortalities of invertebrates also occur. Twenty-two species of phytoplankton have been recorded as dominant or subdominant in blooms, not counting bacteria or unidentified organisms.

Most blooms fall into two categories: firstly, phytoplankton blooms near the mouths of rivers, frequently associated with hydrographic fronts; and secondly, blooms in lagoons or harbours, which start by being dominated by phytoplankton, but in some cases progress to the elimination of all life except for sulphur bacteria.

In many areas, both off river mouths and in lagoons, blooms have been reported to increase in frequency, intensity and duration in relation to increased nutrient input. Off the Nile delta, however, decreased nutrient input since 1960 due to the construction of the Aswan Dam which has led to the disappearance of blooms and to a decline in fisheries.

In the Mediterranean/Black Sea region the number of bordering countries, twenty-one, and the number of languages, at least twelve, used in the literature on phytoplankton blooms, hamper this field of research. Yet aquaculture, at present embryonic or still inexistant in many countries, is now being energetically promoted by national, regional and international bodies. For rational planning, red tides and the threat they pose to Mediterranean and Black Sea aquaculture must be understood. A comprehensive review of lagoonal, coastal and offshore red tides in this region, published in open literature and updated every ten years or so, is much needed.

# RED TIDES AND TOXIC PHYTOPLANKTON ON THE NORTH AND WEST COASTS OF FRANCE

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At least twenty-seven species of phytoplankton have been recorded as dominant or codominant in red tides around the north and west coasts of France. Areas most affected include: certain estuaries; semi-enclosed lagoonal areas; port areas; and fronts between water masses over the continental shelf. In the latter situation only red tides of <u>Noctiluca</u> <u>scintillans</u>, <u>Gyrodinium c.f. aureolum</u> and more rarely <u>Emiliana huxleyi</u> are encountered. Apart from exceptions given below, most of the blooms are without apparent ill effect, and most of the bloom-dominating species are not known to be toxic.

In a single red tide, in June 1979, in the port of Le Havre, a known producer of Paralytic Shellfish Poison (PSP), <u>Protogonyaulax catenella</u> was recorded as co-dominant, but apart from this incident, no PSP producing species has been recorded in high number. No human PSP intoxication has been reported from these coasts either, but since the finding of Diarrheic Shellfish Poisoning (DSP) on the Dutch coast, a surveillance network for both PSP and DSP has been in operation since 1981.

In August 1982 at Antifer (about 20 km north of Le Havre on the English Channel coast), the entire stock of fish in a fishfarm died after the occurrence of a red tide reported to be dominated by <u>Exuviaella sp</u>. and an unidentified dinoflagellate. The Association of Official Analytical Chemists' (AOAC) test, carried out on muscle tissue of sublethally affected fish, proved positive for PSP.

As well as routine monitoring for DSP in molluscs using the suckling mouse test, coastal waters are monitored for the presence of DSP-producing species such as <u>Dinophysis</u>. Concentrations of <u>Dinophysis spp</u>. in excess of 100,000/litre sometimes occur , and occasional closure of certain mollusc fisheries has been necessary when permitted levels of DSP toxin were exceeded.

This report has been summarized principally from information very kindly supplied by Dr. P. Lassus.

# PHYTOPLANKTON BLOOMS AND RED TIDES IN THE FAR EAST COASTAL WATERS OF THE USSR

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Outbursts of phytoplankton growth attaining the strength of "blooms" and red tides have been repeatedly observed in the coastal waters of the far-eastern seas and adjacent water areas. Long-term year-round observations of phytoplankton in bays and inlets in the north-western part of the Sea of Japan were started in 1969, (refs. 2, 3, 4, 6, 9) and short-term surveys have been conducted in the coastal waters of the Bering and Okhotsk Seas and along the Pacific coast of Kamchatka (ref. 5).

Based on long- and short-term studies of phytoplankton in the fareastern seas and adjacent waters of the Pacific Ocean, two types of water blooms have been established: regular seasonal outbursts of phytoplankton which repeat annually with different intensities, and occasional "red tides".

Winter diatom blooms have occurred under ice cover at subzero temperatures of water in the north-western part of the Sea of Japan. An algal bloom occurred yearly, for one month between December and February, in highly productive inlets of Peter the Great Bay, in its freezing part, at water temperatures of -0.2 to  $-1.9^{\circ}$ C and illumination of 2 klx, under a 30 to 60 cm-thick ice cover.

Biomass of microphytic algae was in the range of 10 to 20 g m-3. An intensive growth of diatoms was associated with strong convective mixing of the water column supplying nutrients from bottom layers. The winter bloom can be explained not only by high concentrations of nutrients (ref. 9) but also by a low requirement of illumination by the diatom species and their tolerance to subzero temperatures. The boreoarctic diatom <u>Thalassiosira nordenskioeldii</u>, accounting sometimes for as much as 40 to 80 per cent of phytoplankton biomass, and <u>Chaetoceros debilis</u> and <u>Ch</u>. pseudocrinitus should be noted among the dominant species of the bloom. Th. nordenskioeldii, the species highly adapted to low temperature and illumination, has no real competitor under the ice cover. Therefore, the species can proliferate in the nutrient-rich coastal waters in winter and early spring. In addition, reduced rate of decomposition and depressed grazing by consumers at low temperatures contribute to accumulation of phytoplankton biomass.

An autumn bloom in eutrophic bays of the western part of the Sea of Japan, with microalgal biomass (8 to 19  $gm^{-3}$ ) at the winter bloom level, occurs between mid-September and mid-November. The bloom is favoured by strong autumn winds, and is fed by an upward flow of nutrients and bacterial

mineralization of summer detritus. These autumn and winter-spring blooms result from massive growth of the diatoms <u>Sceletonema costatum</u>, <u>Eucampia</u> <u>zoodiacus</u>, <u>Ch</u>. <u>compressus</u>, etc.

A summer bloom lasting about a month between June and August is observed when the water column is stable and organic matter decomposition and nutrient regeneration are active at high water temperatures. The bloom occurs at coastal upwellings generated by offshore winds and is supported by increased allochtonous nutrient influx in the season of summer rains. Numerous flagellated algae predominate. The densest populations are produced by the smaller dinoflagellates <u>Katodinium rotundatum</u>, <u>Prorocentrum triestinum</u>, <u>P. micans</u> and others, by the cryptomonad genus <u>Plagioselmis</u> and the euglenoid <u>Eutreptia lanowii</u>. In summer, as well as in autumn, the obligatory eutrophic species <u>S. costatum</u> and the boreotropical <u>Ch. affinis</u> predominate among the diatoms.

Since the early 1980s, red tides associated with dinoflagellates <u>Noctiluca miliaris</u>, <u>Alexandrium (=Protogonyaulax)</u> <u>tamarensis</u> etc. and the infusorium <u>Mesodinium rubrum</u> blooms have become more frequent. During 1980-1987, more than ten cases of red tides of different intensities were recorded in the areas of the study, three of them were toxic and resulted in infection of molluscs and in mortality of birds. In April-May 1980 and 1982, strong red tides produced by <u>N. miliaris</u> were observed in inlets of Peter the Great Bay, population density reaching 0.5-0.7 x 10<sup>6</sup> cells per litre. Along with nutrient abundance and high rates of reproduction in the absence of grazing, the factor of primary importance was a delay in transportation of the population from inshore shallows into open waters of the bay because of weakening of offshore winds and a change in their parts (ref. 7).

Five cases of "red blooms" associated with the infusorium M. rubrum have been recorded since 1983. Two blooms occurred in hypereutrophic Avachinskaya Guba Inlet (Kamchatka Peninsular) in September-October 1983 at water temperature of  $9-11^{\circ}$ C and salinity of 2.7% in the surface layer (ref. 5) and in the late November-early December 1984 at water temperature about The second bloom was short-termed and less strong (0.2-0.3 x  $10^6$ zero. cells/l) as compared with the first one (2 x  $10^6$  cells/l); it was obviously due to decreased reproduction rate at low water temperature. A weaker red tide associated with M. rubrum was first observed in the Sea of Japan (Vostok Bay) in August 1985 at water temperature of 21-22°C. It occurred after significant desalination of the surface sea water in the zone of the greatest overland runoff and anthropogenic impact (Kononovalova, Selina, Population density in the zone of the bloom was rather high, 1.8 x 1986).  $10^6$  cells/1. Onshore south winds maintained stability of water column and contributed to the development of the bloom. Distinct "red blooms" caused by <u>M. rubrum</u> were repeatedly observed in August-September, at 8-12°C, in the highly productive Kraternaya Bay (Ushishir Island, the Kuriles), eutrophicated through natural factors. None of these red tides caused any obvious unfavourable effects.

In July-early August 1984 and 1987, however, brown colouring of the water was observed in Avachinskaya Guba Inlet (Kamchatka) at surface water layer temperature of  $14-15^{\circ}$ C and with increasing toxicity of mussels.

An analysis showed an abundance of dinoflagellates of the genus Alexandrium (=Protogonyaulax), "tamarensis" group, <u>A. tamarensis</u> and <u>A. acatenella</u> with population density up to 0.6-0.7 x  $10^6$  cells/1. In July-August 1984, the dominant species was <u>S. costatum</u> (8 to 16 x  $10^6$  cells/1), proliferation of which stimulates growth of most flagellates associated with red tides (ref. 8). This bloom was followed in November-December by a red tide caused by <u>M. rubrum</u> A brown-red water bloom was also observed in July 1986 near the north-eastern coast of Kamchatka (the Bering Sea), at water temperature of  $12 \cdot 14^{\circ}$ C; it caused mortality of marine animals. Here also, water sampling showed high abundance (up to  $1 \cdot 2 \times 10^{6}$  cells/1) of <u>A. tamarensis</u> and <u>A. exavata</u> and extremely poor composition of microplankton.

Considering the blooms described above, both seasonal and cyclic in character, and "occasional" strongly pronounced blooms of the red tide type, one can easily note that all of them occurred in eutrophic waters rich in biogenic elements. Nobody doubts today that the frequency and advance of blooms are directly related to an anthropogenic impact on neretic zones of the sea. It is established that optimal development of a species requires a specific complex or a combination of both biological and physical environmental factors, and the complex seems to develop more readily in nutrient-rich waters.

The author thanks Dr. A.V. Zhirmunsky, the Director of the Institute of Marine Biology for his initiative and inspiration in these investigations.

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#### PHYTOPLANKTON BLOOMS IN KENYA

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Our concern in Kenya today is the persistence of anoxia and cyanobacterial blooms in Lake Victoria since December 1983. These occurrences have now turned into an annual event with losses estimated at over 10,000 tons per year. While the Kenya coast experiences the effects of monsoonal phenomena, no substantial red tide events have been observed. The only reported fish kill along our coast was in 1965 and the annual anoxia in scattered areas seem to be related to the Monsoon.

As phytoplankton blooms are often predictable features of the aquatic habitats, we hope that this workshop will come up with a set of hydrobiological changes that lead to red tides and such related toxicity that can easily be sued by managers worldwide to recognize bloom conditions. Recognition of one or more of the acceptable criteria in this meeting could be sued to qualify or identify bloom conditions in specific regional aquatic habitats where red tide technology is not readily available.

Further, upon examining combinations of environmental conditions most likely to enhance bloom formations in both fresh water and marine environments, a set of commonalities exist. We hope that from the meeting, a combination of technical and managerial expertise will be used in an interdisciplinary fashion to resolve phytoplankton bloom-related problems to benefit all water resource users.

#### RED TIDE PROBLEMS FOR AQUACULTURE IN JAPAN

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In Japan, aquaculture of oysters and "nori" (sea laver) has taken place since before 1940 and culturing of fish and shrimp was developed after 1960.

The total production from aquaculture in 1983 was  $1,153 \times 10^3$  tons, corresponding to 9.6% of the total fish catch of  $11,967 \times 10^3$  tons. The main cultured fishes are yellowtail and red sea bream, and the production in 1983 was  $156 \times 10^3$  tons and  $25 \times 10^3$ , respectively. The aquaculture production of yellowtail was as much as 4 times that of the natural fishery, and the aquaculture production of red sea bream was 1.8 times. Kuruma shrimp culture produced 1,950 tons in 1983. Shellfish culture (oysters and scallops) is also a big industry, together with nori culture. Besides aquaculture controlled by human management, the release of larvae of marine organisms is promoted by the assistance of the Government. In 1984, 1,776 x  $10^6$  scallop larvae and 293 x  $10^6$  Kuruma shrimp larvae were released.

In relation to the development of aquaculture, red tide problems became unavoidable after 1970. In 1972,  $14 \times 10^6$  yellowtail were killed by red tides of <u>Chattonella antiqua</u> in the eastern part of the Seto Inland Sea from the middle of July to August. PSP and DSP also became a serious problem for scallop and oyster culture, mainly in the northern area of Japan from 1975. The establishment of red tide counter measures was requested and a study group was organized in 1976.

Mass mortalities of yellowtail due to <u>Chattonella ssp</u> occurred in 1970, 1972, 1977, 1978, 1979 and 1983 in the Seto Inland Sea. <u>Gymnodinium</u> <u>nagasakiense</u> also killed fish in 1984 and 1985 (Fig. 1). Minimum lethal cell densities of <u>Chattonella antiqua</u> to yellowtail was estimated at 110 cells/ml at 29°C. These cell densities are lower than that in a visible brown patch which occurs at about 1,000 cells/ml.

The shellfish monitoring and investigation systems of red tides in the Seto Inland Sea are now actively working as shown in Figs. 2 and 3. The systems are being conducted in five different regions around Japan. About 150 scientists, plus 200 administrative staff, are engaged at present.







Fig. 2. - Shellfish poisoning monitoring and investigation system in Seto Inland Sea.



Fig. 3. - Information exchange and red tide investigation systems in Seto Inland Sea.

## RED TIDES IN KOREAN WATERS: OCCURRENCE AND COUNTERMEASURES

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In order to predict mechanisms of red tide occurrence and to reduce their damage to marine living resources, comprehensive red tide research and monitoring have been intensively carried out monthly at about 100 stations in shellfish growing areas and around coastal industrial sites, especially Jinhae Bay and its vicinity, since 1972, by the National Fisheries Research and Development Agency (NFRDA). During the monitoring period, plankton samples for red tide organisms are taken and environmental parameters data such as nutrient salts, dissolved oxygen, chemical oxygen demand, suspended solids, transparency, pH, water temperature and salinity are collected from the key stations mentioned above.

Geographical occurrences of Korean red tides were mostly restricted to Jinhae Bay and its vicinity, south coast, until the 1970s. Since 1980, the red tides have extended to other regions such as Ulsan Bay, Inchon Bay, Gamak Bay, Kwangyang Bay, Jinju Bay, etc. where eutrophication has been heavily developed. In 1981, unprecedented huge red tides by monospecific type <u>Gymnodinium nagasakiense</u> occurred in and around Jinhae Bay from July to September and caused mass mortalities of cultured shellfish such as oyster, mussel and arkshell worth approximately US\$ 2,500,000 and other local fishery resources. However, red tide phenomena in and around Masan Bay including Haengam Bay, representing the largest red tide, show long, persistent red tides every year, from April to October, with concentrations over  $10^4$  cells/ml, due to the increasing eutrophication derived from the human induced loading of organic pollutants.

Red tide monitoring activities such as frequent routine observations by research vessels and a remote sensing system would be desirable, however, it is essential to predict the outbreaks of red tides as early as possible, in order to reduce damage to fisheries. The countermeasures against fishery damage due to red tides could be as follows:

- (i) prevention of eutrophication by strengthening of regulatory measures such as specially strict effluent standards and improvement of sewage systems;
- (ii) control of mariculture facilities in appropriate scale;
- (iii) prediction of occurrence and expected duration of red tides and estimated effect to the biota owing to the tides;

- (iv) refuge of culture cages and facilities which are quite simple to move like seed collector of oyster: movement, sinking and covering;
- (v) elimination of red tide organisms: mechanical or chemical treatment;
- (vi) early collection of culture production;
- (vii) intensive monitoring system of red tides;
- (viii) investigation of growth promoting factors of the red tides organisms and their chemical/biological assay;
  - (ix) taxonomy of toxic plankton including cysts involved in the red tides;
    - (x) toxicological study of red tide organisms and assay of PSP on shellfish; and
  - (xi) international red tide newsletter issue.

Monitoring activities for red tides in Korea have now been covered, in serious red tide areas, 5 times a month by research vessels of NFRDA and once a month by helicopter. During the period of June and September the monitoring has been strengthened by a network made up of extension service workers of NFRDA who have been stationed at important fishery villages to make checks every three days (when heavy red tides occur, a check is made every day), take samples of tide organisms and analyse water quality. Aquaculturers have been co-operating with them in cases of heavy red tides.

An information exchange system for red tide prediction is extremely important and should be urgently promoted. A report system for new information concerning red tide outbreaks, such as sea conditions and culture organisms from culture people and members of fisheries cooperative unions, would be more accurately and quickly required for comprehensive analysis with field survey data, because the prediction programme must mainly be attributed to the kind of system mentioned above.

NFRDA usually provide necessary data for prediction and issue the prediction of a red tide outbreak to fishermen by means of radio, TV, newspaper and leaflet and also, directly, by extension service workers. Its contents include main causative organism concentrations, size, expected duration and occurrence of harmful species of the red tide and estimated effect to the biota due to the tide and necessary measures to be taken for counterplan.

Concentration to recognize the formation of red tide as the standard for prediction being practiced, has been mainly based on the cell numbers/ml through the experiences, for example, attentiveness: Over 1,000 cells/ml in case of <u>Gymnodinium nagasakiense</u> red tide and over 10,000 cells/ml in case of <u>Skeletonema costatum</u> and <u>Heterosigma akashiwo</u>, in this case fishery damages might be expected to develop; warning: Over 5,000 cells/ml in case of <u>Gymnodinium nagasakiense</u> red tide, in this case fishery damages followed.

# RED TIDE PROBLEMS AT THE PORTUGUESE COAST

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Red tides along the Portuguese coast have been reported since 1949, but basic studies on this kind of phenomenon only started in 1955 when it was possible to relate bivalves toxicity with dinoflagellate blooms at Obidos Lagoon. These toxic blooms caused not only health problems but also economic trouble to Obidos region as the lagoon was closed to mollusc fisheries for 28 years.

Offshore events are occurring with increasing frequency in restricted areas which we can designate as prone regions. These regions are bays or embayments close to the most industrialized parts of the country (Oporto and Lisbon) or to touristic resorts like Algarve and Nazare where in summer the population can more than double.

Since 1986 the occurrence has been due to the physical transportation of the species to our coast after a major red tide event at Galicia, NW spanish coast. The problem in 1987 made it possible for the first time to confirm the presence of DSP in bivalves from Obidos Lagoon, Figueira da Foz, (Mondego estuary), Aveiro (Aveiro Ria) and Espinho (offshore) due to the presence of the genus <u>Dinophysis</u>, mainly <u>D. sacculus</u>.

This finding was made through a routine monitoring programme carried out at all the coastal zones where mollusc bivalves are of economic importance. In conjunction with this monitoring programme, a toxin detection programme is carried out by the National Health Institute. These programmes include qualitative and quantitative analyses of phytoplankton, isolation of potential toxic species to cultivate and study, observation of chemico-physical parameters of the water column, and harvesting of different bivalve species in order to detect toxins (PSP, DSP) and heavy metals (Hg, Cd).

The aim of these programmes is to maintain sanitary control of bivalves to protect human health and the economy of the fisheries, as well as to reach international requirements for exportation.

## PHYTOPLANKTON BLOOMS: THE PROBLEM OF THEIR OCCURRENCE AND REGULATION

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Phytoplankton blooms are natural phenomena in the sea, exhibiting well-known seasonal and regional patterns in occurrence. Blooms represent pronounced variations in abundance which, in turn, reflect cyclical and spatial differences and variations in regulatory environmental-growth factors such as light, nutrients and temperature. These recruitment growthfactors are poised against loss-factors, such as grazing, advection and sinking. Thus, phytoplankton bloom events represent the changing balance between factors regulating population recruitment and loss. The quantification of these processes represents a classical problem within biological oceanography. The general factors influencing the inception and termination of blooms in the sea have been identified, but our knowledge of the specific causes of total community blooms or of individual species is seriously limited.

This ignorance is exacerbated by the global epidemic of nuisance phytoplankton blooms, now spreading into all biogeographical regions and occurring with increased frequency. Species often either insignificant or previously not found in the local phytoplankton community suddenly are triggered into explosive growth, dominate the community for a variable period of time, then decrease to insignificance. These anomalous blooms may recur periodically in unpredictable fashion. Some of these rogue blooms are nuisance blooms: paralytic shellfish poisoning may result, posing public health problems; some blooms are ichthyotoxic; other blooms cause ecosystem dysfunction due to anoxia. In all instances, economic losses to fishermen can occur. This combination of ecological disruption and economic suffering accompanying such blooms and their global spreading and increased frequency set them apart from normal bloom events in the sea.

The underlying factors regulating normal bloom events probably do not differ from those regulating anomalous and nuisance algal blooms, even though the underlying factors remain enigmatic. However, the global spreading and increased frequency of nuisance blooms suggest that significant environmental changes are occurring globally triggering blooms of selected species. Some of these blooms are aperiodic; others occur more frequently. Some bloom-species are ordinarily relatively unimportant; others more important. It is irrelevant whether the underlying environmental changes, or triggering events, represent subtle changes in water-quality of larger-scale modifications. The more relevant, and major question is whether the required environmental modifications needed to trigger such blooms represent natural variability or anthropogenic

modification. There is limited inferential evidence that both types of environmental change are occurring. Studies are needed to establish the contribution of natural and anthropogenically induced variability as potential causative factors of the spreading, increasingly more frequent anomalous bloom events. This requires investigations on a global scale, both in pristene and anthropogenically modified waters and in different thermal regions.

Specific aspects needing quantification, include: Is the bloom outbreak a response to changes in macro-nutrients? in micronutrients? changes in grazing pressure?

A similar set of questions with regard to the termination of blooms require resolution. Given the complexity of the bloom process, simultaneous observations and field experimentation on a global basis are required, since the prominence of the various regulatory factor-combinations is expected to vary regionally. This would allow a regionally derived synthesis of cause and effect relationships leading ultimately to clearer elucidation of bloom regulatory processes.

It seems reasonable to expect that those factors regulating the spatial distribution, occurrence and abundance of a bloom-species will also influence its temporal variations within a given region. Thus, a regional approach seems the best operational investigative procedure to quantify and understand normal and unusual bloom processes. This synergistic, multiple approach will also help to resolve a fundamental problem with biological oceanography: how are phytoplankton blooms in the sea regulated? Such blooms are an important source of carbon-energy required to maintain marine ecosystems. However, with increasing frequency, an energy-overload accompanies such blooms leading to ecosystem disruption, and even dysfunction.

## PROPOSED NATIONAL TOXIC DINOFLAGELLATE RESEARCH CENTER IN FLORIDA

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The purpose of the proposed center is to promote and accelerate research on toxic dinoflagellates by providing profiled culture material and a focus for multidisciplinary interaction of international and national scientists at the university, government, and private laboratory level. This can be accomplished via on-site facilities for visiting scientists, post doctoral, Ph.D. students, and others, as well as off-site. The center will also offer courses on dinoflagellate biology (including culture and taxonomy) and toxicology for international participants.

The center will include the following components:

Research: Systematics and Evolution, Physiology, Ecology and Toxicology.

Facility: Laboratories, Cultures, Standards. Education: Visiting Scientists and Student Programmes, Courses. Administrative: Management and Advisory.

Administratively, the Center would be unique. There are essentially five co-principal investigators, four are research and one is administrative. The PIs represent the Florida Institute of Oceanography of the State University System, the Department of Marine Resources under the Governor and Cabinet, the University of South Florida, and the University of Miami - a private university. There would be an international advisory board for research that would serve on an rational basis. Currently, there are 40 scientists from the U.S.A., Great Britain, Canada, Spain, France, Germany, Norway, Japan, Italy, Australia, India, Philippines, Guatemala, Puerto Rico, and other geographic areas that have expressed verbal or written interest in collaboration.

If funded, the Center would be supported by NSF for only four years and then the funding would terminate. The State of Florida, the Department of Natural Resources and the State University System, have agreed to put the Center as a top priority, and 1) support a request to the Florida legislature for a DNR/USF/FIO building complex to be constructed by 1992; 2) renovate existing facilities to accommodate the Center until new buildings are complete; and 3) pick up positions and facility costs at the end of the grant.

Facility accommodations will be unique also because of local expertise and already available equipment, including those at a branch of the USF Medical School at All Childrens Hospital in St. Petersburg. Research opportunities are provided in four fields for collaborators.

<u>Systematics and Evolution</u>: Includes profiled clones from different geographic areas characterized by light and electron microscopy of motile and cyst stages, pigment composition and mtDNA analysis and life cycles and reproductive behaviour.

<u>Physiology</u>: Includes nutrient requirements and growth responses under different variables with different strains, and photosynthetic characteristics and photoadaptation under different variables and different strains.

<u>Ecology</u>: Includes vertical migration behaviour under different variables with different strains, allelopathy and competitive strategies under different variables with different strains, and this section is also responsible for maintaining cultures and checking for polyploidy and is the living culture repository.

<u>Toxicology</u>: Includes profiled toxins (IR, UV, NMR, HPLC, and potency assay) available as research tools from different geographic clones, and toxin multiplicity and toxin synthesis under different variables with different strains, including plasmid research as an infective entity below the bacterial level.

Florida is a unique area to do field research because it has <u>Ptychodiscus brevis</u>, <u>Gonyaulax monilata</u>, <u>Gambierdiscus toxicus</u>, <u>Prorocentrum lima</u>, <u>P. concavum</u>, <u>P. mexicanum</u>, <u>P. emarginatum</u>, <u>Coolia monotis</u> and <u>Ostreopsis</u> spp. Florida crosses 6<sup>o</sup> of latitude and has a wide range of habitats inshore and on a broad continental shelf influenced by frontal systems and upwelling. Florida also has the only living coral reef tract in the continental United States.

# PHYTOPLANKTON BLOOMS IN THE WESTPAC REGION: PROBLEMS TO BE CONSIDERED AND EXPERTISE TO BE SHARED

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Phytoplankton blooms in the countries of the WESTPAC region are now becoming more and more important not only because non-toxic seasonal blooms are going on but inasmuch because cases of fish-kills, PSP and DSP have been recorded in areas which never experienced such phenomena in the past. Hence, it is now the time to pay more attention to the blooms in order to prevent harmful impact on consumers and also to reduce economic losses.

Within the region, because of the long concern in red tide problems and high dependence on marine products for consumption, Japan has taken a leading role in terms of progress in scientific studies of the blooms and the toxification. Japan has also developed a very good cooperative system in monitoring.

Along the east coast of the USSR, seasonal red tides resulting from eutrophication have also been reported. Korea has also experienced a wide range of red tides. The blooms from more than 25 species of organisms have been recorded from both toxic and non-toxic species and have in some cases resulted in heavy economic loss. Thus, Korea is now developing a monitoring system. It is also to be noted that the blooms have changed from diatoms into dinoflagellates and seem to be with heavier concentrations, to cover wider areas and to last longer.

China and Hong Kong also have recorded red tides, some of which created fish kill. The blooms were shown to be somewhat related to increased nutrient loads which might be a result of population expansion. Taiwan reported to encounter the first toxic bloom due to conjunction to the culture purple clam.

The Philippines, which was known to have PSP cases recently which caused hardship to the villagers when banning of shellfish collection was enforced, is now conducting more research on the problems. East Malaysia and Brunei reported to have had PSP cases within the past few years. Thailand once reported to have a PSP case, but still cannot identify the causative species to that incident. Even though blooms of non-toxic species still occur, Thailand has not yet found any other new PSP case. More detailed studies are now going on. Indonesia reported red tides of both toxic and non-toxic blooms earlier but so far no monitoring system has been developed and more detailed studies are still going on.

Cases of toxic blooms in the Pacific Islands, for example, Fiji, Palau, Guam, Papua New Guinea, Solomon Islands, etc. have been recorded. Detailed studies in these areas are still very scattered. Concerning New Zealand and Australia particularly, the recent works in Tasmania have shown records of toxic blooms as well.

Focusing on the WESTPAC region, some countries such as Japan and Australia, have more expertise engaged in the study of red tide problems, while other countries are still short of adequate knowledge and studies. It is vital to establish international collaboration among developed and developing countries. The international agencies, such as the IOC should play a very important role in getting the knowledge and expertise from developed countries to be shared by developing countries. This can be done through a series of workshops, training programmes, or even funding graduate studies for personnel from developing countries. International meetings and symposia on red tides can also provoke more interest in the study of this problem in developing countries. Providing adequate literature and equipment for developing countries to work on this problem is also a great help.

It is also to be noted that there are many collaborative programmes among various countries within the region, for example, the ASEAN Marine Science Programme, the ASEA-Australia Co-operative Programme on Living Resources, etc. Such programmes might provide a framework for international co-operation on red tides.

# EXCEPTIONAL PLANKTON BLOOMS IN NORWAY AND ADJACENT WATERS

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A large number of exceptional plankton blooms in Scandinavian waters during the last 10 years have included dinoflagellates, diatoms, flagellates, ciliates and coccolithophorids. Monitoring programmes are now operative in Norway, Denmark and Sweden in order to give information to the shellfish and finfish farming industry. In Norway information on the marine environment, including plankton blooms, is transmitted to the fish farmers, funding and insurance institutions, and other users, by the information network MARINET which is owned and financed by the associations of fish farmers. It is believed that rapid information on harmful situations (forecasting) will reduce losses, e.g. due to noxious organisms.

Regular monitoring in Norway is based on samples collected along the coast by fish farmers and observers and by monthly cruises from Norway to Denmark. There is a testing programme aimed at shellfish toxins (PSP and DSP) using the mouse bioassays. The situation by November 1987 was as follows.

- It is established that plankton blooms may occur in restricted, local areas due to pollution (e.g. the Oslo fjord).
- Plankton blooms may originate outside Norway and be transported to the Norwegian coast by surface currents (e.g. large populations of <u>Gyrodinium aureolum</u> originating from the southern North Sea).
- Algal toxins (PSP and DSP) have been detected in shellfish even when the populations of toxic plankton were small (<u>Dinophysis</u> spp. below 100 cells/l (DSP), <u>Alexandrium</u> <u>excavatum</u> below 10000 cells/l (PSP).
- Blooms of <u>Ceratium</u> spp. are often accompanied by increased numbers of <u>Dinophysis</u> acuta and <u>D</u>. <u>norvegica</u>.
- Dinoflagellate blooms (<u>G. aureolum</u>, <u>Ceratium</u> spp.) may be transported over long distances along the coast by the Norwegian coastal current.
- <u>Prorocentrum minimum</u>, which was reported from Norway for the first time in 1979, has shown a gradual spreading northwards to the Barents Sea and eastwards to the Baltic Sea. Several blooms have been reported in Norway, Sweden and Denmark after 1979.

- <u>Gyrodinium aureolum</u> is established and has formed massive blooms after it was first observed in the area in 1966.
- Fish disease and mortality in fish farms is observed during blooms and occurrences of various algae (see summary in Table 1), mainly dinoflagellates (<u>Gyrodinium aureolum</u>, <u>Alexandrium excavatum</u> (Faeroe Islands), <u>Ceratium</u> spp., and <u>Polyknikos</u> sp.), flagellates (cf. <u>Heterosigma akashiwo</u> in Iceland, <u>Distephanus speculum</u> in Denmark, <u>Eutrepticella Gymnastica</u> in Norway), and diatoms (mixed populations in the spring bloom).
- Based on experimental work and field observations the critical concentrations of <u>G. aureolum</u> are indicated and used as a basis for warning/forecasting.
- Remote sensing device (in vivo fluorescence) is tested on anchored buoys as part of a plan for automatic monitoring along the coasts (net ocean data, e.g. temperature, salinity, oxygen, chlorophyll, current speed).

There are ongoing red tide research projects at the universities of Oslo, Trondheim and Bergen on general biology of bloom organisms and special biochemical aspects (Trondheim), with projects on algal toxins (DSP, PSP) at the Norwegian Veterinary College (Oslo) and at a research group in Trondheim (CHIRON, OCEANOR, SINTEF). Monitoring of blooms is operative at the south coast by the Fisheries Directorate through the biological station at Flodevigen and on the west and north coast by OCEANOR as a project for the fish farmers associations. In both projects fish farmers, marine biologists, district colleges and other institutions constitute the observation network. The results, when appropriate, are transmitted through the information network of MARINET.

TABLE 1Summary of algae reported to have noxious effects on farmed fishin Norway, and observed symptoms on atlantic salmon

## <u>GYRODINIUM AUREOLUM</u> - dinoflagellate

Low concentrations (less than 1 mill. cells/1)

no effects observed

Moderate concentrations (1-10 mill/1)

- reduced appetite
- some damage in the gills
- swimming near the surface
- Large concentrations, brown surface water (more than 10 mill/I) - no appetite
- abnormal, irregular swimming behaviour
- signs of oxygen deficiency
- gill damage, swelling of primary lamellae, respiratory epithelium of secondary lamellae sloughing
- reduced osmoregulation

<u>GYMNODIMIUM GALATHEANUM</u> - dinoflagellate

Effect on larger fish unknown, probably similar to G. aureolum, but larger concentrations necessary in order to harm fish

## ALEXANDRUIM EXCAVATUM - (Gonyaulax excavata) - dinoflagellate

Large concentrations

- no appetite
- swimming near the surface, "apathetic"
- gill damage, sloughing and necrosis
- paralysis (?)

<u>COLACIUM</u> sp. - green parasitic flagellate, primarily freshwater

Attached to the skin of smolt during adaptation to saltwater

- small colonies of cells cause point hemorrhages
- colonies usually near fin bases
- algae disappears when salinity is increased

<u>PHAEOCYSTIS POUCHETTII</u> - gelatinous colonies of non-motile cells Undefined illness and mortality during blooms of this species

# CHAETOCEROS spp. - diatom colonies

Large concentrations

- bleeding in gills caused by cuts and wounds due to the silica spines of the diatom cells
- clogging of gills

<u>ALL BLOOMS</u> - diatoms, flagellates, mixed blooms

- Spring and summer-autumn blooms, discoloured water
  - reduced appetite
- fish trying to escape

## PHYTOPLANKTON BLOOMS AND RED TIDES: EFFECTS ON FISH RECRUITMENT

# Alan W. White

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It is an accepted tenet among fisheries scientists that events which occur during the oral life stages of fish are the major determinants of year-class strength. Only a minuscule percentage of newly hatched larvae reaches the juvenile stage as most larvae die from starvation or predation. The degree of success of recruitment of larval fish to the population of juveniles is dependent upon a number of factors. One of the major factors is how well the timing of spawning coincides with the annual blooms of phytoplankton and zooplankton, which of course provide food for the larvae. A well-documented example is the relationship between recruitment of Northern anchovy (<u>Engraulis Gymnodinium splendens</u>) off the coast of California.

The association of exceptional phytoplankton blooms and red tides with mass kills of adult fish, wild and cultured, in many areas of the world is well known. Kills result from oxygen depletion, ichthyotoxicity, or physiological or mechanical stress. Whether extensive kills of adult wild fish, such as those reported during <u>Gyrodinium aureolum</u> blooms in northwestern Europe and during <u>Ptychodiscus brevis</u> blooms in the Gulf of Mexico, have significant impact on fisheries productivity in these areas is unknown. Given the tremendous fecundity of fish, long-lasting effects of toxic blooms and red tides on fish production may be rare. However, recurrent toxic blooms may perhaps influence stock sizes of fish stocks in certain areas.

Regarding fish larvae, it is difficult to assess whether the overall net effect of exceptional algal blooms and red tides on recruitment (used in this sense to mean recruitment of larvae to the population of juveniles) is positive or negative. Blooms and red tides serve as pulses of carbon into marine systems. If the causative organism is not toxic or if the bloom does not result in anoxia, then the increased primary and secondary production translates into increased food for fish larvae, thus conceivably resulting in a positive effect on recruitment. If, on the other hand, the bloom is toxic or causes anoxia, then mass kills of larvae may occur, conceivable resulting in negative effects of recruitment.

There is not enough field or laboratory information available to be able to estimate to what extent the above scenarios occur. Experimental evidence on effects of toxic, red-tide organisms on fish larvae is limited. Results of one study, presented at the International Symposium on Red Tides, Tamatsu, 10-14 Nov. 1987, indicate that some larvae can be killed by eating the toxic dinoflagellate, <u>Gonyaulax excavata</u> or zooplankton which, in turn,

have eaten <u>G</u>. <u>excavata</u>. Further, calculations from the study suggest that in nature only a few <u>G</u>. <u>excavata</u> cells constitute a lethal meal for a first-feeding larva, and only a few dozen copepods constitute a lethal meal for older larvae.

Since <u>Conyaulax excavata</u> can kill larvae, it is reasonable to ask if the other red-tide algae that contain saxitoxin and related toxins (i.e., <u>G. catenella</u>, <u>Pyrodinium bahamense</u> var. <u>compressa</u>, and <u>Gymnodinium</u> <u>catenatum</u>) can too. Investigations on the effects on fish larvae of these algae, as well as other fish-killing algae such as <u>Gyrodinium aureolum</u>, <u>Chattonella antiqua</u>, <u>Heterosigma akashiwo</u>, <u>Ptychodiscus brevis</u>, etc., should be conducted.

Although the methodology presents a challenge, field studies of the behaviour and survival of fish larvae during toxic algal blooms and red tides should be included in the biological oceanographic examinations of these events. To my knowledge, no studies of this sort concerning fish larvae have been done.

## MEDITERRANEAN RED TIDES

#### <u>T. Wyatt</u>

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Red tides in the Mediterranean are largely of the "trivial" kind, e.g. concentrations of phytoplankton and frequently <u>Noctiluca</u> along hydrographic fronts, and in bays and other sheltered areas. They are frequent in the Gulf of Valencia and between Barcelona and Lansa on the Spanish coast, and along the coast of Yugoslavia in situations like Kastela Bay, Pula Harbour, and the Gulf of Kotov.

Bacterial red tides occur in landlocked lagoons on the French and Italian coasts. Species of <u>Thiocapsa</u>, <u>Desulfobacter</u> and <u>Desulfovibrio</u> are involved.

Significant changes seem to have taken place in Greek waters in the last decade, where <u>Ptychodiscus brevis</u> has bloomed, and been associated with fish mortality. An event of this kind in 1978 was attributed to eutrophication.

Blooms of <u>Mesodinium</u> and <u>Gymnodinium</u> are also known in Izmir Bay (Western Turkey) and may offer parallels to those recorded in the Rias Bajas of northwest Spain.

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# EFFECTS OF FRONT FORMATION ON RED TIDE OCCURRENCE

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It has been said that the red tide occurrence is related to the surface convergence such as siome, streaks and fronts because the plankton is passive to the water movement and lighter than the sea water. However, there has been some confusion on the definition or terminology of such surface convergence.

Yanagi (1987) proposed a suitable classification of siome, streaks and fronts as shown in Fig. 1. The surface convergence has to last for more than one week to develop a plankton patch of a normal cell density of 10 cells/ml into the cell density at red tide of 1000 cells/ml assuming a doubling time of one day. From this viewpoint, only the tidal front has a possibility to be directly related to red tide occurrence of large scale in coastal areas because the life time of streaks range from several hours to one day. The estuarine front and the thermal effluent front exist too near to the coast and the thermohaline front exists only in winter. The tidal front is generated in a transition zone between the water that is vertically mixed by tidal stirring and the stratified water caused by heating through the sea surface in summer. The location of a tidal front is controlled by the variations in tidal mixing caused by spatial changes in tidal current amplitude U and the depth H, that is, the critical parameter is  $log(H/U^3)$ .

For example, the red tide of Noctiluca was found along the tidal front in Osaka Bay, Japan as shown in Fig. 2.



Fig. 1. - Classification of siome, streaks and fronts. Siome: surface convergence larger than the diffusion. Streak: surface convergence within the same water mass. Front: surface convergence between two different water masses.



Fig. 2. - Distribution of log (H/U<sup>3</sup>) in Osaka Bay, Japan and the location of tidal front from NOAA 9 i.r. image (left). The location of red tide Noctiluca from LANDSAT 3 MSS image (right).

# INSTRUMENTAL ANALYSES OF DINOFLAGELLATE TOXINS

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A highly sensitive and specific analytical method was developed for the determination of okadaic acid (OA) and dinophysistoxin-1 (DTX1), the main toxins responsible for diarrhetic shellfish poisoning. The toxins. which bear a carboxylic group, were esterified with 9-anthryldiazomethane and the resultant fluorescent derivatives were determined by high performance liquid chromatography (HPLC) after clean up on a disposable cartridge column (Sep-pak Silica, Waters). Satisfactory separation of the toxin derivatives was achieved on a reversed phase column (Develosil ODS) using acetonitril-methanol-water (8:1:1) as a moble phase. The excitation and emission wavelengths were set at 365 and 412 nm, respectively. The minimum detection level was about 1 pmol. The high sensitivity of the method enabled us not only to confirm the toxigenicity of Dinophysis spp. but also to determine the levels and profiles of the toxins with a small number of cells (100-1000) collected under a microscope. Thus the method provides an efficient means to test the toxicigenicity of species incapable of growing in laboratories. The method was also useful to distinguish different strains of Prorocentrum lima by toxin profiles.

The fluorometric HPLC method for determination of paralytic shellfish toxins was also improved. The method is capable of analyzing toxin profiles with a few hundred cells of dinoflagellates and thus saves the time and labor needed to prepare cultures. Another advantage of the instrumental assay is the easiness with which it determines sulfamate toxins, which are difficult to analyze by mouse assays due to their instability and low potency.

# AUTOMATED ANALYSIS OF PHYTOPLANKTON AND BLOOMS

## Clarice M. Yentsch

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Vast improvements in optics, electronics, stain utilization, and immunofluorescence result in the ability to analyze particles and cells of a natural population by flow cytometers/cell sorters within minutes. Advancements impossible by other means of analysis include: quantification of multiple parameters, analysis on individual particle basis, analysis at rapid rates at thousands of particles per second, good sensitivity, statistical precision, cell sorting capability and extended data analysis.

Flow cytometers/cell sorters have provided biological and optical oceanographers with an opportunity for new flexibility and experimentation. This is characterized by rapid measurement of two to six parameters made simultaneously on large numbers of individual particles. Knowledge of variability can advance our information base. Information compression as well as information expansion relating size/light scatter and fluorescence parameters are presented.

In a static sea, one would expect that productivity would be steady-state and directly proportional to sunlight in the ocean surface layer. The dynamics of currents, tides and topographic mixing serve to inject nutrients and stir the plankton into variable light and nutrient environments. Thus the distribution of photosynthetic production, and its producers, varies by several orders of magnitude over the globe. One wants to know what kinds of particles these are. Scientists have subdivided these into several groups, with coccolithophores, dinoflagellates, blue-green algae (cyanobacteria), and diatoms being the most numerous.

<u>Coccolithophores</u>: A satellite 500 miles above the ocean's surface senses phenomenal reflectance in each of its visible wavelength channels. Researchers in the lab study cultures to estimate the pattern of magnitude of nature's contribution to "acid rain," and geological information relating to our changing climate and global ocean flux.

<u>Dinoflagellates</u>: A bioluminescent glow is emitted in the wake of a submarine "hiding" at the thermocline. Geologists core the ocean basin to examine for dinoflagellate resting cysts, in an effort to position oil exploration. Cycles of both the motile and resting stages of species sometimes bloom and cause toxic "red tides".

<u>Cyanobacteria</u>: At sea, measurements on single cells indicate a marked pigment change both horizontally and vertically within the oceans' illuminated layer. In the laboratory, the pigments are extracted and used to tag antibody reagents of major utility in biomedical research.
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<u>Diatoms</u>: Turbulent upwelling regions and other mixed waters are characterized by cold, nutrient-rich waters at the surface. Sea surface temperature data are now available to several fishing vessels and fishing ports in California and Rhode Island.

Conclusion: Knowing "how many" of "who" are "where", "when", in our oceans is important. Past methods involving preservation and/or extraction, manipulation, filtration, incubation are ridden with artifacts. Microscopic methods are slow and tedious. Present-day tools of discovery have far greater powers of resolution than those available to Darwin. Clear impressions of the variability in productivity are emerging by satellite imagery and received shipboard. Side-by-side, there are automated instruments to enumerate thousands of individual micron-sized particles <u>in</u> <u>situ</u> per second. These are now the frontiers.

What engineering feats permit novel <u>in situ</u> ventures with microscopic particles? The advent of flow cytometry and sorting. Explorations employing flow cytometry/sorting are currently on-going in several laboratories in many fields of research. There are at least 16 instruments in North America, Japan, and Europe dedicated to aquatic research.

Flow cytometry (FCM) is literally the measurement of cells in a flow system. Inert particles as well as cells can be easily measured. Single-celled organisms common in the oceans range in size from <1.0 um to greater than 100 um expressed as cell diameter. If expressed as cell volume, this is approximately  $0.5 \text{ um}^3$  to  $500,000 \text{ um}^3/\text{m}!$  Metabolism is related to cellsize. By defining the limits of variability, we are able to make some generalizations with confidence, in fact, to compress information. By making observations of a population on a particle-by-particle basis, the variability within a population is easily assessed. In addition, another level of resolution may be obtained through the simultaneous measurement of two or more parameters for each particle. With flow measurements, statistically significant numbers of particles may be analyzed for a large range of particle concentrations in near real time.

The more sophisticated instruments go beyond the mere multiple simultaneous parameters. Laminar flow aligns the sample stream containing the particles into an interrogation region which permits sensitive, precise quantification of fluorescence intensity,  $90^{\circ}$  light scatter (SSC) and forward angle light scatter (FALS).

Although the purchase price of automated equipment is high, it is cost effective and permits rigorous sampling which can help to quantify bloom conditions in time and space.

# SURVEILLANCE OF OCEAN BLOOMS

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There exists a strong correlation between regions of high primary production and toxic outbreaks of algae. This leads one to believe that the appearance of these toxic blooms is a part and parcel of oceanographic processes which occur on a planetary scale. It is believed that most blooms result from enrichment and the answer for their genesis of blooms is physical/chemical interactions; physical forces resulting from tides, wind stress etc. are the prime candidates. Although one cannot dismiss the fact that blooms are the result of natural oceanographic phenomena, the question arises whether or not the increased frequency of blooms is due to long term climatological change and/or to the influence of human activities. It is recognized that the two are not unrelated. Given the inadequacy of shipboard observations the only reasonable approach is a commitment of long term surveillance by use of satellite imagery to establish the frequency of blooms in all oceans. It is suggested that the IOC could identify and commission regional satellite centers to search through their archives to; log blooms and the frequency of occurrence in specific locations, and 2) 1) to assess regional and temporal ocean-climate interactions associated with these blooms.

# ANNEX III

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(2) Attended subgroup discussion on ecology

(3) Attended subgroup discussion on toxicology

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# ANNEX IV

#### POTENTIALLY BLOOM-FORMING PHYTOPLANKTON SPECIES

Table 1. RED TIDE CAUSATIVE ORGANISMS Bacteria Pseudomonadomycetes Rhodobacteriales Chromatium Phytoplankton Cyanophyceae Chroococcales Microcystis Nostocales Trichodesmium, Oscillatoria, Aphanizomenon Anabaenopsis, Anabina Cryptophyceae Cryptomonadales Chroomonas Dinophyceae See Table 3 Bacillariophyceae Centrales Cyclotella, Skeletonema, Thalassiosira, Coscinodiscus, Leptocylindrus, Rhizosolenia, Eucampia, Bacteriastrum, Chaetoceros Pennales Asterionella, Fragilaria, Nitzschia Raphidophyceae See Table 2 Haptophyceae (Prymnesiophyceae) Isochrysidales Emiliania, Pleurochrysis Prymnesiales Chrysochromulina, Prymnesium, Phaeocystis Euglenophyceae Eutreptiales Eutreptia, Eutreptiella Euglenales Euglema, Trachelomonas Prasinophyceae Pyraminonadales Pyramimonas, Prasinocladales <u>Tetraselmis</u> Chlorophyceae Volvocales Dunaliella, Oltmannsiellopsis, Chlamydomonas, **Brachiomonas** Protozoa Ciliophora Prostomatida Mesodinium (=Myrionecta)

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Table 2. RED TIDE CAUSATIVE RAPHIDOPHYCEAE

Class Raphidophyceae Order Raphidomonadales Family Vacuolariaceae <u>Fibrocapsa japonica</u> <u>Chattonella antiqua</u> <u>C. marina</u> <u>C. subsalsa</u> <u>Heterosigma akashiwo</u>\*

\* Often confused with Olisthodiscus luteus

# Table 3. RED TIDE AND/OR TOXIC DINOFLAGELLATES

Class Dinophyceae Order Prorocentrales Family Prorocentraceae Prorocentrum balticum -P. concavum Ρ. compressum <u>P.</u> dentatum <u>P.</u> gracile \*<u>Р.</u> lima \*<u>Р.</u> mexicanum <u>P.</u> <u>micans</u> <u>P.</u> minimum <u>P.</u> sigmoides <u>triestinum</u> <u>P.</u> Order Dinophysiales Family Dinophysiaceae \*Dinophysis acuminata \*<u>D.</u> acuta <u>caudata</u> D. <u>fortii</u> \*<u>D.</u> \*<u>D.</u> mitra \*<u>D.</u> <u>norvegica</u> \*<u>D.</u> <u>tripos</u> Order Gymnodiniales Family Gymnodiniaceae \*Amphidinium carterae <u>conradii</u> <u>A.</u> klebsii \*<u>A.</u> Cochlodinium catenatum helicoides <u>C.</u> \*<u>С.</u> polykrikoides Gymnodinium albulum \*<u>G.</u> <u>breve</u> \*<u>G.</u> catenatum <u>lacustre</u> <u>G.</u> <u>G.</u> mikimotoi \*<u>G.</u> nagasakiense <u>G.</u> <u>sanguineum</u> <u>G.</u> <u>simplex</u> \*Gyrodinium aureolum <u>G.</u> <u>falcatum</u> <u>G.</u> fissum <u>G.</u> flavum <u>G.</u> instriatum Family Lophodiniaceae Katodinium glaucum Family Polykrikaceae Pheopolykrikos hartmanii

Polykrikos kofoidii P. schwartzii Family Pronoctilucaceae Entomosigma peridinioides Oxyrrhis marina Order Noctilucales Family Noctilucaceae <u>Noctiluca</u> <u>scintillans</u> (<u>=miliaris</u>) Order Peridiniales Family Calciodinellidaceae Scrippsiella hexapraecingula <u>trochoidea</u> **S**. Family Ceratiaceae Ceratium furca <u>fusus</u> <u>C.</u> <u>C.</u> trichoceros Family Gonyaulaceae Alexandrium balechii minutum <u>A.</u> Gonyaulax polyedra polygramma <u>G.</u> <u>G.</u> spinifera <u>G.</u> triacantha <u>G.</u> verior Protoceratium reticulatum \*Protogonyaulax acatenella <u>P.</u> affinis \*<u>Р.</u> <u>catenella</u> <u>cohorticula</u> \*<u>Р.</u> <u>P.</u> peruviana \*<u>Р.</u> <u>tamarensis</u> Pyrodinium bahamense var. compressa P<u>.</u> <u>b.</u> <u>bahamense</u> <u>v.</u> Family Ostreopsidaceae \*<u>Ostreopsis</u> ovata siamensis \*0. \*<u>Coolia monotis</u> Family Peridiniaceae Cachonina hallii <u>Heterocapsa</u> triquetra Peridinium bipes <u>P.</u> <u>cunningtonii</u> <u>P.</u> gregarium \*<u>Р.</u> hangoei <u>P.</u> penardii \*<u>Р.</u> polonicum guinquecorne <u>P.</u> Family Triadiniaceae Triadinium pseudogoniaulax \*Gambierdiscus toxicus

Country	Year this report submitted				Rapporteur			
Institution Type*	Address	<u></u>	Tel.	T1m.	Tlx.	Fax	Personnel	Expertise
Type Codes to be followe	ed			Experti	se Codes			
A - Taxonomic Expertise			*	*a – Phy	toplankt	on Iden	tification g	eneral

- A Taxonomic Expertise
- **B** Toxicological Expertise
- C Ecological Expertise
- D Hydrographic Expertise
- .E Algal Culture Expertise
- F Fish Pathological Expertise
- G National Co-ordinating Centre
- II Other control agency (Fisheries)
- I Other control agency (llealth)

- \*\*a Phytoplankton Identification general
  - b Taxonomic specialist (specify group)
  - c Toxicologist
  - d Ecologist
  - e Physiologist
  - f Physical or chemical oceanographer
  - g Algologist
  - h Fish qnd Shellfish Pathologist
  - i Statutory control of fisheries
  - j Bioassay qnd Chemical Analyses

No.	Title	Publishing Body	Languages	No.	Title	Publishing Body	Languag
32 Suppl.	Papers submitted to the UNU/IOC/Unesco Workshop on International Co-operation in the Development of Marine Science	IOC, Unesco Place de Fontenoy 75700 Paris, France	English	45	IOCARIBE Workshop on Physical Oceanography and Climate Cartagena, Colombia, 19-22 August 1986	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
	and the Transfer of Technology in the Context of the New Ocean Regime Paris, 27 September-1 October 1982			46	Reunión de Trabajo para Desarrollo del Programa «Ciencia Oceanica en Relación a los Recursos No vivos	IOC, Unesco Place de Fontenoy 75700 Paris, France	Spanish
33	Workshop on the IREP Component of the IOC Programme on Ocean Science in Relation to Living Resources (OSLR)	IOC, Unesco Place de Fontenoy 75700 Paris, France	English		en la Región del Atlantico Sudoccidental Porto Alegre, Brazil 7-11 de Abril de 1986		
34	Halifax, 26-30 September 1983 IOC Workshop on Regional Co-operation in Marine Science in the Central Eastern Atlantic	IOC, Unesco Place de Fontenoy 75700 Paris, France	English French Spanish	47	IOC Symposium on Marine Science in the Western Pacific: The Indo-Pacific Convergence Townsville, 1-6 December 1986	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
35	(Western Africa) Tenerife 12-17 December 1983 CCOP/SOPAC-IOC-UNU Workshop	IOC, Unesco	English	48	OCARIBE Mini-Symposium for the Regional Development of the IOC-UN (OETB) Programme on "Ocean Science in Relation	IOC, Unesco Place de Fontenoy 75700 Paris, France	English Spanish
	on Basic Geo-scientific Marine Research Required for Assessment of Minerals and Hydrocarbons in the South Pacific	Place de Fontenoy 75700 Paris, France		49	to Non-Living Resources (OSNLR)" AGU-IOC-WMO-CPPS Chapman Conference: An International Symposium on "El Niño" Guyaquil, Ecuador, 27-31 October 1986	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
36	Suva, Fiji, 3-7 October 1983 IOC/FAO Workshop on the Improved Uses of Research Vessels Liebon 20 Marks	IOC, Unesco Place de Fontenoy 75700 Paris, France	English	50	CCAMLR-IOC Scientific Seminar on Antarctic Ocean Variability and its Influence on Marine Living Resources, particularly Krill	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
36 Suppl.	Papers submitted to the IOC-FAO Workshop on Inproved Uses of Research Vessels	IOC, Unesco Place de Fontenoy 75700 Paris, France	English	51	(organized in collaboration with SCAR and SCOR) Paris, France, 2-6 June 1987		Frindlich
37	Lisbon, 28 May-2 June 1984 IOC/Unesco Workshop on Regional Co-operation in Marine Science in the Central Indian Ocean	IOC, Unesco Place de Fontenoy 75700 Paris, France	English		Processes in the South Pacific Island Nations, Lae, Papua-New Guinea, 1-8 October 1987	Place de Fontenoy 75700 Paris, France	Linguist
37 Suppl	and Adjacent Seas and Gulfs Colombo, 8-13 July 1985 Papers submitted to the IOC/Unesco Workshoo on Bedional Co-oneration	IOC, Unesco	English	52	SCOR-IOC-Unesco Symposium on Vertical Motion in the Equatorial Upper Ocean and its Effects upon Living Resources and the Atmosphere	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
oopp.	in Marine Science in the Central Indian Ocean and Adjacent Seas and Gulfs Colombo, 8-13 July 1985	75700 Paris, France		53	Paris, 6-10 May 1985 IOC Workshop on the Biological Effects of Pollutants Och 11.29 August 1986	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
38	IOC/ROPME/UNEP Symposium on Fate and Fluxes of Oil Pollutants in the Kuwait Action Plan Region Resrat, ira, 8-12 January 1984	IOC, Unesco Place de Fontenoy 75700 Paris, France	English	54	Workshop on Sea-level Measurements in Hostile Conditions Bidston, UK, 28-31 March 1988	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
39	CCOP (SOPAC)-IOC-IFREMER- ORSTOM Workshop on the Uses of Submersibles and Remotely Operated	IOC, Unesco Place de Fontenoy 75700 Paris, France	English	55	IBCCA Workshop on Data Sources and Compilation Boulder, Colorado, 18-19 July 1988	IOC, Unesco Place de Fontenoy 75700 Paris, France IOC, Linesco	English Foolish
40	Vehicles in the South Pacific Suva, Fiji, 24-29 September 1985 IOC Workshop on the Technical Anaptis of Esugami Analyses	IOC, Unesco Place de Fontenov	English	30	of Penaeld Prawns in the Indo-West Pacific Region (PREP) Cleveland, Australia, 24-30 July 1988	Place de Fontenoy 75700 Paris, France	L'Agnor
	Prediction and Communications Sidney, B.C., Canada, 29-31 July 1985	75700 Paris, France		57	IOC Workshop on International Co-operation in the Study of Red Tides and Ocean Blooms Takamatsu, Japan, 16-17 November 1987	IOC, Unesco Place de Fontenoy 75700 Paris, France	English
40 Suppl.	IOC Workshop on the Technical Aspects of Tsunami Analyses, Prediction and Communications Submitted Papers Sidney, B.C., Canada, 29-31 July 1985	IOC, Unesco Place de Fontenoy 75700 Paris, France	English				
41	First Workshop of Participants in the Joint FAO/IOC/WHO/IAEA/UNEP Project on Monitoring of Pollution in the Marine	IOC, Unesco Place de Fontenoy 75700 Paris, France	English				
	Environment of the West and Central African Region (WACAF/2) Dakar, Senegal, 28 October - 1 November 1985						
42	IOC/UNEP Intercalibration Workshop on Dissolved/Dispersed Hydrocarbons in Seawater Bermuda, USA, 3-14 December 1984 (in press)	IOC, Unesco Place de Fontenoy 75700 Paris, France	English				
43	IOC Workshop on the Results of MEDALPEX and Future Oceanographic Programmes in the Western Mediterranean Venice, Italy, 23-25 October 1985	IOC, Unesco Place de Fontenoy 75700 Paris, France	English				
44	IOC/FAQ Workshop on Recruitment in Tropical Coastal Demersal Communities Ciudad del Carmen, Campeche, Mexico, 21-25 April 1986	IOC, Unesco Place de Fontenoy 75700 Paris, France	English (out of stock) Spanish				
44 Suppi.	IOC/FAO Workshop on Recruitment in Tropical Coastal Demersal Communities - Submitted Papers Ciudad del Carmen, Campeche, Marino 21-25 April 1986	IOC, Unesco Place de Fontenoy 75700 Paris, France	English				