Intergovernmental Oceanographic Commission
Training Course Reports

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First IOC/WESTPAC Training Course on Monitoring of PSP Plankton and Shellfish Toxicity

School of Fisheries Sciences
Kitasato University, Japan
July 1995

Second IOC/WESTPAC Training Course on Species Identification of Harmful Microalgæ

Asian Natural Environmental Science Center
The University of Tokyo, Japan
February 1997

Third IOC/WESTPAC Training Course on Species Identification of Harmful Microalgæ

Asian Natural Environmental Science Center
The University of Tokyo, Japan
August 1997

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ABSTRACT

Two training courses on Species Identification of Harmful Microalgae were held at ANESC (Asian Natural Environmental Science Center) of the University of Tokyo on 28 February - 8 March and 22-30 August, 1997. Before these courses a training workshop on Monitoring of PSP Plankton and Toxin Monitoring was held at School of Fisheries Sciences of Kitasato University on 17-21 July, 1995. The courses and workshop were one of the important activities planned and implemented by the WESTPAC HAB project (Harmful Algal Blooms in the Western Pacific). Expenses were provided by the Japanese Government through the funds in trust to the Intergovernmental Oceanographic Commission (IOC) of UNESCO, and partially by ANESC of the University of Tokyo and School of Fisheries Sciences of Kitasato University.

The participants included twenty-two people from eleven countries for the 1995 Workshop, ten from eight countries for the 1997-February Course, and fifteen from countries for 1997-August Course. They were from China, Fiji, Indonesia, Korea, Malaysia, New Zealand, Philippines, Russia, Thailand, and Vietnam in WESTPAC area and from Croatia, Guatemala and Latvia.

The Courses and the workshop were organized by Dr. Yasuwo Fukuyo (ANESC, the University of Tokyo), in cooperation with Ms. Miyuki Kuranishi (Japanese Ministry of Education, Science, Sports and Culture, Tokyo) and Dr. Shigeki Mitsumoto (WESTPAC Secretariat Office, Bangkok). Dr. Masaaki Kodama (School of Fisheries Sciences, Kitasato University), Dr. Yasukatsu Oshima (Faculty of Agriculture, Tohoku University), Dr. Arnulfo N. Marasigan (Aquaculture Institute, University of the Philippines in the Visayas, Iloilo, Philippines) and Mr. Tumpak Sidabutar (Research and Development Centre for Oceanology, Ambon, Indonesia) were invited to deliver lectures.

The 1995 Workshop was held in order to increase awareness of the importance of a regular monitoring of PSP plankton and their toxins. The 1997 Courses were more specific, as they were held to improve identification skills on harmful microalgae through lectures and practical laboratory exercises to enable participants to detect their occurrence and mitigate their harmful effects. In particular, the Course was focused on identification of toxic and harmful dinoflagellates by using compound light microscopes which are commonly available in the countries of the participants.

During the Workshop and Courses several methods for observating dinoflagellates and the environment in which they occur were explained and demonstrated by the lecturers. Microscopic observation on plankton samples brought by participants were carried out.

The training provided will be used to design and implement a monitoring programme on harmful microalgae in the area of participant's countries. The training activity will also focus on improving related human resources as well as technology and knowledge transfer in order to further develop the capability in WESTPAC countries for management of HAB events resulting from the additional trained personnel who attended the workshop courses.

1. FIRST IOC/WESTPAC TRAINING WORKSHOP ON MONITORING OF PSP PLANKTON AND SHELLFISH TOXICITY

1.1 BACKGROUND AND OBJECTIVES

The occurrence of PSP has been increasing in WESTPAC countries. As research programs develop, and as more data are collected, more areas affected by paralytic shellfish poisoning (PSP) are recognized.

The number of victims by poisoning, and amounts of economic loss in coastal fisheries are increasing. At the Second Session of the IOC Sub-Commission for the Western Pacific held in Bangkok, Thailand, 25-29 January, 1993, and at the joint Scientific Seminar of River Inputs/Harmful Algal Bloom/Continental Shelf Circulation Project Groups of WESTPAC held in Bali, Indonesia, 20-21 November, 1994, the WESTPAC-HAB project group recognized the importance of regular training activities to develop human resources to minimize PSP tragedies.

To take advantage of the presence of many scientists that were to attend the Seventh International Conference on Toxic Phytoplankton held at Sendai, Japan, 12-16 July, 1995, the present workshop entitled "Monitoring of PSP Plankton and shellfish Toxicity" was planned. The workshop was included as one of the supplemental activities in the educational and scientific elements of IOC Harmful Algal Programme.

The objectives of the training workshop were to improve the skills of the participants and to provide them with updated scientific information concerning monitoring methods that are used for dinoflagellates that cause PSP and contaminate shellfish. Furthermore, it was intended to encourage the individual participants from the various countries to design and establish a monitoring programme for PSP occurrence in their home countries. Participants were also encouraged to organize local or regional training workshops on PSP and to encourage development of scientific networks of experts on harmful algae. The WESTPAC-HAB project group shall assist the local activities in various ways such as sending an expert mission to the local or regional training workshop and providing reference publications and teaching materials.

1.2 PARTICIPANTS

Twenty-two participants from eleven countries attended the workshop, including seventeen participants from seven WESTPAC member countries namely: China, Indonesia, Malaysia, Philippines, Russia, Thailand and Vietnam. Lectures, laboratory, and field work were organized by Dr.Yasuwo Fukuyo (the University of Tokyo), Masaaki Kodama (Kitasato University) and Yasukatsu Oshima (Tohoku University), with the help of Dr. Matsuoka of Nagasaki University and staff of Kitasato University: Dr. Ogata, Dr. Kotaki, Dr. Sato, and Mr. Koike. Section 1.5 contains the list of participants, lecturers, instructors and guest lecturers. All participants have been working in the field of biology, chemistry and oceanography of harmful algae.

Five participants: Mr. Wang, Mr. Sidabutar, Ms. Noor, Ms. Bajarias and Ms. Huyen were supported by IOC. Expenses of the other participants and lecturers was supported by the Organizer of the Seventh International Conference and the University of Tokyo. Expenses of laboratory and field works were kindly provided by the School of Fisheries Sciences, Kitasato University.

1.3 CONTENT OF THE WORKSHOP

1.3.1 Opening

The training workshop was opened at 09:30 hours on 17 July, 1995, by Dr.Yasuwo Fukuyo, the leader of WESTPAC-HAB project team at the School of Fisheries Sciences, Kitasato University.. Mr. Fukuyo welcomed participants on behalf of the IOC and expressed his appreciation to Dr. Helle Ravn, an IOC Associate Expert on Harmful Algal Blooms, for her efforts in organizing the training workshop. He explained the importance of monitoring species of plankton that cause PSP and shellfish toxicity to detect PSP and to understand the blooming and spreading mechanisms and he stressed that the Workshop would provide a good opportunity not only for the study of monitoring techniques, but also for establishing contacts among scientists inside and outside the WESTPAC area.

Dr. Helle Ravn thanked the organizers on behalf of the IOC and explained the mechanism of the IOC and various HAB related activities, especially educational, scientific, and operational elements of the IOC-HAB Programme.

1.3.2 Outline of the programme

The following subjects were covered during the five days of the training workshop:

(i) sampling methods for water, plankton, sediment and shellfish;
(ii) identification and counting of dinoflagellates plankton;
(iii) identification and counting of dinoflagellate cysts;
(iv) measurement of PSP toxicity by mouse bioassay;
(v) measurement of PSP toxicity by HPLC.

For the lectures three manuals were used as textbooks:

(i) Manual for Modern Dinoflagellate Cyst Sudy,
(ii) Sandard Mouse Bioassay for Paralytic Shellfish Toxins,
(iii) Manual for PSP Toxin Analysis by High Performance Liquid Chromatography.

In addition to the manuals, several reference books such as "Red Tide Organisms in Japan" and "Biology, Epidemiology and Management of *Pyrodinium* Red Tides" were loaned to the participants. Some pieces of laboratory items including counting chambers, slide glasses, plastic pipettes etc. were provided.

The trainees were satisfied with the teaching materials and local arrangements. Some of the trainees expressed their plan to hold a local training workshop on a this topic and requested WESTPAC and IOC to support them by sending an expert mission and reference materials. All the trainees also requested WESTPAC to hold regular training workshop on related HAB subjects.

1.3.3 Closure

The training course was closed on 21 July, 1995. The chief organizer, Dr. Yasuwo Fukuyo congratulated the participants for the successful completion of the training course and thanked IOC for helping to organize and support it. He also expressed his sincere thanks to the lecturers and the supporting staff for their contribution and the trainees for their co-operation. He hoped that the knowledge and techniques they had acquired would be useful and helpful in their future work at home. He further suggested that WESTPAC would support their future work in various way and have a regular training workshop once a year.

1.4 CONCLUSION

The training workshop was well-organized and successfully conducted in a friendly atmosphere. As most of the trainees had difficulty in identifying toxic plankton in their regions, they studied very hard to acquire knowledge of taxonomy. Reference books were very useful for that purpose. Due to the limitation of the duration of the workshop, it was impossible for them to acquire complete understanding of the toxonomy of HAB organisms , but they could at least gain necessary and adequate knowledge and techniques for monitoring work.

All participants recognized the importance that similar training activities should be held in the WESTPAC regions continuously.

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1.6 DETAILED PROGRAMME

17 July 1995		
08:30-09:00	Registration	
09:00-09:40	Opening of the Workshop	
	Welcome Address	Fukuyo
	Message from IOC	Ravn
	Technical and Practical Information	Ogata
09:40-10:00	Coffee Break	
10:00-11:30	Self Introduction	Participants
11:30-12:00	IOC/UNESCO and its HAB Programme	Ravn
13:30-15:00	Shellfish poisoning and their causative organisms	Fukuyo
15:00-15:30	Coffee Break	
15:30-17:00	Intoxication mechanism of shellfish and importance of toxicity monitoring	Kodama
18 July		
08:30 - 12:00	Field Trip to Ofunato Bay	
	Collection of Samples	Ogata/Kotaki/Sato/Koike
	Demonstration of Sampling Technique	Fukuyo/Matsuoka
13:00 - 14:00	Visit of Iwate Prefectural Seedling Center	
14:00 - 15:00	Short trip from Ofunato to Kamaishi	
15:00 - 17:00	Visit of Iwate Prefectural Fisheries technical Center	
17:00 - 18:30	Free time at Kamaishi City	
18:30 - 20:30	Welcome Dinner	
19 July		
09:00 - 10:00	Guidance to laboratory work	
10:00 - 10:20	Coffee break	
10:20 - 12:00	Technical Session: Each trainee takes one of the programmes	
	Programme 1: quantitative analysis of PSP plankton contains talks and technical demonstration of following subjects:	
	identification and counting of dinoflagellates	Fukuyo
	identification of dinoflagellate cysts	Matsuoka
	Programme 2: shellfish toxicity measurement contains talks and technical demonstration of following subjects:	
	measurement of PSP toxicity by mouse bioassay	Kodama
	measurement of PSP toxicity by HPLC	Oshima
13:30 - 17:00	Technical Session:	
18:00 - 20:00	Identification of Dinoflagellate	Fukuyo
20 July		
09:00 - 12:00	Technical Session: on the different programmes	
13:30 - 17:00	Technical Session:	
18:00 - 20:00	Identification of Dinoflagellate	Fukuyo
21 July		
09:00 - 10:00	Identification of Dinoflagellate	Fukuyo
10:00 - 10:20	Coffee break	
10:20 - 12:00	Identification of Dinoflagellate	Fukuyo
13:30 - 15:00	Culture of Dinoflagellate	Koike
15:00 - 15:30	Coffee break	
15:30 - 17:00	Closing of the workshop	Fukuyo
	Message from the Organizer	Fukuyo
	Award of Certificate to Participation	Fukuyo/Kodam
18:00 - 21:00	Farewell dinner	

2. SECOND IOC/WESTPAC TRAINING COURSE ON SPECIES IDENTIFICATION OF HARMFUL MICROALGAE

2.1 BACKGROUND

The marine environment is a major source of food in WESTPAC countries and the increasing incidence of harmful and toxic algal blooms have been a major health and economic concern in the region. To date, the increasing population with its corresponding demand for food puts enormous pressure for expansion of aquaculture in coastal areas of WESTPAC countries. Therefore this increases the risk of coastal eutrophication, with microalgal blooms as one of its latent effects. In Southeast Asia, for example, human deaths have been reported after consumption of mussels harvested from mariculture in addition to natural ones. Restriction and banning of harvesting of bivalves brought economic dislocation to small scale shellfish growers. These events due to harmful algal blooms could be mitigated, if there could be an increased awareness and concerted national and international efforts to understand the nature of harmful algal blooms. Accumulation of information and development of human resources capable of monitoring for the occurrence of harmful microalgae and toxic shellfish are fundamental.

The WESTPAC-HAB project group recognizes the importance of regular training activities on biological, oceanographic and chemical aspects related to the problems. In the first stage of successive training courses the group gave the highest priority to one of the biological aspects, development of identification skill and taxonomic knowledge. In the second stage, the trainees attending the course are expected to assist/hold training activities in their home regions in co-operation with the WESTPAC-HAB project group.

2.2 OBJECTIVES

The objective of the Training Course was to improve the participants taxonomic skills on harmful microalgae in order to enable them to detect their occurrence and mitigate their harmful effects. The training experience obtained will be used to design and implement a monitoring programme and basic study. The training activity focused on improving related human resources, as well as technology and knowledge transfer in order to develop capacity in WESTPAC countries for the management of HAB events.

2.3 PROGRAMME

The Course was organized by Dr. Yasuwo Fukuyo and Dr. Arnulfo N. Marasigan in co-operation with Ms. Miyuki Kuranishi (Japanese Ministry of Education, Science, Sports and Culture, Tokyo, Japan) and Ms. Naoko Ichiyama (IOC, Paris, France) and co-hosted by Asian Natural Environmental Science Center, the University of Tokyo, Japan. Dr. Masaaki Kodama (School of Fisheries Sciences, Kitasato University) was invited to deliver a lecture.

Since 1985, the main trainer had conducted various introductory training courses and scientific surveys in the WESTPAC region such as Malaysia, Indonesia, Thailand, Vietnam and the Philippines. The training course on species identification was the most fundamental one, but would require several kinds of resources such as extensive reference materials and adequate instruments. So at this point, Japan would be the logical choice for the course. Depending on the availability of the resources, other member countries will be able to host the training course for HAB. Thus, this may be one of the outcomes of the present training course.

The effective planning of HAB monitoring and ecological studies in developing WESTPAC countries depends on increasing manpower skills in the fundamentals of taxonomy and biology of HAB species and the practical aspects of sampling techniques and microscopic analyses. The topics covered in the lectures were the taxonomy and ecology of dinoflagellates and monitoring techniques and provided the trainees with hands-on experience in the techniques used in HAB species identification. The complete Course Programme can be found under section 2.6.

The Training Course is aimed at participants who have already a basic knowledge of phytoplankton taxonomy and who are familiar with the use of a regular compound microscope. Among twenty applicants, ten people from eight countries were admitted to the course. Priority was given to younger scientists who are involved in plankton monitoring. The list of participants is given under section 2.5 with the list of lecturers.

During the lecture, technical manuals and detailed photomicrographs and line drawings of dinoflagellates were presented to the trainees to assist in species identification. Several books such as "Red Tide Organism in Japan (Fukuyo *et al.*, 1990)" were provided to each trainee as a guide and reference during classroom sessions. The laboratory was designed to reinforce the lectures by letting the students advance from basic microscopy to acquisition of skills in advanced photomicrograph techniques for DIC (Differential Interference Contrast) and epiflourescence equipment. These techniques were used by the trainees to identify species in samples from their countries. The laboratory was supervised by Drs. Fukuyo and Marasigan and assisted by Mr. Yoshida and Dr. Yoo. Flexibility was built into the training course by conducting consultations with the trainees after the practical sessions and the laboratory was kept open until late in the evening for the trainees to carry on with their specific laboratory work. The trainees were given lists of references from which they chose the titles for photocopying to be taken back to their countries. A training kit was provided at the start of the course containing IOC-UNESCO Manual of Harmful Marine Algae, the proceedings of the 7th International Conference on Toxic Phytoplankton (Harmful and Toxic Algal Blooms), a set of photo-slides of HAB and HAB events, and color plates of dinoflagellates.

In the introductory talks by participants, the present condition of HAB occurrence and related research and study in each country was presented (section 2.7). The trainees recognized that HAB events had been occurring widely and spreading gradually and, in order to mitigate the consequence, the future close cooperation on the research and study was most important. All the participants expected the International Programme on HAB by IOC and WESTPAC-HAB programme implemented by IOC subsidiary body WESTPAC could play a leading role for the activity.

2.4 EVALUATION

Questioners were given to the participants at the end of the course. The summary of the responses are shown in the following page. Phrases in comments for some question (A.6, A.7 etc.) are also listed. It was a contrast between the knowledge of the lecturers which was highly rated, 'very good', and the assistance provided wherein the lowest score, 3, was given by two participants.

	Mean Score	Lowest Score
A. Course content		
1. Are the topics of the course sufficient to meet the objectives?	3.7 (Good)	3
2. Did the lectures meet your expectations/needs?	3.8 (Good)	3
3. Did the practicals help you acquire new techniques?	4.0 (Very good)	2
4. Did the practicals make you understand/related to the lectures?	3.7 (Good)	2
5. Are the topics and practicals well organized?	3.7 (Good)	2
6. What other topics do you suggest for lectures/practicals:		
'cyst identification'		
'cyst sampling techniques'		
'factors affecting toxin production'		
'other microalgal group such as cyanobacteria and diatom'		
'electron microscope technique'		
'microalgal culture establishment and maintenance'		
7. What did you learn new and useful to your work?		
'observation on armored dinoflagellates using fluorescence microscopes'		
'identification of other toxic microalgae other than *P. bahamense*'		
'new knowledge on how to use some advanced microscope such as DIC and Axiophot 2'		
'new technique for photo-taking using DIC, Phase-contrast, epifluorescence'		
'I have increased my knowledge on morphology of dinoflagellates from this course...'		
8. What modifications to the course do you suggest?		
'course duration longer than 10 days'		
'field work to learn on-site techniques'		
9. Will you apply the knowledge you learn from the course? How?		
'I will use the knowledge in the monitoring plankton in my country'		
'I would like to start a monitoring programme together with other universities and agencies'		
'I will teach my colleague these new knowledge and techniques in our future work'		
'I would like to conduct echo seminar'		
'I will make a check-list of dinoflagellate in my coastal waters'		
B. Facilities and arrangements		
1. How do you rate the lecture facilities?	4.1 (very good)	3
2. How do you rate the laboratory facilities?	4.2 (very good)	3
3. How do you rate the handouts?	4.3 (very good)	3
4. How do you rate the accommodation?	4.4 (very good)	3
Other comments:		
C. Others		
1. Are the lecturers knowledgeable?	4.5 (very good)	4
2. Did the support staff provide?	4.2 (very good)	3
3. Are the lecturers approachable?	4.2 (very good)	3
4. Did the lecturers make the topics interesting?	4.3 (very good)	4
5. Did the lecturers/staff provide personal attention to your questions and needs?	4.3 (very good)	3

Other comments: 'prepare a form of description for participants to answer when observing phytoplankton samples' 'the color printouts, slides and transparencies given are very useful....'
'prepare a few samples of which names are unknown to participants but known by trainers for morphological observation and critical identification'

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2.6. DETAILED PROGRAMME

28 February 1997	
09:00-10:00	Orientation of the university campus
10:00-10:45	Opening of the Training Course
	Welcome address by the Director of ANESC, UT (Dr. Manabu Tanaka)
	Introduction and mechanics of the training course (YF)
11:00-11:30	LE: International cooperation on the study of harmful microalgae (YF)
13:00-16:00	LE: Sampling strategies and design (AM)
16:00-16:30	Short presentation and introductory talks by participants (1)
16:30-17:00	Orientation on laboratory equipment and facilities
1 March	
09:00-11:00	LE: Facts of HAB (YF)
11:00-11:30	LE: Introduction on microscopy (Olympus Co.)
13:00-14:00	LE: Principals and practice of photomicrography using DIC (Olympus Co.)
14:00-15:00	TS: Photomicrography using DIC (Olympus Co.)
15:00-16:15	TS: Sampling techniques for quantitative and qualitative analysis (AM)
16:15-17:00	Short presentation and introductory talks by participants (2)
2 March	
09:00-10:00	LE: Issues and problems in HAB species identification (YF)
10:00-11:30	LE: Taxonomy and species identification of Prorocentrales (YF)
13:00-14:30	LE: Taxonomy and species identification of Dinophysiales (YF)
14:30-16:15	TS: Identification of Prorocentrales & Dinophysiales (YF, AM)
16:15-17:00	Short presentation and introductory talks by participants (3)
3 March	
09:00-11:30	LE: Taxonomy and species identification of Gymnodiniales (YF)
13:00-17:00	TS: Identification of Prorocentrales & Gymnodiniales (YF, AM)
	TS: Analysis of own samples (YF,AM)
	LE: Use of reference publications (YF,AM)
	RD: Difficulties in species identification (AM,YF)
4 March	Free day
5 March	
09:00-11:30	LE: Taxonomy and species identification of Gonyaulacales and Peridiniales (YF)
13:00-17:00	TS: Observation of thecal plate tabulation (YF, AM)
6 March	
09:00-10:30	LE: Taxonomy and species identification of Gonyaulacales and Peridiniales (YF)
10:30-11:30	RD: Difficulties in species identification (AM)
13:00-17:00	TS: Species identification (YF, AM)
7 March	
09:00-10:00	LE: Species identification of harmful dinoflagellates (YF)
10:00-11:30	TS: Species identification (YF, AM)
13:00-17:00	LE: Toxin chemistry and toxicology (MK)
	RD: Difficulties in toxicology (KM, YF)
8 March	
09:00-11:30	Report on species identification achievement by participants
13:00-15:00	RD: Future cooperation on monitoring activities
15:00-17:00	Closing of the Training Course

Lectures: Yasuwo Fukuyo (YF), Masaaki Kodama (MK), Arnulfo N. Marasigan (AM)
LE: lecture, TS: technical session, RD: Round table discussion

2.7 COUNTRY REPORTS

1. *INDONESIA* : HAB and Aquaculture in Indonesia by Ms. Hikmah Thoda

Shrimp ponds in Indonesia are currently experiencing problems on low production due to mass moralities. Shrimp processors informed us the occurrence of white spots in shrimp heads which may be due to bacterial infection. Our laboratory is responsible for water quality monitoring in shrimp ponds. Water samples we took from shrimp ponds which had shrimp kills showed the presence of *Pseudo-nitzschia, Gymnodinium, Noctiluca, Protoperidinium* and *Scrippsiella.* These findings came from samples we took from Kawal shrimp ponds.

2. *KOREA* : HAB events and researches in Korea by Ms. Hae-Ok Lee and Ms. Jae-Yeon Park

In April 1986, a poisoning incident occurred for the first time near Pusan, Korea, from the consumption of mussels, *Mytilus edulis.* Since then, HAB is considered one of the major environmental problem in Korea. Studies on HAB ecology and toxin had been pursued since then. The toxin of PSP was found in sea mussels, scallops, oysters and venus clams among the 28 species screened. PSP was detected mainly during May to June along the southern coast of Korea. The PSP toxin are produced by *Alexandrium tamarense.* Recently in 1995, *Cochlodinium* red tide occurred in the south coasts of Korea on a large scale and it continued for over 3 months. Economic loss to mariculture estimated at more than 1 million U$ resulted from mass kill of flatfish and shellfish. In 1996 during the early month of May, a poisoning case was reported near Chichundo in Chinhae Bay. So we consider HAB problem as an urgent problem to control. In Korea, clay spread on areas affected by HAB to control algal bloom wherein the clay stick to the cell walls of HAB organism which result to increased sinking rate of HAB organisms. The Ministry of Science and Technology provides financial support to HAB researches, for example, development of biological methods to control outbreak of red tide at Seoul National University and physiological and biochemical responses of toxic algal species affected by environmental stresses at Hanyang University, Seoul. Continuous culture of *Alexandrium tamarense* will be used to investigate the effects of various nitrogen and phosphorus levels and the various rate of population growth to the kinetics of PSP production.

3. *MALAYSIA* : Red Tides in Malaysia by Ms. Aileen Tan Shau-Hwai

Red Tides in Malaysia have occurred along the west coast of Sabah and Brunei Bay, since 1976. In Peninsular Malaysia, the first recorded incidence of a red tide occurrence was in Telok Kumbar, Penang in August 1978. Table 1 shows the red tide occurrences in Malaysia. Recently, in Peninsular Malaysia, the occurrence of paralytic shellfish poisoning (PSP) was reported in Malacca Straits in 1993. A second incidence in 1994, in a mussel growing area, again in Malacca, suggests a possibility of Red Tide organisms re-occurring in the area. Currently, Peninsular Malaysia has no management measures, in the form of an action plan, to overcome any adverse effects of a harmful algal bloom or Red Tide event.

University Sains Malaysia has been working on the identification of all local phytoplankton species from local waters as well as culturing the phytoplankton for food source used in mariculture as well as pollution research.

Owing to the increase occurrence of Red Tides in Malaysia, University Sains Malaysia intends to initiate monitoring programmes as well as studying Red Tide problems in Malaysia. The programmes would include studies on the dynamics of phytoplankton especially that of the dinoflagellates, the determination of the surface mixed layer, chlorophyll concentrations, nutrients such as phosphates and nitrates, plankton and benthos composition as well as identification of cysts of toxic dinoflagellates. Research which investigates the conditions which act as triggers to Red Tide blooms would also be looked into, and prediction models for Red Tides would be developed.

With our participation in IOC/WESTPAC Training Course on "Species Identification of Harmful Microalgae", Universiti Sains Malaysia aims to initiate monitoring programmes, as well as correlating the effects of the toxin of harmful microalgae in aquaculture; of course with the help and collaboration from other countries with similar interests. Special focus will be aimed at molluscs aquaculture (mussels and oysters) because these two molluscs are commercially cultured in areas which were once reported of Red Tide occurrences (Pulau Betong in Penang; and Malacca) (Table 2).
Record of Red Tide occurrence in Malaysia.

DATE	SITE	ORGANISM	IMPACT
Feb.-May '76	Kota Kinabalu, Sabah	*Pyrodinium*	202 cases food poisoning, 7 dead, mortality of aquatic life
May '80	Brunei Bay, Sabah	*Pyrodinium*	30 affected, 2 dead
Jan. '83	Gaya Island, Sabah	*Pyrodinium*	31 affected, 3 dead
Jan. '84	Gaya Island, Sabah	*Pyrodinium*	-
Sept. '85	Kimanis Bay, Sabah	*Pyrodinium*	Toxin in oysters
Dec. '87	Sabah waters	*Pyrodinium*	*Sardinella* caused death of cats
Jan. '91	Gaya Island, Sabah	*Pyrodinium*	-
Aug. '78	Telok Kumbar,Penang	*Noctiluca*	Fish kills
Nov. '78	Pulau Betong/Kuala Sungai Pinang, Penang	*Noctiluca*	-
Oct. '79	Telok Kumbar, Penang	*Noctiluca*	-
Dec. '79	Johore Straits, Johore	-	-
March '81	South China Sea, Terengganu	-	-
Mar., May, Jul., Sept., Nov. '83	Johore Straits, Johore	*Hornellia*	Fish, prawns, crab mortality
Jan-Feb '83	Johore Straits, Johore	-	prawn mortality
June '90	Sungai Dindings, Perak	-	Sores in fish, mussel mortality
Nov. '93	Sungai Sebatu, Malacca	*Alexandrium* *Gymnodinium*	6 cases food poisoning
April '94	Sungai Sebatu, Sungai Rambai, Malacca	*Alexandrium* *Gymnodinium*	-

4. *PHILIPPINES* : HAB in the Philippines by Mr. Ruel Almoneda

Among the ASEAN countries, the health and economic impacts of paralytic shellfish poisoning caused by blooms of *Pyrodinium bahamense* var. *compressum* in the Philippines is most devastating. Since 1983, more than 50 deaths had been associated with the consumption of shellfish from contaminated bays. To date, the most critical bays for HAB are Manila Bay, Carigara Bay, Zambales and Negros. During the monitoring activities done in these bays species of *Alexandrium*, *Gymnodinium*, *Dinophysis* were observed from water samples. Last February to March 1996, blooms and toxin from *Gymnodinium catenatum* in Manila Bay resulted to serious health problems and shellfish harvest was imposed. In one of our surveys along the coasts of Negros Island, the dinoflagellate *Alexandrium* was found in high densities. The detection of species other than *Pyrodinium* point to the need for upgrading of skills in species identification for fisheries personnel involved in HAB monitoring. There are indications also that HAB problems may not be limited onlyThtotoManila Bay but to the other bays in the Visayas region as well. The regular monitoring of Manila Bay, Carigara, and Negros Island had likely resulted to the decline in the reported deaths from PSP. The monitoring program in the Philippines can still be expanded and improved through continued training and acquisition of hydrographic equipments.

5. *RUSSIA* : Events of blooms in the Far Eastern seas of Russia by Ms. Tatyana Orlova

The occurrence of about 20 species of microalgae known to be toxic has been established in the last 10 years in the Peter the Great Bay (Sea of Japan) and along the eastern coasts of Kamchatka on the basis of long and short term phytoplankton observations. More than half of these species may cause water blooms especially intensive in the sea port zones of Vladivostok (Amursky Bay) and Petropavlovsk-Kamchatsky (Avachinskaya Guba Inlet), and also in the zone of Bering Sea shelf near the Korjak Mountains. The cases of water blooms caused by harmful species were especially numerous in Amursky Bay in summer and autumn 1987-1992. Red tides caused by dinoflagellates *Gymnodinium mikimotoi* (up to 1 million cells/l), *Prorocentrum minimum* (about $8x10^6$ cells/l) and representatives of raphidophytes, *Heterosigma akashiwo* and *Chattonella* spp., were observed quite often. Intensive bloom of toxic diatom *Pseudo-nitzschia multiseries* was marked in the beach zone of Vladivostok in June 1991, 1992 being maximum in the Sport Harbour - nearly 35 $x10^6$ cells/l. Widespread red tides caused by *Alexandrium tamarense* accompanied by marine animals mortality were observed in Bering Sea along north-eastern coasts of Kamchatka Bay in 1986, 1988, 1990. Representative of *Alexandrium* were the causative dinoflagellate in several local water blooms in Avachinskaya Guba Inlet at this period. Outburst of *Dinophysis* were regularly observed in Avachinskaya Guba Inlet and Amursky Bay in summer and autumn mainly of *D. acuminata* (up to 0.1 - 0.5 x 10^6 cells/l). Nevertheless, the cases of human poisoning and mortality were not marked at this period.

The main cause of origin and growth of the dangerous water blooms number is obviously hyper-eutrophication of coastal waters due to human activities but other factors and their combinations are possible.

6 *VIETNAM* : Harmful algae and red tide in Vietnam by Mr. Chu Van Thuoc

Studies on harmful algae and red tide in Vietnam had just started a few years ago. There are few information as to the presence of harmful algal species. There were few reports on algal bloom, but poisoning from shellfish consumption had not been reported. The preliminary surveys in Vietnam's coastal areas showed the presence of potential harmful algae such as: *Prorocentrum micans, P. mexicanum, P. lima, Dinophysis caudata, D. miles, Gonyaulax spinifera, G. verior, Noctiluca scintillans, Chattonella* and *Alexandrium.* Blooms of diatoms such as *Skeletonema costatum, Chaetoceros* spp., *Bacteriastrum* spp., *Asterionella* and dinoflagellates, namely, *Ceratium furca* and *C. fusus*, blue green algae, *Trichodesnium erythreaum*, had been found in North to South Vietnam. At present, a monitoring station had been establish for marine environmental quality. The study of phytoplankton is included in the monitoring. However, these activities are still preliminary in nature. The difficult problem right now is the lack of research equipment like modern microscopes and experienced staff which hamper the expansion of phytoplankton and toxin studies. In the ASEAN-Canada program for marine sciences in Vietnam, harmful and red tide study is included in the research program. This is will be the first step to the improvement of our HAB studies.

3. THIRD IOC/WESTPAC TRAINING COURSE ON SPECIES IDENTIFICATION OF HARMFUL MICROALGAE (August 1997)

3.1. BACKGROUND

The marine environment is a major source of food in WESTPAC countries and the increasing incidence of harmful and toxic algal blooms have been a major health and economic concern in the region. To date, the increasing population with its corresponding demand for food puts enormous pressure for expansion of aquaculture in coastal areas of WESTPAC countries. These therefore increases the risks of coastal eutrophication with microalgal blooms as one of its latent effects. In Southeast Asia, for example, human deaths have been reported after consumption of mussels harvested from mariculture in addition to natural ones. Restriction and banning of harvesting of bivalves brought economic dislocation to small scale shellfish growers. Red tides caused by noxious plankton often result mass mortality of fish in mariculture cages. These events due to harmful algal blooms could be mitigated, if there could be an increased awareness and concerted national and international efforts to understand the nature of harmful algal blooms. Accumulation of information and development of human resources capable of monitoring on the occurrence for harmful microalgae and toxic shellfish are fundamental.

The WESTPAC-HAB project group recognized the importance of regular training activities on biological, oceanographic and chemical aspects related to the problems and gave the highest priority to one of the biological aspects, development of identification skill and taxonomic knowledge. There have been already one workshop conducted in July 1995 and two courses, including the present one, in February and August 1997, but it is obvious that the number of skilled scientists are still not sufficient, comparing to the vast scientific fields. The WESTPAC-HAB project group members expect that the trainees attended to the Courses could assist/hold regional training activities in their home area with cooperation and assistance of WESTPAC-HAB project in order to develop and strengthen the human resources in the area.

3.2. OBJECTIVE

The objective of the Training Course was to improve the participants taxonomic skills on harmful microalgae in order to enable them to detect their occurrence and mitigate their harmful effects. The obtained training experience will be used to design and implement a monitoring programme and basic study. The training activity focused on improving related human resources as well as technology and knowledge transfer in order to develop capacity in WESTPAC countries for the management of HAB events.

3.3. PROGRAMME

The Course was organized by Dr. Yasuwo Fukuyo, Dr. Arnulfo N. Marasigan and Mr. Tumpak Sidabutar in cooperation with Dr. Shigeki Mitsumoto (WESTPAC Secretariat Office, Bangkok) and Ms. Miyuki Kuranishi (Japanese Ministry of Education, Science, Sports and Culture, Tokyo, Japan) and co-hosted by Asian Natural Environmental Science Center, the University of Tokyo, Japan. Dr. Masaaki Kodama (School of Fisheries Sciences, Kitasato University) was invited to deliver a lecture.

Since 1985 the main trainer had conducted various introductory training courses and scientific surveys in the WESTPAC region such as Malaysia, Indonesia, Thailand, Vietnam and the Philippines. Training course on species identification was the most fundamental one, but would require several kinds of resource materials such as extensive collection of publications and preserved plankton samples and adequate instruments. So at this point, Japan would be the logical choice for the course. Depending on the availability of the resources, other member countries will be able to host the training course for HAB. Thus, this may be one of the outcomes of the present training course.

The effective planning of HAB monitoring and ecological studies in developing WESTPAC countries depends on increasing manpower skills such as possession of strong fundamentals in the taxonomy and biology of HAB species and the practical aspects of sampling techniques and microscopic analyses. The topics covered in the lectures were the taxonomy and ecology of dinoflagellates and monitoring techniques. The practical work allows the trainees hands-on experience on the techniques used in HAB species identification. The complete Course Programme can be found in the section 3.6.

The Training Course is aimed at participants who have already basic knowledge of phytoplankton taxonomy and who are familiar with the use of regular compound microscopes. Among 28 applicants, 15 peoples from 9 countries were admitted in the Course. Priority was given to younger scientists who are involved in plankton monitoring. The list of participants is given in the section 3-5 with the list of lecturers.

During the lecture, technical manuals and detailed photomicrographs and line drawings of dinoflagellates were presented to the trainees to facilitate species identification. Several books such as "Red Tide Organism in Japan (Fukuyo *et al.*, 1990)" were provided to each trainee as a guide and reference during classroom sessions. The laboratory work was designed to reinforce the lectures by allowing the students to advance from basic microscopy to acquisition of skills in advanced photomicrograph techniques for DIC (Differential Interference Contrast) and epiflourescence equipment. These techniques were used by the trainees to identify species in samples from their countries. The laboratory exercises were supervised by Drs. Fukuyo, Marasigan and Sidabutar with assistance of Mr. Yoshida and Dr. Yoo. Flexibility was built into the training course by conducting consultation with the trainees after the practical sessions and the laboratory was kept open until late in the evenings for the trainees to carry on with their specific laboratory work. The trainees were given lists of references from which they chose the titles for photocopying to be taken back to their countries. A training kit was provided at the start of the Course which contained IOC-UNESCO Manual of Harmful Marine Algae, the proceedings of the 7th International Conference on Toxic Phytoplankton (Harmful and Toxic Algal Blooms), a set of photo-slides of HAB and HAB events, and color plates of dinoflagellates.

In the introductory talks by participants, the present condition of HAB occurrence and related research and study in each country was presented (section 3.7). The trainees recognized that HAB events had been occurring widely and spreading gradually and, in order to mitigate the consequence, the future close cooperation on the research and study was most important. All the participants expected the International Programme on HAB by IOC and WESTPAC-HAB programme implemented by IOC subsidiary body WESTPAC could play a leading role in the activity.

3.4. EVALUATION

Questionnaires were given to the participants at the end of the Course. The summary of the responses are shown in the following page. Phrases in comments for some question (A.6, A.7 etc.) are also listed.

	Mean Score	Lowest Score
A. Course content		
1. Are the topics of the course sufficient to meet the objectives?	4.1 (Very good)	2
2. Did the lectures meet your expectations/needs?	3.9 (Good)	2
3. Did the practicals help you acquire new techniques?	3.7 (Good)	1
4. Did the practicals make you understand/related to the lectures?	3.9 (Good)	2
5. Are the topics and practicals well organized?	3.6 (Good)	2

6. What other topics do you suggest for lectures/practicals:
   'cyst identification' 'cyst sampling techniques'
   'cyst ecology' 'electron microscope technique'
   'technic for photo-taking' 'field monitoring method'
   'microalgal culture establishment and maintenance' 'identification of diatoms'
   'toxin analysis by mouse assay and HPLC'
7. What did you learn new and useful to your work?
   'Use of microscope, especially for photo-micrographs'
   'important characteristics for species identification'
   'identification of other toxic *Alexandrium*' 'thecal plate morphology of dinoflagellates'
   'new technique for photo-taking using DIC, phase-contrast, epifluorescence'
   'new technique for observation of armored dinoflagellates using fluorescence microscopes'
8. What modifications to the course do you suggest?
   'provision of practical guide to observe important taxonomic criteria for identification'
   'field work to learn on-site techniques'
9. Will you apply the knowledge you learn from the course? How?
   'I will teach my colleague these new knowledge and techniques in our future work'
   'I will make an extention work to pass the knowledge to aquaculter farmers and fishermen'
   'I will use the knowledge to isolate specimen for identification'
   'I will clarify flora of dinoflagellate in my coastal waters'
   'I will make a data-base of HAB occurrence and educational campain'
   'I will organize local and regional seminar'
10. What specific problem do you have in your country which make HAB study difficult?
   'shortage of finance' 'lack of information'
   'lack of facilities such as microscope' 'lack of experts'
11. If you want to hold a training course, what is the location, duration and topics?
   'Japan, Denmark, USA, 1-3 months, topics should cover all aspects of HAB'
   'Japan, cyst identification, toxin analysis'
   'WESTPAC Countries' 'subjects related to ciguatera'
   'Outside Japan, because price of goods are so expensive'
   'Kota Kinabalu, 5 days, water quality analysis, toxin analysis and species identification'

	Mean Score	Lowest Score
B. Facilities and arrangements		
1. How do you rate the lecture facilities?	4.1 (Very good)	2
2. How do you rate the laboratory facilities?	3.9 (Good)	1
3. How do you rate the handouts?	3.9 (Good)	2
4. How do you rate the accommodation?	3.9 (Good)	2

Other comments:
   'In order to facilitate visa and passport application, the invitation letter should arrive earlier.'
   'Air ticket should be sent by mail, not through air line office'

	Mean Score	Lowest Score
C. Others		
1. Are the lecturers knowledgeable?	4.4 (Very good)	3
2. Did the support staff provide?	4.1 (Very good)	1
3. Are the lecturers approachable?	4.4 (Very good)	3
4. Did the lecturers make the topics interesting?	4.3 (Very good)	2
5. Did the lecturers/staff provide personal attention to your questions and needs?	4.6 (Very good)	3

3.5 LIST OF PARTICIPANTS AND LECTURERS

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3-6 DETAILED PROGRAMME

22 August 1997

09:00-10:00	Registration
10:00-10:45	Introduction and mechanics of the training course (YF)
(11:00-11:30	LE: Introduction on harmful microalgae (YF)
13:00-14:30	LE: Biology of dinoflagellates (AM)
14:45-16:00	LE: Strategy and methodology of monitoring (TS)
16:00-17:00	Short presentation and introductory talks by participants (1)
17:00-17:30	Break
	Orientation of Ueno Area (Busy market area)

23 August

09:00-11:30	LE: Taxonomy of harmful microalgae (1)(YF)
13:00-14:30	TS: Monitoring methods of harmful microalgae (AM, TS, YF)
14:45-16:00	TS: Morphological observation of harmful microalgae (YF, AM, TS)
16:00-17:00	Short presentation and introductory talks by participants (2)

24 August

09:00-11:30	LE: Taxonomy of harmful microalgae (2)(YF)
13:00-14:30	TS: Methods for species identification (YF, AM, TS)
14:45-16:00	TS: Morphological observation of *Pyrodinium* (YF, AM, TS)
16:00-17:00	Short presentation and introductory talks by participants (3)

25 August 25

09:00-11:30	LE: Taxonomy of harmful microalgae (3)(YF)
	TS: Morphological observation of *Pyrodinium* and *Alexandrium* (YF, AM, TS)
13:00-16:00	LE: Biochemistry of microalgal toxins (KM)
16:00-17:00	Short presentation and introductory talks by participants (4)
17:00-19:00	Beer Party

26 August Free day

27 August

09:00-11:30	LE: Taxonomy of harmful microalgae (4)(YF)
	TS: Morphological observation of *Prorocentrum* and *Dinophysis* (YF, AM, TS)
13:00-17:00	TS: Morphological observation of benthic dinoflagellates (YF, AM, TS)

28 August

09:00-11:30	TS: Species identification (YF, AM, TS)
13:00-17:00	TS: Species identification (YF, AM, TS)
	Round table discussion: Difficulties in species identification

29 August

09:00-11:30	TS: Species identification (YF, AM, TS)
13:00-17:00	TS: Species identification (YF, AM, TS)
	Round table discussion: Difficulties in species identification

30 August

09:00-11:30	Report on species identification achievement by participants
13:00-15:00	Round table discussion: Future cooperation on monitoring activities
15:00-15:30	Closing of the Course

Lectures: Dr. Yasuwo Fukuyo (YF), Dr. Arnulfo N. Marasigan (AM), Dr. Masaaki Kodama (MK), Dr. Tmpak Sidabutar (TS)

3.7 COUNTRY REPORTS

1. *CHINA*: The Ecology and Occurrence of Harmful Algal Blooms In the South China Sea

Chang-jiang Huang
Hydrobiological Institute, Jinan University

As same as many countries over the world, red-tides and harmful algal blooms increasingly spread around the South China Sea, especially in the embayments where fish and shrimps are intensively raised since 1980s, and most of the occurrences made a lot of economy loss. With the support of NSF of China, therefore, a major project was carried out to study the outbreak mechanisms of harmful algal blooms from 1990 to 1994.

From 1990 to 1993, over 10 embayments in SCS were investigated, which includes Dapeng Bay, Daya Bay and Shenxhen Bay. Among them, Dapeng Bay was the main location for HAB occurrence. 378 species of phytoplankton were found in these areas. Among them, the main causative species of HABs in SCS included approximately 20 species as shown in Table 1. Of them, *Alexandrium tamarense*, *Gymnodinium catenatum*, and *Chattonella marina* are toxic cyst-forming species.

From 1981 to 1994, over 146 red tides have been recorded in SCS. The accrual incidents may be more than that because of the lack of regular monitoring system. 24 blooms occurred in Dapeng Bay, SCS, during April 1990 to June 1992. The most prevalent were *Noctiluca* blooms, which accounted for 55% of blooms recorded. Toxic algal blooms were also reported in recent years. As a result of eating toxic shellfish *Mytilus viridis*, 2 people died in Daya Bay in 1992.

Besides zoo- and phyto-plankton composition and distribution, the subjects studied also included cyst composition, distribution and physiological features. For example, 33 cyst types were found in Dapeng Bay, Daya Bav and the mouth of the Pearl River, SCS. Cyst germination was successful for some species, such as *Alexandrium tamarense* and *Scrippsiella trochoidea*. For dinoflagellates in SCS, nutrient depletion is the main inducing factor in cyst formation. N-depleted and P-depleted mediums were used to induce cyst formation of *Alexandrium* and *Scrippsiella*. The results indicated that nitrogen was the main factor in inducing cyst formation.

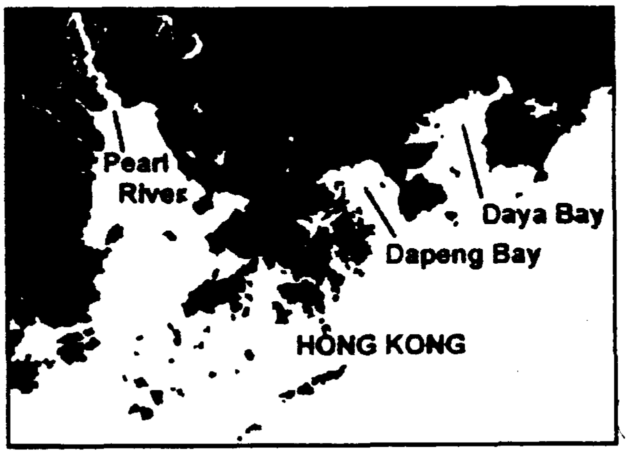

Figure 1. Important area for HAB study

Though the project covered most of fields of red-tides science, it was very difficult

Table 1 HABs causative species in South China Sea

Alexandrium catenella	*Noctiluca scintillans*	*Thalassiosira nitzschia*
Alexandrium leei	*Prorocentrum sigmoides*	*Thalassiosira rotula*
Alexandrium tamarense	*Pseudonitzschi delicatissima*	*Thalassiosira subtilis*
Chaetoceros affinis	*Pseudonitzschia pungens*	
Chattonella marina	*Rhizosolenia styliformis*	
Distephanus speculum	*Rhizosolenia alata* f. *gracillima*	
Gonyaulax polygramma	*Scrippsiella trochoidea*	
Gymnodinium catenatum	*Skeletonema costatum*	
Leptocylindrus danicus		
Nitzschia lorenzianus		

to clear the mechanism of red-tides and HAB blooms. Only the ecological results of *Noctiluca* population could explain partly why the blooms occurred so prevalently in Dapeng Bay. From 1990 to 1992, 13 *Noctiluca* blooms were recorded. The bloom is often large-scaled and may be distributed in a wide area. A *Noctiluca* bloom covered most of Dapeng Bay in 1991. A warm and windless weather seemed to be the key factors for the occurrence of *Noctiluca* blooms. And during a neap, the *Noctiluca* blooms tend to happen more easily.

Since the red-tides and HBA blooms have been causing an environmental, economical and social problem greater and greater, a new major project was supported again by NSF of China to study the mechanism of red-tides and HBA blooms occurred in the waters of aquatic farms from May of this year. The studied water areas of the new project include Bohai Bay in North China Sea and Daya Bay in South China Sea. A regular sampling including zoo- and phyto-plankton, water and sediment has been doing in a 3-days interval from June of this year, and will be extended to May of 2000.

2. *FIJI*: Fish Poisoning in Fiji - A Summary

Aisake T. Batibasaga
Ministry of Agriculture, Fisheries and Forest

Dependence on fisheries resources, as a readily available protein source in Fiji, and elsewhere in the Pacific region continues to increase, and fishing for all coastal people is a way of life. Poisoning through fish consumption or other reef resources are therefore not uncommon. There had been occasional lethal cases in the last previous years from the consumption of fish, reef crabs, hawksbill turtles, bivalves and nereid worms in Fiji.

There are reef fish species which are obligatively ciguatoxic such as puffer fishes (*Arothron* sp.), moray eels (*G. undulatus*), Maori seaperch (*Lutjanus rivulatus*) and flowery cod (*E. fuscogattatus*). The level of ciguatoxins is often related to seasonal changes, whilst other species toxicity may be restricted to certain areas of Fiji, and not being implicated in other regions.

The last reported cases of human deaths resulting from fish consumption in Fiji was in 14 December, 1996, where 4 adult males died from the consumption of yellow tail barracudas (*Sphraena forsten*) from the island of Rotuma (1230.0'S, 177'05.0'E), whilst in August 1995, 3 people (2 children: women) died from eating the many-spotted reef crab (*Zosimus aeneus* Linnaeus), and on 19 Feb 1995, 2 children died as a result of the consumption of HawkSbill turtle (*E. imbricata*) from Northern Vanua Levu (16'07.4'S, 179'57.0'W). The suspected causes of poisoning in the Hawksbill turtles from this region could be due to increased accumulation of heavy metals in turtle tissues - since the impacted area is in close proximity to an old Copper and Gold mine, which closed down from 1969, but is now being re-opened.

Fish poisoning in some regions here in Fiji have been associated with the annual rising of the nereid worm (*Eunice viridis*) from the bottom of the sea, commonly known in Fiji as "balolo", between October and November each year, after which many of the fish in the surrounding reef systems are found to be toxic. However, there is no scientific evidence associating "balolo" with fish poisoning.

In Fiji, about 5 types of fish poisoning are recognized, with more than 20 fish species implicated :

1. Poisoning by Puffer fish (Tetradon poisoning) particularly by *Arothron* species which are eaten by coastal people.
2. Poisoning by Clupeids (locally known as Daniva poisoning) - the principal implicated species is *Herklotsichthys quadrimaculatus* ('daniva').
3. Scombrid poisoning : Tuna and mackerels which often result from poor refrigeration or processing and canning.
4. Mullet poisoning : associated with or after annual rising of the Nereid worms from the sea.
5. Ciguatera poisoning : (commonly observed with reef fish species, the causative organism being the dinoflagellate *Gambierdiscus toxicus*

Ciguatera is a toxin that is present in a wide variety of fishes, but only reaches its highest and sometimes fatal concentration in carnivorous fishes at the top of the food chain. *G. toxicus* will colonise the bare surfaces of rocks, piers, corals, shipwrecks, and blades of seagrass and algae, especially after major habitat disturbances or ecological degradations - such as after cyclones and reef blastings. The dinoflagellate is eaten along with filamentous algae or seagrass by herbivorous fishes, which would in turn be eaten by carnivorous fishes such as barracudas, trevallys and emperors.

If a fish consumed is ciguatoxic, the symptoms will show up with 1-12 hours of eating. Common symptoms found, which would vary widely between different peoples, are :

i. Tingling of lips and tongues (burning sensation when in contact with water)
ii. Reversal of the sensation of hot and cold
iii. Muscular weakness and pain in joints
iv. Vomiting / nausea
v. Diarrhea
vi. Shortness of breath
vii. Cardiac arrest
viii Skin rashes or irritation
ix Memory disturbance / lapse
x. Depression
xi Paresthesia (of hands and lips)
xii Headaches and sweating
xiii. Abdominal pains

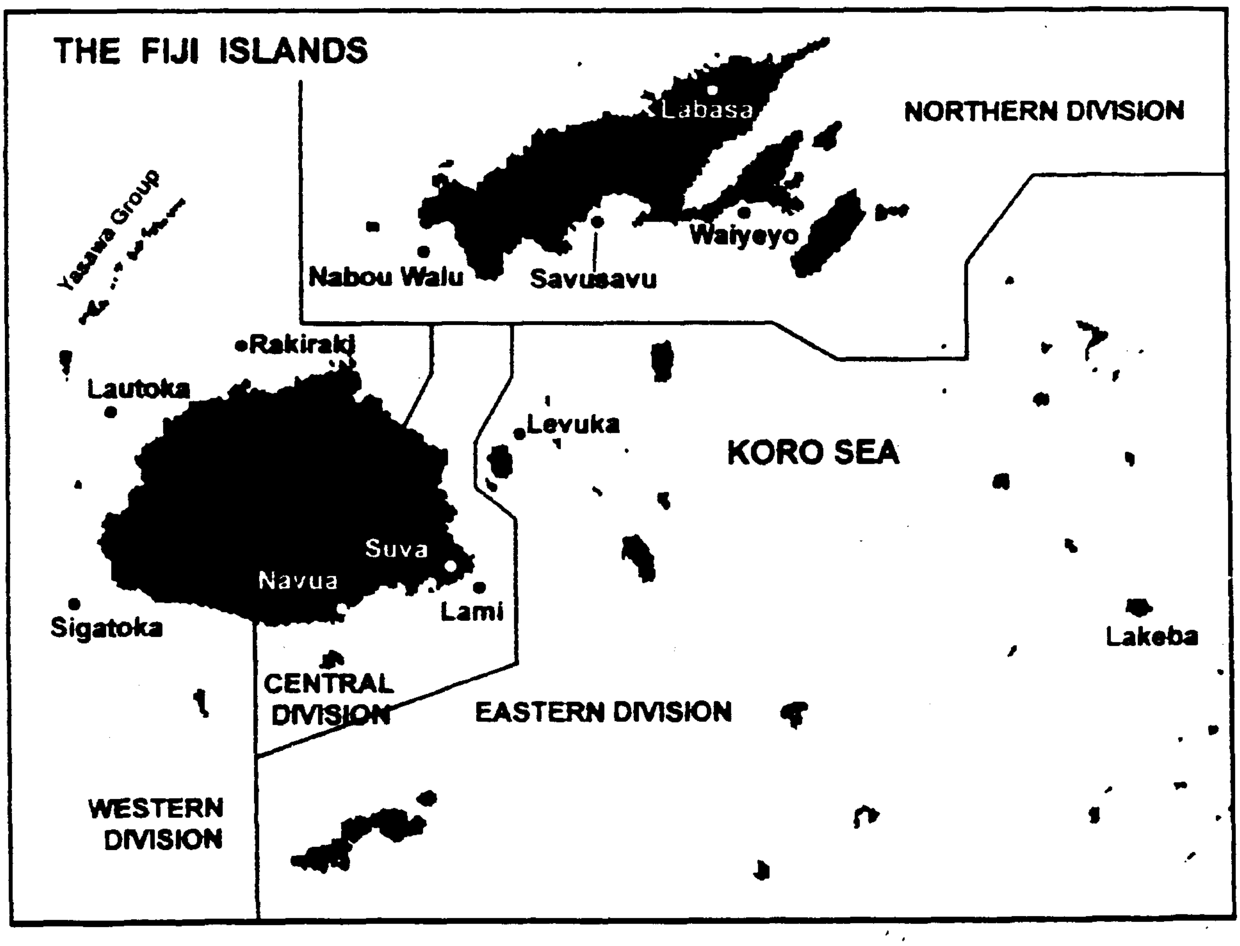

Figure 2. Fiji Islands

Fish that have been known to be ciguatoxics in Fiji are shown in Table 2. Traditionally the Red Snapper, *Lutjanus bohar* (Forskal) is the most suspected fish in Fiji, particularly towards the end of the year when many people will refuse to eat it. It is indeed banned from sale in municipal markets, and is usually not sold by fishermen. Fish of the Serranid family are highly targeted food fishes, and are moderately abundant but are not commonly implicated with ciguatoxins as they do in Tahiti.

Table 2 Ciguatoxigenic fish

COMMON NAME	SCIENTIFIC NAME	FIJIAN NAME	TIME OF THE YEAR TOXIC	REGION
Red bass	*Lutjanus bohar*	Bati	Seasonal	certain areas/ South Fiji
Blackspot snapper	*Lutjanus fulviflamma*	Tina ni Kake	seasonal, and all year round in some regions	all Fiji Waters.
Coral Trout	*Plectropomus leopardus*	Donu	seasonal	Certain parts of Fiji (Eastern)
Lyretail Trout	*Variola albimarginata*	Donu mata ni siga	all year around	all Fiji Waters
Giant/Undulated Moray Eel	*Gymnothorax javanicus* *G. Undulatus* (other morays)	Dabea	all year around, also only some areas where it is taken as food.	all Fiji Waters
Arothron pecies (Tetradonids)	*Arothron mappa*, *A. immaculatus*, and other species	Sumusumu	all year around	all Fiji Waters
Nereid worms	*Eunice viridis*	Balolo	October - Nov.	all Fiji Waters
Yellow-tail Barracuda	*Sphraena forsteiri*	Ogo	Seasonal/ warmer seasons	all Fiji Waters
Sweetlip Emperor	*Lethrinus miniatus*	Dokonivudi	all year around	Kubuna and Vatu-i-ra Passage
Giant Sweetlips	*Plectohinchus obscurum*	Dreke ni Toga	occassional	concides with 'balolo' -Eastern Division, particularly Lomaiviti Group
Yellowlip Emperor	*Lethrinus xanthochilus*	Kacika	seasonal, occasional in some areas	parts of Southern Viti Levu.
Maori Seaperch	*Lutjanus rivulatus*	Regua	seasonal, coinciding with "balolo rising"	certain areas of the Fiji Group.
Flowery cod	*Epinephelus fuscogattatus*	Delabulewa	all year around	all Fiji Waters
Herrings	*Herklotsichthys quadrimaculatus*	Daniva	all around the year	infected through-out Fiji Waters

During the past few years, it has been observed that the most toxic fish in Fiji were the Barracuda (particularly the yellowtailed *Sphyraena forsteri*), which are highly abundant schooling species.

Larger fish of a species (and larger species, especially high-level carnivores) are more likely to be toxic, and to have higher concentrations of the toxins. Toxicity of fish is not entirely dependent on species but on the association of each fish to impacted or toxic areas of a reef system .

An estimated 300-350 cases of ciguatera fish poisonings are not reported or not medically treated every year in Fiji, the majority of cases being in outer island.

An ecological survey of the presence of *G. toxicus* (and two other related unicellular algae) on benthic macroalgae were carried out through the Fiji Islands by Inoue and Raj (1985) showed that *G. toxicus* was common in all areas surveyed, suggesting the possibility of "blooms" when conditions become favorable for growth.

Sampling stations taken in this study were selected principally in or around coral reef passages, where *G. toxicus* and other dinoflagellate species were usually found higher number in other surveys, particularly for those taken in Tahiti and other islands in French Polynesia, where *G. toxicus* was first established as the causative organism for fish poisoning, in 1968.

Cases of ciguatera poisoning have been reported around the Fiji Islands ,after reef blasting incidence from the 1980s in the Eastern Fiji Group, particularly in Southern Lau; and the impacts have also been observed to increase after repeated cyclone occurrence within these areas, and a few shipwrecks around each islands (Fig. 2). Traditionally the Red Snapper, Lutjanus bohar (Forskal) is the most suspected fish in Fiji, particularly towards the end of the year when many people will refuse to eat it. It is indeed banned from sale in municipal markets, and is usually not sold by fishermen. Fish of the Serranid family are highly targetted food fishes, and are moderately abundant but are not commonly implicated with ciguatoxins as they do in Tahiti. During the past few years, it has been observed that the most toxic fish in Fiji were the Barracuda (particularly the yellowtailed *Sphyraena forsteri*), which are highly abundant schooling species.

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3. *INDONESIA*: Red Tide in Indonesia

Rose O.S.E. Mantiri
Sam Ratulangi University

In Indonesia Red Tide has not been well known by people, except for scientists and people who are interested in and concerned about this issues. Some people know from incidents happened in their areas and also people who got affected. Most people think that red tide only occur in temperate waters, and it is impossible to occur in tropical waters such as Indonesian waters. This is because of not so much information about this issues published either in magazines or newspaper.

Yet, this phenomenon has already occurred in some places of Indonesian waters since 1979 (Dahril, 1981). It might have occurred long time before but people did not know or realize about it. Seagel and Corrales (1995) reported about harmful algal bloom occurred in 13 areas in Indonesian waters such as North Aceh, Lampung, Jakarta Bay, Bali, Flores, North Sumbawa, Sepatik Island, Ujung Pandang, Kao Bay, Piru, Ambon Bay, and Biak (Fig. 3). Some sites and species are confirmed, but some are not. As you can see from the map, Indonesia is much surrounded by waters, and has many islands (about 13.000 islands with only 5 big islands). It is located in between Australia and Philippines, and very close to Malaysia and Papua New Guinea.

The first report on Red Tide in Indonesia, and particularly in western Indonesian waters, was published by Dahril (1981). He reported that there was a water discoloration occurred in the middle of September 1979 in Jakarta Bay. He took some samples at two sites, and found out that at the first site the causative organisms were *Skeletonema costatum* and *Gymnodinium* sp. Some other species were *Prorocentrum minimum*, *P. micans*, *Peridinium* sp., *Ceratium furca*, *Dinophysis* sp, and *Tintinnopsis* sp. At the second site, he also found that *Skeletonema costatum* was the dominant species mixed with Chaetoceros sp., *Tintinnopsis* sp., and *Prorocentrum micans*.

Eight years later, in the same waters (Jakarta Bay), Adnan (1989) reported that the causative organism caused a water discoloration in that waters again was *Noctiluca scintillans*. Besides this species, *Skeletonema costatum*, *Chaetoceros* sp., *Thalassiothrix* sp., *Coscinodiscus* sp., *Ceratium* sp., *Dinophysis* sp., and *Peridinium* were also found. She also reported that at the same time a mass mortality of demersal fishes and other benthic organisms were recorded from the same waters. There were some cases also occurred in the other part of western Indonesian waters as Seagel and Corrales (1995) reported. In eastern Indonesian waters, the first event recorded was in North Flores (Adnan, 1984). It was reported that in November 24, 1983, four people died and 191 got sick after eating fishes which were caught in Lewotobi in November 21. In November 27, the same species of fishes (*Sardinella* sp. and *Selaroides leptolepis*) caught 1 km from the first area made forty five people sick. *Pyrodinium bahamense* was thought to be the cause of the incidence.

A report on the occurrence of *P. bahamense* in eastern Indonesian waters was reported by Wiadnyana, et al (1996). They reported that this toxic dinoflagellate bloomed in Kao Bay at March 1994, changing the water to a brownish-red. Then few months later, in July 1994, in Ambon three children died and thirty three people showed PSP symptoms after eating shellfish *Hiatula chinensis* collected from Ambon Bay. They also reported that *P. bahamense* was detected in low concentration

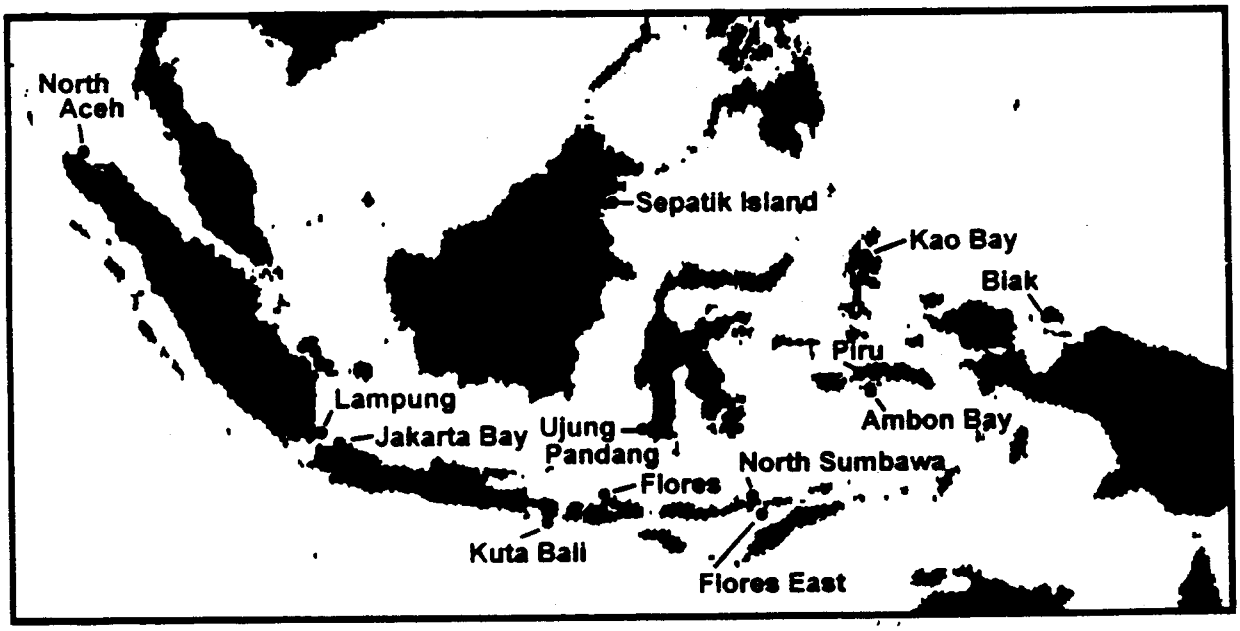

Figure 3. Location of HAB occurrence

and no PSP incidence in Ceram and North Irian Jaya. This dinoflagellate was found in areas near mangrove swamps. There were some cases also occurred in the other part of eastern Indonesian waters as Seagel and Corrdes (1995) reported. No such events has occurred in North Sulawesi waters, but some reports on phytoplankton composition in the areas were written by Mantiri (1995-1997). She reported that the organisms found could be one of the red tide species, such as *Coscinodiscus* sp., *Ceratium* sp., *Chaetoceros* sp., *Dinophysis* sp., *Peridinium* sp., *Pseudonitzschia* sp., *Skeletonema* sp., *Thalassiothrix* sp. and some others.

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4. *KOREA*: Present status of red tide in Korea

Jang, Dong-Hyuk and Kim Yeon-Jae
Han Yang University

Red tide have occurred every year in the southern coast of Korea ever since it was reported at first in 1967. Non-toxic diatom bloom was the main stream before the 1980's, but after that causative species was replaced to toxic dinoflagellates. Month of red tides occurrence becomes gradually early (Table 3, 4) and damages caused by it have increased in scale. Red tides have brought out fish kills, partly in connection with oxygen deficiency and partly with other causes like disease (Table 5, 6). Moreover poisoning episode by consuming shellfish containing algal toxins frequently occurred in Korea. Therefore studies were carried out on physiology of toxin production of red tide causative species, red tide monitoring and taxonomy of harmful algae.

On March 1986 in Pusan, scores of people were poisoned after eating mussel, two of them died. It was proved that the accident resulted from Amnesic Shellfish Poisoning (ASP). In 1987, approximately 20 persons were poisoned and two persons died after eating poisonous shellfish in Gam Chun Bay, Korea. On 16 February 1992, an illness similar to Amnesic Shellfish Poisoning (ASP) beset two persons after eating six canned smoked oysters. On 26 March I 992, symptoms similar to the 16 February attack showed both persons after eating seafood gumbo.

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Table 3. Red tide occurrence in 1995 and 1996

Year		1995	1996	Increase
Number of occurrence		123	188	65
Period (month)		5-11	2-12	
Number of causative organisms		19	23	4
	February	-	1	1
	April	-	6	6
	May	4	5	1
	June	10	23	13
	July	10	47	37
Frequency	August	10	6	-4
	September	88	96	8
	October	-	1	1
	November	1	2	1
	December	-	1	1

Table 4. Red tide organisms and their occurring season (month) in 1995

Species	Total case number	Occurrence season (month)										
		F	M	A	M	J	J	A	S	O	N	D
Gymnodinium sanguineum	23	1	-	1	1	5	10	1	-	1	2	1
Prorocentrum sp.	5	-	-	1	-	1	3	-	-	-	-	-
Pseudonitzschia pungens	2	-	-	1	-	-	-	-	1	-	-	-
Leptocylindrus danicus	3	-	-	1	-	-	-	1	1	-	-	-
Noctiluca scintillans	10	-	-	2	-	3	4	-	1	-	-	-
Heterosigma akashiwo	24	-	-	1	1	14	6	-	2	-	-	-
Eutreptiella gymnastica	3	-	-	1	2	-	-	-	-	-	-	-
Prorocentrum minimum	1	-	-	-	1	-	-	-	-	-	-	-
Skeletonema castatum	7	-	-	-	1	1	5	-	-	-	-	-
Alexandrium sp.	1	-	-	-	1	-	-	-	-	-	-	-
Prorocentrum triestinum	2	-	-	-	-	1	1	-	-	-	-	-
Ceratium furca	25	-	-	-	-	1	21	3	-	-	-	-
Prorocentrum micans	2	-	-	-	-	2	-	-	-	-	-	-
Eucampia zodiacus	2	-	-	-	-	1	-	-	1	-	-	-
Chaetoceros spp.	11	-	-	-	-	-	9	-	2	-	-	-
Gymnodinium sp.	3	-	-	-	-	-	3	-	-	-	-	-
Fibrocapsa japonica	2	-	-	-	-	-	2	-	-	-	-	-
Prorocentrum dentatum	1	-	-	-	-	-	1	-	-	-	-	-
Thalassiosira spp.	3	-	-	-	-	-	1	1	1	-	-	-
Rhizosolenia fragilissima	1	-	-	-	-	-	-	1	-	-	-	-
Cochlodinium polykrikoides	86	-	-	-	-	-	-	-	86	-	-	-
Cylindrotheca sp.	1	-	-	-	-	-	-	-	1	-	-	-
Stephanopyxis sp.	2	-	-	-	-	-	-	-	2	-	-	-
Total	220	1	0	8	7	29	66	7	98	1	2	1

Table 5 Amount of economic loss by red tides (unit: hundred million won)

Year	1990	1992	1994	1995
Amount	4	194	5	842

Chun Bay, Korea. On 16 February 1992, an illness similar to Amnesic Shellfish Poisoning (ASP) beset two persons after eating six canned smoked oysters. On 26 March I 992, symptoms similar to the 16 February attack showed both persons after eating seafood gumbo.

Studies in relation with HAB are made progress by national institute research and university in Korea. It was centered upon several issues such as prevention of red tide organism, monitoring of HAB, mechanism of toxin production and qualitative or quantitative determination on algal toxins. Investigation into the agent responsible for seafood poisoning events was carried out lively in several laboratories. Especially in the study of harmful algae need for clearing of algal toxin production is increased because that it is related with loss of lives. These studies were carried out on toxic dinoflagellate, for example *Alexandrium tamarene*. In order to make clear relation of toxicity and environmental conditions continuous culture is essential. The toxicity of the extracts was investigation of the toxicity and toxin types in organisms growing in different nutrient conditions. Also different toxin compositions of seasonal samples of *A. tamarense* in Masan Bay was analyzed with HPLC. A searching of bacterial - algal interaction on toxin production. development of nucleic acid probe is necessary in future study.

Table 6 The present condition of economic loss (unit: million won) in several districts in 1995

Area	Total		Fish		Shellfish	
	Damaged Fisheries house	amount	Damaged Fisheries house	amount	Damaged Fisheries house	amount
Yeo Su	23	537	7	335	16	202
Yeo Chun	698	10,153	432	6,369	266	3,784
Go Heung	1	3	1	3	-	-
Jang Heui	6	1,596	3	1,544	3	52
Wan Do	94	9,301	82	8,817	12	484
Total	822	21,590	522	17,068	297	4,522

5. *MALAYSIA*: Harmful algal blooms in Malaysia

Ann Anton
Universiti Malaysia Sabah

Most of the HABS in Malaysia occur off the west coast of Sabah, East Malaysia, along a 300 km stretch of coastline. The causative organism is the dinoflagellate, *Pyrodinium bahamense* var *compressum*, which has resulted in more than 300 paralytic shellfish poisoning (PSP) cases and several deaths since its first (and worst) outbreak in May 1976 (Ting 1989). Ever since then, sporadic outbreaks occur several times a year at different locations during which time shellfish become toxic even without visible blooms.

The toxic shellfish are reported from many locations, particularly at Sipitang, Kuala Penyu, Binsuluk, Kota Kinabalu and Kota Marudu, which are classified as high risk areas (Fig. 4). Various clams, oysters, cockles and mussels (including *Perna viridis*, *Anadara* spp, *Atrina* sp., *Oliva* sp. and *Donax*. spp.) have been reported to accumulate the PSP toxin. Certain fish species, viz. the sardines (*Sardinella* spp.) and the round scads (*Decapterus* spp), which are caught in abundance in the Kota Kinabalu and Papar area were also found to accumulate the toxin.

Monitoring of HABS in Malaysia is the responsibility of the Sabah State Fisheries Department, while the ban is imposed by the Health Department. The monitoring programme consists of monthly (weekly during bloom periods) shellfish and plankton sampling in several locations in the west coast. Toxin determinations of the shellfish are carried out using the mouse bioassay technique by the Fisheries Department. The purpose of the monitoring programme is mainly

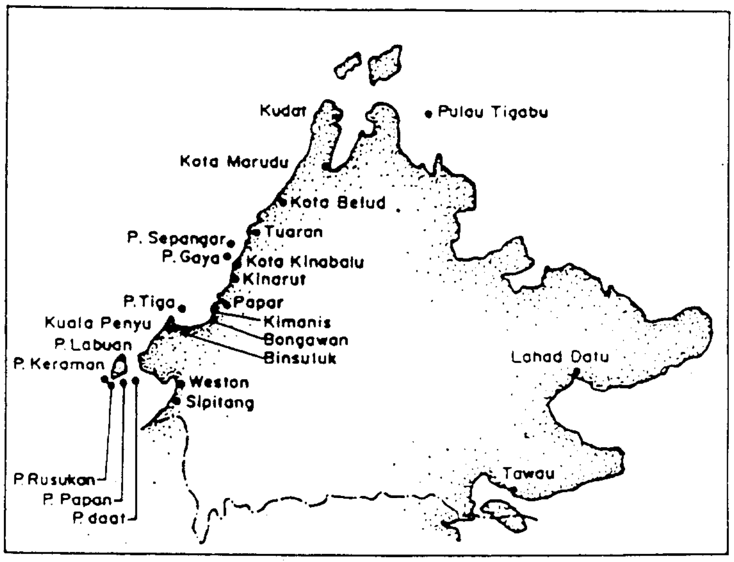

Figure 4. Geographical distribution of toxic shellfish in Sabah

to impose the ban in order to protect the consumers, as well as to prevent the aquaculture farmers from selling contaminated shellfish. No attempt has been made to determine the causal factors of the red tides in Sabah.

Up to date, very few and isolated research has been carried out on the red tides in Sabah. Usup et al (1987, 1988) reported the occurrence of *Pyrodinium* red tides in Brunei Bay, Teluk Kimanis and in Sepangar Bay, around Kota Kinabalu, besides some studies on the characterization of the PSP toxin and its properties (Usup *et al.* 1995). Presently, there are concerted efforts by a team from the Universiti Malaysia Sabah to study the ecology of the *Pyrodinium* populations in the west coast of Sabah, to correlate it to toxin levels in the sea and in the shellfish in order to determine the causal factors responsible for the bloom. It is expected that sediment analyses for *Pyrodinium* cysts will also be undertaken in order to obtain more information about the organism and to determine the frequency of previous blooms. Laboratory cultures will eventually be used to test some of the findings obtained from the Weld.

In addition to *Pyrodinium*, research on organisms causing ciguatera poisoning are also being investigated. While in Peninsular Malaysia, studies following incidences of PSP reported in the Sebatu, Melaka, have led to the identification of *Alexandlium tamiyavanichii* as the possible PSP producing organism (Anton *et al.* 1995, Anton and Mohd-Noor 1996a, 1996b). The incidence was first reported in 1993 and consequently in 1994, where 6 people were hospitalized, but no mortalities were reported. The density of *A. tamayavanichii* in the waters, however, were extremely low ranging between about 30 - 390 cells l^{-1}. The population showed seasonality with peaks in densities occurring in July and October. Further investigations, however, are needed to confirm that this species was the cause of the PSP reported. Other HAB species that were identified in this study were *Prorocentrum micans, Dinophysis caudata, Gonyaulax spinifera, Gymnodinium catenatum, Noctiluca scintillans* and *Ceratium furca*. No other occurrences have been reported since then.

In conclusion,, HAB studies in Malaysia are in its infancy. More studies are required in order to understand the ecology and biology of the causative organisms which are required for the development of strategies in the management of red tides. In addition, there should be stronger collaboration between the agencies responsible for monitoring and the researchers in order to have a full understanding of the cause and effects of the HABs. Only then can the health problems caused by red tides be under control with proper early warning signs and an efficient monitoring network be established to prevent further moralities. In addition, through well-planed educational programmes, there is a need for an awareness to the HAB issues in order that the problems associated with it be solved.

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6. *THAILAND*: Harmful Algae in Thailand

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Red tides are a well-known phenomenon which occur around the world. They are important because of their effects on fisheries, public health and the marine environment. The occurrence of a red tide is often followed by mass mortality of fish and shellfish. In some cases it is associated with poisoning of humans by shellfish. Mollusks filter toxic phytoplankton and accumulate toxins within the body. Warm-blooded animals that feed on those toxic shellfish subsequently became ill or die.

In Thailand, the red tide phenomenon has been recorded by researchers in many academic institutes, government agencies and private fisheries farms. They usually appear in coastal waters, and particularly in the inner part of the Gulf of Thailand. The phenomenon was frequently observed in the four major rivers; Bang Pakong, Chao Phraya, Tha Chin and Mae Klong. Blue-green algae (*Trichodesmium*), diatoms (*Coscinodiscus, Rhizosolenia, Skeletonema*) and dinoflagellates (*Ceratium, Gymnodinium, Noctiluca, Prorocentrum*) are the dominant species of phytoplankton found in these algal blooms (Suvapepun 1982, 1992).

The first record of a red tide in Thailand was published by Charenphol in 1952. Six years later, he reported a bloom caused by a dinoflagellate, *Noctiluca*, in the inner part of the Gulf of Thailand. Blue-green algae, *Trichodesmium*, was also observed offshore from Chumporn Province in Southern Thailand (Charenphol, 1958). At that time, mass mortality of fish was observed and anoxia was thought to be the cause of death. However this could not be confirmed since there was no data on water quality.

Data on extensive red tide phenomena were reported again in 1981 at Tha Chin estuary (Chernbamroong and Tharnbupha, 1981) and later in the inner Gulf of Thailand (Suvapepun, 1982; Suvapepun et al., 1984). However no shellfish poisoning was reported. The first case of shellfish poisoning was reported at Pranburi River, Prachuabkirikan Province on the west coast of the Gulf (Tamiyavanich *et al.*, 1985). After that occurrence intensive studies were carried out by various groups. Phytoplankton samples were collected from Pranburi river one month after the incident. It was the first time that the toxic species of marine dinoflagellate, *Alexandrium tamarense* was observed in Thailand (Fukuyo, *et al.*, 1989). Monitoring of red tide phenomena and phytoplankton revealed two potentially toxic species, *A. tamarense* and *A. cohorticula*. A unialgal culture of these two species was successfully established in the laboratory. No toxicity was found in the former species using a mouse bioassay and an LC analyzer of paralytic shellfish poisoning (PSP) (Kodama *et al.*, 1987) but the latter species was highly toxic (Kodama *et al.*, 1988). The toxic strain of *A. tamarense* which isolated from Ang Sila, Chonburi Province was reported in 1990 (Pholpunthin et al., 1990). After the occurrence of PSP in Thailand, two red tide workshops were held in Thailand to provide basic knowledge and understanding on red tide phenomena and toxic phytoplankton.

Long term studies of plankton blooms in the Gulf of Thailand were recently reviewed (Piumsomboon *et al.*, 1995). A 3-year project on diversity of marine dinoflagellates in the Gulf of

Thailand is being conducted. This project is supported by Programme for Biodiversity Research and Training (BRT), Thailand (Pholpunthin, 1997-2000). Toxic phytoplankton are kept in culture at Prince of Songkla University and Chulalongkorn University.

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7. *THAILAND*: Red Tide in Chon Buri Coastal Area

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Most of red tide occurrences in the inner Gulf of Thailand were caused by the excessive bloom of phytoplankton during the rainy season and were found near the river mouths. From many reports, the most areas affected are the east coast of the inner Gulf of Thailand which extends from Chon Buri Bay to Amphur Sattahip. The slope along the east coast is not steep and the surface current is influenced by the monsoon. There are two monsoons in the Gulf. One is the northeast monsoon which causes the direction of surface current turn anticlockwise, from the north and east coast to the southern part of the inner Gulf of Thailand, occurring in winter season from November to

January. Another is the south-west monsoon which turns the direction of surface current to clockwise, occurring in summer season from March to May, while it has started in February and passes the north of the Gulf of Thailand through Hua-Hin, Sattahip and Si-Chang Island. There is wind from southward to west in May, and goes on in September (Figure 5). There are 4 main rivers running to the inner Gulf. They are the Mae Klong River (Samutsongkram Province), the Tha-Chin River, the Chao Phraya River (Samutprakarn Province) and the Bangpakong River (Chachernsoa Province).

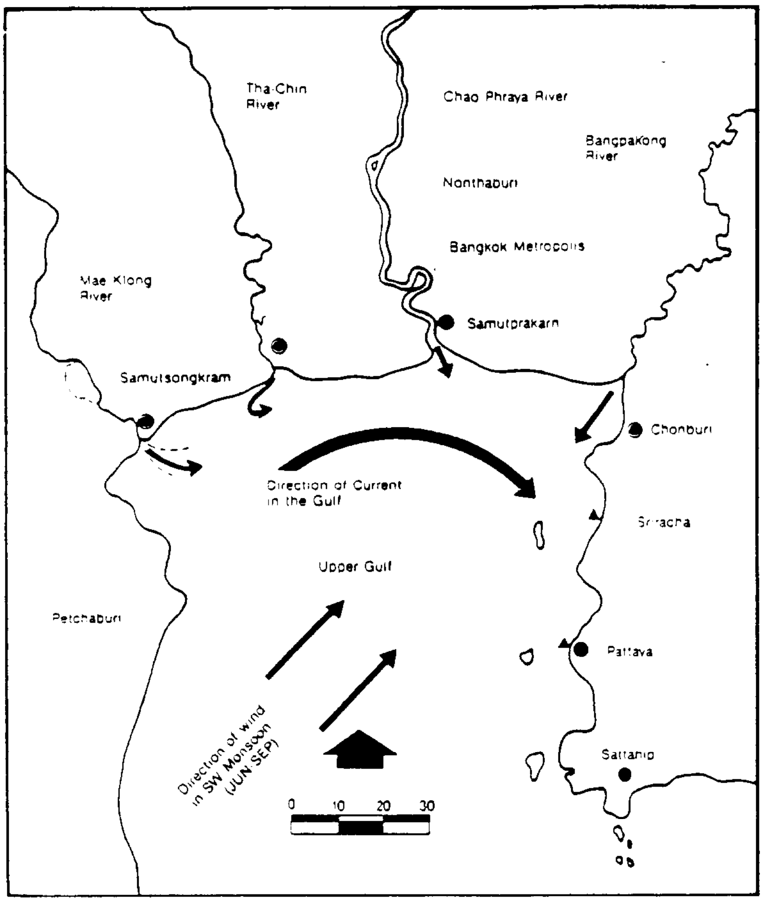

Figure 5. Inner part of the Gulf of Thailand

Chon Buri is an important province located in the East Coast of the Gulf of Thailand, which a total coastline of approximately 156 kilometers. It comprises valuable environmental resources of the area which includes beach resort, important fisheries, agricultural and industrial areas. It locates near Bangpakong estuary. These areas have influenced high river runoffs that could result in high nutrient contents in the estuarine environment, the untreated domestic wastewater were discharged into the river, especially in rainy season.

Red tide phenomena along the coastal area of Chon Buri Province had first recorded in 1985, but was not serious problem. It recently becomes apparent as a problem (Table 7). Especially two events that occurred in mid-August 1991 along the coastal area from Bang Saen Beach to Udom Bay. This was caused by a bloom of dinoflagellates, *Noctiluca* sp. and occurred again covering an area from Ang Sila to Sri Racha. The bloom of *Noctiluca* sp. on these two occasions were serious, affected the environment and people living in the area. Numerous fishes and other marine animals died, a bad smell was observed which deteriorated both seawater and the beach (Sirirattanachai and Thongra-ar, 1994 and Lirdwitayaprasit *et al.*, 1995). Recently, the bloom of *Noctiluca* sp. have been observed more frequently. They occurred many time along the coastal waters fróm Sri Racha to Mung Mai during 1992-1993, especially in August 1992, July 1993, August 1993 and October 1993, but the

Table 7 Occurrence of red tides in Chon Buri coastal area

Date	Affected Area	Causative Organisms
August 1991	BanSaen Beach to Udon Bay	*Noctiluca* sp.
July 1992	Ang Sira to Sri Racha	*Noctiluca* sp
August 1992	Sri Racha to Mung Mai	*Noctiluca* sp.
August 1992	Ang Sira	*Ceratium furca*
October 1992	Chon Buri Bay	(no specimen)
May 1993	Sri Racha	*Noctiluca* sp.
June 1993	Leam Chabang to Pataya	*Noctiluca* sp.
July 1993	Sri Racha to Mung Mai	*Noctiluca* sp.
August 1993	Sri Racha to Mung Mai	*Noctiluca* sp.
October 1993	Ang Sira	*Ceratium furca*
July 1995	Bang Saen Beach	*Noctiluca* sp.
October 1995	Bang Pra	*Ceratium furca*
July 1995	Bang Saen Beach	*Noctiluca* sp.

bloom were not serious to cause fishes and other marine organisms die. Furthermore, the bloom of *Ceratium furca* were also found along this area, especially at Ang Sila in August 1992 and October 1993 (Thongra-ar *et al.*, 1995).

In 1995, the baseline study of red tide from the Bang Pakong River Mouth to Sri Racha, Chon Buri Province was investigated during the period from July to December. The percentage composition of dinoflagellate was higher in July and October, constituting between 60-98% of the total phytoplankton populations. The presence of four genera of possible harmful dinoflagellates were found as follows: *Noctiluca, Ceratium, Dinophysis* and *Peridinium*. Among them *Noctiluca* was dominant species, followed by *Ceratium* and *Dinophysis* respectively (Thongra-ar *et al*, 1996).

In 1997, the red tide have occurred along Bang Saen Beach since early July until now which cause serious problems because of the death of fishes and other marine organisms. These occasions were serious during 4-5 July and 12 August which affected the local fishermen, aquaculturists, health and tourism. The occurrences of the red tide in Chon Buri coastal area are summarized

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8. *VIETNAM*: Harmful Algal Blooms in Vietnam

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Recent eutrophication in coastal waters by strong increasing amount of nitrate and phosphate with increasing temperature of surface layer is a dangerous phenomenon. Those conditions are all known to be contributor to blooming of harmful plankton. So that the studies on HAB not only belong to different countries in the world, but also to Vietnam. During three years (from 1995 to now) the Vietnamese Government ready to permit my Institute to create monitoring system along coast line from north to middle center of Vietnam (containing 5 stations). While harmful phytoplankton is studied as one of the parameters of sea water to evaluate quality of marine environment. We begin our studies step by step. In addition, we also have been received many helps from IOC/WESTPAC. Now there are a lot of documents, which help our identification of harmful phytoplankton species easier and we already have good opportunities to take part in some training courses. But we still lack experts in this field, so that our results are preliminary.

The study to make a list of harmful phytoplankton species in Vietnam is not complete. The blooms of phytoplankton have only reported little and their effects as shellfish poisoning, fish mortality and people death never dealt in any report.

In framework of some projects on study of phytoplankton have showed that some harmful species were discovered in Vietnam. They belong to some genera such as *Prorocentrum, Dinophysis,* *Alexandrium, Gonyaulax* and *Gymnodinium*. But their density is low (usually 10^2 - 10^3 cells/l). However, the harmful plankton monitoring in my Institute within limit. Phytoplankton samples are collected once in every three months, because of limitation of governmental budget for monitoring. So that we are very difficult to discover HAB events, which may occur in short time.

But some bloom events have occurred by diatoms such as *Pseudonitzschia* and *Chaetoceros*, dinoflagellates such as *Ceratium furca*, and blue-green alga *Trichodesmium erythraeum*. They are recorded in some reports. The causes of abundance and their effects are not studied. The study on harmful plankton species is necessary to make a complete list of harmful species and determine their effects on ecosystem.

In addition, the analysis of toxin by mouse method and cultures of some plankton species are needed to carry out on base-line to prepare for PSP monitoring programme as soon as possible.

9. *INDIA*: Occurrence of phytoplankton blooms along the Indian coast: A review

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Phytoplankton blooms mainly of non-toxic organisms are common along the east and west coasts of India and the red tide organisms reported to be responsible are *Noctiluca scintillans, Trichodesmium erythraeum, Coscinoduscus* sp, *Asterionella glacialis, Fragilaria oceanica, Ceratium* sp, *Chaetoeeros* sp. and *Rhizosolenia* sp. *Biddalphia* sp , *Skeletonema costatum* and *Thalassiosira subtilis* are known to bloom occasionally at restricted areas (Mathew et al, 1988).

Subrahmanyan (1954) provided a detailed description of the flagellate *Hornellia marina*. now confirmed to be *Chattonella marina* (Imai, 1990) and it is the only detailed account on the life history and ecology of bloom forming organism from Indian waters. Non-toxic flagellates (Hornell, I 917), *T. erythraeum* (Chacko, 1942; Chidambaram & Unny, 1944) and *Noctiluca* (Hornell, 1910; Aiyar, 1936; Bhimachar & George, 1950) have been reported to be associated with fish mortalities. Depletion of oxygen in seawater and mechanical obstructions to fish movement due to slime production during the swarming and decay of these organisms appear to be responsible for fish mortality in these cases.

Along the west coast of India, regular blooming of *N. scintillans* is observed during or immediately after the onset of monsoon while *T. erythraeum* blooms during the later part of North-East monsoon (Nair *et al*, 1992). Blooms of *N. scintillans* reported from the coastal waters of Goa (Devassy & Nair, 1987; Devassy, 1989) caused green coloration due to the presence of the flagellate *Pedinomonas noctilucae* in large numbers. Mid-water trawls during this period showed substantial fall in fish catch. Blooming of *T. subtilis, S. costatum* and *Gonyaulax* spp were observed in the coastal waters of Bombay affected by anthropogenic wastes from urban and industrial settings (Neelam *et al*, 1997).

Along the east coast, the diatom *A. glacialis* bloomed in the Rushikulya estuary (Panigrahy & Gouda, I 990) and coastal waters of Kalpakkam (Satpathy & Nair, 1995) during the summer months of April and May. In the Gopalpur estuary, this species was followed by the bloom of *Peridinium* (Gouda and Panigrahy, 1996). Diatoms were completely absent during the *N. scintillans* bloom in the coastal waters of Kalpakkam (Sargunam et al, 1989). Intertidal hard bottom communities were observed to be affected by this phenomenon resulting in disappearance of grazers like limpets. Also, experimental teak wood panels showed a decline in the settlement of cyprid larvae during this period (Sasilcumar *et al*, 1989).

Only a few reports are available on toxic red tides and resulting mortality of marine animals and shellfish poisoning in India. Incidence of Paralytic Shellfish Poisoning (PSP) and Diarrhetic Shellfish Poisoning (DSP) from the coast of Karnataka was reported by Karunasagar *et al* (1984, 1989a, b). Shrimp mortality in aquaculture ponds associated with occurrence of algal species like .*Gymnodinium* sp, *Prorocentrum* sp, *Noctiluca* sp, *Ceratium* sp, *Lyngbya* sp, *Schizothrix* sp and *Synechococcus* sp was recently reported (Karunasagar *et al*, 1997). Dinoflagellate cysts belonging to the genus *Alexandrium* and *Gymnodinium* were examined from estuarine and offshore sediments around Mangalore, SW coast of India (Godhe *et al*, 1997).

Aquaculture practices in India are fast increasing and an in-depth study on the occurrence, taxonomy, diversity, life history, ecology and physiology of bloom forming organisms along the Indian coast is essential to understand the existing situation and develop measures to counteract the possible effects (both toxic and non-toxic) resulting from the global spreading of bloom forming organisms.

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ANNEX I

Lecture Note: Sampling Strategies and Methodologies for HAB Monitoring

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Harmful algal blooms, particularly those causing paralytic shellfish poisoning, are the major concern among the Southeast Asian countries in the Western Pacific region. The increased in human activities such as industrialization and aquaculture along the coastal areas of the region have invariably led to eutrophication of the coastal waters because of drastic increase of nutrient (nitrogen, phosphorus and organic substances) input. For example, in the Philippines there are several bays which face the problem of outbreaks by *Pyrodinium bahamense* var. *compressum* and among these are Manila Bay where industrial and high urban population are located and Maqueda Bay where extensive aquaculture of green mussels are located. The inner Gulf of Thailand is plagued with the occurrence of annual bloom of *Noctiluca scintillans*. Lirdwitayaprasit *et al.* (1995) attributed this phenomena to the increase in nutrients particularly, nitrate and phosphate, from fishpond discharges around the Gulf of Thailand. On the other hand, most Southeast Asian countries rely on the marine environment for protein source and, therefore, the impacts of harmful algal bloom should be reduced to acceptable health and economic levels through a "well focused HAB monitoring programme" (Andersen, 1996). The satisfactory implementation of HAB monitoring and ecological studies usually depend on planning which include definition of goals, logistical and operational considerations. The Intergovernmental Oceanographic Commission (IOC) of UNESCO have published comprehensive and practical aspects of HAB monitoring: *Manual on Harmful Marine Microalgae* IOC Manual and Guides No. 33 (Hallegraeff *et al.*, 1995) and *Design and Implementation of some Harmful Algal Monitoring Systems* IOC technical Series No. 44 (Andersen, 1996). The present article is prepared to supplement the above two articles for participants of the IOC-WESTPAC Training Course.

1. GOALS OF THE HAB MONITORING AND ECOLOGICAL STUDIES

Monitoring programs should yield data and information that can be used by the government health and fisheries regulatory agencies which draw up contingencies such as warnings and restrictions on fishing in affected areas and consumption of fisheries products, i.e., mostly bivalves during algal blooms. Ecological studies are part and parcel of the scientific efforts of government agencies and academic institutions that lead to the understanding of the processes or dynamics of algal bloom. The types of studies may be for short or long term periods in certain coastal areas.

For discussion purposes let us cite some specific goals for certain types of activities:

(a) baseline information studies - Carry out phytoplankton and benthic microalgal samplings to prepare a listing of harmful algal species present in the bay(s) and the offshore areas. Monitor the occurrence of the species included in the Table 4 of the IOC Technical Series no.44 (Andersen, 1996) carefully. Conduct toxicity study in shellfish and fish by mouse bioassay and, if possible, by high performance liquid chromatograph (HPLC) to evaluate type and levels of toxins. Draw a map of presence-absence and distribution of toxic dinoflagellates.

(b) ecological studies – Undertake sample collection to determine phytoplankton/dinoflagellate abundance, diversity and species succession in a particular bay, cove or aquaculture area. Collect also regularly sediment samples for resting cyst analysis in order to evaluate relationship with phytoplankton distribution; simultaneously undertake hydrographic surveys to determine water quality, movements and relationships to patterns of dinoflagellate distribution and bloom.

(c) advanced study on ecological processes - Place mesocosms in selected sampling stations to determine plankton and cyst behavior such as daily vertical migration and germination of cysts *in situ*. Take water and phytoplankton samples to map out coastwise transport or determine effects of local hydrographic factors and any anthropogenic activity;

2. LOGISTICAL CONSIDERATIONS

The logistical status of HAB monitoring program and ecological studies largely depends on government funding and support. The HAB monitoring program is important for countries such as those in WESTPAC, which have a long coast line with high marine production. But except for a few areas where tragedy has occurred, most of countries have various excuses for not implementing a programme. Therefore, information network among fisheries and academic institutions is very important to collect, evaluate and disseminate HAB data. Among the institutions one or two laboratories could be the focal point for HAB studies or monitoring. Such laboratories should have basic or advanced capabilities for studies on biological, ecological or chemical aspects of HAB. Two levels of facilities could be suggested:

Level 1 - basic and modest but sufficient

(a) Plankton Sample -
qualitative analysis of HAB organisms: plankton nets, at least two pieces of net with mesh sizes 20 *um*.
quantitative analysis of HAB organisms: at least 3 units of water bottles – van Dorn, Niskin, or Nansen samplers; glass bottles are also necessary for concentrating the samples.
microscopes with photomicrograph system

(b) Hydrographic data -
salinity: salinometer or titration equipment (handy refractometer cannot be recommended, as the measurement is too rough)
temperature: mercury thermometer
location: magnetic compass
surface water movement (water current): by optical observation (putting a small piece of white paper on the surface)
nutrients such as nitrate and phosphate: spectrophotometer

(c) Toxin data -
toxins in shellfish and/or plankton: mouse bioassay (observation of symptom)

(d) Others -
refrigerator to keep samples

Level 2 - advanced configuration and sophistication based on capabilities of human resources and objectives of the laboratory; the list may therefore vary

(a) Sample collection -
boat with winch
SCUBA apparatus
water bottle samplers
plankton nets

(b) Hydrographic data -
portable GPS for station reckoning
current meter
fluorometer
light meter
temperature - salinity meter, CTD meter
auto nutrient analyzer

(c) Toxin data -
HPLC with toxin standard
microscopes -
photomicrograph system
phase contrast, differential interference contrast, epifluorescence attachment
electron microscope
sonicator - water bath type or probe type

(d) Data analyses -
computor

3. OPERATIONAL CONSIDERATIONS

The hydrography of the HAB affected area will be among the primary factors that will influence the strategies and conduct of the implementation for HAB monitoring programs. Before taking any monitoring studies, it is best to acquire a navigation chart to get insights on sediment types, water movement patterns and water depth. Conduct of interview with local fishermen can provide tentative information on water movements and climatic variations in the sampling area. Information concerning areas from where a bloom starts and terminates, or areas where toxic shellfish are collected, is also very important for determination of sampling sites.

4. SAMPLING STRATEGIES

The Phytoplankton Manual published by UNESCO (1978) provides short and concise guidelines in sampling design and data analyses which can be applied to HAB monitoring, however, for advance topics the works of Pielou (1974) and Gauch (1982) should be consulted. Prior to the design of a study or monitoring program, a concise statement of the problem or objective should be made by investigators, since this will prevent inefficiencies such as excessive number of samples which although increases accuracy, but has no ecological meaning. Once the objectives had been well defined, preliminary samplings can be done to determine whether the target phytoplankton community is homogenous in structure and composition. Phytoplankton distribution is rarely random but usually follows environmental gradients such as salinity, depth, temperature, and water movement. The sampling stations and design should be able to acquire sufficient environmental data for HAB evaluation and management purposes. The critical environmental parameters are tabulated as follows;

Physico-chemical Parameter
Vertical gradients: water and bottom sediment
temperature
salinity
nutrient – NO_3^--N, NO_2^--N, NH_4^+-N, PO_4^{---}-P, Silicate
chlorophyll *a*
dissolved oxygen
light intensity

Biological Parameters
- Phytoplankton
- Zooplankton
- Benthos
- Fish.

After preliminary sampling and identification of tentative environmental factors that affect distribution, decisions as to the placement of sampling stations can be made and a sampling design be formulated. This design can be shown to a Statistician or oceanographer for modification. On the other hand, some trade off have to be done mostly whether the sampling method and frequencies are affordable, availability of manpower and sufficiency of sampling time.

4.1 SAMPLING STATIONS – HORIZONTAL OR SPATIAL DISTRIBUTION

Franks (1995) in the IOC-UNESCO Manual on Harmful Marine Microalgae had presented in details hydrographic factors to be considered in the location of sampling stations. Sampling should be done at more than one location. Samples taken from one station only would lack information on the spatial distribution and magnitude of algal bloom. For horizontal distribution large area can be reduced for most HAB species since they are mostly found in coastal areas. However, as a rule of thumb the entire length of a line transect or grid would depend on the capability and safety of the boat used for sampling. So in the case of the Philippines where motorized banca has mostly 18 horsepower engines, usually 5 kilometers from the shoreline is considered safe during fair weather. The methods cited below are tried and tested sampling design for phytoplankton ecological studies. These methods allow the systematic or regular collection of data and can cover the study area evenly and give accurate results.

a. Line transect method - in line transect method the direction of the line runs across a particular environmental gradient, e.g., salinity, type of sediment, current or even depth. This allows samples taken from various communities and environments present. Sampling stations not less than three are placed at regular interval within a transect.

b. Grid method - this is usually applicable for mapping or spatial distribution of the target species. Sampling stations are normally placed in grids, which clarify pattern of distribution as response to environmental gradient. Efficiency is increased when a certain area of the coast or bay has been identified for recurrence of blooms.

c. Stratified sampling - not commonly used but has the advantage of both systematic and random sampling. The basic approach is to divide the study area into geographic compartments and randomly sited samples are taken from each compartment (Gauch, 1982).

d. Vertical sampling - the interval of sampling depth obviously depend on the depth and tidal fluctuations of the study area. For shallow waters less than 5 meters only surface and bottom samples would be sufficient, while deeper ones would need interval at certain depths. Depth and strongness of thermocline, salinocline and pycnocline, however, should be taken in account for determination of sampling depth in stratificated area. As in highly polluted embayments an oxygen depletion layer with high concentration of ammonium-nitrogen and phosphate-phosphorus develops near bottom, and special preparation is often necessary in order to prevent artifacts by the unusual condition.

4.2 SAMPLING PERIOD

There are coarse scale and fine scale distribution of organisms thus the sampling frequency would largely depend on the objective of the study. If, for example, vertical migration of a dinoflagellate is being studied, then a 24-36 hours continuous sampling at every 2m-interval depth should be conducted. It is also recommended that the sampling period should coincide with the tidal phases, e.g, ebb tide and high tide since water movement and flushing in small bays are largely influenced by tidal movement. On the other hand, if a bloom had occurred, a daily sampling of the species will yield patterns on movements over a certain area and the stages of the population cycle and individual cell cycle of a dominant dinoflagellate.

In conclusion, the success of a sampling design may be attributed to a well planned programme that considers the concise statement of the objective or goal and due regard to logistical and operational aspects of implementing a HAB monitoring program. Experience and 'educated guess' inputs carried out in the selection of a design would also help in making the design practical and simpler.

5. SAMPLING TECHNIQUES

5.1 FIELD SAMPLING

(a) Sample collection

The distribution of phytoplankton in coastal environments is rarely homogenous since these organisms respond to physico-chemical factors. Sampling devices used for distribu-tion determination of phytoplankton are plankton nets, water bottles or pumps. For practical purposes we will deal only with nets for quantitative purposes. Water bottles of 1-2*l* capacity are commonly used for either phytoplankton or physico-chemical analyses.

(i) Nets

Net types can vary from simple to complex ones but the most common and widely used design is the simple conical net. For HAB studies it is necessary to use a fine mesh, 20-40 *um*, nets. For a net bucket a sophisticated heavy metal one is ideal, as it is heavy (net sinks in a short time) and easy to handle. But a short plastic pipe with close end, which has a screw to attach to the net, can be the alternate. But for the latter a heavy weight must be tied to the net, but not direct to the net cloth. As fine mesh nets have low filtering rates and the disadvantage of clogging and backflow, towing speed should be slower than 50 cm/sec. A small mouth net about 20-30 cm is sufficient for HAB species collection. The procedure of the vertical haul sampling is as follows:

1. First determine the depth of the sampling area by consulting navigation chart and trial sampling using net with calibrated in meters nylon rope can be done to determine actual depth. Water current can produced slack in the rope.

2. Once the depth is determined, lower the net until the bottom is hit by the weight; do not let the net rest in the bottom since the sediments can produce clogging of the net.

3. Slowly and gently haul the net to prevent overflow and damage.

4. Once the net is on board, open the net bucket and transfer concentrated plankton in sample bottle about 200 ml capacity.

5. Close the net bucket and wash the net with seawater just enough to fill the net bucket. Pour content in the sample bottle. Do the rinsing of the net three times to get the phytoplankton that attached to the net.

6. It is advised that at least two vertical hauls be made.

(ii) Water Bottle Sampler

Several types of water bottles can be used to collect discrete and representative water sample from a given depth. The two common design are: i) reversing bottle: both ends of the water bottle are closed when a messenger or weight strikes or get in contact with the releasing mechanism and the reversing water bottle swing 180 degree and the reversing movement causes the top and bottom of the bottle, ii) non-reversing bottle - Van Dorn and Niskin bottles have the top and the bottom rubber-caps held by a clamp producing a tension of the rubber connecting this through the bottle. When the messenger hit or strike the releasing mechanism, the clamps release then rubber tension and the caps are pulled into the opening which closes the both ends of the bottle. The procedure of the water sampling is as follows:

1. Check the Van Dorn water sampler if the rubber tensioner is loose or not. Be sure the water cock is closed to prevent water leakage. If the bottle is in working condition, attached the water bottle to the rope or winch with its clamp.

2. Open both ends by first pulling one of the rubber stopper and hooking the rubber tensioner to one end of the releasing mechanism. Once this had been done, turn the bottle upside down and pull the other stopper and hook its rubber tensioner to the other end of the releasing mechanism.

3. Lower the bottle at the desired depth after which connect the messenger to the rope and release it downward.

4. After releasing the messenger, try to hold the rope in which the water sampler is attached and feel if the messenger had struck the releasing mechanism.

5. Pull up the rope to get the bottle and once on board. Place the mouth of the sample bottle (0.5 - 1.0 *l* capacity) at the tip of the stop cock. Open the stop cock to release the water content to the sample bottle. Add formalin to preserve the phytoplankton sample. Sometime the messenger slides past to the releasing mechanism if the rope is not perpendicular and at an angle due to the presence of water current.

6. repeat the above process (2-5) as necessary depending on the amount of samples needed in your study.

(b) Sample preservation

Phytoplankton in general can be preserved with either formalin, Lugol's solution or glutardehyde. However, if the target species is a naked or athecate dinoflagellate, the only way to do microscopic study is to analyze the fresh specimen without any preservative.

(i) Formalin

Formalin is the popular and commonly used preservative. It is readily available but please use only enough because formalin is a carcinogenic substance. A 2-10% concentration for the sample will be enough for preservation. In general amount of formalin should be changed depending on the amount of organizms. For a water sample with small amount of plankton, add about 2 ml of formalin to 100 ml sample at first, and after microscopic observation add about 10 ml of formalin to 100 ml sample. For net haul, add about 50-70 ml formalin or about 1/3 volume of the net concentrate.

(ii) Lugol's solution

Use the Lugol's solution only for water samples and not for net hauls, because preservation ability is limited comparing with other preservatives. Slowly add the solution to the water sample just enough to produce a weak brown color usually 0.4-0.8 ml to 200 ml sample. Shake well. Please use only clear glass bottle and avoid plastic container since this will react with iodine solution. Plankton cells are stained in dark-brown, and the color helps detection of cells under microscope even in low magnification. But the color often disappear if sample id exposed to strong light such as sun shine.

The composition of the Lugol's solution is as follows. Dissolve them to 1 liter of distilled water.
100g KI
50g iodine crystals

(iii) Glutaraldehyde

Glutardehyde have been very strong and unpleasant smell but are useful for preserving naked or athecate dinoflagellates. Samples should be analyzed as soon as possible, because preservation ability is limited comparing to formalin. Samples preserved with glutaraldehyde can also be used for SEM analyses. To a known volume of seawater sample add concentrated glutardehyde to make 2-5% solution. In order to keep the sample for rather long time for further detailed study, it is recommended to add 10 % formalin too.

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ANNEX II

Technique for the Observation of Dinoflagellate Thecal Plates

Almost all genera are classified according to thecal plate configuration (see Fig.7, 8) in armored dinoflagellates. For some species thecal plate ornamentation such as smooth, mesh and porous surface with or without spine is useful information which helps critical identification. Therefore the observation of thecal plate characteristics is inevitable for armored dinoflagellates, but the observation is sometimes very difficult because of their transparence. Modification to the regular light microscopic observation method is necessary and the methods modified are subdivided into three groups.

The first method is utilization of new style microscope that has phase contrast or differential interference contrast equipment. The attachment enhances fine difference of thecal plate condition into visible clear picture with gradation of black/white or color. However the attachments are rather expensive, sometimes more than microscope itself.

The second is staining of thecal plates. Von Stosch (1969) invented useful staining solution for thin thecal plates, but it contains strong acid that causes rust of lens. Imamura and Fukuyo (1987) modified the composition to avoid the unpleasant side effect, and now it is rather widely used because any new attachment is not necessary. The composition of the IF (Imamura and Fukuyo) staining solution and stepwise procedure are as follows;

2.6 g	Iodine
5.0 g	Potassium iodine
4.0 g	Chloral hydrate
10 ml	Distilled water

1. Prepare a full-, a half- and a quarter-strength solutions by diluting with distilled water. Each solution of 10 ml may last for several years and it is recommended to keep them in dark place.
2. Isolate a cell in question from a plankton sample by capillary pipette under microscope. To avoid contamination of other species, a single cell must be sucked carefully. (Fig 6, A)
3. Place the sucked cell in a droplet of filtered seawater on a slide glass. (Fig 6, B)
4. Place a cover slip over the droplet carefully so as not to lose the cell. If the cell has no protoplasm, proceed the step E. If the cell has protoplasm that disturb observation, thecal plates must be separated. Add a droplet of ca.5% sodium hydrochlorite solution from a margin of the cover slip. At the same time draw excess water from opposite margin to help the penetration of the solution (Fig 6, C). All the processes should be carefully done under microscope.
5. Press and crush the cell by gently pressing the cover slip with a needle (Fig 6, D). Theca may open along sutural line and turn into several fragments that contain several thecal plates. If the 5% solution is too strong, all the plates detach and analysis of thecal

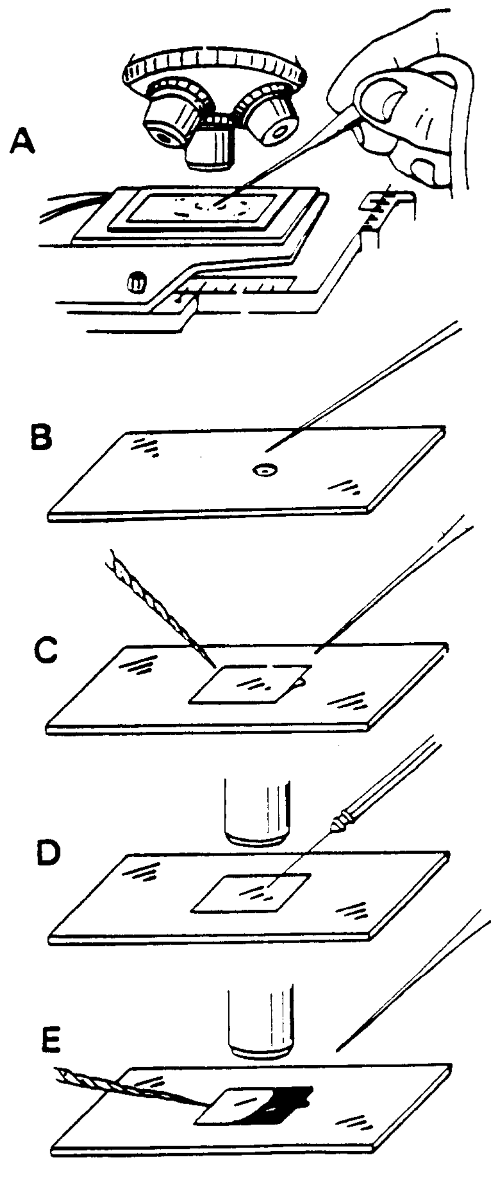

Figure 6. Procedure of staining

plate configuration becomes very difficult. Therefore the strength and amount of sodium hydrochlorite solution may be changed by experience.

6. If the taxonomic characteristics (configuration, ornamentation) of the thecal plates are not clearly observed, add a droplet of the IF staining solution from a margin of the cover slip and let it penetrate using similar technique as the step 4.
7. The stain does not last long, shorter than one day, as iodine color disappear by light. The observation and photo-taking must be finished in a short time.

If a microscope with fluorescence system is available, the observation becomes much easier. Cellufluor (Polysciences, formerly known as Calcoflour White M2R), the fluorescent stain for cellulose and other β-linked glucans, can be used to bind the thecal plates of armored dinoflagellates (Fritz and Triemer 1985). Protoplasm is not stained, therefore it does not require process of thecal plate dissection as is essential in the IF staining method. Cellufluor staining enables observation of the theca lying behind protoplasm. The only deficiency is that the fluorescence microscope is still expensive and not yet commonly used for plankton works.

1. A stock solution of 10 mg/ml of Cellufluor is made with distilled water and placed in a refrigerator until use. This stock solution is diluted with distilled water to make working solution of 0.1 - 100 μg/ml as final concentration.
2. There are two methods: the first is suitable for natural plankton samples preserved with formalin. The second is good for cells with chloroplasts such as cultured autotrophic species, as they have auto-fluorescence.

a. Simple method

This method is useful for samples that has low number of dinoflagellate. Place a droplet of water sample on a glass slide, and then put a cover slip over the droplet carefully. Add a droplet of Cellufluor solution from a margin of the cover slip. At the same time draw excess water from the opposite margin to help the dispersion of the fluorescent stain and leave for few minutes. After this, add a droplet of distilled water on one end and draw for washing at the other end in similar way as the previous process. Observe under fluorescence microscope arranged for UV excitation (wave length 340-400 nm) and thecal plates illuminate blue (w.l. 400-440 nm). Long and strong UV irradiation causes fluorescence reduction.

b. Chlorophyll extract method

Fluorescence produced vary depending on the thickness of thecal plates, and usually the plates of cultured cells are thin and weak. Moreover, cultured cells of autotrophic species have strong red auto-fluorescence even after fixation. Auto-fluorescence disturbs blue fluorescence from thecal plates. So the previous method is not applicable for cultured sample, and auto-fluorescence should be removed. Prior to the stain application, chlorophyll should be extracted by the methanol.

Cells are pipetted into 15 ml centrifuge tube and fix with 1% glutaraldehyde solution for 30 min. After the fixation, the fixed cells are collected by hand centrifuge for a few minutes and the supernatant is discarded. Cold methanol is poured into the tube with the cell-pellet and the tube is placed in a refrigerator for one night. The methanol is then replaced with 10 ml distilled water using centrifugation. About 100 μl of solution of Cellufluor staining solution is added to tube and leave for 30 min in the dark. After staining, the supernatant containing Cellufluor solution is removed using centrifugation and the pellet is centrifuged again for washing with distilled water. The pellet is resuspended in distilled water for observation. Observe under fluorescence microscope in the same manner as in the previous method.

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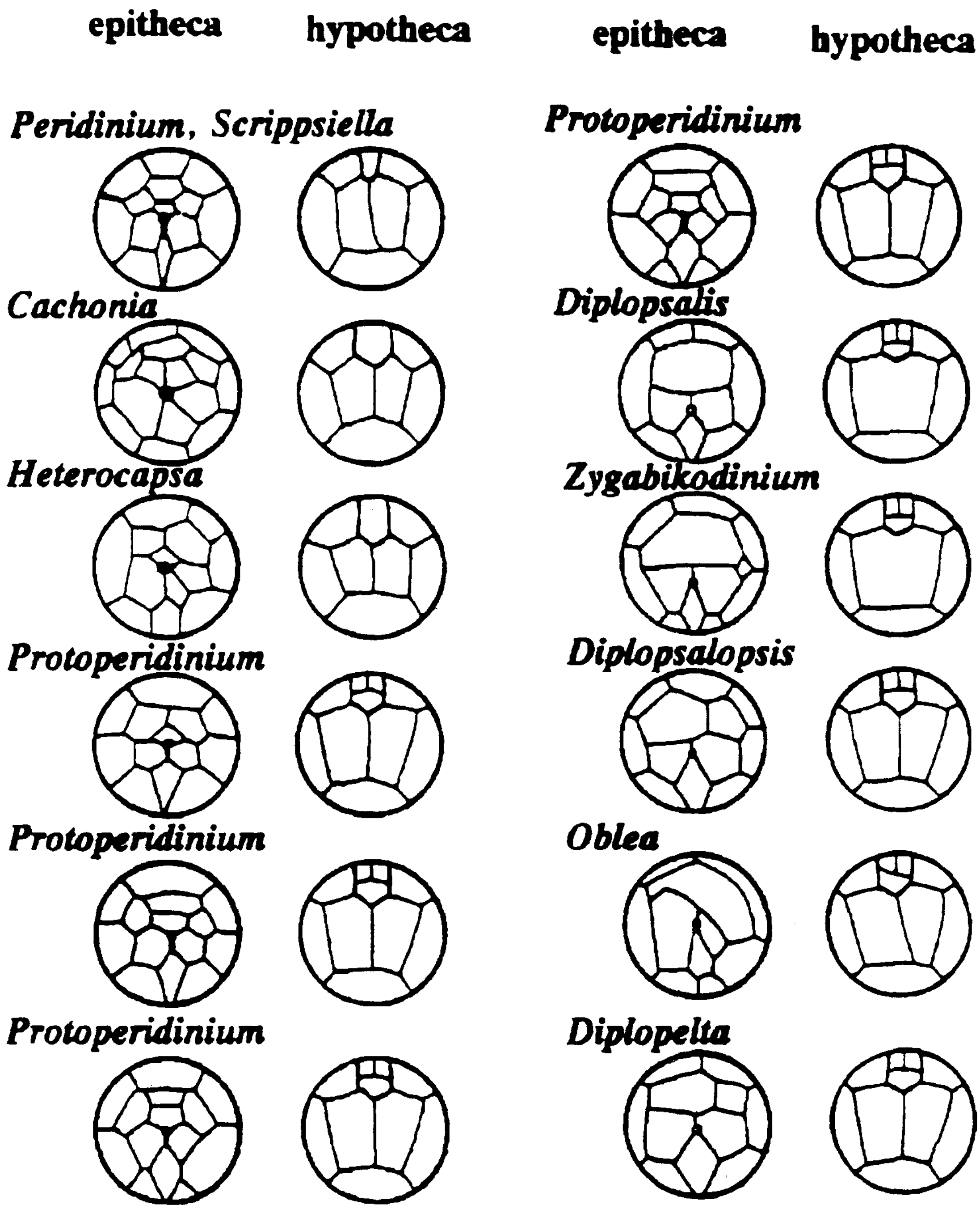

Figure 7 Thecal plate tabulation of genera belonging to the Order Peridiniales

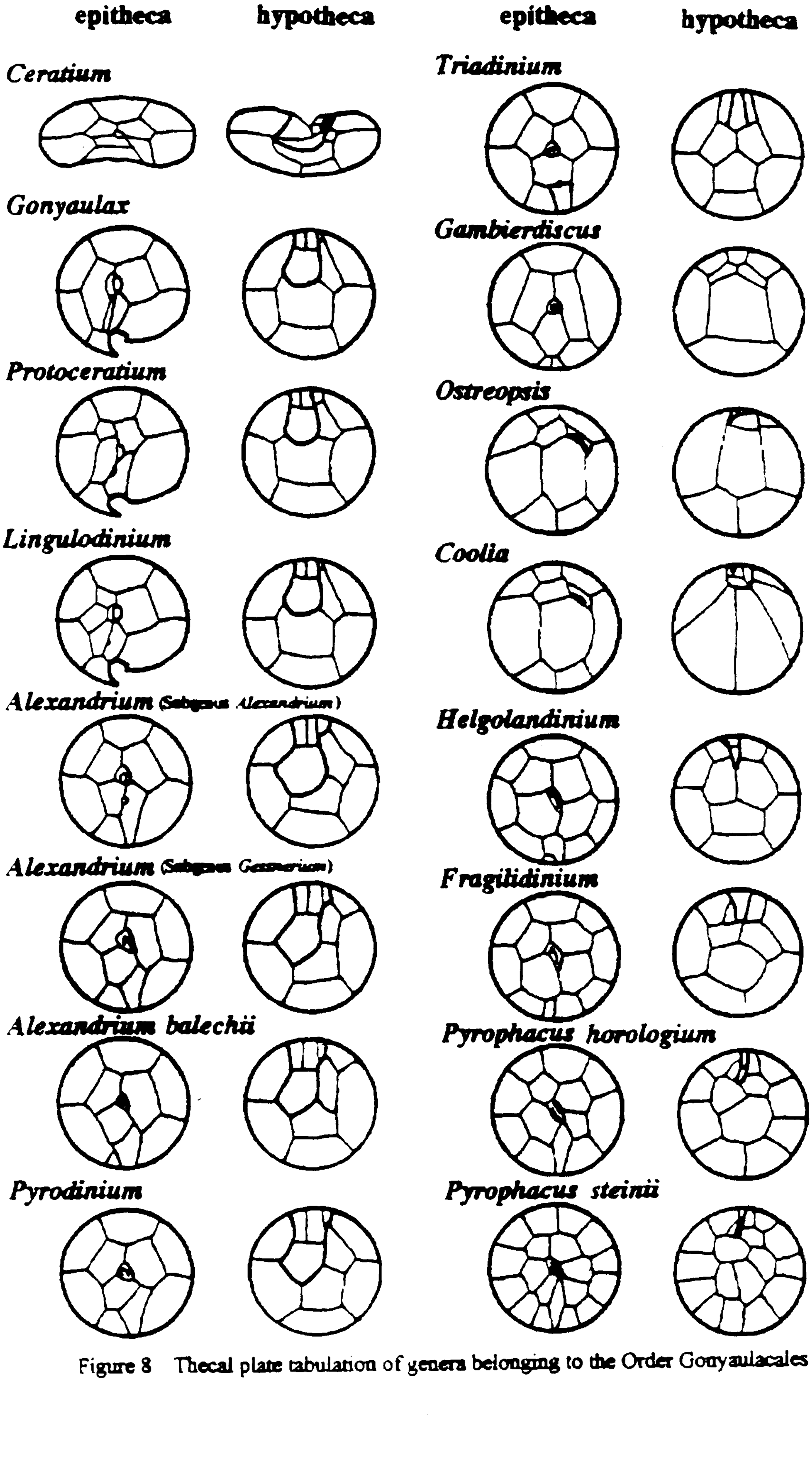

Figure 8 Thecal plate tabulation of genera belonging to the Order Gonyaulacales

ANNEX III

Form for Morphological Observation and Species Identification

1. Name of sampling area

2. Date of sampling

3. Environmental Condition

3.1	Fresh water	Brackish water	Sea water
3.2	Eutrophic area	Oligotrophic Area	Others
3.3	Temp	Salinity	
3.4	Chemical parameter (such as N,P, O_2)		

4. Condition of sample

Fresh   Preserved by

5. Morphology of dinoflagellates in doubt

5-1	Size(n>10)	Length	Width	D/V Depth
5-2	Color of cell			
5-3	Armored	Unarmored		
5-4	(For armored species)			

1. Shape and position of apical pore plate
2. Number of apical plates
3. Number of anterior intercalary plates
4. Number of precingular plates
5. Number of cingular plates
6. Number of sulcal plates
7. Number of postcingular plates
8. Number of posterior intercalary plates
9. Number of antapical plates
10. Plate formula
11. Plate configuration
12. Position of cingulum
13. Plate ornamentation (surface marking)

5-5 (For unarmored species)

1. Position of cingulum
2. Displacement of cingulum
3. Degree of Cingular Overhang
4. Position of sulcus
5. Position of nucleus
6. Ridge on cell surface
7. Special organella

6. Species name

7. Comments (useful reference for identification)