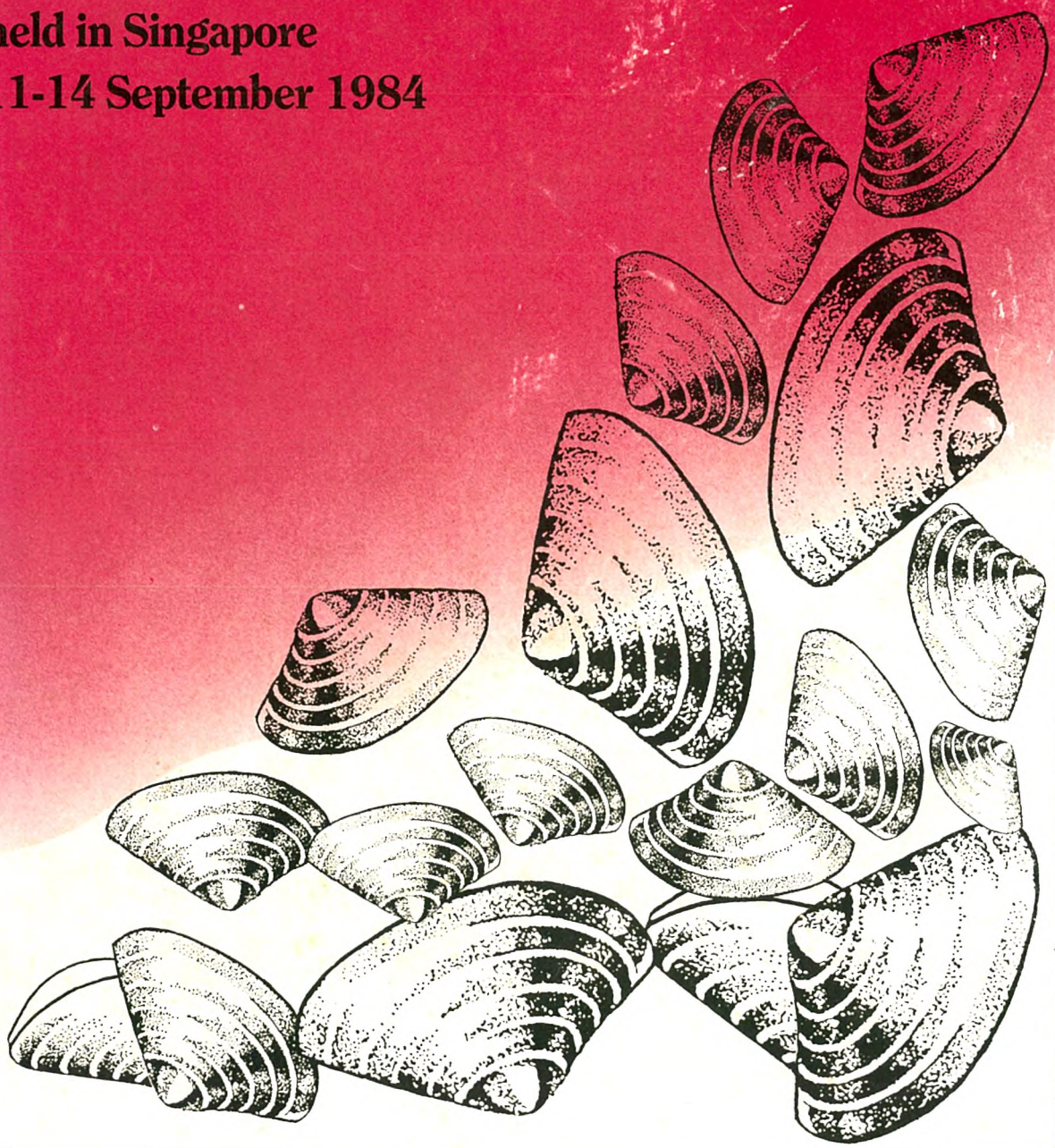
Toxic Red Tides and Shellfish Toxicity in Southeast Asia

Proceedings of a consultative meeting held in Singapore 11-14 September 1984



Toxic Red Tides and Shellfish Toxicity In Southeast Asia

TOXIC RED TIDES AND SHELLFISH TOXICITY IN SOUTHEAST ASIA

Proceedings of a consultative meeting held in Singapore 11 — 14 September 1984

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The Southeast Asian Fisheries Development Center (SEAFDEC) is a technical organisation devoted to the accelerated development of fisheries in the region. The member countries of SEAFDEC are Japan, Malaysia, Philippines, Singapore and Thailand. SEAFDEC has three Departments, namely, the Aquaculture Department in the Philippines; the Training Department in Thailand; and the Marine Fisheries Research Department in Singapore.

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Foreword

Shellfish, such as mussels, oysters, and clams, are important seafoods from the rich coastal waters of Southeast Asia. In recent years, the production of bivalves in particular has increased through the development of improved aquaculture in the region.

Certain shellfish and fish, however, can carry substances that are poisonous to humans and animals as a result of their accumulating toxins produced by a small number of species of dinoflagellates. Red tides have been associated with dinoflagellate blooms that have caused alarm among government bodies because of the grave public health and economic problems that paralytic shellfish poisoning (PSP) and associated phenomena may bring.

The Council of SEAFDEC at its sixteenth meeting in 1983 agreed that a consultative meeting be held on shellfish toxicity, the results of which should be submitted to the Council for consideration. The meeting, organized jointly by the Southeast Asian Fisheries Development Center (SEAFDEC) and the International Development Research Centre (IDRC) of Canada was held in Singapore from 11 to 14 September 1984. It was attended by government officials and researchers from SEAFDEC and ASEAN member countries as well as by SEAFDEC and IDRC staff members. The objectives of the meeting were to review the status of shellfish toxicity in Southeast Asia; discuss ways and means for the study and control of shellfish toxicity; and prepare a proposal on a multidisciplinary approach for appropriate measures to protect consumers of fishery products.

Although expertise in this specific field is limited and only a moderate amount of technical information from the region as a whole was available, the meeting was able to formulate a number of recommendations for future action. It called for the setting up of a rapid warning system within and among countries on the occurrence of PSP or other toxicity problems. One of the immediate needs is for training to be provided to upgrade skills and ensure regional consistency in approach; this could be done most appropriately by a regional program. It was noted that, in Southeast Asia, *Pyrodinium bahamense* var. *compressa* appears to be the main organism causing PSP.

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Recommendations and Conclusions

Recommendations and Conclusions

The occurrence of paralytic shellfish poisoning (PSP), usually in conjunction with visible red tides in a number of countries of the region, was documented. In a number of cases, human deaths and widespread illness were noted. Governments of the region are concerned about this alarming situation. It is apparent that one of the major causative organisms is *Pyrodinium bahamense* var. *compressa* and that it may be distributed throughout the region. At present, there seems to be no method of predicting when or where similar occurrences may take place in the region in the future. It was recommended, therefore, that the following points require immediate action by governments and concerned institutions in the region.

Regular Standard Monitoring

Most countries that have now experienced PSP problems have instituted a monitoring program. It is recommended that all countries establish a regular monitoring program including the collection of fortnightly or monthly plankton samples, sediment samples, and oceanographic data. It is also recommended that a standardized routine for this monitoring be established to allow between country/institution comparisons. This approach will be important to document the occurrence and distribution of toxic dinoflagellates in the region on a long-term basis.

Provision of appropriate technical literature for identification of the causative agent is needed. Because PSP phenomena have a bearing on fisheries development and aquaculture programs and in certain countries support for research on PSP is still limited, international organizations should assist in making the required research programs possible. Consideration should also be given to the establishment of a regional short training course in this subject to ensure a consistent approach. Cyst collection and identification from sediment samples may provide useful information on the causative organism(s), particularly where red tide or PSP problems have not yet been documented. Technical expertise from countries outside the region with experience on red tides will likely be required. It was noted, however, that it is considered important that expertise within the region in these disciplines needs to be developed as soon as possible.

Standard Bioassay

The mouse bioassay technique followed by the AOAC has been set up by a number of countries to assess the presence of toxins. Some countries within the region have used other techniques or modifications of this bioassay. It is recommended that at least one institution in all countries set up the standard mouse bioassay. Standardized toxicity testing is necessary for between country/institution comparisons. Analysis should include testing for the presence of other toxins, such as DSP, which to date has not been documented in the region.

Information Exchange

A rapid-warning system from country to country on the occurrence of PSP or other toxicity problems is recommended. Regular exchange of information between scientists and fisheries managers/administrators both within countries and between concerned countries of the region is recommended. Regional organizations such as SEAFDEC or ICLARM might cooperate with the newsletter planned by the WESTPAC task team by providing background literature, manuals, identification sheets, etc. Subsequent meetings/workshops to update the PSP status should also be considered.

Governments should develop warning and information-exchange systems for all concerned fisheries, public health, and appropriate local groups to create a clear understanding of the problem and the need for government intervention to prevent continued loss of lives.

The recommended second priority actions include the following.

Research on the Biology, Ecology, and Culture of the Causative Agent

It should be possible to isolate and culture *Pyrodinium bahamense* var. *compressa* in the laboratory. It is recommended that at least one group of researchers in the region attempt this work with appropriate safeguards against the release of the organism into coastal waters.

Research on Possible Detoxification Methods

Depuration has been tried by some groups of researchers in the region and further testing of a variety of methods should be attempted to find a possible practical and economic detoxification procedure.

Compilation and Examination of Trends in Environmental Oceanographic Data Associated with the Occurence of PSP

This is a long-term project that may provide clues concerning possible trigger mechanisms of PSP and red tides and may assist in developing capability for predicting the times and areas of likely red tide or PSP occurrence.

Medical and Public Health Considerations

Public health is of primary importance but it has been given a lower priority at this meeting as little can be done other than the banning of sale or consumption of fishery products from affected areas. Information on the recommended first-aid treatment should be widely distributed (e.g., Appendix 6). Use of traditional local remedies, such as the drinking of coconut milk and sugar, were noted. It is not clear how effective these remedies are although any remedy that voids the body of the toxic agents quickly (e.g., vomiting) is likely to be helpful.

Summaries of Discussions

Summaries of Discussions

Identification of Causal Organisms

The session opened by noting that there are few phycologists involved in the identification of dinoflagellates causing red tides. It was also noted that the literature on the systematics and taxonomy of phytoplankton in Southeast Asia is limited.

In present-day approaches, phytoplankton taxonomists use electron microscopy, a constraint for Southeast Asian workers. The participants were encouraged to identify their specimens by themselves first, before sending them to another taxonomist for identification.

The session then proceeded to discuss four subtopics: (1) confirmation of dinoflagellates involved in red tide and paralytic shellfish poisoning (PSP) in their respective areas, (2) importance of preserved materials, (3) presence of cysts in mud samples, and (4) how to get specimens identified.

Confirmation of Samples to Date

There is little doubt that the majority of cases of red tide and PSP in the region involve *Pyrodinium bahamense* var. *compressa;* in other areas, e.g., Thailand, it is still unconfirmed. Those involved in the work did their own preliminary identifications but opted to send their samples to established taxonomists for confirmation. A recent case of PSP in Thailand may have been caused by another organism but identification of the organism is unconfirmed.

It was agreed that the difficulty experienced in general taxonomic work on dinoflagellates (and even diatoms) is compounded by the nonavailability of library materials. It was also noted that there is no phytoplankton monograph for this region.

In view of a lack of expertise in taxonomy in some Southeast Asian countries, participants agreed on the need for a training program that would equip them with the basic prerequisites for taxonomic work. Participants also asked if they could be provided with a list of experts to whom their specimens could be sent for confirmation.

Importance of Preserved Materials

The significance of preserved materials was emphasized. Preservatives can keep the dinoflagellates in good condition for years, and they maintain the protoplast in its normal shape. The formula for one such preservative is as follows: 1 part formalin, 1 part glacial acetic acid, add to plankton samples at 2-3%.

The maintenance of live cultures is important not only for taxonomic work but also for more specialized studies on physiology and biochemistry of toxins. It was observed that the maintenance of pure cultures of diatoms is comparatively easier than that of dinoflagellates, having kept many unialgal cultures. There is a need for training within the region concerning isolation of the organisms, maintenance, etc.

Cysts in Mud Samples

Knowledge of the cysts of red-tide species is important. It may prove to be a key to predicting future red-tide occurrences and even PSP. At this stage, a standard method

should be adopted for uniformity and constancy of comparison. Becuase adequate separation of cysts from mud is difficult, procedures were described that may be useful. The use of a sonicator was also discussed for preliminary separation. It has one disadvantage though in that it works well with thick-walled cysts but not with thin-walled cysts.

With regard to cysts of *Pyrodinium bahamense* var. *compressa,* their existence has been noted in Steidinger's redescription of the species but there were no accompanying photographs. There are some reports of persons having seen cysts, including those in the process of germination.

Collection of Samples

Generally, samples may be obtained in two ways (1) with sampling bottles and (2) with small-mesh plankton nets. The second method has some disadvantages because the total volume of water filtered is difficult to quantify and some smaller cells may go through the mesh. For quantification or phytoplankton profile analysis, it is better to use water bottles.

It is desirable to obtain samples from several depths, appropriately spaced between the surface and bottom. The fact that *P. bahamense* var. *compressa* occurs in discrete bands was noted by several participants.

Occurrences of Red Tides/PSP

The topic was introduced with (1) an overview of areas where red-tide problems were increasing and (2) the dinoflagellates involved, such as *Gyrodinium aureolum* which is now prevalent in northwestern Europe. Hydrographic factors have proven important. Increased pollution almost certainly accounts for some of the increased incidence of red tides in some areas, although the role of pollution in other areas is not well defined.

Geographic Factors

There seems to be two situations — one involving cosmopolitan *Pyrodinium bahamense* var. *compressa* and one involving localized phenomena, as in the Thailand case. In the central Philippines, *Pyrodinium bahamense* var. *compressa* could have been carried around in the ballast of coastal vessels and in the water leaked into small fishing boats. On a larger scale, it was suggested that the dinoflagellates could have travelled in the equatorial current from Palau to the Philippines causing the 1983 red tide there. Similarly, a late 1975 bloom in Papua New Guinea might have been transported to Sabah (Malaysia) and Brunei causing the early 1976 red tides there. Finally, the role of ballast water, especially oil tankers, was discussed in terms of the possibility of transporting organisms from one area to another.

On the origin of the species in the region, it may be (1) introduced in recent years (via currents from South America, for example); (2) endemic, thus more attention is now being paid to blooms; or (3) endemic, with population size affected by long-term environmental cycles.

On the role of enrichment, one school of thought is that "coastal" red tide organisms find the concentration of metal ions too high in seawater. Bloom areas appear to be where organic nutrients in river inputs can chelate the metals, especially cooper and iron.

Seasonality

Some time was spent considering the role of regional current systems and monsoon systems, especially the effects of wind and rainfall. The evidence was ambiguous with respect to all factors.

Toxin Problems

Shellfish

PSP and DSP: Shellfish toxicity in Southeast Asia appear to be caused mainly by PSP. The problem with DSP is not widespread and was only reported in Thailand's inner gulf region. An observation was made on the similar symptoms that arise from illness caused by consuming food contaminated by certain *Vibrio* spp. and DSP. The need to collect and preserve water samples early for identification was pointed out.

Contamination: Contamination occurs in the whole shellfish, especially in the viscera area and hepatopancreas region. The case of sea snail, *Oliva* sp., contaminated by paralytic shellfish toxins in Sabah was noted with interest as this could possibly be the first record of a grazer causing PSP. It was suggested that the snails consumed the cysts of the causative dinoflagellate, *Pyrodinium bahamense* var. *compressa,* while feeding after the red tide outbreak.

Kills: There is still no evidence of direct kills of shellfish by paralytic shellfish toxins. The Sabah bloom in 1976 killed shellfish, corals, and fish probably by oxygen deficiency. In Thailand, it was noted that mussel larvae failed to settle on poles during a *Noctiluca* bloom. The possibility of observing the effect of red tide through observations on the behavioural responses of the shellfish, e.g., shell gaping and extended siphon, was mentioned.

Retention: Retention of paralytic shellfish toxins in shellfish may be as long as several months, as experienced for mussels in the Philippines. In Sabah, a 3-month retention period for oysters was reported, taking place during the rainy season with normal water exchange in the sea. An extended retention period would have serious economic repercussions for the farmer. Long-term toxin retention in shellfish in certain parts of Canada was cited. Another consideration is the ingestion of the toxic dinoflagellates in low concentrations and retention of the toxins by shellfish over long periods, as in the case of the alaskan butter clam in western Canada. In such cases, no red tides are visible in the water and yet shellfish toxicity remains. It is, therefore, useful to monitor waters and shellfish regularly, including samples from different depths.

Finfish

Ciguatera: This toxin is produced by benthic dinoflagellates found in coral areas. The fat-soluble toxin goes through the lipid fraction of the food web. A case of ciguatera poisoning due to consumption of red snapper caught in southern Sulu Sea was noted. Otherwise, the problem does not appear to be widespread in Southeast Asia.

PSP: Toxicity from shellfish is caused by paralytic shellfish toxins found in the viscera. Fish flesh is normally toxin free and safe for human consumption. So far, all bioassays of fish flesh from dying and dead fish affected by red tide have yielded negative results.

Contamination: Contamination occurs mainly in the viscera of the fish. However, the potency of the toxins is maintained when whole fish are eaten after being pickled or cooked in vinegar, as reported in the Philippines. Usually, the contaminated fish are plankton feeders, e.g., *Rastrelliger* spp., *Sardinella* spp., etc.

Kills: Fish kills may be mainly due to the secondary effect of oxygen depletion in the bloom area. However, some fish could be killed directly by the toxins, as reported for Canadian waters.

Other Marine Organisms

Examples of squid toxicity are scant. An alleged case of toxic squid from Thailand found in a consignment to Italy in March 1983 was mentioned. This resulted in the banning of all imports of seafood from Thailand by Italy although the claim could not be substantiated by Thai fisheries officials. In the Philippines, it was reported that a woman died from eating whole pickled squid during the peak of a red tide bloom. Reference was made to toxins found in squid viscera during a menhaden kill in New England, USA, at the peak of a red tide bloom there.

It is important not to view the adverse effects of toxic dinoflagellate blooms in isolation, as toxins can reverberate their effect through the food web. It was noted that the sea snail *Oliva* sp. and a brachiopod have been implicated in PSP.

Oxygen Problems

Bottom water oxygen depletion has been responsible for catastrophic kills of marine animals, for example, during *Ceratium* blooms off the coast of New Jersey (eastern United States). In the case of Sabah in 1976, *Pyrodinium bahamense* var. *compressa* bloomed and dead fish were found in the water below 10 m probably due to anoxia. In Thailand, a bloom of *Ceratium furca* in September 1983 in estuarine water caused depletion of oxygen to 2 mL/L and it was found that fish in traps were killed in 12 hours.

Because there is an impact on fisheries resources, it is necessary to establish monitoring techniques to reduce damage. The monitoring should include measurements of chemical, physical, and biological parameters.

Economic Impact Of Red Tide/PSP

The session opened with a note regarding the difficulty of putting actual figures/values on the economic impact of red tides/PSP. Although scientists usually don't get involved in this aspect, information on its effects is necessary for managers responsible for instituting projects/plans/programs.

There are two major concerns on the economic impact of red tide/PSP, namely the effects of the toxins themselves and the effects as a result of anoxia. With regard to the latter, deaths/kills of varieties of organisms have short term implications for local ecosystems and food sources.

Items discussed in relation to the first concern (toxins) included the following.

Shellfish Bans

Marketing of shellfish is definitely affected whenever shellfish bans are imposed. Bans also result in loss of jobs/unemployment for fishermen directly involved with production of shellfish. Other people/industries involved in related shellfish activities, such as marketing, processing, ice making, middlemen, and suppliers (bamboo and other materials for culture) are also affected. Unemployment becomes a liability to the government.

Bans also pose problems for international trade and discourage expansion of the local fishing industry and development in aquaculture.

Finfish

Kills of adults/larvae may have effects on the populations over the years, in certain areas, and on certain species. Fish landings may decline. An example was the decrease in herring catch from the Bay of Fundy 3-4 months after a fish kill in 1976. The settling of dead herring on the bottom of the bay caused other fish to avoid the area although it is not known by what mechanism.

Consumer Wariness

This often results in a lack of confidence not only with respect to shellfish but fish and other seafoods in general. During the early 1970s, when some contaminated shellfish from New England found their way into New York markets, bans were imposed for several weeks on almost all seafood commodities as a result of consumer misinformation and sensationalized media coverage. It was mentioned that newsclippings of 41 press releases over a period of 6 months in the Philippines gave conflicting accounts or information. Reporters themselves are often misinformed regarding what red tide/PSP really is, for example, a headline in Sabah read "R.T. virus in Sabah." In some areas where literacy is low and information is disseminated by word of mouth, confusion also results. It is more difficult to regain consumer confidence once it's lost. To correct this problem, awareness campaigns regarding red tide/PSP should be initiated, especially in Southeast Asian countries. This may be done by way of manuals, handouts, leaflets, or other conservative (nonsensational) approaches.

There was a loss of about ₱500000 (US$25000) during a 2-week ban on mussels from Maqueda and Villareal Bays in the Philippines. From mid-July 1983 to mid-March 1984, there was an estimated loss of about ₱10 million. The impact of red tide (*Noctiluca*) on shrimp farms in the inner Gulf of Thailand during May 1983 — March 1984 resulted in the reduction of normal yields of ฿30000/rai/15 days (2.5 rai = 1 ha) to ฿5000/rai/15 days. There are economic effects of red tide but actual figures are few and difficult to come up with as was already pointed out at the start of the session. Future studies and considerations should include economic impact and the effect of red tide on aquaculture activities.

A positive note on red tide/PSP is that dinoflagellate blooms are good sources of carbon to the marine food web (dinoflagellate → zooplankton → herring). Blooms of *Gonyaulax* in eastern Canada between June and September increase summer plankton productivity. Herring catches in the Bay of Fundy may be related to the intensity of dinoflagellate blooms.

The associated medical cost for people affected by poisonings was also recognized but, on the other hand, red tide liabilities may be turned into assets if benefits (pharmaceuticals) may be discovered in the future.

Corrective Measures

Surveillance and Bans

The present status of monitoring suspect areas and regulations during bans were discussed.

In Sabah, fortnightly sampling of plankton is carried out and mouse bioassays of bivalve shellfish are made at the same time. In the event of potential outbreak situations, sampling frequency is increased. There is no surveillance in other parts of Malaysia or Indonesia. In the Philippines, monthly plankton monitoring in three areas has been maintained by the Bureau of Fisheries and Aquatic Resources since the 1983 red tide there. Shellfish and finfish are examined for the presence of *Pyrodinium* in their intestines. When and if cells are found, bioassay work commences and will be carried out by the Bureau of Research Laboratory of the Philippines Department of Health. The College of Fisheries of the University of the Philippines has carried out mouse bioassays occasionally since 1980 for tetrodotoxin and preliminary investigations of PSP in crabs but these are not coordinated with the Department of Health.

In Thailand, the 1983 fatality triggered research activities by several government departments and universities, resulting in a committee of interested groups formed

under the Department of Health, Chulalongkorn University Marine Science Department, the Fisheries Department and the Department of Health are carrying out regular monitoring. Monthly plankton surveys of river mouths are made by the Marine Fisheries Division. In Singapore, fortnightly monitoring of water quality and toxin in mussels (mouse bioassay) is carried out. In Brunei, fortnightly plankton monitoring has been carried out since the first outbreak in 1976. Mouse bioassay work is carried out when *Pyrodinium* is detected in plankton samples.

Bioassay Facilities

A problem in some countries is access to standard toxin (saxitoxin) solutions. Healthy mice of the correct type are also often lacking.

In the Philippines, it has so far proven impossible to obtain government permission to import the toxin. It is felt that this can be overcome, however. Sabah and Brunei had no such difficulty. The American source of toxin solutions is Cincinnati, Ohio.

A much more concentrated powdered toxin is available but its acquisition is difficult. Obtaining sufficient mice of suitable strains does not seem to be a problem in any of the countries concerned. These are not, in general, the AOAC variety, but it was reported that there is only moderate variation in the sensitivity to toxins of the various strains.

Calibration of the mouse strain used with the standard toxin solution is necessary. It was noted that bioassay facilities may be located within health or fisheries agencies in different countries. Cooperation between the two is necessary, but often difficult. Finally, a "fly-bioassay" project appears to be nearing finalization that may reduce the need for special mice.

Detoxification Attempts

No efficient detoxification process to date is known and this is clearly an area for further research. The work done in the Philippines on detoxification of contaminated mussels by depuration using ozone was noted. The cost and time involved for the process may, however, inhibit its wider use. Detoxification efforts for shellfish in Japan and Canada using ozone have, however, been unsuccessful so far.

Depuration work in Singapore using ultraviolet sterilization concerned sanitation aspects.

Information Dissemination

Two kinds of communication and information exchange are needed — that for the public and that for researchers and managers.

It was recalled that WESTPAC (Western Pacific Group) of the Intergovernmental Oceanographic Commission (IOC) had decided to produce a quarterly newsletter for researchers, following their regional red tide workshop (Sydney, Australia, 18-20 June 1984).

Contacts between governments when outbreaks are discovered have been at an informal level and some national and regional coordination may be needed.

The types of pamphlets for public dissemination were discussed. There was debate as to whether information should be directed to the public at large or only, for example, village leaders, fisheries managers, or doctors.

Immediate Research Needs

Identification: Help from experts is needed in identifying phytoplankton. Expertise should eventually be developed within the region. External assistance is needed initially and WESTPAC is planning a 1-week training course in identification and bioassay in Thailand, March 1985. There is also a lack of useful literature on this

subject. To make toxic dinoflagellate identification easier, some fairly simple sheets are adequate for fisheries biologists. At a second level a more detailed monograph is required on regional phytoplankton by specialists.

Sampling Methods: Techniques differ between the various countries of the region. It was a general consensus that using water bottles is the most reliable for enumeration purposes. Use of a net (20 μm) is recommended for bulk collections for toxin analysis. For *Pyrodinium,* it was felt useful to carry out visual inspections by diving where a bloom is suspected, because of marked layering observed for this organism.

Culture: Culture methods are well known and should be able to be successfully applied with practice. Training may be included in the WESTPAC course referred to earlier.

Toxins: Toxin analysis is difficult but scientists elsewhere are interested in cooperation, for example, Dr. Yasumoto at Tohoku University, Sendai, Japan.

Distribution: A regional survey was suggested to ascertain the spread of suspect dinoflagellates.

Prevention: Physical processes are involved in red tides. It was pointed out that simply opening a sandbar (as in the case of the 1983 incident in Thailand) or damming an inlet (as in the case of *P. bahamense* var. *bahamense* in a Jamaican Bay) may improve conditions.

Regional Papers

Occurrences of Red Tide in Brunei Darussalam and Methods of Monitoring and Surveillance

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Introduction

Negara Brunei Darussalam is situated in the northwest of Borneo Island and lies between Sarawak to the southwest and Sabah to the northeast. It lies between latitudes 4° N and 5° 05' N and longitudes 114° 04'E and 115° 22'E.

To the northeast of Brunei Darussalam, lies Brunei Bay and Brunei River estuary. These are fringed by mangroves and form the basis of an important prawn and seasonal *Rastrelliger* fishery.

The deep channel entering the bay between Pulau Muara Besar and Tanjong Trusan and the dredged areas in the Muara Port area are relatively deep but generally the water in the inner bay area varies between 2 and 6 m. Fine sands, silts, and muds with a high organic content comprise the sediment. Salinity in the bay varies from 15–28 ppt, but this fluctuates depending on tidal activity and freshwater discharge from rivers.

The coastline fringes the South China Sea and is about 110 km long. The bottom sediments further from the coast consist of mud and sandy mud. Nearer the coast are sand, coral, and rocky patches. A 100-fathom isobath lies offshore and varies in distance from 56 km from the western end and 95 km from the eastern end of the country. Fishing in the South China Sea is largely concentrated close to the shore in open boats. A few fishermen also operate further out to sea using traps and long lines. There are also some trawlers and purse seiners that are engaged in a wider range of offshore activities than those conducted from open boats.

In terms of winds, the northwest monsoon occurs between December and March; the southwest monsoon occurs between July and October. Between these periods, variable winds blow from any direction but usually from southwest through west to north and from north through east to south.

Red Tide Occurrence in 1976

On 11 March 1976, Fisheries Department biologists were conducting unrelated work at sea when they noticed an extensive reddish-brown discolouration 4 nautical miles north-northeast of Muara Port. This was suspected as being a planktonic bloom and samples were collected. Subsequent microscopic examination of the samples showed the presence of heavy concentrations of a marine dinoflagellate that was later identified as *Pyrodinium bahamense* Plate (1906). Although red tide was unknown in Brunei at that time, its potentially toxic nature when concentrated was recognized and the Director of Medical Services was advised of the situation. A statement warning the public not to eat shellfish was broadcast over the radio and television the same evening.

On the same day, five patients sought treatment at Bandar Seri Begawan General Hospital for complaints of numbness and tingling of the lips and tongue, giddiness, bitter taste in the throat, weakness of limbs, fatigue, and tingling and numbness in the fingers and toes. The patients had earlier consumed chub mackerels (*Rastrelliger* sp.) and scads (*Selar* sp.).

News also reached Brunei that four children had died and many more people had been reported ill in Sipitang (on the eastern side of Brunei Bay), Sabah. The children died after consuming a meal of bivalve molluscs (Roy 1977). Although the news was rather sketchy, concern was expressed by the Brunei authorities over the above happenings in Brunei and Sabah. A possible link between the incidents and the appearance of large patches of red tide was suspected.

The usual way for PSP to occur in humans is through consumption of contaminated shellfish. Thus, the complaints outlined above by patients

encountered in Brunei after consuming fish seemed a bit odd. This was explained, however, by the fact that in Brunei it is usual to consume part of the gut and internal organs either with the flesh or as a separate dish with rice. *Rastrelliger* sp. is a plankton feeder and could have ingested the toxic dinoflagellates before being caught.

Once the danger was recognized, the Fisheries and Medical Departments undertook action to achieve the following objectives: (1) to inform the public on the nature and possible dangers of red tide, (2) to undertake measures to prevent further poisoning, (3) to identify the organism concerned, (4) to survey and monitor the occurrence, (5) to undertake toxicology tests on various related food items for possible contamination, and (6) to obtain further information on the phenomenon.

It was observed that the blooms were close to the surface. Their intensity was greatest between 08:30 hours and 10:30 hours. The sizes of the blooms varied; an extensive bloom measured was 9.6 km in length and 1.6 km in width (Fig. 1).

The causative organism was originally identified to be a specises of *Gonyaulax*. It was eventually identified by Dr. Karen Steidinger of the Florida Department of Natural Resources, USA, to be *Pyrodinium bahamense* Plate (1906).

Beales (1976) reported that the blooms appeared in phases as follows:

11-15 March: The initial dense blooms that originated to the northeast of Pelong Rocks were blown slowly onto the beaches by the prevailing winds.

16-19 March: New blooms appeared to the north and northeast of Pelong Rocks. These were either dispersed or blown ashore by 25 March.

5-12 April : Further blooms appeared in the area of Pelong Rocks. These slowly dispersed.

14-27 April: Visible blooms were observed in the inner part of Brunei Bay, at first covering large areas and later becoming compressed into compact and very dense patches under the influence of the complex water movement in the area.

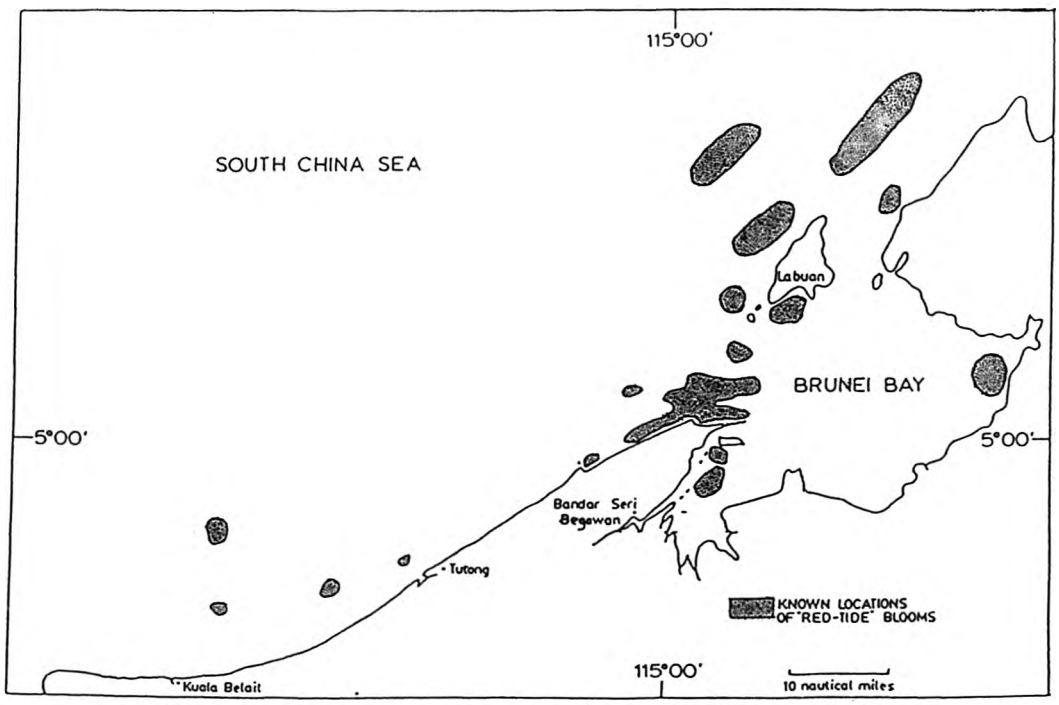

Fig. 1. Known locations of red tides (stippled) in Brunei's coastal waters and adjacent areas, 1976 red-tide occurrence (adapted from Beales 1976).

4- 6 May: Further new blooms to the north of Pelong Rocks occurred on 4 May and small patches were noted coming ashore at Pulau Punyit. These had disappeared by 6 May.

It was reported by oil-rig helicopter pilots that the bloom was very extensive, covering large parts of the water of northwest Borneo. To get an idea of the size of the bloom, it stretched from approximately Kudat in North Sabah to the west end of Brunei, a distance of more than 200 km.

The weather during the period of the occurrence was fine. The days were sunny and the seas calm. A light wind blowing from the north-northeast and becoming stronger in the afternoon was evident.

There was little evidence of large-scale fish kills, although it must be mentioned that a few hundred specimens of *Lethrinus* and *Stolephorus* spp. were found dead along the seashores and in the port area during the early phase of the red tide. At Pelong Rocks, where most of the dense blooms occurred, evidence of damage to the fish or corals during or after the event was lacking. The points of origin of the majority of the blooms were centred around the shallow areas bordering the channel entering Brunei Bay.

The red tide was last detected on 6 May 1976, but toxicology tests were continued on specimens until 21 August 1976, after which it was announced that it was safe to eat molluscs again. Since the first recorded occurrence of red tide in 1976, the Fisheries Department has continued to conduct an annual vigilance during which it advises the public to be on the lookout for red tide.

Red Tide Occurrence in 1980

Following the Fisheries Department's effort to increase public vigilance toward red tide, reports of red tide occurrence were received again on 28 April 1980. The reports that were received from various sources claim that red tide blooms were observed in the Brunei Bay area. These reports were confirmed to be correct when Fisheries Department personnel collected samples at the reported area on the same day. Preliminary examinations of the samples have indicated that the causative organism was similar to the dinoflagellate that caused the red tide in 1976.

A warning was first issued to the public on 29 April 1980 about the presence of the toxic dinoflagellates and the public was warned against consuming molluscs, especially mussels and other bivalves. Owing to this early action, there were no incidents of PSP reported in Brunei during the 1980 red-tide incident.

Plankton samples were again sent to Dr. Karen Steidinger of the Florida Department of Natural Resources for identification. The causative organism was again positively identified as *Pyrodinium bahamense* var. *compressa.*

During this occurrence, the following actions were taken to ensure that the public was protected from the dangers of the red tide: (1) continuous surveillance and monitoring of the presence of the organism responsible, (2) toxicology tests on various seafood items for possible contamination, and (3) inform the public of the dangers involved.

The 1980 blooms were less visible on the surface, being most densely concentrated around 10 m depth (Fig. 2). Observations made on 1 May showed that large quantitites of toxic dinoflagellates were present. Blooms were still reported around mid-May and daily plankton sampling showed considerable amounts of *Pyrodinium bahamense* var. *compressa* had also appeared within the confines of Muara Port and the Brunei River estuary.

There was a distinct decline in the presence of the organism around the week of 20 May in the waters off Muara and the Brunei River estuary. Reports were also received that blooms had been observed off Seria in the western end of the state. These reports, however, could not be confirmed.

On 19 June, a large bloom about 6-15 nautical miles wide and with a streaking pattern was sighted in the northern part of Brunei Bay. By 7 July, there was a decline in the abundance of toxic dinoflagellates in plankton samples and in September the Fisheries Department reduced the routine daily sampling to once a week.

Routine plankton samples collected in November and December indicated that toxic *Pyrodinium bahamense* var. *compressa* was absent in the stations sampled, whereas samples collected in January and February 1981 again contained the causative organism around Pelong Rocks. Plankton samples collected in March 1981 still showed low concentrations of *P. bahamense* var. *compressa.* This trend of the occasional and sparse presence of toxic *Pyrodinium bahamense* var. *compressa* continued until July 1981, after which the toxic dinoflagellate was absent.

Methods of Monitoring

Plankton Sampling

Plankton sampling by boat was the most widely used method of monitoring the presence of red-tide organisms. For this reason, stations were

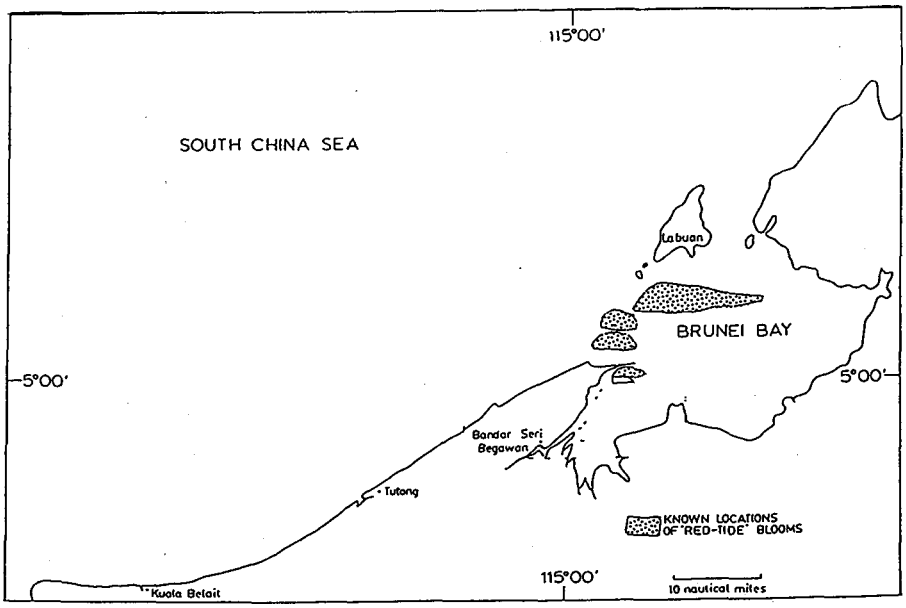

Fig. 2. Known locations of red-tides (stippled) in Brunei's coastal waters and adjacent areas, 1980 red-tide occurrence.

established at representative locations and samples were taken at these stations for comparative assessments. Quantative sampling was not possible using the equipment available. For horizontal sampling, a net was towed just below the water surface at a speed of about 3 knots for about 3-5 min. Vertical sampling was carried out at a depth of 15 m. The samples were preserved in 5% formalin and examined in a laboratory.

Because it was not possible to undertake quantitative sampling, subjective terms of "dense" or "diffuse" were utilized in cases of visible blooms and "present" or "absent" in cases where blooms were not readily visible.

Aerial Surveillance

Aerial surveillance was used extensively during both the 1976 and 1980 red-tide occurrences. At this time, state armed forces helicopters were made available for surveillance purposes, especially during the critical initial and latter phases of the occurrences. In addition to the armed forces helicopters, near-shore waters were surveyed using helicopters belonging to Brunei Shell Petroleum Limited and fixed-wing aircraft of the state airline, Royal Brunei, on their normal routes. Observations were also obtained from these sources.

For aerial surveys, the best height for observation was found to be around 333 m. At this height, a wider area can be viewed. The best time for observing the bloom is between 08:30 and 10:30 hours because these are the times during which the blooms are nearest the surface and most intense. Polarizing sunglasses were found to be useful for making visual observations and polarizing filters aided in recording the blooms on colour film.

Toxicology Tests

In view of the associated toxic nature of red tides, toxicological tests were carried out to ascertain the effect of red-tides on fish and shellfish. In the initial stage of the 1976 red tide occurrence, the samples were sent to Singapore for testing by the Department of Pharmacology of the Univesity of Singapore. In the meantime, a mouse bioassay unit was established at the Brunei Medical Department to undertake the investigations during the latter part of the occurrence. For the 1980 occurrence, toxicological investigations were carried out by the Fisheries Department.

The method utilized involved intraperitoneal injection of supernatant extracted from suspected fish and shellfish samples into healthy mice. The time of death was then recorded and converted into toxin content with the aid of a conversion factor obtained with standard solutions of paralytic shellfish poison. Paralytic shellfish poison standard solution was obtained from the Division of Criteria and Standards, Environmental Control Administration, Rockville, MD, USA. The method is described in detail in Horwitz (1970).

Table 1 provides a summary of the 23 samples found toxic during the 1976 red-tide occurrence. Table 2 provides a summary of the results of the

bioassays carried out during the 1980 red-tide occurrence. The locations from which the specimens were collected are shown in Figs. 3 and 4. Values greater than 80 μg/100 g flesh are considered harmful. This level of toxin has been referred to by Clem (1979) and Hurst (1979).

Discussion

The first occurrence of red tide in 1976 overwhelmed the tiny state. During the first week, a total of 52578 kg of seafoods, with a retail value of approximately B$198000, were seized from the markets and condemned. Swimming on beaches exposed to the South China Sea was also banned. These actions, however, could have been responsible for the low incidence of PSP. Only 14 cases of PSP were recorded during this occurrence.

More information was available for the red tide occurrence in 1980. As a result, only the consumption of molluscs, especially bivalves, was advised against.

It is worth mentioning that there were several significant differences in the features of the red tides that occurred in 1976 and 1980. Whereas the 1976 red tide manifested itself very prominently in streaks, the 1980 occurrence was much more diffused and in most instances the organisms were not visually evident even though they were present in the samples. The earlier red tide was observed to be concentrated on the surface, whereas the 1980 red tide was evident much deeper and in some instances was detected only on vertical plankton samples taken from a depth of 15 m. They were found to be most dense at about 10 m below the surface of the water. In most of these instances, the causative organism was not detected in horizontal tows. Even though no quantative samplings were undertaken during either of the 1976 or 1980 occurrences, it was evident that the 1976 occurrence was more widespread and dense than the latter. During the first occurrence, the weather was fine and the seas were calm. The reverse held true for the 1980 incident, however, with the days being rainy and overcast and the seas rough.

Aerial surveys proved to be an essential part of the monitoring and surveillance of the visible blooms of the red tide. With this type of surveillance, a wider area could be covered, thus facilitating the reporting procedure. In addition, the shape and extent of the bloom can be recorded more accurately. Furthermore, this type of surveillance is quicker than surveillance by boat but has the disadvantage of being expensive as well as presenting difficulties in the collection of samples.

Both methods of surveillance have their advantages and disadvantages but utilized in conjunction with each other they have proven to be very useful. During the initial and latter phases of the occurrences, it is important to ensure that reports of its existence or absence be accurately confirmed. Thus, when a report was received, both helicopters and boats were dispatched to the general area. As soon as the bloom was sighted from the helicopter, a flare was dropped to mark the exact location for the benefit of the people in the boat. This in itself reduces the search time and provides more time for sampling other locations.

Toxicological tests were conducted on various seafood items but efforts were concentrated on species that have been implicated to accumulate toxins or thought to cause PSP.

Toxicology tests involving intraperitoneal injection of mice have provided good indicators of the toxin contents of the seafoods sampled. However, the following disadvantages to this method (Taylor and Seliger 1979) should be recognized: (1) the need to maintain a mouse colony, (2) mice must be in the 19-21 g weight range, (3) death time is subjective, (4) limits of sensitivity are dependent on mouse strain, and (5) assays are not linear (death time vs toxic level).

Although Brunei Darussalam does not have a mollusc industry to protect, the appearance of red tides in 1976 and 1980 has introduced a new limiting factor to the adoption of mollusc culture. Past investigations into the culture of *Perna viridis* have been encouraging.

The authors thank the air force of the Royal Brunei Armed Forces for the considerable assistance rendered in carrying out aerial monitoring surveys. Thanks are also extended to Dr. Karen A. Steidinger of the Florida Department of Natural Resources for identifying *Pyrodinium bahamense* var. *compressa*.

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Table 1. Summary of toxic samples; red-tide occurrence 1976 (adapted from Beales 1976).

Date	Sample	Location	Toxicity MU/100 g meat
12 March	*Rastrelliger* sp. (Rumahan)[a]	Inner Bay	99
12 March	*Sardinella* sp. (Tamban)	Inner Bay	193
29 March	Gastropod (Tekuyong)	Sg. Bangau	71
29 March	Gastropod (Tekuyong)	Pulau Kitang	663
29 March	Gastropod (Tekuyong)	Tanjong Batu	876
29 March	Gastropod (Tekuyong)	Pulau Pepatan	296
29 March	Lamellibranch (Teritip)[b]	Pulau Chermin	589
29 March	Crab (Ketam)	Sg. Raya	339
29 March	Lamellibranch (Biluyan)[c]	Kg. Masjid Lama	847
29 March	Lamellibranch (Tiram)[d]	Kg. Masjid Lama	57
29 March	Lamellibranch (Karakas)	Kg. Masjid Lama	293
27 April	Lamellibranch (Kunau)[c]	Pulau Bedukang	436
27 April	Lamellibranch (Kunau)	Pulau Muara Besar	218
27 April	Penaeid Prawn (Udang)	Inner Bay	190
1 May	*Rastrelliger* sp. (Rumahan)	Inner Bay	478
2 May	*Rastrelliger* sp. (Rumahan)	Inner Bay	314
2 May	Lamellibranch (Tiram)	Pulau Chermin	1351
2 May	Lamellibranch (Teritip)	Sg. Teritip	864
6 May	Lamellibranch (Teritip)	Sg. Teritip	2310
12 May	Lamellibranch (Tiram)	Pulau Chermin	20
12 May	Lamellibranch (Teritip)	Sg. Teritip	935
15 May	Lamellibranch (Tiram)	Pulau Muara Besar	274
15 May	Lamellibranch (Biluyan)	Pulau Muara Besar	2060

[a]Brunei Malay names are given in brackets.
[b]Teritip: mangrove oyster.
[c]Biluyan and Kunau: clams.
[d]Tiram: oysters.

Table 2. Summary of toxicity tests; red-tide occurrence 1980.

Date	Specimen	Location	Toxin content μg/ 100 g meat
24 June	*Saccostrea cucullata* (Teritip)	Belangkas Jetty, Muara	52
4 July	*Arcuatula arcuatula* (Kupang)	Tanjong Bakalan	nt
12 July	*Perna viridis* (Kupang hijau)	Raft at Serasa	200
19 July	*P. viridis* (Kupang hijau)	Raft at Serasa	94
30 September	*P. viridis* (Kupang hijau)	Raft at Muara	138
6 October	*S. cuccullata* (Teritip)	Belangkas Jetty, Muara	64
28 October	*S. cucullata* (Teritip)	Belangkas Jetty, Muara	nt
28 October	*Meretrix meretrix* (Kunau)	Pulau Muara Besar	nt
28 October	*Anadara granosa* (Tembayangan)	Pulau Muara Besar	108
28 October	*P. viridis* (Kupang hijau)	Raft at Muara	71
26 November	*S. cuccullata* (Teritip)	Belangkas Jetty	nt
26 November	*M. meretrix* (Kunau)	Pulau Muara Besar	nt
26 November	*A. granosa* (Tembayang)	Pulau Muara Besar	83
26 November	*M. viridis* (Kupang Hijau)	Fisheries Station, Muara	64
4 February	*S. cucullata* (Teritip)	Belangkas Jetty	70
4 February	*M. meretrix* (Kunau)	Pulau Muara Besar	nt
4 February	*A. granosa* (Tembayangan)	Pulau Muara Besar	188
19 February	*S. cucullata* (Teritip)	Belangkas Jetty	66
19 February	*M. meretrix* (Kunau)	Pulau Muara Besar	nt
19 February	*A. granosa* (Tembayangan)	Pulau Muara Besar	92
3 March	*S. cucullata* (Teritip)	Belangkas Jetty	66
3 March	*M. meretrix* (Kunau)	Pulau Muara Besar	nt
3 March	*A. granosa* (Tembayangan)	Pulau Muara Besar	184

Notes: nt: not toxic. *S. cucullata* was nontoxic by April 1981, but *A. granosa* was reported to be toxic until at least October 1981.

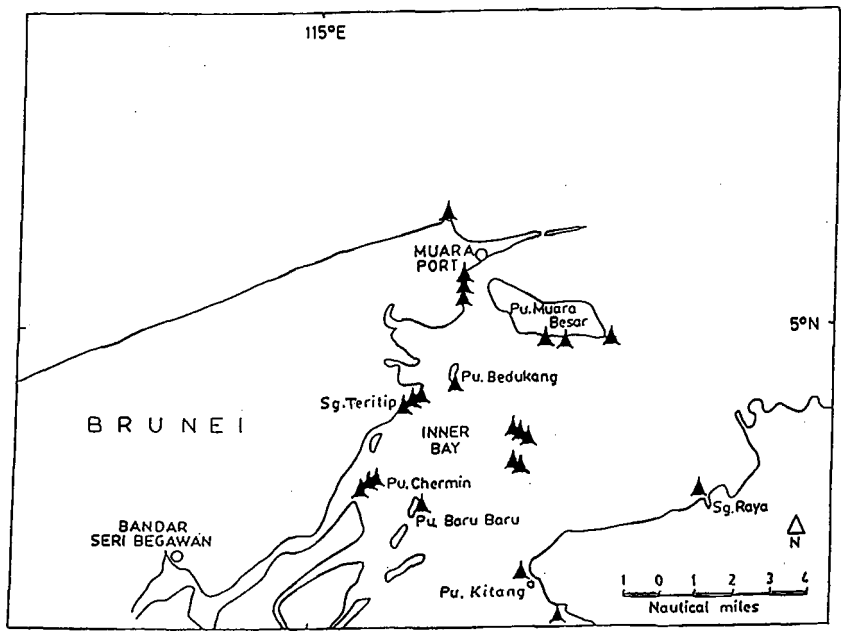

Fig. 3. Collection sites of toxic samples (dark tringles) in the inner bay area of Brunei, 1976 red-tide occurrence (adapted from Beales 1976).

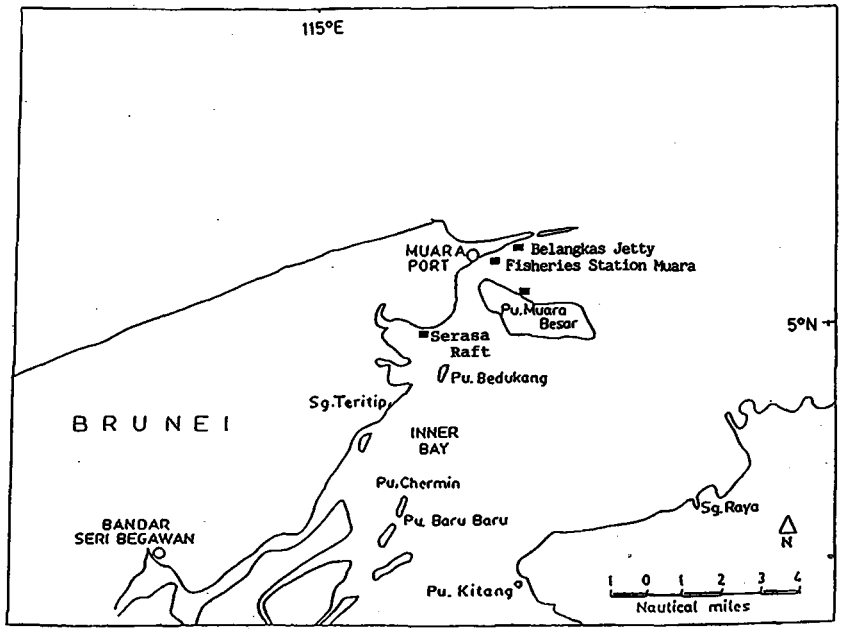

Fig. 4. Collection stations (boxes) of toxic samples in Brunei Darussalam, 1980 red-tide occurrence.

Distribution of Dinoflagellates at Jakarta Bay, Taman Jaya, Banten, and Benoa Bay, Bali: A Report of an Incident of Fish Poisoning at Eastern Nusa Tenggara

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Introduction

"Red tide" is the name given to the phenomenon of red discolouration of marine and estuarine waters caused by a bloom of single-celled plants. The colour of the water can range from blood red to orange or brown in daylight and is often visible as phosphorescence at night (Carey 1983). Harmful red tides cause severe damage to local fishery resources, especially cultured oysters, mussels, and fish.

Some of the dinoflagellates associated with the red discolouration in coastal waters have been identified as *Noctiluca miliaris, Gymnodinium, Cochlodinium, Ceratium, Prorocentrum, Peridinium,* and *Goriyaulax.* Steidinger (1979) (as cited by Estudillo 1984) reported in her taxonomic account of toxic dinoflagellates that there are less than 20 dinoflagellate species known or thought to produce toxins. Unprecedented red tides have occurred all over the world, such as in Korean waters (Park 1982; Lee and Huh 1983). Park noted that the red tide in Jinhae Bay, Korea, due to *Gymnodinium* type 65, occurred during the period July to September 1981. Lee noted that the red tide at Jindon Bay, caused by *Ceratium furca,* occurred in August 1980. In Harima Nada, Japan, the main cause of red tides was *Chatonella antiqua* (Hada) Ono in 1977, 1978, and 1979. In each case the red tide was associated with mass fish mortality (Okaichi et al. 1981). In the Philippines, the main cause of a red tide was *Pyrodinium bahamense* var. *compressa.* Paralytic shellfish poisoning (PSP) was reported for the first time in late June to early September 1983 in Maqueda Bay of Western Samar (Estudillo 1984). In Hong Kong, red tides have been caused by *Gymnodinium* in clear water and *Trichodesmium* in offshore waters (Lam 1984). In Papua New Guinea, the major causative organism of red tides was *Pyrodinium bahamense* var. *compressa* (Maclean 1984).

In some Indonesian waters, phytoplankton populations generally consist of diatoms and dinoflagellates; diatoms are dominant, whereas dinoflagellates are very rare. Under certain conditions, however, unprecedented blooms, such as *Noctiluca* have occurred. Praseno and Adnan (1978) reported on a bloom of *Noctiluca* in May 1976 in Jakarta Bay. At that time the abundance of *Noctiluca* was 2×10^3 cells/ L.

This paper deals with an incident of fish poisoning in East Nusa Tenggara in November 1983 and the distribution of dinoflagellates in Jakarta Bay, Taman Jaya, and Benoa Bay.

Incidence of Fish Poisoning

An incident of poisoning due to ingestion of clupeid fish was reported in East Flores, the province of East Nusa Tenggara. This incident occurred on 24 November 1983 and resulted in four deaths and 191 people becoming ill. The incident began on 21 November when many fish were caught at Lewotobi, Wulanggitang, East Flores. On 24 November, after eating the fish, four people died. On 27 November, the same fish were caught by fishermen about 1 km from the previous area at Loowuran. After consuming the fish, 45 people became ill, suffering from numbness; dizziness; tingling sensations of the lips, tongue, and throat; and difficulty in breathing. The water and fish samples were identified at the National Institute of Oceanology. The fish were identified as *Sardinella* spp. and *Selaroides leptolepis.* The colour of the

water samples was similar to the colour of the contents of the gut of the fish, reddish-brown. Unfortunately, the organism responsible for discolouring the water could not be identified because of insufficient preservation. It is possible that the organism was *Pyrodinium bahamense* var. *compressa,* which has been the cause of red tides in the Philippines and in Papua New Guinea. This was the first incident recorded, however, in East Indonesia.

According to Wyrtki (1961, Plates 4 and 5), in August the surface currents flow from east to west, i.e., Pacific Ocean to Papua New Guinea to Arafura Sea to Banda Sea to Flores Sea to Java Sea to China Sea. Due to surface circulation, upwelling occurs in Arafura Sea and Banda Sea.[1]

In October, surface currents still flow from east (South Pacific) to west but the middle portion of the current moves back. From the China Sea and Pacific Ocean, currents flow through Sulu Sea down Macassar Strait to Flores Sea and some to Sawu Sea (East Nusa Tenggara).

It is thought that the red tide in East Nusa Tenggara might have been related to water movement, which could increase water enrichment. It is also thought that the cyst of *Pyrodinium bahamense* might be endemic and, given the proper conditions, would grow in most areas in Southeast Asian waters. Thus, the red tide in East Nusa Tenggara might have come from Philippine waters or Papua New Guinea: Lewotobi is located at the foot of Lewotobi mountain which faces the Sawu Sea.

From personal communications, the following incidences of red tide have been described.

(1) In a bay in Halmahera Sea, many fish were killed in July 1981. The fishermen there said that this occurs annually and is a natural phenomenon.

(2) In Jakarta Bay/Pari Island, an unusual condition occurred that caused the water to become dirty. This was due to a *Trichodesmium* bloom that took place over a large area. Prior to the bloom, the water had been calm, following rainfall in September 1982.

(3) At Kuta Beach, Bali, in November 1983, many dead fish were found along the coast. It is possible that the fish might have been thrown away by fishermen or they may have, in fact, died from other causes.

[1]Nontji (1975) described an increased plankton biomass and high rates of primary production in the region in July, August, and September due to nutrient enrichment resulting from the upwelling.

Materials and Methods

Phytoplankton samples were taken from three areas: monthly from Jakarta Bay from October 1982 to September 1983; twice from Taman Jaya, December 1983 and July 1984; and twice from Benoa Bay, Bali, June and December 1982.

The surveys were conducted using motorized boats. The phytoplankton samples were collected using a net 120 cm in length, a diameter of 31 cm, and a mesh size of 75μm. The net was towed horizontally for a duration of at least 5 min. A TSK flowmeter was used to determine the amount of water filtered through the net. Settling volume was measured after 24 hours of settlement had taken place in a 50-mL graduated cylinder. Identification and counting of cells was carried out on a fraction and poured into a "Sedgwick-Rafter Cell," which was observed under a microscope at magnification 15x10 (Davis 1955; Newel and Newel 1979; Yamaji 1966; and Wickstead 1965).

Results and Discussion

There were only four genera of dinoflagellates in Indonesian waters: *Noctiluca, Ceratium, Dinophysis,* and *Peridinium. Noctiluca* was the dominant genera, whereas *Dinophysis* and *Peridinium* were rare and even absent at many stations. The four general fluctuated in a similar manner in Jakarta Bay. The peaks of the fluctuations occurred from October to December 1982 and June to September 1983. From January to May 1983, only *Noctiluca* was found (Adnan 1984). About a mile from the shoreline, *Noctiluca* reached its highest concentration in July 1983. The concentration was 8×10^2 cells/L, nutrient concentrations were low, and temperature, salinity, and pH were relatively high. Also at this station, the following diatoms were found in high concentrations: *Skeletonema costatum,* 2.3×10^3 cells/L; *Chaetoceras* sp., 8.7×10^2 cells/L; and *Coscinodiscus,* 1.6×10^2 cells/L.

In the area where *Noctiluca* was abundant, the water was dirty and covered with small green leaves. This condition was similar to that which occured in Thai waters but was different from that which occurred in Japan and New Zealand. In Japan and New Zealand, for example, when *Noctiluca* was in bloom, the colour of the water was red (S. Sudara and T. Okaichi, personal communication, 1984). Okaichi and Sachio (1976) in their study on *Noctiluca miliaris* noted that ammonia was the principal toxin associated with red tides of *Noctiluca.* They found that the acidic extract of *N.*

miliaris was not only toxic enough to kill fish immersed in the extract but also to kill mice when injected intraperitoneally.

Five species of *Ceratium* were found in Jakarta Bay, *C. azonicum* and *C. macroceras* being the most common; the others are *C. externum, C. lineatum* and *C. imaginor* (Adnan 1984). On 21 January 1983, the concentration of *C. azonicum* was very high, 3.1 x 10^2 cells/L, whereas the concentration of *C. macroceras* was 2 x 10^2 cells/L. There was only one species of *Dinophysis* in the area, *D. homunculus,* which occurred in January 1983 in small numbers, 20 cells/L. Also, only one species of *Peridinium* was found, *P. depressum,* again in small numbers. Near the shoreline, *P. depressum* on 23 September 1983 had a concentration of 50 cells/L. Besides the dinoflagellates, *Oscillatoria* was found at all stations from October to November 1983. The highest concentration occurred in August, about 2 miles from the shoreline, 7 x 10^2 cells/L.

The phytoplankton populations in Taman Jaya were much higher than in Benoa Bay, Bali (Adnan 1984). In Taman Jaya, *Noctiluca* dominated the area. The highest concentration occurred in December 1983 near the shore, 2.7 x 10^2 cells/L. This was followed by *Thalassiothrix* sp. at 2.5 x 10^2 cells/L. The samples in December 1983 and July 1984 also contained large quantities of *Rhizosolenia* and *Chaetoceros.* In Benoa Bay, the samples also contained these except *Rhizosolenia,* but additionally contained *Streptotheca, Coscinodiscus, Ceratium, Nitzschia, Bacillaria* and *Hemialus.* The abundance of phytoplankton in Benoa Bay was greater in December 1982 than June 1982.

Conclusions and Suggestions

Dinoflagellates in Jakarta Bay, Taman Jaya, and Benoa Bay, Bali, in general, showed the usual species composition. There was no evidence of a red tide.

In East Nusa Tenggara, East Indonesian waters, in November 1983, an unprecedented event occurred, namely fish poisoning, which killed four people. It is hoped, therefore, that a research project can be carried out to study the occurrence of red tides and PSP in eastern Indonesian waters.

The Governor of the Province of East Nusa Tenggara, Kupang, and Dr. Purwito Martosubroto, Director, Balai Penelitian Perikanan Laut Jakarta, are acknowledged for their report of the incident of fish poisoning at East Nusa Tenggara. Dr. Aprilani Soegiarto, Director, National Institute of Oceanology-LIPI, is also acknowledged for his guidance. Thanks are also expressed to Drs Kasijan Romimohtarto, O.H. Arinardi, and A.B. Sutomo for their suggestions and help.

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Shellfish in Indonesia

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Introduction

Indonesia is an expansive archipelago consisting of more than 13000 islands and a long coastline that under the new law of the sea exceeds 80000 km. Its coast is endowed with extensive tidal mud flats, bays, estuaries and lagoons that support large populations of shellfish of commercial significance. These resources have so far been only lightly tapped by low-income small-scale coastal fishing communities as the demand for molluscs is generally limited and rather localized. In 1981, total shellfish production stood at 50947 metric tons, valued at Rp8657 million (Table 1).

In the Fourth Five-Year Development Programme of the Government of Indonesia, increases in shellfish production will be brought about by the farming of the species in a suitable environment using applicable technology and directing the benefits to the needy rural coastal communities. Increases in production will help compensate for production losses arising from the banning of the trawl, on the one hand, and provide additional animal protein supplies to the people at large.

Present Shellfish Production

Species

Table 1 shows the eight important taxa (including cephalopoda) produced from 1977-1981. The three cephalopods, however, are incidental to trawl and seine catches, whereas the others are collected using various manual methods. Species of commercial significance include cockles (*Anadara* spp.), clams (*Meretrix* spp, and others), mussels (*Mytilus* spp.), and oysters (*Crassostrea* spp. and others). As development progresses, other commercial taxa will be stressed. For cultivation purposes, a list of 30 potential commercial species have been identified during a nationwide survey in 19 areas (Table 2, Fig. 1).

Table 1. Production and value[a] of shellfish, 1977-1981.

Molluscs	1977		1978		1979		1980		1981	
	Tonnage	Value	Tonnage	Value	Tonnage	Value	Tonnage	Value	Tonnage	Value
Cupped oyster	1274	645	186	23	912	566	1141	643	1131	642
Scallops	79	6	453	40	484	87	166	49	225	25
Hand clams	2702	64	4319	95	2556	166	2281	171	2053	200
Blood cockles	31360	546	40980	1074	32183	1140	32383	1245	37410	2896
Squid[b]	7088	1414	8691	1937	12812	3243	11142	3950	8867	4170
Cuttle fish[b]	2396	406	1804	364	1827	538	1995	847	862	391
Octopus[b]	-102	21	65	12	37	8	54	14	44	11
Others	839	99	1838	870	258	60	736	199	355	323

Source: Fisheries Statistics of Indonesia (1981).
[a]Million Indonesia rupiahs.
[b]Cephalopoda.

Table 2. Cultivable shellfish collected from surveyed areas.

	Banten Bay	Kenjeran	Pasuruan	Probolinggo	Socha Kwanyan	Demak	Tayu	Rembang	Jepara	Tan. Balai	Tangerang	Pari Island	Lombok	Bima	Sape	Kupang	Maros	Lokotoi
Anadara granosa		x	x	x	x	x	x	x	x	x				x				
A. inflata		x	x	x	x	x	x	x	x	x							x	
A. nodifera		x	x	x	x	x	x	x	x	x	x					x	x	
A. indica										x	x							
A. antiquata		x	x	x	x	x	x	x										
A. pilula		x	x				x	x										
A. maculosa												x	x		x	x	x	x
A. pausigranosa														x				
Meretrix lyrata		x	x	x	x	x	x	x	x	x			x	x		x		
Mytilus viridis	x	x	x	x	x				x	x	x							
Musculista senhausia							x	x										
Crassostrea cucullata		x	x	x	x	x			x		x		x	x		x	x	
Pinctada maxima																		x
P. margaritifera												x	x					x
P. martensii	x									x					x			x
Pteria penguin												x						x
Placuna placenta		x	x			x										x		
Polymela coakans														x				
Lucina edentula														x				
Mactra maculata													x					
Soligna madriata														x				
Lutria maxima														x				
Gafrarium gibbia																x		
Tellina sp.			x	x	x	x				x		x				x		
Atrina spp. (Pinna)												x			x			
Pecten sp.										x		x				x		
Cardium flavum		x	x									x			x			
Tapes sp.		x	x	x	x				x				x		x			
Cleone isabellina			x															
Tridacna sp.												x			x			

Note: Refer to Fig. 1 for location of areas surveyed.

Collection Method

Present methods of collecting shellfish can be broadly classified as follows:

(1) Collecting cockles and clams on mud flats during low tide using a skateboard (Fig. 2), to cover a wide area, and a spade or spatula for digging (Fig. 3).

(2) Collecting cockles and clams during low tide on foot using a spade or spatula for digging, in which case the area covered is restricted.

(3) Collecting cockles and clams in substrate of soft-bottomed seabed using a wooden- or bamboo-handled hand dredge from a boat (Fig. 4).

(4) Collecting cockles and clams in substrate of soft-bottomed seabed using a dredge towed from a sailboat (Fig. 5).

(5) Collecting cockles and clams in substrate of soft-bottomed seabed using a rake attached to a collecting bag (Fig. 6).

(6) Diving or wading to collect green mussels and oysters from submerged hard substrates.

Production and Value

Table 1 gives the production and value of eight taxa of molluscs for the period 1977-1981. As there seems to be no consistent trend in production, it is assumed that the supply and demand trend at the time fluctuated inconsistently throughout the period. This inconsistency is a reflection of the harvesting methods utilized, which are dependent upon the weather. The highest production occurred in 1978 and amounted to 58336 tons.

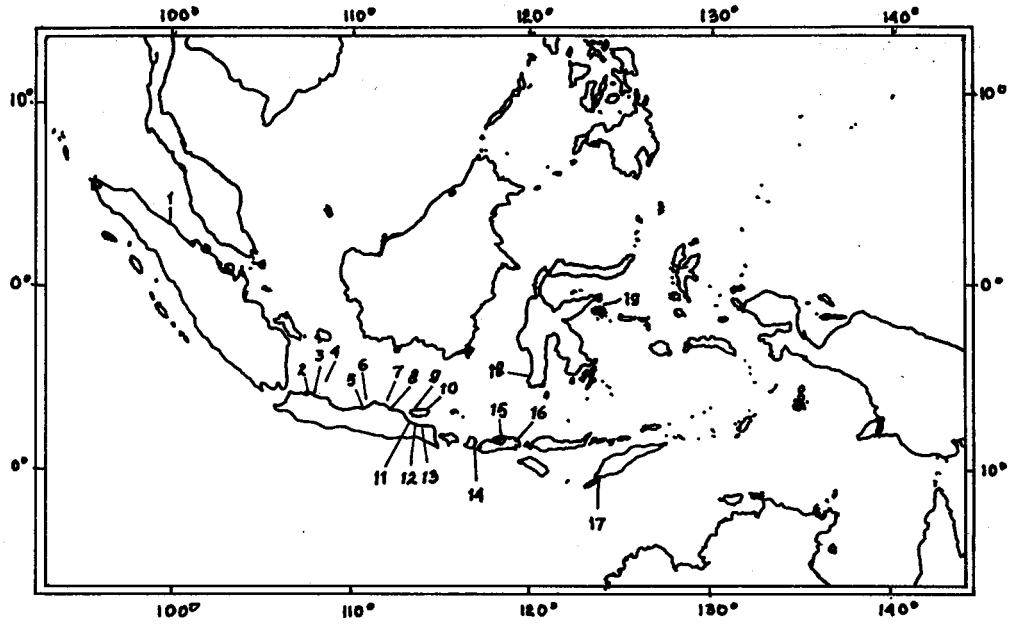

Fig. 1 Locations of sites surveyed.

1. Tanjung Balai, North Sumatra
2. Banten Bay, West Java
3. Ketapang Bay, West Java
4. Pari Island, Jakarta
5. Demak, Central Java
6. Jepara, Central Java
7. Tayu, Pati, Central Java
8. Rembang, Central Java
9. Desa Soca, Madura Is., East Java
10. Kwanyar, Madura Is., East Java
11. Kenjeran, Surabaya, East Java
12. Pasuruan, East Java
13. Probolinggo, East Java
14. Lombok Island, West Nusa Tenggara
15. Bima Bay, Sumbawa Is., West Nusa Tenggara
16. Sape Bay, Sumbawa Is., West Nusa Tenggara
17. Kupang, East Nusa Tenggara
18. Maros, South Sulawesi
19. Lokotoi, Banggai, Central Sulawesi

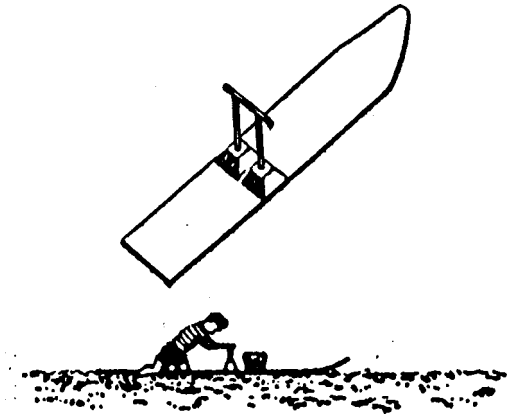

Fig. 2. Wooden skateboard.

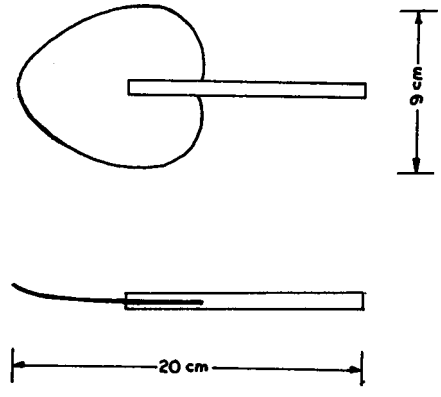

Fig. 3. Spatula used for digging bivalve.

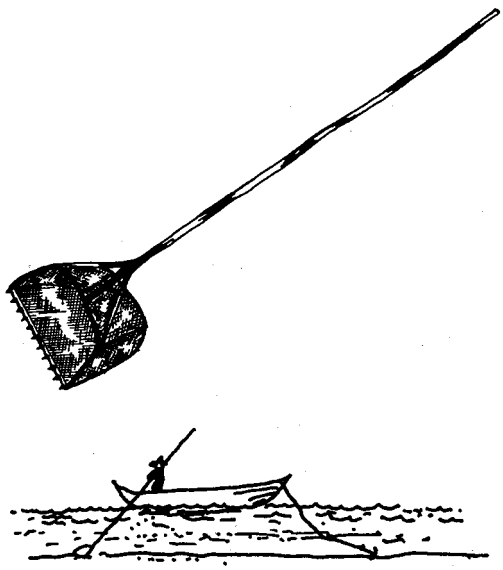

Fig. 4. Hand dredge.

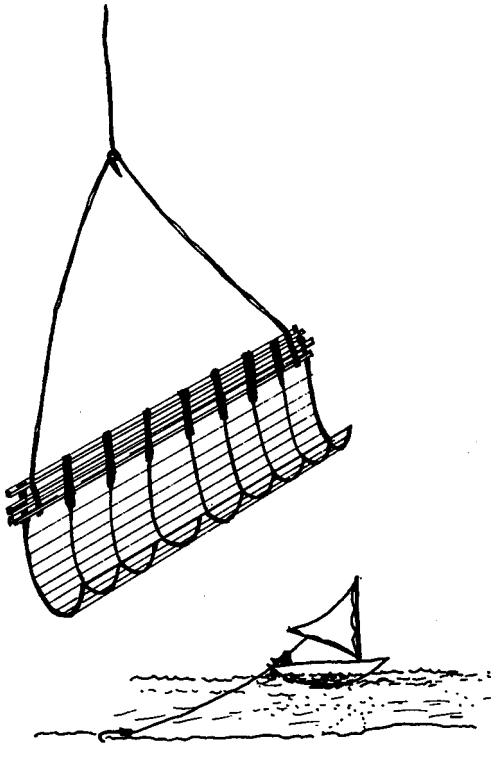

Fig. 5. Dredge towed by sailboat.

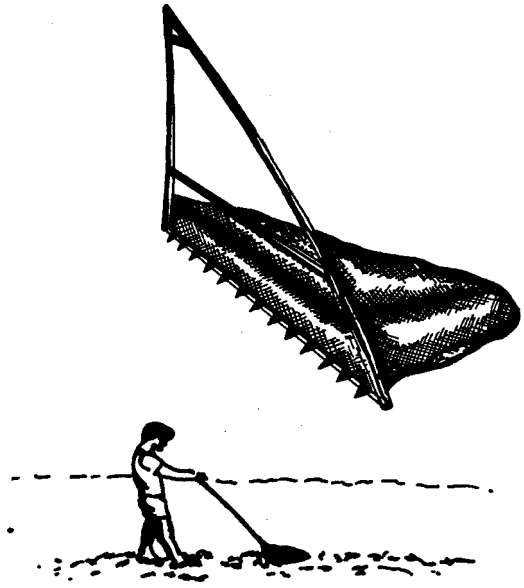

Fig. 6. Rake with collecting bag.

Regarding the value of the shellfish, the average value increased from approximately Rp 70 per kg in 1977 to Rp 170 per kg in 1981. In this tabulation, the cephalopod taxa are incidental to fishing gear and are not used for comparison.

Utilization

Other than the three cephalopods, whose disposal pattern and practice follow those of finish, the molluscs produced are mainly utilized fresh, some dried, by communities next to the place of capture. To relay the product beyond that would require post-harvest development considerations.

All molluscs produced are utilized for human consumption. The *Pinctata* oysters are used for pearl culture. The shells of some molluscs are used for making ornamental handicrafts, a practice that merits further development.

Discussions

The present Five-Year Development Programme calls for increases in fish production. It is anticipated that the development of shellfish farming would significantly contribute toward this end. Farming enables much greater control over the harvesting and production schedules, making systematic post-harvest development undertakings possible and more manageable. Thus, market development and product utilization would be possible. Successful shellfish farming also protects

the wild shellfish stock from overexploitation and the environment in the substrate, which forms the habitat of many species.

From the viewpoint of socioeconomic development of the primary sector, shellfish farming is also expected to contribute towards the betterment of small-scale fishing and rural coastal communities by creating meaningful job opportunities and uplifting the present adverse socioeconomic status of these communities.

Recognizing these benefits, the Government of Indonesia has conducted various preliminary investigations into the future prospects of seafarming as a whole. Of particular note is the preparatory assistance in seafarming development carried out by the Directorate-General of Fisheries of the Ministry of Agriculture supported by the United Nations Development Programme (UNDP) and the Food and Agriculture Organization of the United Nations (FAO). The assistance of the Japan International Cooperation Agency (JICA) to the Marine Fisheries Research Institute of the Agency of Agriculture Research and Development of the Ministry of Agriculture is also important.

In a forthcoming 3-year seafarming development project, the Directorate-General of Fisheries, supported by FAO and UNDP, will undertake the introduction and development of seafarming systems and technologies applicable to Indonesia. In this project, shellfish will be included.

Because shellfish, being filter feeders, are prone to the adverse effects of some pollutants, the project plans to work on post-harvest development problems as well as the depuration of the products to ensure health standards and edible quality. To protect shellfish culture grounds, the project will also look into pollution aspects, particularly the toxic effects of spilled oils, oil dispersants, industrial and domestic sewage effluents, and toxins in natural or induced dinoflagellate blooms.

Status of Shellfish Toxicity and Related Problems in Malaysia

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Introduction

The major species of molluscan shellfish exploited in the coastal waters of Malaysia include bivalves such as the cockle (*Anadara granosa*), mussel (*Perna*[1] *viridis*), and oysters (*Saccostrea*[2] *belcheri, S. cucullata, Ostrea folium*), and gastropods as such as *Cerithedia obtusa*. There are, of course, a host of other species of bivalves exploited at subsistence levels.

In 1982, the entire coastal zone of Malaysia yielded about 57000 metric tons of shellfish (Anonymous, in press). Cockles formed the bulk (86%) of this yield. They are reared in culture beds in the Straits of Malacca mainly in the mud flats off the states of Perak, Penang, and Selangor. Currently, much importance has been accorded to the expansion of the cockle industry, in view of the great potential shown by this bivalve for large-scale production in the inshore coastal waters.

Shellfish form less than 10% of the gross marine fishery production in Malaysia; nevertheless, they are consumed widely in the country, particularly by the coastal population.

Shellfish Toxicity

The frequency of occurrence of shellfish toxicity in Malaysia has not been established. However, there are records of isolated cases of what is known as paralytic shellfish poisoning (PSP) and some cases of viral gastroenteritis, i.e., if the latter can be considered in the context of shellfish toxicity. This does not exclude the probable occurrence of numerous other cases of shellfish poisoning in the past, cases that were either not related to shellfish toxicity in terms of a medical diagnosis, or those that did not seek appropriate medical care and hence were unknown.

The first reported case of PSP in Malaysia was that which occurred in the state of Sabah between January and April 1976, following an outbreak of "red tide" in the coastal waters of the state (Roy 1977). Of a total of 202 cases of food poisoning, seven (all children ranging in age from 4-11) died. The species of shellfish consumed was not established; however, the red-tide organism was identified by the Marine Research Laboratory in St. Petersburg, Florida, as *Pyrodinium bahamense* var. *compressa* Böhm, a dinoflagellate that is known to carry biotoxins that can accumulate in shellfish (Jothy 1982).

A second outbreak of "red tide" was recorded in Sabah from April to June 1980, causing two deaths from PSP (Maclean, this volume).

Paralytic shellfish poisoning struck the state of Sabah again from November 1983 to March 1984. Of the 25 persons known to have consumed shellfish, locally called "kalasiu" (*Oliva* spp.),5 (all children ranging in age from 3-9) succumbed to the poisoning. Normally, the shellfish concerned is reported to be found buried in 1.0-1.5 cm of sand in the intertidal zone of the beach. There were no indications of a red-tide phenomenon during this period.

Red Tide

The phenomenon of red tide has, for a long time, been known to occur in subtropical waters,

[1] *Perna* = *Mytilus.*
[2] *Saccostrea* = *Crassostrea.*

particularly in Florida and Japan, during the summer months. This phenomenon has, in recent years, shown up in the warmer tropical seas of Papua New Guinea in 1972 (Maclean 1973), in Brunei (Beales 1976) and Malysia since 1976, and in the Philippines in 1983 (Hermes and Villoso, in press).

Red tides in Malaysia have been observed in the coastal waters of Sabah and Penang and in the Straits of Johor. The causative organism in all cases was a dinoflagellate. In the case of Sabah, it was identified as *Pyrodinium bahamense* var. *compressa* Böhm, a toxin-bearing dinoflagellate, whereas that recorded in Penang and the Johor Straits was *Noctiluca miliaris* Suriray, which has not been known to carry any toxins.

The Penang and Johor phenomena were observed to be triggered by a period of heavy rainfall, which may have caused nutrient enrichment in the surrounding coastal waters, possibly emanating from terrestrial runoff. Fishermen have reported reduced catches during such phenomena and this may be due to oxygen deficiency in the water, which may have driven the fish to safer grounds. Unlike the Sabah phenomena, there have, thus far, been no cases of shellfish toxicity in the Penang and Johor phenomena.

Research

There has been very little research in Malaysia in the field of shellfish toxicity. Research has been confined to mouse bioassays, carried out by the Sabah Fisheries Department on a routine basis, and by the Sabah Medical and Health Services on an ad hoc basis, i.e., as and when cases of paralytic shellfish poisoning are reported in the state.

Following the second attack of shellfish poisoning in Sabah, in early 1984, the Fisheries Division of the Ministry of Agriculture, having become aware of the seriousness of the matter, set up a national working group in May 1984 to plan a program to carry out in-depth studies into the problem of shellfish toxicity in Sabah in an attempt to avoid future incidents of human fatalities arising from the consumption of shellfish. The working group comprises staff from Universiti Sains Malaysia, Penang; Universiti Pertanian Malaysia, Serdang; Fisheries Research Institute, Penang; Institute of Medical Research, Kuala Lumpur; Sabah Fisheries Department; and Sabah Medical and Health Services.

Efforts are currently under way to formulate a multidisciplinary study of Sabah's coastal waters covering research in the following areas: oceanography (coastal current patterns, nutrients); plankton regime (dinoflagellates); benthos (dinoflagellate cysts); shellfish biology and distribution; biotoxins in plankton and shellfish; pharmacology of the biotoxins (bioassays); and immunology.

I wish to thank the Director General of Fisheries, Malaysia, for allowing me to participate in this workshop and the International Development Research Centre (IDRC), Canada, for its sponsorship.

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Red Tide and Paralytic Shellfish Poisoning in Sabah, Malaysia

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Introduction

The coastal waters of Sabah are subject to sporadic blooming of toxic dinoflagellates. The various toxins from these dinflagellates are accumulated by bivalve molluscs of commercial value, such as clams, mussels, oysters, and cockles, which filter feed on these algae.

Toxic red-tide bloom is a comparatively recent phenomenon in Sabah. The first and by far the worst outbreak occurred in 1976. On 15 January of that year nine cases of suspected shellfish poisoning occurred in Kampung Maruntum, Putatan, near Kota Kinabalu, that resulted in two deaths. On 15 March 1976, 186 victims of what appeared to be mass food poisoning occurred in Sipitang, a village near Brunei, after eating bivalves found dead but freshly washed up onto the beach. One hundred and five people were hospitalized and four children died (Roy 1977).

As early as February 1976, dense patches of red water had been seen by scuba divers near Kota Kinabalu. However, it was not until the occurrence of mass food poisoning in Sipitang after consuming bivalves in early March that the red water and subsequent fish kills that the events were linked.

During the peak of the red tide in late March 1976, nearly the whole of the west coast of Sabah was affected (Fig. 1). Several aerial surveys were carried out during the peak period to monitor the situation. The bloom subsided in the middle of May 1976.

The movement of the red patches was from the southern to the northern part of Sabah and disappeared in the Kudat area. The geographical focal point of interest was the waters around the island of Labuan, which was the area affected most and where red tide blooms occur annually. Since 1981, however, no red tide has been seen or reported.

Recent Cases of Paralytic Shellfish Poisoning

The problems of forecasting toxicity have been further confounded by the recent discovery that shellfish can become toxic without any visible planktonic bloom. Even though no red tide was observed since 1981, certain species of shellfish still remain toxic. On 23 November, 1983, four children died and five others were hospitalized after they had eaten a meal of shellfish (*Atrina* sp.) that were found washed ashore at Kampung Binsuluk, 124 km from Kota Kinabalu.

On 7 January, 1984, two children died after a meal of cockles (*Anadara* sp.) and a certain rare species of rock oysters that had been washed ashore along the beach at Pulau Gaya near Kota Kinabalu. Six others were given emergency treatment.

Again, on 15 March 1984, another five children died and three others were hospitalized following a meal of sea snails (*Oliva* sp.) collected from the beach at Bongawan 58 km from Kota Kinabalu.

Mouse bioassay tests indicated that the shellfish involved in the three incidents were highly toxic and dangerous for human consumption (202-638 μg/100 g). However, the nature and origin of the neurotoxins have yet to be determined. It has been speculated that the toxins originated from toxic dinoflagellate cysts left behind during previous red-tide blooms (Dale et al. 1978). Unfortunately, Sabah lacks the personnel and means to carry out further research into PSP problems.

Toxicological Work

Mouse bioassays, according to AOAC methods, are being used in all toxin determinations and the standard safety level (400 MU/100 g) is based on Canadian epidemiological studies (AOAC 1975).

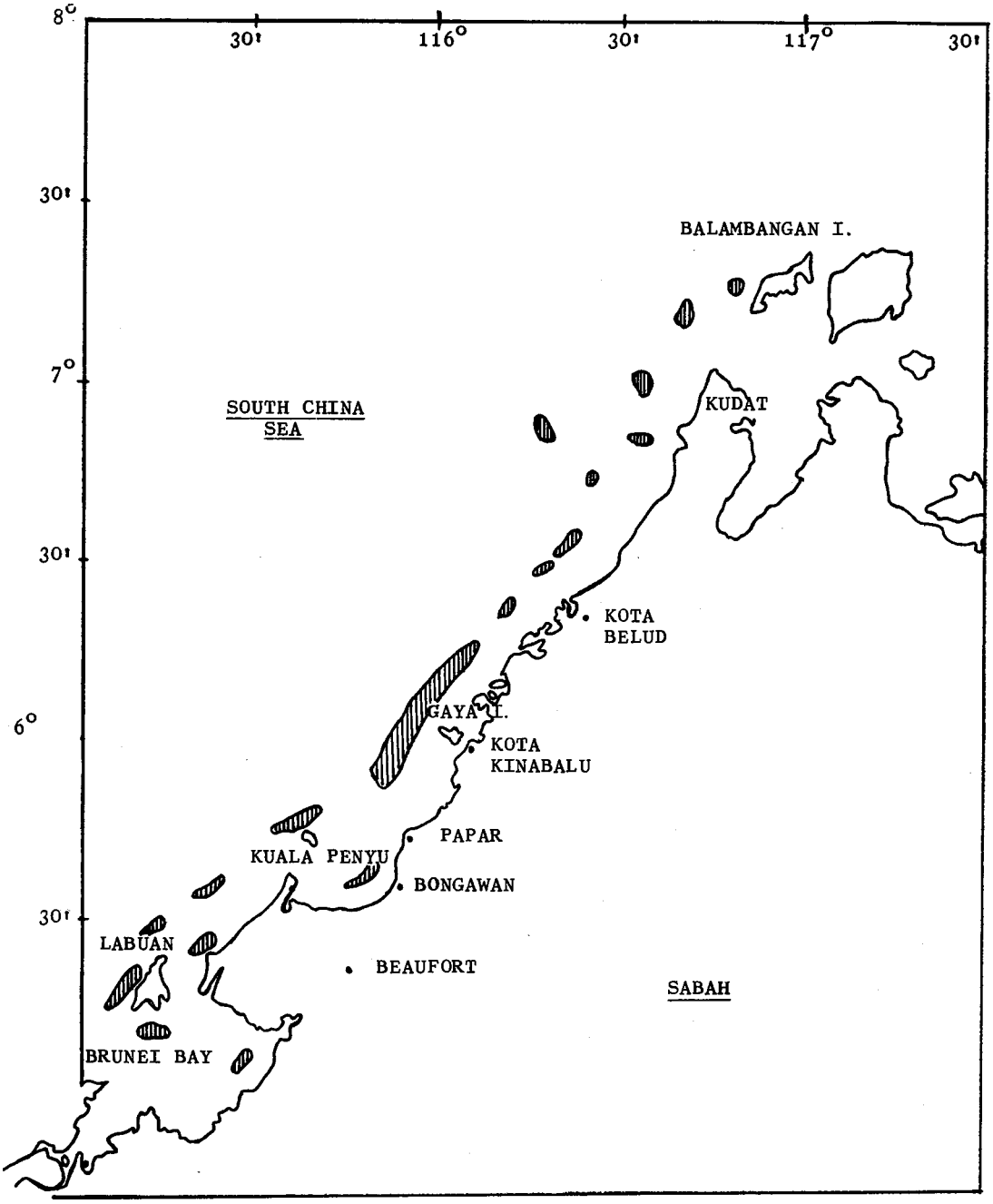

Fig. 1. Areas affected by red tide, March-May 1976.

Table 1. Toxicity levels of paralytic shellfish poison found in fish and cephalopoda following a red-tide occurrence in March 1976.

Genus	Date	Collection Site	Mouse units per 100 g[a]	Remarks
Nemipterus spp.	1.4.76	Near Gaya Island	Not detectable	Entire fish used
Decapterus spp.	1.4.76	Near Gaya Island	Not detectable	Entire fish used
Sciaena sp.	7.4.76	Marudu Bay	Not detectable	Entire fish used — dead fish found floating
Mixed species	11.4.76	Tg. Aru Beach near Kota Kinabalu	Not detectable	Entire fish used — dead fish washed up on shore
Nemipterus spp.	12.4.76	Near Gaya Island	Not detectable	Entire fish used.
Caranx spp.	22.4.76	Near Denawan Island	Not detectable	Entire fish used.
Priacanthus spp.	22.4.76	Near Tiga Island	Not detectable	Entire fish used
Sepia spp.	22.4.76	Near Tiga Island	Not detectable	Entire fish used
Saurida spp.	22.4.76	Near Tiga Island	Not detectable	Entire fish used
Sphyraena spp.	22.4.76	Near Denawan Island	Not detectable	Entire fish used
Pseudorhombus spp.	22.4.76	Near Tiga Island	Not detectable	Entire fish used
Gills from bottom feeders	22.4.76	Tiga Island	175 (5 nos.)	—
Apolectus spp.	2.5.76	Kota Kinabalu (K.K.) fish market	Not detectable	Entire fish used
Stomateus spp.	5.5.76	K.K. fish market	Not detectable	Entire fish used
Arius spp.	12.5.76	K.K. fish market	Not detectable	Entire fish used
Caranx spp.	6.5.76	K.K. fish market	Not detectable	Entire fish used
Gills from filter feeders	6.5.76	K.K. fish market	Not detectable	—
Sardinella spp.	12.5.76	K.K. fish market	Not detectable	Entire fish used
Ilisha spp.	12.5.76	K.K. fish market	Not detectable	Entire fish used
Rastrelliger spp.	12.5.76	K.K. fish market	Not detectable	Entire fish used
Caranx spp.	12.5.76	K.K. fish market	Not detectable	Entire fish used
Stomateus spp.	25.5.76	Kudat fish market	Not detectable	Entire fish used
Rastrelliger spp.	25.5.76	Kudat fish market	Not detectable	Entire fish used
Caranx spp.	25.5.76	Kudat fish market	175 (5 nos.)	Death of mouse disimilar to toxin death
Nemipterus spp.	25.5.76	Kudat fish market	Not detectable	Entire fish used
Sepia spp.	25.5.76	Kudat fish market	Not detectable	Entire fish used
Arius spp.	25.5.76	Kudat fish market	Not detectable	Entire fish used
Megalops spp.	25.5.76	Kudat fish market	Not detectable	Entire fish used
Guts from *Stomateus* spp.	25.5.76	Kudat fish market	Not detectable	—

[a]Numbers in parentheses refer to number of mice used. If number not indicated, 1 mouse was used as the control and 2 mice were used for testing.

However, in emergency cases when mice stocks were running low, young chicks were used as substitutes. The mice used are Porton strain and the conversion factor for this strain has been worked out according to AOAC methods as 1 mouse unit = 0.253 μg toxin. The main purpose of this investigation is to determine the suitability for consumption of marine food organisms found in certain coastal areas.

All results for the toxin testing of seafood over the 1976-1977 period are presented in Tables 1, 2, and 3. Table 4 summarizes some of the more recent analyses. All of the fish and cuttle fish examined (Table 1) showed barely detectable levels of toxin. Of the 15 samples of prawn analyzed (Table 2) only two showed barely detectable levels of toxin. Analysis also showed that none of the lobsters and crabs contained a toxin level in excess of 400 MU/100 g (Table 2). Results in Table 3 indicated that most of the shellfish in affected areas were highly toxic and dangerous for consumption.

Worthy of note is the fact that the sea snail *Oliva* sp. was found to be toxic and was responsible for five fatalities recently (Table 4). This species of

Table 2. Toxicity levels of paralytic shellfish poison found in crustacea following red-tide occurrence in March 1976.

Species	Date	Collection Site	Mouse units per 100 g[a]	Remarks
Portunus pelagicus	4.5.76	Kota Kinabalu (K.K.) market	175 (5 nos.)	Entire body used
	6.5.76	K. K. Market	134 (8 nos.)	Entire body used
	13.5.76	K. K. Market	Not detectable	Entire body used
	18.5.76	K. K. Market	151	Entire body used
	19.5.76	K. K. Market	Not detectable	Entire body used
	22.5.76	K. K. Market	Not detectable	Flesh only
	22.5.76	K. K. Market	288	Gills only
	22.5.76	K. K. Market	328	Guts only
	24.5.76	K. K. Market	Not detectable	Flesh only
	24.5.76	K. K. Market	Not detectable	Guts only
	24.6.76	K. K. Market	Not detectable	Flesh only
	24.6.76	K. K. Market	Not detectable	Gills only
	24.6.76	K. K. Market	234	Guts only
	28.6.76 to 1.7.76	Brunei Bay	Not detectable	Flesh only
		Brunei Bay	193 (7 nos.)	Gut only
Mangrove crabs	24.6.76	K. K. Market	Not detectable	Flesh only
	24.6.76	K. K. Market	175 (3 nos.)	Gills only
	24.6.76	K. K. Market	239 (5 nos.)	Gut only
Panulirus versicolor	5.5.76	Labuan processing plant	Not detectable	Cleaned and forzen tail
	5.5.76	K. K. Market	175 (5 nos.)	Entire lobster used
	19.5.76	K. K. Market	Not detectable	Tail only
	19.5.76	K. K. Market	184	Body and legs
	24.5.76	K. K. Market	Not detectable	Tail only
	24.5.76	K. K. Market	175 (7 nos.)	Body only
	25.6.76	Kudat Market	Not detectable	Tail only
	25.6.76	Kudat Market	Not detectable	Body only
Panulirus longipes	5.5.76	K. K. Market	211 (5 nos.)	Entire lobster
	19.5.76	K. K. Market	Not detectable	Tail only
	19.5.76	K. K. Market	177	Head and legs only
Penacidae	March	Frozen tails for export — Labuan	Not detectable	—
	March	Frozen tails for export — Labuan	Not detectable	—
	End March	Frozen tails for export — Labuan	Not detectable	—
	7.4.76	Marudu Bay	Not detectable	Body only
	10.4.76	Frozen tails for export — Labuan	175 (3 nos.)	—
	10.4.76	Frozen tails for export — Labuan	Not detectable	—
	11.4.76	Brunei Bay	Not detectable	Body only
	End April	Frozen tails for export — Labuan	Not detectable	—

Table 2. (continued)

Species	Date	Collection Site	Mouse units per 100 g[a]	Remarks
Penacidae	End April	Frozen tails for exports — Labuan	Not detectable	Tail only
	4.5.76	K. K. Market	Not detectable	Tail only
	5.5.76	K. K. Market	Not detectable	Tail only
	25.5.76	Kudat Market	Not detectable	Tail only
	25.5.76	Kudat Market	268 (5 nos.)	Body only
	24.6.76 to	Brunei Bay	Not detectable	Body only
	28.6.76	Brunei Bay	Not detectable	Body only

[a]Numbers in parentheses refer to number of mice used. If number not indicated, 1 mouse was used as the control and 2 mice were used for testing.

Table 3. Toxicity levels of paralytic shellfish poison found in shellfish following red-tide occurrence in March 1976.

Species	Date	Collection Site	Mouse units per 100 g[a]	Remarks
Anadara spp.	29.4.76	Kuala Kinarut	1552	Entire fish used
	25.5.76	Kudat	175 (3 nos.)	Entire fish used
	2.6.76	Kuala Kinarut	195 (7 nos.)	Entire fish used
	4.7.76	Kota Belud Tamu	175 (5 nos.)	Entire fish used
	13.3.77	Tuaran Tamu — from Gaya Island	Not detectable	Entire fish used
		From Gaya Island	Not detectable	Entire fish used
	23.3.76	Gaya Island	—	Entire fish used
Atrina spp.	4.6.76	Sapangar Island	162 (5 nos.)	Abductor muscle only
	4.6.76	Sapangar Island	4864 (5 nos.)	Mantle only
	4.6.76	Sapangar Island	4873	Body including digestive gland
	16.7.76	Sapangar Island	203 (5 nos.)	Abductor muscle only
	16.7.76	Sapangar Island	731	Mantle only
	16.7.76	Sapangar Island	4160 (5 nos.)	Body including digestive gland
	7.9.76	Sapangar Island	292 (5 nos.)	Abductor muscle only
	7.9.76	Sapangar Island	2588 (5 nos.)	Mantle only
	7.9.76	Sapangar Island	3861 (4 nos.)	Body including digestive gland
	29.9.77	Sapangar Island	Not detectable	Abductor muslce only
	29.9.77	Sapangar Island	225	Mantle only
	29.9.77	Sapangar Island	300	Body including digestive gland
	9.10.77	Tiga Island	Not detectable	Abductor muslce only
	9.10.77	Tiga Island	1440	Mantle only

Table 3. (continued)

Species	Date	Collection Site	Mouse units per 100 g[a]	Remarks
Atrina sp.	9.10.77	Tiga Island	529	Body including digestive gland
Buccinacea (Dog Whelk)	30.5.76	Tg. Aru Beach near Kota Kinabalu	Not detectable	Entire fish used
Donax spp.	29.4.76	Kuala Kinarut	6776	Entire fish used
	2.6.76	Kuala Kinarut	199 (5 nos.)	Entire fish used
Gafranium spp.	29.4.76	Kuala Kinarut	165 (4 nos.)	Entire fish used
	2.6.76	Kuala Kinarut	Not detectable	Entire fish used
Geloina similis	11.6.76	Mengkabong estuary	175 (5 nos.)	Entire fish used
Lambis lambis	30.5.76	Tg. Aru Beach	175 (3 nos.)	Entire fish used
	13.3.77	Tuaran Tamu — from Gaya Island	Not detectable	Entire fish used
Lethophaga spp.	11.6.76	Mengkabong estuary	Not detectable	Entire fish used
Meretrix spp.	29.4.76	Kuala Kinarut	1055 (5 nos.)	Entire fish used
	2.6.76	Kuala Kinarut	175 (3 nos.)	Entire fish used
	6.6.76	Tuaran Tamu	Not detectable	Entire fish used
	28.6.76	Tg. Pajar, Sipitang	Not detectable	Entire fish used
	28.6.76	Tg. Pajar, Sipitang	Not detectable	Entire fish used
	28.6.76	Tg. Pajar, Sipitang	Not detectable	Entire fish used
	28.6.76	Tg. Pajar, Sipitang	Not detectable	Entire fish used
	13.3.77	Tuaran Tamu — from Gaya Island	2140	Entire fish used
	27.3.77	Tuaran Tamu — from Gaya Island	—	Entire fish used
Placuna placenta	5.5.77	Mengkabong estuary	Not detectable	Entire fish used
Saccostrea cucullata	24.5.77	Tawau (east coast)	Not detectable	Entire fish used
	25.5.77	Mengkabong estuary	298	Entire fish used
Telescopium telescopium	11.6.77	Mengkabong estuary	Not detectable	Entire fish used
Giant clam	4.5.77	K. K. Market	149 (7 nos.)	Digestive gland hadn't been removed before sale but filter already had
	6.5.77	K. K. Market	Not detectable	Abductor muscle only
	6.5.77	K. K. Market	Not detectable	Mantle only
Coral-living type clam	4.6.77	Sapangar Island	Not detectable	Abductor muslce
	4.6.77	Sapangar Island	178 (5 nos.)	Mantle and body only
Giant clam	4.6.77	Sapangar Island	311 (5 nos.)	Abductor and mantle only
	4.6.77	Sapangar Island	9920	Other body parts
	23.6.76	K. K. Market	Not detectable	Abductor muscle
Coral-living type clam	23.3.77	Gaya Island	Not detectable	Abductor muscle
	9.9.77	Tiga Island	Not detectable	Abductor muscle only
	9.9.77	Tiga Island	Not detectable	Mantle only
	9.9.77	Tiga Island	Not detectable	Body and digestive gland only
Bunkul[b]	6.6.76	Tuaran Tamu	Not detectable	Entire fish used
Pahat-Pahat[b]	6.6.76	Tuaran Tamu	Not detectable	Entire fish used
Kanjapan[b]	6.6.76	Tuaran Tamu	Not detectable	Entire fish used

[a]Numbers in parentheses refer to number of mice used. If number not indicated, 1 mouse was used as the control and 2 mice were used for testing.

[b]Local Malay name (clam).

Table 4. Toxicity levels in shellfish following recent PSP.

Genus	Date	Collection Site	Mouse units per 100g [a]
Crassostrea sp.	29.11.83	Kulau Penyu	1300
Meretrix sp.	29.11.83	Kinarut	Not detectable
Gafranium sp.	29.11.83	Kinarut	Not detectable
	30.11.83	Pulau Daat	220
	30.11.83	Pulau Papan	Not detectable
Anadara sp.	10.12.83	Kuala Penyu	Not detectable
Crassostrea sp.	10.12.83	Kuala Penyu	1500
Anadara sp.	19.12.83	Kuala Penyu	Not detectable
Meretrix sp.	19.12.83	Kuala Penyu	Not detectable
Crassostrea sp.	12.1.84	Kuala Penyu	600
	14.1.84	Pulau Gaya	2500
Anadara sp.	14.1.84	Pulau Gaya	800
Snail (unidentified)	14.1.84	Pulau Gaya	Not detectable
Crassostrea sp.	16.2.84	Kuala Penyu	262
Anadara sp.	21.2.84	Pulau Gaya	290
	5.3.84	Pulau Gaya	Not detectable
Snail (unidentified)	5.3.84	Pulau Gaya	Not detectable
Oliva sp.	17.3.84	Bongawan	2525
Crassostrea sp.	20.3.84	Kuala Penyu	Not detectable
Anadara sp.	23.3.84	Pulau Daat	Not detectable
Crassostrea sp.	23.3.84	Pulau Daat	Not detectable
	23.3.84	Pulau Papan	Not detectable
Pitar sp.	23.3.84	Kawang	Not detectable
	26.3.84	Kimanis	Not detectable

[a]Numbers in parentheses refer to number of mice used. If number not indicated, 1 mouse was used as the control and 2 mice were used for testing.

shellfish is found buried under 1.25 cm of sand along the beach and its abundance is reported to be seasonal. These sea snails are occasionally washed up on shore and villagers have eaten them in the past without apparent ill effects.

Hydrographic Surveys

Hydrographic surveys were carried out in conjunction with plankton trawl and underwater observations of marine life in coral reefs in areas affected by red tide during March/April 1976. Water samples were collected from various depths, including surface and bottom samples. Parameters examined include pH, temperature, salinity, and dissolved oxygen.

The surface temperature was around 28.5°C, dropping fairly rapidly to 26°C at 5 m depth and 24.0°C at 10 m. There was no further decrease in temperature below this depth. The pH was fairly constant at 8.1.

Surface salinity ranged from 33 ppt near the coast to 35 ppt in the open sea. The vertical gradient of salinity was small and generally an increase of 1 ppt was observed around a depth of 20-30 m, below which it remained fairly constant.

The drop in dissolved oxygen with depth was fairly dramatic. Near Gaya and Sepangar Islands, the surface dissolved oxygen was around 4.1 mL/L. At 20 m it dropped to 3.0 mL/L and at 30 m values were generally less than 0.5 mL/L. At 1 m above the sea floor, dissolved oxygen was practically depleted and values as low as 0.03 mL/L were recorded. Oxygen depletion could either be caused by excessive red-tide blooms, which use up all available dissolved oxygen at night, or by the decomposition of dead marine organisms killed by the red tide.

Underwater Observations after the Occurrence of Red Tides

Underwater observations carried out in March/April 1976 of the coral reefs off Gaya Island, where large patches of red tide had earlier been reported, revealed that virtually all the corals appeared to be dead and were covered with a thick black deposit. Apart from one or two live anemones, no other live invertebrates were seen. Observations revealed dead hydroids, sponges, molluscs, crustaceans, and echinoderms (starfish, sea urchins, and sea cucumbers). No fish were seen

below 10 m and a distinct smell of hydrogen sulphide was noticed around 12 m depth and below, indicating anaerobic conditions. The absence of both live and dead fish below 10 m suggests that live fish moved away to more favourable areas as conditions in the vicinity of the red tide deteriorated. A 0.5 hour experimental trawl in the vicinity yielded only about 1 kg of fish. Trawls conducted in other affected areas also yielded very poor catches. However, bottom dwelling sedentary animals, being unable to move, were extremely vulnerable to sudden adverse changes in environmental conditions and many of them might have fed directly on the toxic red-tide organisms and died. The low oxygen values near the sea floor could also have increased the mortalities.

Plankton Studies

Numerous samples of plankton were collected and studied during the several red-tide outbreaks. The samples consisted mainly of surface horizontal hauls using a fine phytoplankton net. Occasionally, surface samples were obtained by simply scooping with a bucket. Several subsurface samples were also collected by divers towing the plankton net by hand at the appropriate depth where dense clouds of phytoplankton were seen. Other samples were collected by simply opening polyethylene bags under water by divers. Microscopic examination of the samples revealed that most samples consisted mainly of one particular species of dinoflagellate. Samples collected from dense patches of red water contained only this species and nothing else. The species was positively identified by Prof. F.J.R. Taylor and later confirmed by Dr. K.A. Steidinger as *Pyrodinium bahamense* var. *compressa,* the same species that was found in New Guinea (Maclean 1979).

Monitoring of Red Tides

The 1976 red tide caught Sabah totally unprepared. Few people in Sabah had heard of red tide before. Through valuable experience gained from the first and subsequent red-tide outbreaks, however, the Department of Fisheries is now ready to cope with the situation with more confidence. The most urgent problem was and still is the determination of the suitability for consumption of marine food, particularly shellfish from previously affected areas. As in most countries affected by toxic red tides, the department has PSP surveillance programs using standard procedures for the bioassay of toxin levels and a standard quarantine level based on Canadian epidemiological studies. Admittedly, there is little that can be done to prevent or control the outbreak of red tide; however, should any sign of an impending outbreak be detected, the general public could be given early warning, thus eliminating any unnecessary loss of lives.

Conclusion

Further studies need to be carried out by qualified personnel into the reasons why some species of shellfish become toxic from time to time even though no red tide is seen or reported. More specifically, the origin and nature of the toxins should be investigated. The possibility of resting cysts as the source of toxins should be fully investigated. Hopefully, such research can shed further light onto the problem of PSP forecasting in this region.

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Lethal Effect of Paralytic Shellfish Poison (PSP) from *Perna viridis*, with Notes on the Distribution of *Pyrodinium bahamense* var. *compressa* During a Red Tide in the Philippines

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Introduction

Paralytic shellfish poisoning (PSP) was a relatively unknown phenomenon in the Philippines until a toxic dinoflagellate bloom occurred in July/ August 1983. Generally, people are victimized by toxic dinoflagellates for the following reasons:

(1) The poison does not affect the physiology of the shellfish; hence, no distinguishing characteristics can be observed between poisonous and nonpoisonous shellfish.

(2) The poison is heat-stable; hence, canned and processed shellfish may still contain up to 50% of the poison even if the product is heated to 115°C (Schantz 1973).

(3) The concentration of dinoflagellates in seawater can reach toxic levels before their presence is detected macroscopically, through the discolouration of the sea known as "red tide."

In the Philippines, several additional factors can be cited. There is general lack of awareness of the danger of PSP, especially in remote fishing villages. Mussels, e.g., *Perna virdis,* although not a traditional seafood item in the Philippines, have become very popular as a relatively low-priced source of protein. Methods of preparation include, in addition to boiling and broiling, curing in vinegar (pickling). Thus, through the consumption of various types of shellfish, the accumulated dinoflagellate toxin reaches the human body (Fig. 1).

Other important aspects include problems connected with the dissemination of information and warnings in a developing archipelagic country, and enforcement of fishing bans and restrictions, which are imposed only after several persons are reported to have become intoxicated. The profit obtained from shellfish beds may be given prime consideration even to the detriment of the consuming public.

A significant factor contributing to the gravity of poisoning cases is the lack of systematic analysis of the true cause of death and the unavailability of accurate reports on the affected areas. Often, several people will die before the cause of death is associated with PSP.

This study was conducted mainly to determine the potency of the dinoflagellate toxin accumulated in green mussels, *Perna viridis.* As the ability of the shellfish to accumulate toxins may be largely dependent on its filtration rate and the density and distribution of toxic dinoflagellates, the results of a plankton survey on the distribution and abundance of the red tide causing algae are presented. Preliminary investigations on the effect of varying pH's on the toxicity of the crude toxin extract were likewise conducted.

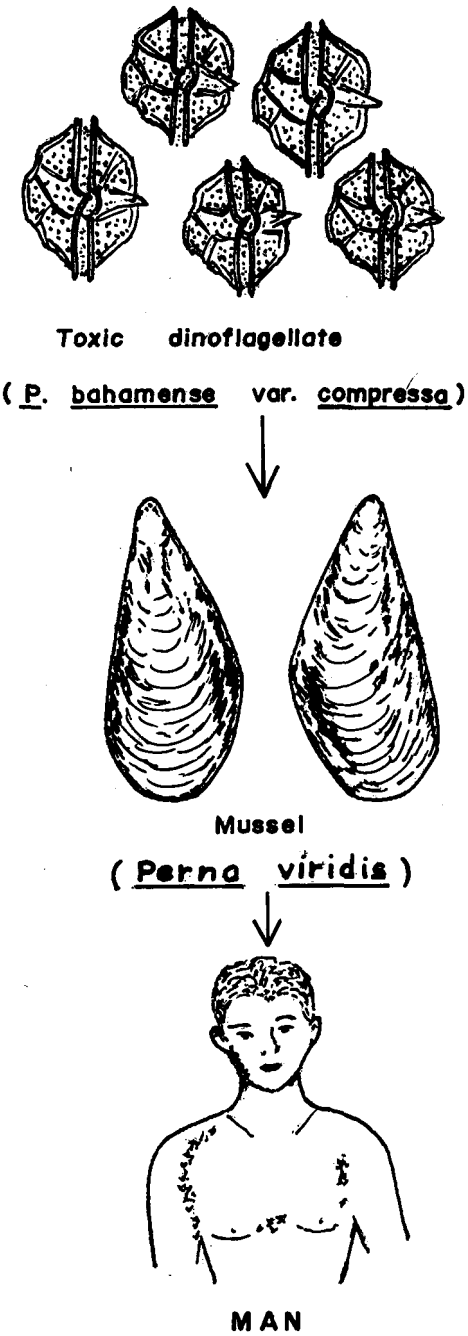

Fig. 1. Pathway of human intoxication through ingestion of toxic dinoflagellate.

Review of Literature

Visible discolouration of the seawater surface, changing the colour from blue to a variety of shades, attributed to the bloom of dinoflagellate single-called algae, but not exclusively, is called red tide. Some of the more notorious dinoflagellates, the "gonyaulacoid" forms, are capable of producing very potent low molecular weight toxins. These thecate dinoflagellates, among them *Gonyaulax* spp. and *Protogonyaulax* spp., have been known to occur in temperate waters of the Atlantic and Pacific Oceans (Steidinger et al. 1980).

Pyrodinium bahamense var. *compressa,* a related form with unpredictable toxic nature, having nontoxic as well as extremely toxic strains (Hashimoto 1979; Shimizu 1979), was previously reported from several Indo-West Pacific areas like Papua New Guinea (Maclean 1975, 1977; Worth et al. 1975), Brunei and Sabah (Beales 1976), and Palau (Kamiya and Hashimoto 1978; Harada et al. 1982). *P. bahamense* var. *compressa* was identified from plankton samples taken in August 1983 in the eastern central Philippines (Hermes and Villoso 1983). Based on the presence of *P. bahamense* var. *compressa* and cases of intoxication reported by local newspapers, a map of affected locations was produced (Fig. 2).

Shellfish, which filter water containing toxic dinoflagellates, become intermediary consumers called "transvectors." The binding sites for the toxin are to be found in the dark gland or hepatopacreas of mussels (Sommer and Meyer 1937; as cited by Schantz 1973). Schantz and Magnusson (1964) noted that there are marked differences among species in toxin distribution in the tissues and toxin retention. *Mytilus edulis* accumulates the toxin mainly in the digestive gland and retains the toxin for about 2 weeks. Prakash et al. (1971) observed that the soft-shell clam *Mya arenaria* also concentrates the toxin in the digestive gland during summer, but mostly in the gills during autumn. Alaska butter clam *Saxidomus giganteus* stores the toxin predominantly in the siphon. Green turban shell *Turbo marmorata* stores it in the viscera. Constant toxic levels can be maintained over a considerable period of time.

Studies on the composition of these paralytic shellfish poisons have revealed that the toxin component is either a single neurotoxin or a composite of related neuromuscular toxins (WHO 1977; Hashimoto 1979; Shimizu 1979). During the recent *Pyrodinium*-related Palauan dinoflagellate toxin study, Harada et al. (1982) obtained a composite of five toxins, namely saxitoxin, neosaxitoxin (together comprising 78% of the toxicity),

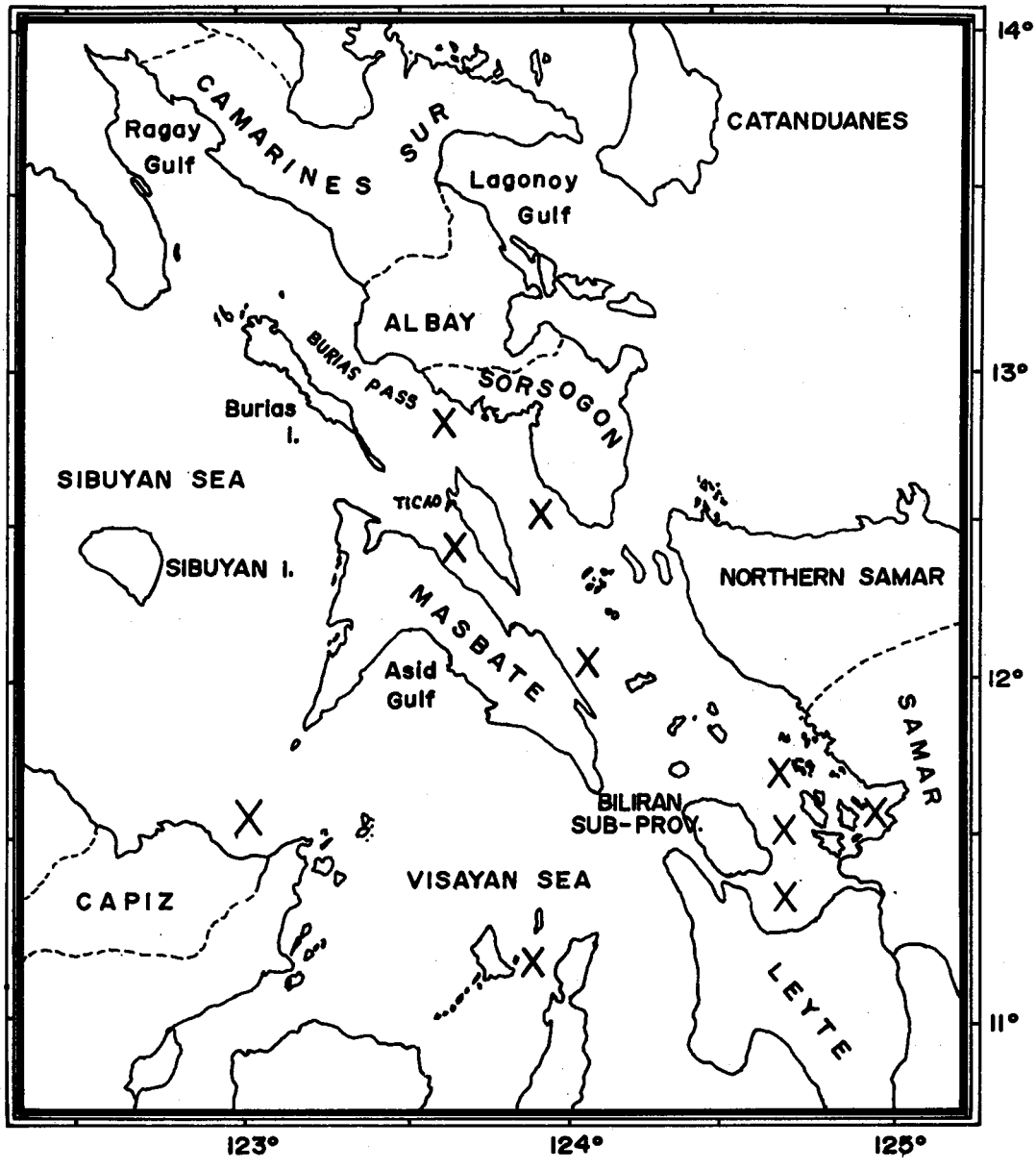

Fig. 2. Eastern central Philippines, areas affected by the red tide/ PSP (X).

gonyautoxin V, and two unidentified toxins, tentatively coded PBT_1 and PBT_2. Saxitoxin is the most potent neurotoxin found in temperate dinoflagellate blooms (Shimizu 1979). Saxitoxin is a dibasic salt that is very soluble in water. As a hydrochloric salt, the formula is $C_{10}H_{17}O_4N_7$. 2 HCl, with a molecular weight of 372. The toxicity is reportedly enhanced at an acidic pH (Hashimoto 1979).

Bates and Rapoport (1975) noted that human consumption of shellfish contaminated with saxitoxin causes poisoning and sometimes death. They estimated the human lethal dose as approximately 1 mg orally. The specific toxicity, however, may vary from 30 mouse units (MU) to 5000 MU/mg. The United States Food and Drug Administration (FDA) has set a maximum level of poison in fresh, frozen, or canned shellfish of not more than 400 MU (about 80 μg) per 100 g of shellfish tissue (Schantz 1973).

Halstead (1965) described the symptoms of paralytic shellfish poisoning. As a a neurotoxin, the poison blocks the propagation of impulses at the neuromuscular synapses, due to the interference with non-permeability of membranes.

Methodology

Crude Toxin Extract Preparation

Crude toxin extracts from *Perna viridis,* collected from Maqueda Bay, Samar, were provided by the Southeast Asian Fisheries Development Center (SEAFDEC). The extracts were prepared using the standard method for the analysis of paralytic shellfish poisons (Horwitz 1980). The sampling of the mussels was carried out several days after the earliest reports of a red tide in the area.

The extracts were divided into two lots. Lot 1 remained untreated, whereas lot 2 was subjected to varying pH levels (1-14).

The stock solution of crude mussel extract was diluted into 1:1, 1:3, and 1:4 proportions using triple distilled water. Another 14 subsamples of 5 mL each were placed in sterilized vials. Calibration for each pH level (1, 2, 3, . . . , 14) was accomplished by titrating with HCl and NaOH solutions. Volumes of the added acid or base solutions were recorded to determine the degree of dilution. Measurement of pH was carried out using a digital Jenco pH meter. To maintain toxin stability, all toxin samples were stored at chilling temperature (3-4°C).

A blank was prepared and histopathological examination was conducted to ascertain the true cause of death of the mouse after injection (i.e., whether due to the acidic/basic nature of the solution or to the manner of toxin administration). The whole toxin extraction process was carried out except for the inclusion of mussel flesh. A solution containing 100 mL of 0.1 *N* HCl was boiled for 5 min with constant stirring and subsequently cooled. The pH was adjusted to between 2.0 and 4.0 (never greater than 4.5) by titration of acid solution. The acidified solution was transferred into a volumetric flask and diluted to 200 mL. The blank solution was also subjected to dilution and different pH levels.

Bioassay

The standard method specifies that white mice weighing between 19 and 21 g be used (WHO 1977; Horwitz 1980). The weight of mice used for this particular experiment ranged from 9-24 g; hence, a correction factor for mice weighing less than 19 g (see Appendix 4) was used to obtain the true death time and a precise measurement of toxicity.

Four mice per dilution, per pH level, were intraperitoneally injected with a known volume of prepared crude toxin extract. The volume injected was adjusted using a correction factor of 0.05. The correction factor was obtained through ratio and proportion.

The time of death from the moment of injection to the last yawning pant movement was carefully observed and measured using a stopwatch with lap functions (to correct the premature indications of last yawning pant movement).

Mouse units (MU), which represent the strength of the crude toxin extract, were computed using Sommer's table of mouse equivalents (ME), and the formula:

$$\text{MU/100 g mussel flesh} = \text{ME/mL} \times F \times VE$$

ME/mL = see Appendix 4
F = dilution factor
VE = total volume extracted

Biological Aspects

Plankton samples and data were gathered in Samar Sea and adjacent affected areas from 4-12 August 1983. Several sampling methods were used in the quantitative assessment of the distribution and abundance of the dinoflagellate.

A 20-cm bongo sampler with a cylindrical-conical net of 47 μm mesh size (Fig. 3), fitted with a flowmeter, was used in double oblique tows to the sea bottom following the procedure of Smith and Richardson (1977) and Hermes and Dizon (1982). Due to the very shallow topography of Maqueda Bay, a hand-held 30-cm Marutoku net of about 70 μm mesh size for vertical tows was used there. In addition, water samples using Nansen bottles were taken at all stations at standard depths of 0, 10, 20,

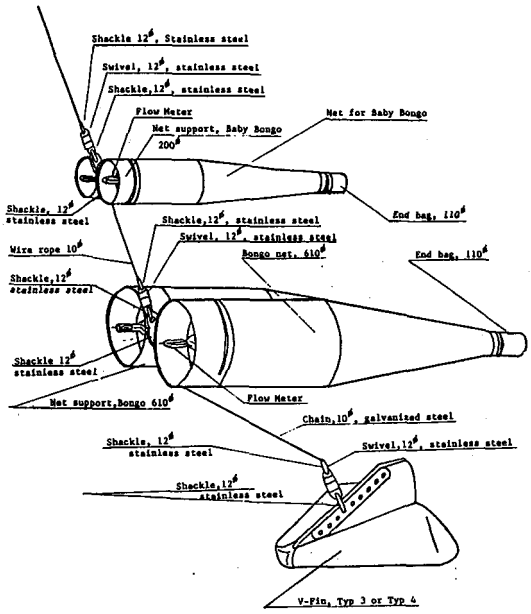

Fig. 3. Bongo nets for plankton collection (reproduced from Alu-Bau GMBH)

30, and 50 m, wherever possible according to bottom profile. Physicochemical parameters (temperature, salinity, and dissolved oxygen) were measured. The quantitative assessment of the plankton samples was based on aliquot counts under a WILD stereomicroscope at magnifications of 40 - 80 x. For the bongo net collection, subsamples of several 100 cells per 0.1 mL were counted; the results of the water-bottle collection were established from 0.1 and 0.5 mL aliquots.

Results and Discussion

The bioassay of crude toxin extract showed a lethal effect that ranged from 349-532 MU/100 g mussel (69-106 μg/100 g), as shown in Table 1. Extracts from mussels collected on 13 August, with a maximum potency of 532 MU (106 μg), exhibited an LD_{50} of 354 MU (70 μg), with 2 min being the fastest time of death recorded. Extracts collected from 13 and 15 August exceeded the maximum tolerable limit of 400 MU/100 g mussel set by the United States FDA. It should be noted, however, that extracts with mouse units lower than 400 MU were also observed to be potent.

Fluctuations in the lethal effect of crude toxin, as shown in and Table 1, can be attributed to the area of collection and varying densities of toxic dinoflagellates. The number of poisonous organisms and amount of water filtered by the

Table 1. Lethal effect of crude toxin extract from *Perna viridis*.

Collection date (August)	MU/100g	μg/100 g
6	361	72
7	363	73
12	386	77
13	532	106
14	350	70
15	441	88
26	349	69

shellfish determine the amount of poison in the shellfish (Schantz 1973). About 1 week before the collection of mussels, the abundance of *P. bahamense* in Maqueda Bay ranged from 1000-6700 cells/L (Figs 4 and 5)). Much higher concentrations of the toxic dinoflagellate were at the same time recorded from the adjacent and considerably larger areas of Samar Sea and Carigara Bay. Peak values of 760000 and 980000 cells/L were observed (Tables 2 and 3). *M. edulis*

Table 2. *Pyrodinium bahamense* cell counts in Samar Sea and adjacent areas, based on double oblique tows with a 20-cm bongo net of 47 μm mesh size (cells/L).

Carigara Bay	
Station 1	1.8×10^5
2	1.5×10^5
3	7.6×10^5
4	1.1×10^5
5	1.0×10^5
6	1.7×10^5
Western Central Samar Sea	
Station 7	1.0×10^5
8	1.8×10^5
9	7.3×10^4
10	1.0×10^5
11	1.4×10^5
Northern and Eastern Samar Sea	
Station 13	8.7×10^3
14	1.3×10^4
15	4.9×10^4
16	3.3×10^5
17	2.1×10^5
18	1.1×10^4
20	3.3×10^5
Masbate Pass	2.5×10^3
Burias Pass South	2.0×10^4
Burias Pass North	1.0×10^4

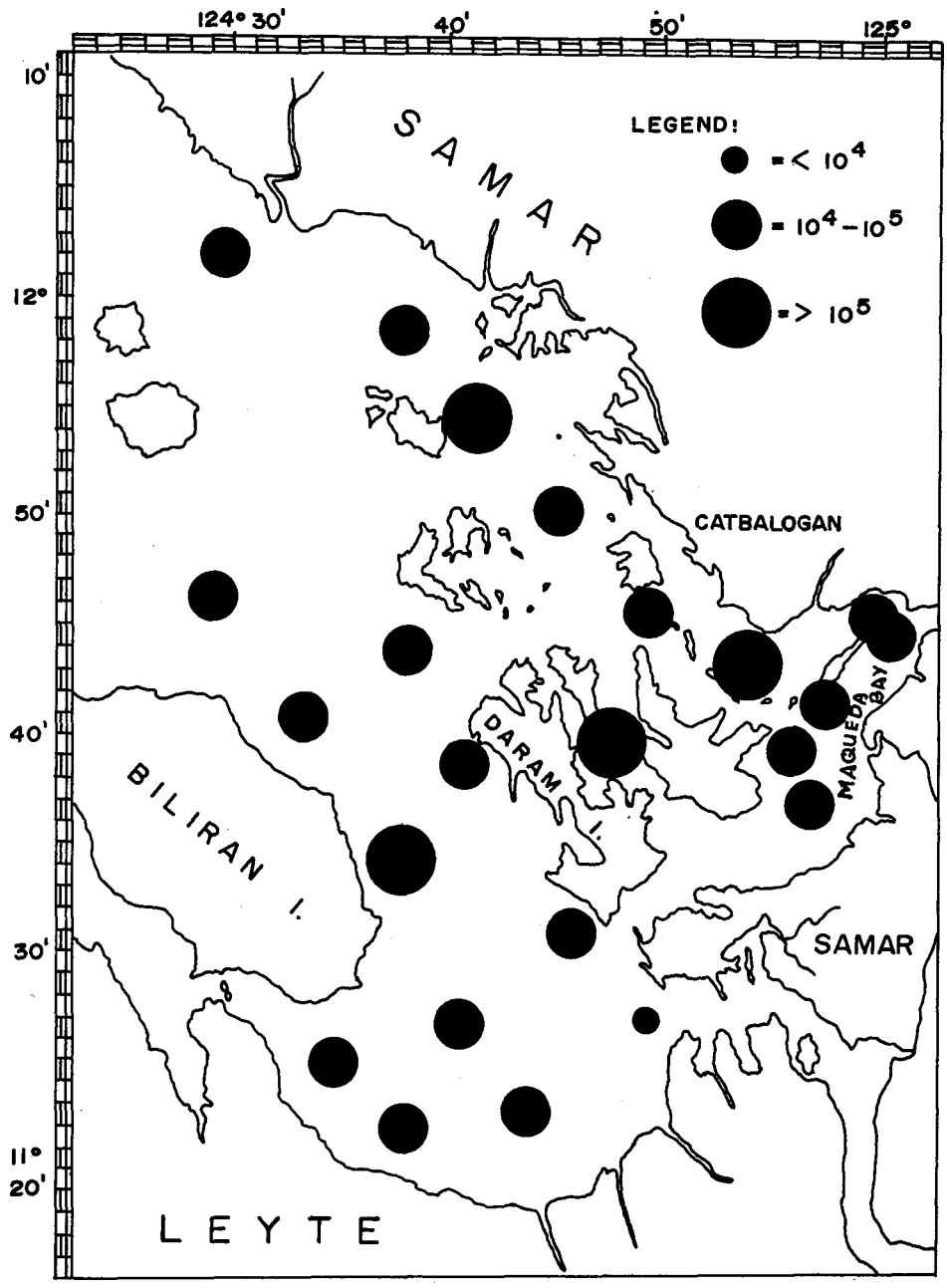

Fig. 4. P. bahamense *surface (0-1 m) densities (cells/ L) in Samar Sea, Carigara and Maqueda bays; based on Nansen Water Bottle samples.*

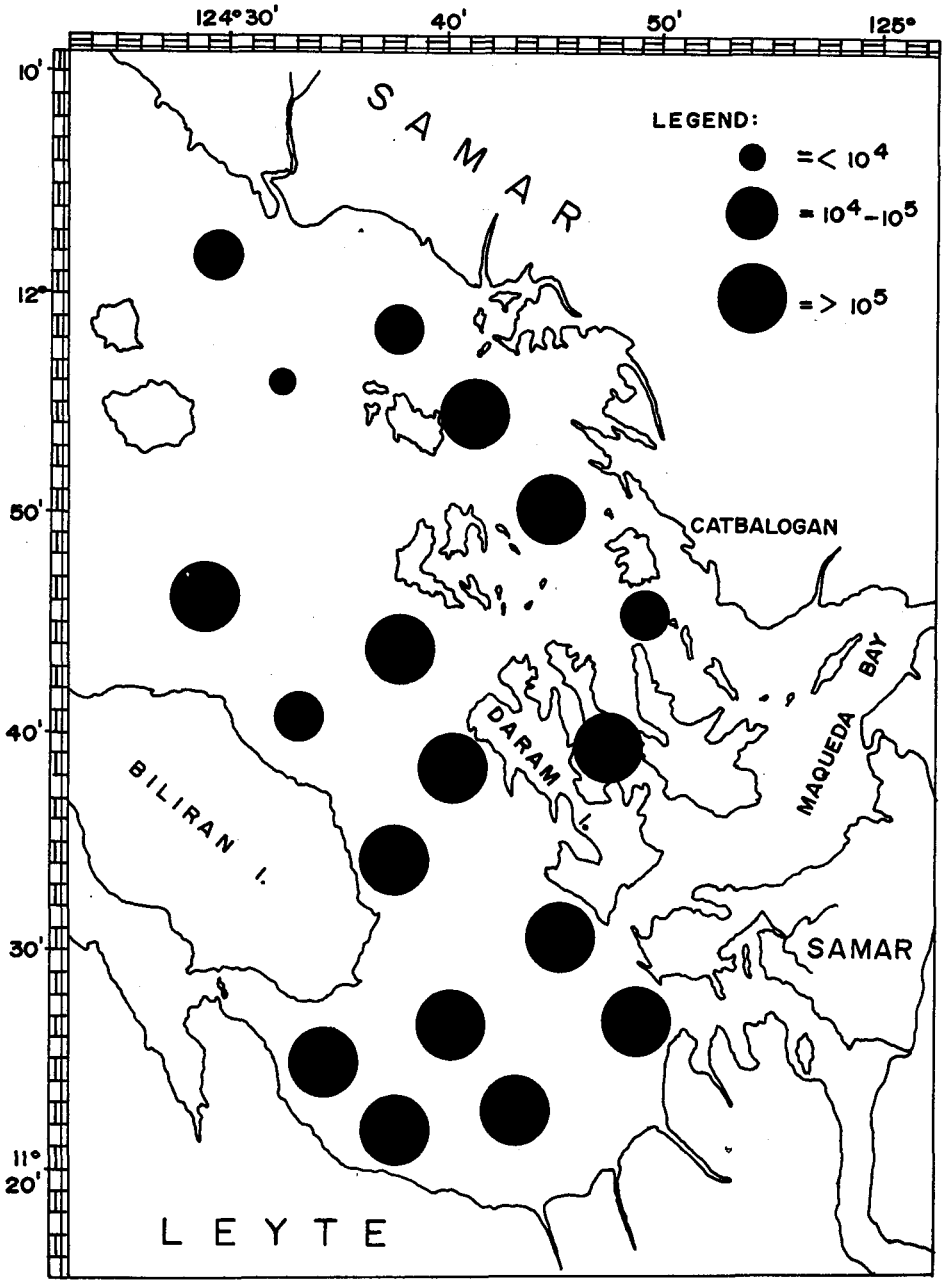

Fig. 5. P. bahamense *densities (cells/L) in Samar Sea and Carigara Bay based on double oblique tows with a 20-cm bongo sampler (0.047 mm).*

Table 3. *Pyrodinium bahamense* cell counts in Samar Sea at three depths, based on Nansen Water Bottle samples (cells/ L).

Station	0 m	10 m	20 m
1	5.5×10^4	1.3×10^5	5.7×10^4
2	4.0×10^4	4.2×10^4	1.5×10^5
3	1.3×10^4	1.8×10^4	2.6×10^4
4	5.3×10^4	—	2.6×10^4
5	9.6×10^3	4.9×10^3	2.8×10^3
6	1.8×10^4	1.4×10^4	1.7×10^4
7	1.9×10^5	5.0×10^3	3.3×10^4
8	1.4×10^4	1.2×10^4	1.2×10^5
9	4.4×10^4	—	7.7×10^3
10	5.8×10^4	7.2×10^4	3.4×10^4
11	1.3×10^4	1.7×10^4	1.3×10^4
14	1.1×10^4	4.8×10^3	8.0×10^4
15	1.4×10^4	3.3×10^4	3.3×10^3
16	9.8×10^5	—	1.5×10^3
17	4.4×10^4	7.5×10^4	—
18	2.1×10^4	7.5×10^3	1.2×10^4
19	1.7×10^5	9.3×10^3	—
20	1.7×10^5	8.0×10^4	—
Near 20	3.6×10^5	3.4×10^5	

filtering *G. catenella* becomes dangerously toxic for human consumption at a cell concentration of 200000/ L (Schantz 1973). The varying distribution of the dinoflagellate and related hydrographic parameters are discussed in a separate paper (Hermes et al. 1984). The slight variations in the toxic level of the shellfish extracts observed with this experimental design do not allow conslucions to be made with regard to natural detoxification, but may be attributed to the amount of toxin accumulated by the mussel at the time of collection.

The comparative lethal effects of crude toxin extracts subjected to different pH levels indicate a maximum toxicity of 784 MU at a pH of 3.0. The lethal effect gradually decreases with an increase in pH to neutral; the amount of toxin present in the extract does not allow the detection of pH effects at more basic pH levels (Fig. 6). The result implies that the toxicity is reduced at alkaline pH levels.

Furthermore, it is noted that the use of two different methods of water collection for plankton quantification give noticeable variations in the total number of plankton per liter (Table 4).

Table 4. *P. bahamense* abundance (cells/ L) in Maqueda Bay. A: based on Nansen Water Bottle samples; B: based on 30 cm Marutoku vertical tow net (70 μm).

Station	A	B
D	1.0×10^4	40
E	2.7×10^4	490
F	3.5×10^4	510
G	6.7×10^4	7900
H	2.1×10^4	50

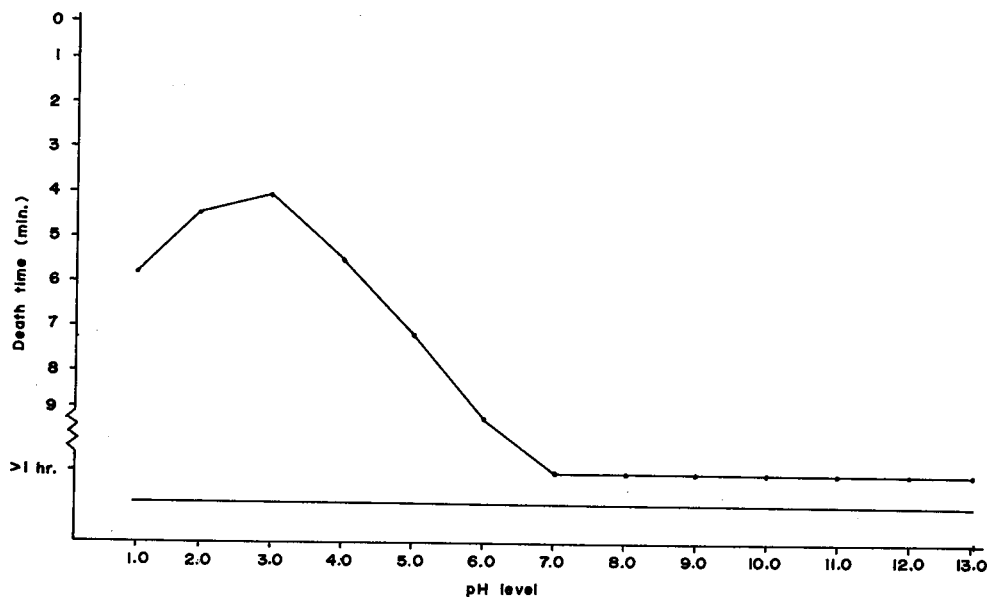

Fig. 6. Effect of pH on the toxicity of crude Perna viridis *extract.*

Limitations

The experiments were conducted without the use of standard saxitoxin. Availability of standard saxitoxin would have made the study more conclusive (with respect to the actual concentration of saxitoxin in the extract). The LD_{50}, or the amount of toxin that kills half of the experimental mice, was not determined for all samples due to financial constraints.

Recommendations

It is recommended that further studies on methods for reducing toxin potency within a reasonable time period and on a reasonable scale be conducted. Studies that involve the use of high pH values to destroy the potency of the toxin yet retain the quality of the mussel should likewise be undertaken. More studies on factors that may trigger red tides and maintain blooms of toxic dinoflagellates are still needed. Monitoring of plankton for the presence of toxic algae and shellfish for accumulated toxicity by bioassay will have to be carried out routinely until other methods become available (e.g., chemical methods). Medical research to develop an antidote to the toxin is also for paramount importance.

The authors wish to express their deepest graduate to Roger Gacutan of SEAFDEC for providing the toxin extracts; Eric Villoso and Tom Jamir for their cooperation in the plankton survey; Rex Gaddi for his support during the bioassay; and Firmo Estocapio for the illustrations. Lastly, our sincerest thanks are extended to Dr. Florian M. Orejana for the use of facilities at the Institute of Fisheries Development and Research.

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Red Tides and Paralytic Shellfish Poisoning in the Philippines

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Introduction

Toxic marine dinoflagellate blooms have been recognized for many years to have a significant impact on the utilization of shellfish resources and human health because of the problem of paralytic shellfish poisoning (PSP), which occurs in many areas of the world and seems to be intensifying and spreading (Prakash et al. 1971; Taylor and Seliger 1979). Less than 20 dinoflagellate species are known or thought to produce toxins (Steidinger 1979).

Many filter-feeding shellfish, such as clams, mussels, and scallops, feed on several of these red tide forming toxic dinoflagellates and accumulate the toxins in their tissues without themselves being affected (Prakash et al. 1971; Twarog 1974; Hashimoto 1979). As a consequence, it may become a serious problem when the affected shellfish are eaten by warm-blooded animals that are particularly sensitive to the toxin. In extreme cases, several species of clams and mussels may prove fatal if eaten by man. To date, research efforts to develop an antidote for the toxins have been relatively unsuccessful (Yentsch and Incza 1980) and the only effective control measure is the closure of the affected areas to shellfish gathering.

Paralytic shellfish poisoning has long been known as a serious problem in four regions of the world, namely Europe, North America, the Pacific coast, and Asia (Twarog 1974). There are medical records of over 1650 cases of this food poisoning worldwide, which have resulted in at least 300 fatalities (Dale and Yentsch 1978). Jay (1970) asserted that paralytic shellfish poisoning in man has a mortality rate close to 10% and as much as 22% in some areas.

Most recently, red-tide outbreaks were reported in tropical Indo-Pacific countries such as Papua New Guinea (1972 until early 1976), Brunei and Sabah (March-May 1976) and the Philippines (mid-1983).

The major species involved in the tropical Indo-Pacific red tides was the armoured, bioluminescent dinoflagellate *Pyrodinium bahamense* Plate 1906, which was recently reclassified as *Pyrodinium bahamense* var. *compressa* Böhm 1931 (Steidinger et al. 1980), a species that is closely related to *Protogonyaulax* (= *Gonyaulax* spp.) (Fig. 1), and was unreported in the region before 1971 (Maclean 1979). The organism was first described from Waterloo Lake, a small, shallow, saline lagoon in Nassau, Bahamas, in 1906 (Beales 1976) and its previous known distribution was restricted to the tropical and subtropical waters of the Carribean Sea (particularly Jamaica and Puerto Rico), eastern Pacific Ocean, Red Sea, Persian Gulf, and North Atlantic Ocean (Wall and Dale 1969) where it was reported to be nontoxic (Beales 1976). It has now, however, been found to be responsible for fatal paralytic shellfish poisonings in Papua New Guinea (Worth et al. 1975), Brunei and Sabah (Beales 1976), and the Philippines (Estudillo 1984).

Harmful dinoflagellate blooms of *Pyrodinium bahamense* var. *compressa* and the paralytic shellfish poisoning it causes were experienced for the first time in Philippine waters when they occurred along the coast of Eastern Visayas northwestward to the coast of Masbate and Sorsogon during the period from late June to early September 1983 (Fig. 2). In the latter part of September, cases of paralytic shellfish poisoning involving one fatality were reported in Western Visayas.

Severely affected by the red tide were the mariculture project farms in Maqueda Bay and Villareal Bay (western Samar), where green bay

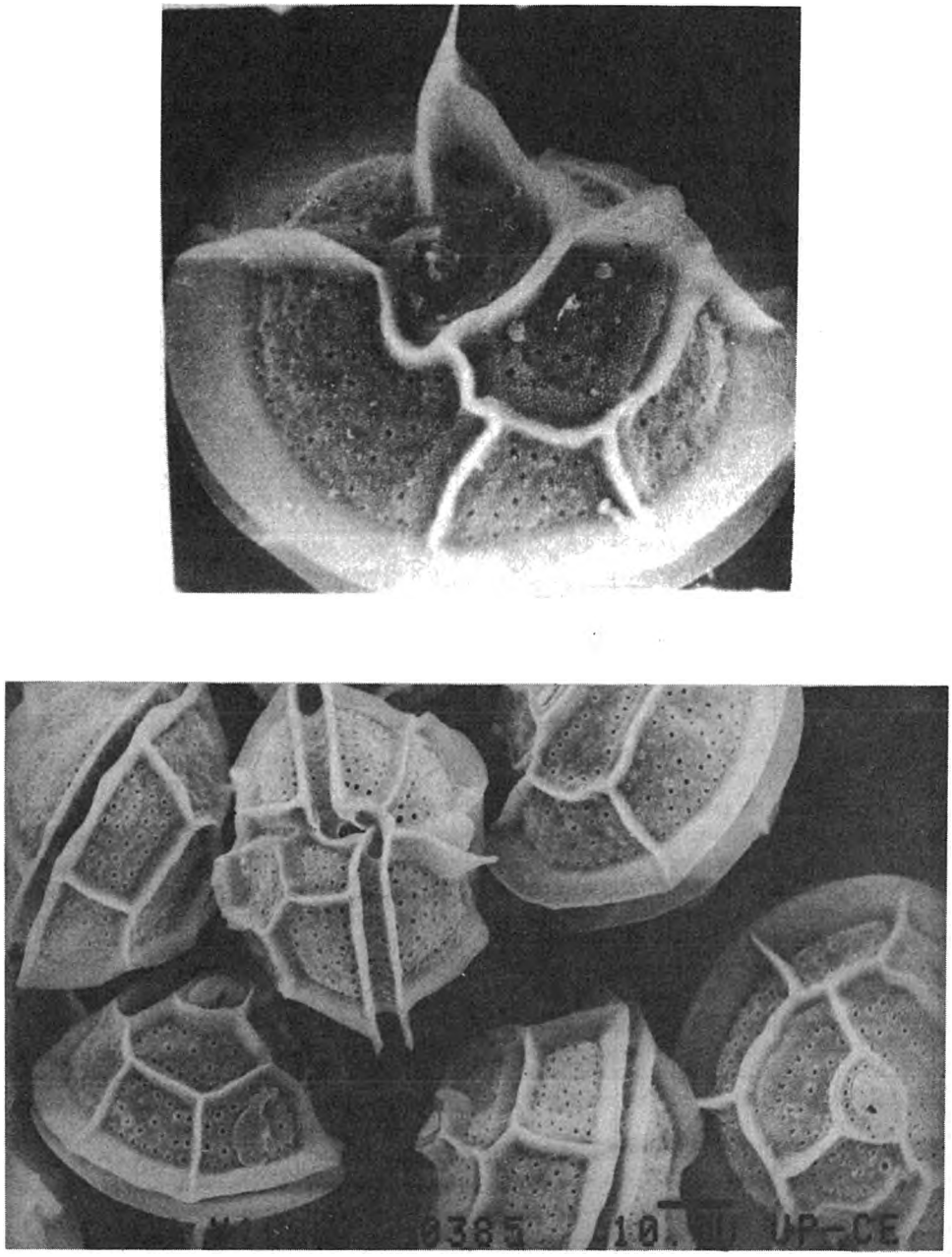

Fig. 1. Pyrodinium bahamense *var.* compressa *Böhm 1931 taken by scanning electron microscope (through the courtesy of the Research Institute for Tropical Medicine and the U.P. College of Engineering).*

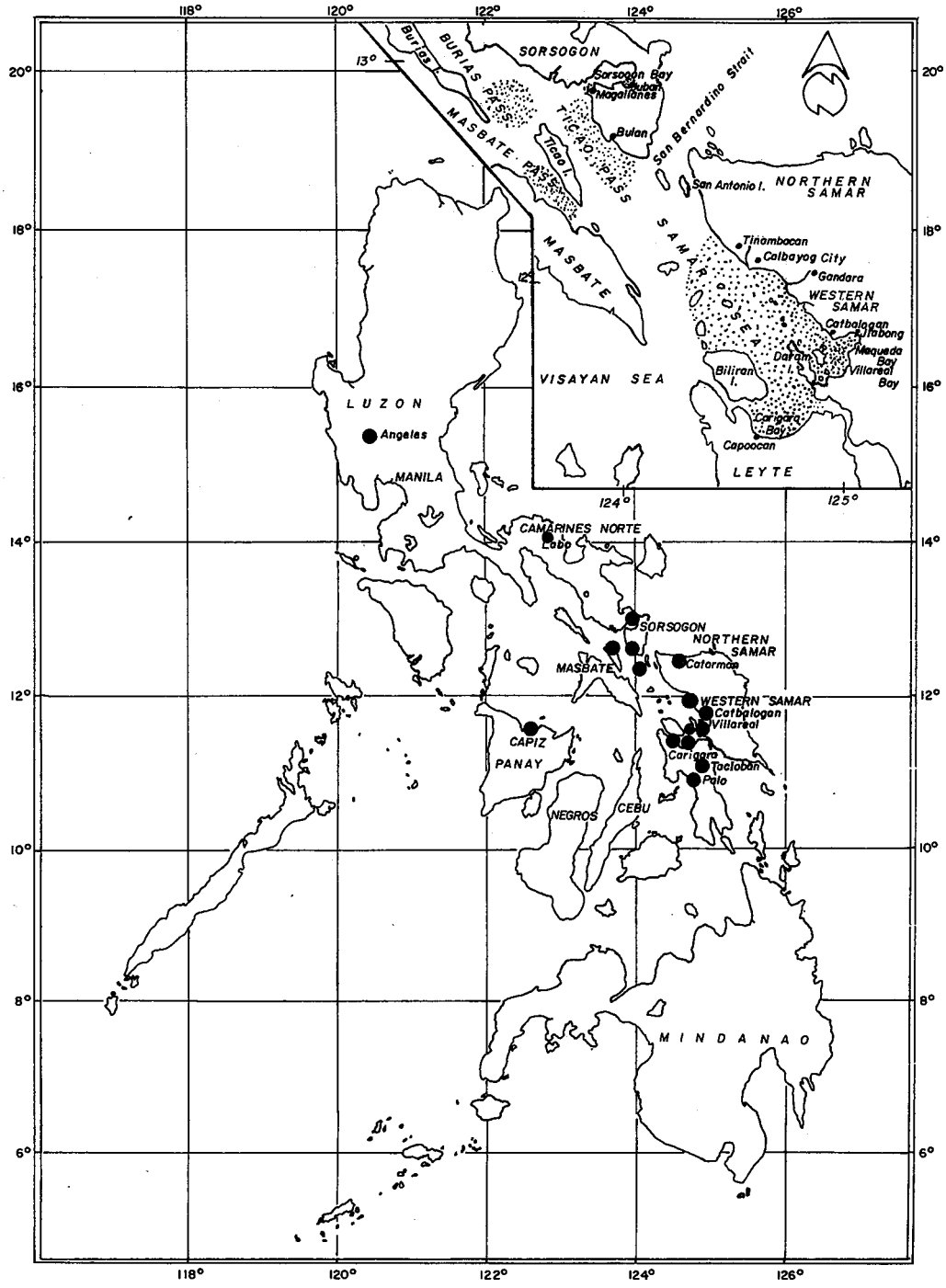

Fig. 2. Map showing the red tide survey area. Shadowed circles indicate areas where paralytic shellfish poisoning cases have been reported. Areas affected by the Pyrodinium *blooms (red tide) are stippled.*

mussels (locally called "tahong") or *Perna viridis* Linne (= *Mytilus smaragdinus*) are being cultured. Commercial harvest, sales, and consumption of mussels and other shellfish were banned for almost 8 months, which resulted in great economic loss. Paralytic shellfish poisoning reports have also shown that many people had been taken ill: a total of 278 cases were reported involving 21 deaths, most of them were due to the ingestion of the green mussels from Maqueda Bay and Villareal Bay.

The extensive toxic dinoflagellate blooms in Eastern Visayas and along the nearby coast provided an opportunity for the Bureau of Fisheries and Aquatic Resources (BFAR) to monitor and investigate the blooms.

This paper deals with the type of monitoring and investigative work conducted from 22 July 1983 until the blooms of the toxic dinoflagellate completely disappeared from the waters of the affected areas and from the gills and viscera of the mussels. The results of the toxicological analysis carried out on the mussels are also presented in this paper.

Methodology

Two aerial observations were carried out to assess the location, configuration, and possible spreading of the red tides. These were carried out using light aircraft from the Eastern Command of the Philippine Air Force in Tacloban City. In addition to these, sighting reports from commercial Philippine Airline flights were also recorded.

Eighteen oceanographic stations were established in Carigara Bay and the eastern and southern parts of Samar Sea where water samples were collected at standard depth intervals of 2 m in the upper 10-m layer and every 5 m thereafter using Nansen reversing water bottles provided with protected and unprotected reserving thermometers. Temperature readings were recorded at each depth and water samples were collected and analyzed for salinity and dissolved oxygen employing the Knudsen-Mohr and modified Winkler methods respectively.

Tansparency readings were taken using the standard Secchi disc. Sea surface and meteorological observations were also recorded.

Vertical plankton hauls were made using a closing plankton net measuring 30 cm in mouth diameter, 110 cm in total length, and with a 90 μm mesh size. Five sets of plankton data, according to sampling depths, were obtained, i.e., 5-0, 15-10, 25-20, 35-30, and 35-0 m, or from near the bottom to the surface if the depth of the station is shallower than 35 m. The samples collected were preserved in 10% formalin solution.

Plankton samples were examined for toxic dinoflagellates using a Nikon bacteriological microscope (model SC). Counting was carried out on a 1 mL aliquot part placed into a Sedgwick-Rafter counting chamber. Numerical estimates were made based on the diameter of the mouth of the net, sampling depth, and volume of the plankton samples. Counts were then converted to number of *Pyrodinium* cells per litre of water filtered by the net.

Regular plankton samplings (both vertical haul and surface tow) in Maqueda Bay and Villareal Bay and approaches were conducted with the same plankton net, but only for qualitative analysis.

Trawling operations, using a German-type net with dragging time ranging from 15 min to less than 2 hours, were made in Carigara Bay, eastern and southern parts of Samar Sea, and its adjoining waters for the purpose of collecting samples of fish and invertebrates for gut content analysis to determine the extent of contamination and identify potentially toxic seafoods.

Fish and invertebrate collections in the shallow waters of Samar Sea, Maqueda Bay, and Villareal Bay, on the other hand, were made by taking samples from the catch of subsistance fishermen operating in the area. Samples of fish and other marine products were also obtained from the markets and fish-landing centres.

Counting and recording of *Pyrodinium* cells in the gut of fish and invertebretes were carried out by removing the gut/ viscera from the fish/ invertebrate and dissecting it. The entire gut contents were then placed on a glass slide and spread out. The number of *Pyrodinium* cells were then counted under a microscope and qalified into various categories.

Bioassay tests were carried out on mussel samples collected monthly (or sometimes twice a month) from a number of mussel farms along the coastal area of Maqueda Bay and Villareal Bay. Fresh mussel samples were sent regularly to the Bureau of Research and Laboratories (BRL) of the Ministry of Health in Alabang, Muntinlupa, Metro-Manila, for "mouse bioassay" testing. The calculation of the toxin level in micrograms per 100 grams of shellfish meat, as suggested in the standard assay method of the Association of Official Analytical Chemists (Horwitz 1975), was not possible in the present study due to a lack of paralytic shellfish poison standard solution. Determination of the presence and strength of the toxin in the tissue of mussels was, therefore, attempted (using the Bureau of Research and Laboratories "standard" procedure) in terms of

percentage mice mortality and death time. For each monthly test, about 200 mice were injected intraperitoneally with shellfish extract and observed together with the control mice, which were injected with acidified water.

Results and Discussion

Chronological Observations of the Occurrence of Red Tides

The first recorded case of paralytic shellfish poisoning involved two deaths and occurred in the early morning of 21 June after a family of eight ingested mussels collected from Jiabong in Maqueda Bay, where the dinoflagellate bloom was first discovered on 10 July (Fig. 3).

The spreading of the visible red tide was observed on 15 July, apparently associated with typhoon "Bebeng", which occurred from 13-15 July, when maximum rainfall (113.6 mm on 13 July, 109 mm on 14 July, 20 mm on 15 July); maximum wind force (4-8 m/sec) in southwesterly and northwesterly directions; and maximum cloudiness (7.6-8.0 units) were recorded. Tremendous discharge from rivers into the sea was observed during this period. As a result, the red tide spread out and was seen from commercial flights as orange-red patches along the 70-km stretch of western Samar coastline from Villareal to Gandara (Fig. 4).

The peak of the visible red tides occurred in the latter part of July (Fig. 5). The fading of the visible red tides started in early August, which could be due to the continuous sunny days with only occasional rain showers, calm sea surface conditions, minimum wind force (1 m/sec), and variable winds (W, NW, SW, SSW) in the area.

In early August, visible blooms were observed to be absent from the open waters of western Samar and they seemed to concentrate very close to the coastline, particularly near the mouths of river systems. Plankton samples taken along the western Samar coastline from Catbalogan to Tinambacan showed live but somewhat sluggish *Pyrodinium* cells. The chain formation of cells were also observed to be diminishing. The longest chain of the organism observed during its peak occurrence in July was 10 cells, compared with only 6 cells in mid-August (Hermes 1983).

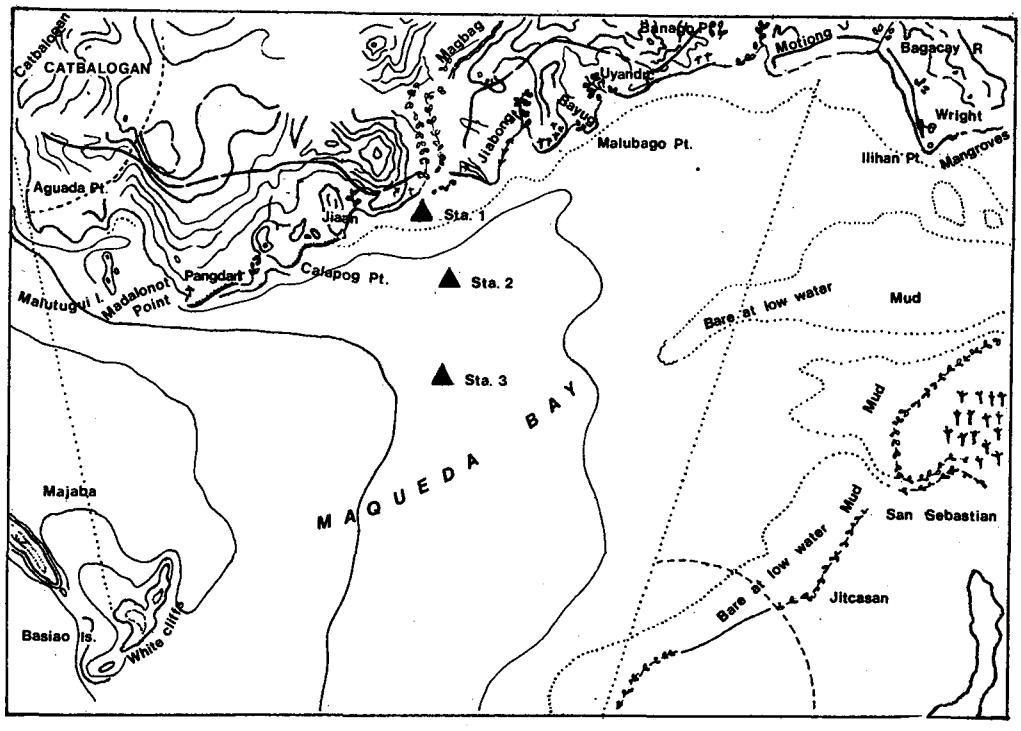

Fig. 3. Locations of plankton stations (dark triangles) on Jiabong side of Maqueda Bay from which toxic dinoflagellate samples were taken, 10 July 1983 (Estudillo 1984).

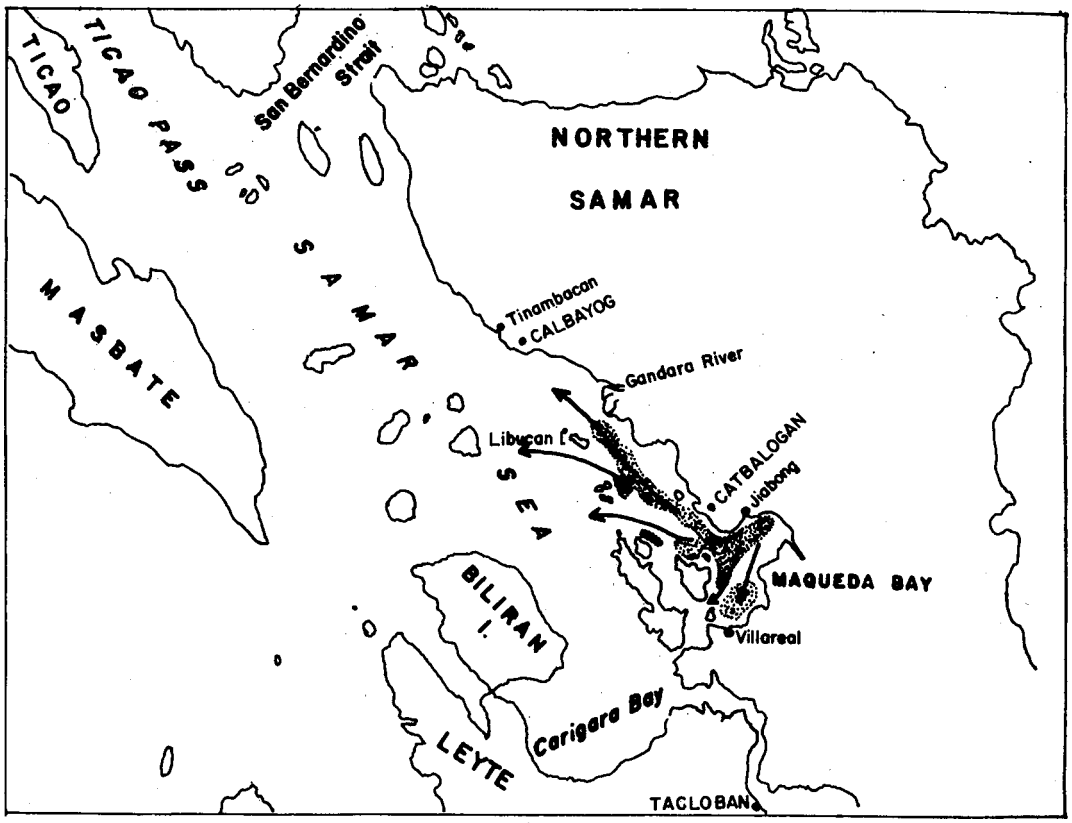

Fig. 4. Distribution of red tide patches (dotted) in Maqueda Bay and approaches from incidental aerial observations — 0900 hours, commercial PAL flight, Tacloban City to Manila, 18 July 1983. Arrows indicate possible movements of dinoflagellate blooms (Estudillo 1984).

During the second week of August, no visible blooms were observed along the western Samar coastline from Maqueda Bay to Tinambacan, except in the area southwest of Tagdarano Island, south of Calbayog City, and near the mouth of the Gandara River, where visible blooms in small patches were noticed.

A plankton sample that was collected near the mouth of the Gandara River showed that *Pyrodinium* comprised about 90% of the total number of plankton organisms. Heavy blooms in patches were also observed at about 1 nautical mile off Biliran Island, between Amambahag Point and Caibiran River (southern part of Samar Sea).

On 13 and 14 August, visible blooms totally disappeared from the southern part of Samar Sea, Carigara Bay, and the entrance of Maqueda Bay. However, plankton samples in Maqueda Bay were still dominated by *Pyrodinium,* followed by *Noctiluca* and *Skeletonema.* On the other hand, *Pyrodinium* completely disappeared from the plankton samples from Zummaraga Channel, except at its entrance where a visible bloom in the form of a streak still remained.

In mid-August, red tide totally disappeared from Maqueda Bay, Villareal Bay, and Samar Sea, but *Pyrodinium* remained a constituent of the plankton population, although in an insignificant quantity.

Plankton samplings were taken at eight stations along the coast of Sorsogon and Ticao Pass that showed the presence of an insignificant number of *Pyrodinium* cells. The dinoflagellate was observed to be present in the gut of the Indo-Pacific mackerel (over 200 cells per individual fish) caught in Ticao Pass (off Bulan, Sorsogon). Slightly discoloured water was noticed on 21 August along the beach of Magallanes, Sorsogon, where a large amount of *Pyrodinium* was found in the plankton sample collected from the area.

The visible blooms and the causative

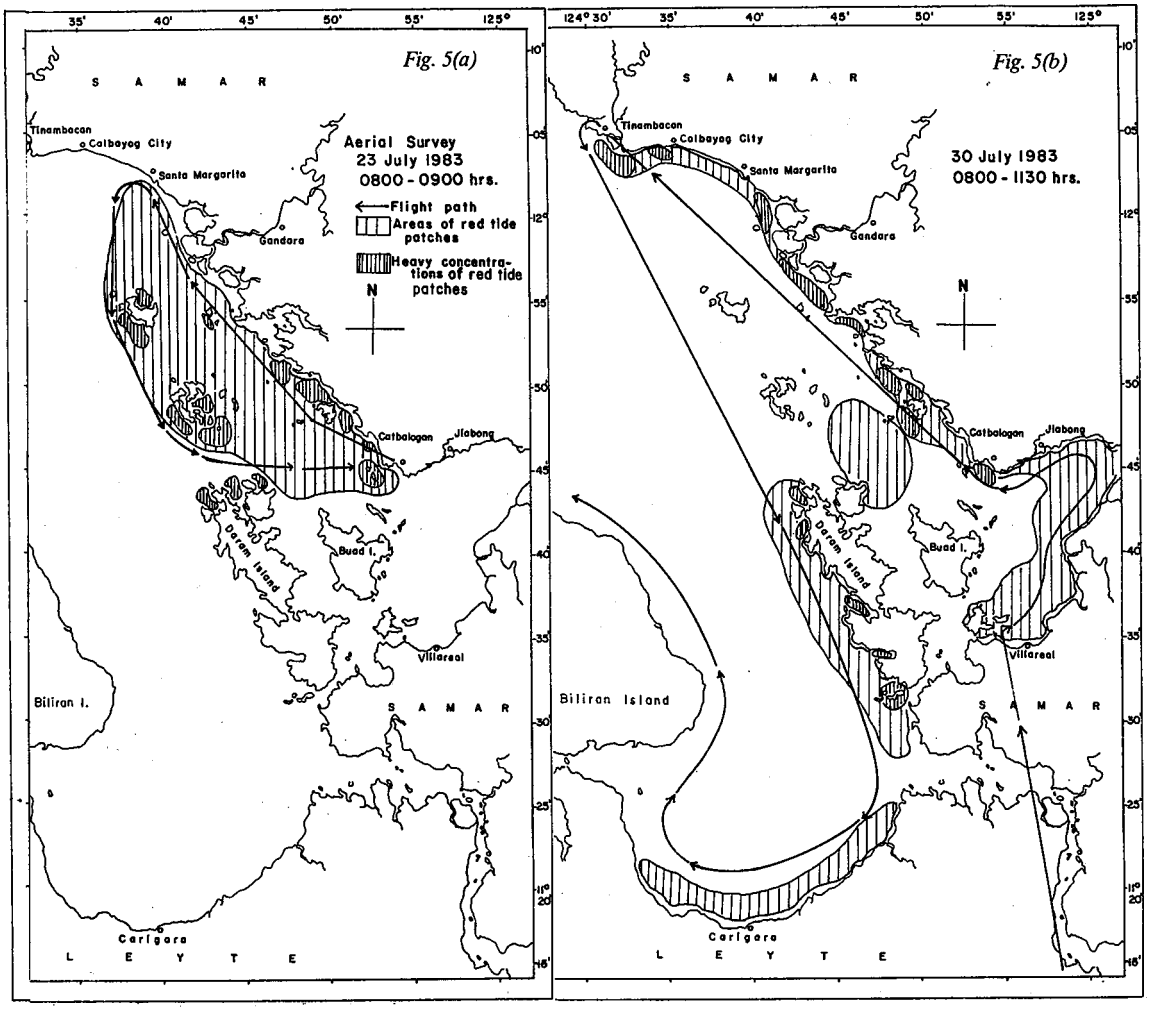

Fig. 5. Distribution of red-tide patches as viewed during aerial surveys on (a) 23 July and (b) 30 July 1983.

dinoflagellate had totally disappeared from Maqueda Bay, Villareal Bay, Carigara Bay, and Samar Sea by the end of August.

On 7 September, follow-up monitoring of Maqueda Bay, Villareal Bay, Samar Sea, and Carigara Bay was conducted and showed once again the complete absence of red tides. Subsequently, blooms of *Noctiluca scintillans* appeared after the toxic red tide blooms. Yellow and greens streaks, or patches of the *Noctiluca* blooms, were observed in Maqueda Bay and Villareal Bay. Blooms of *Noctiluca* were also observed in the eastern Samar Sea, Ticao Pass, and Masbate Pass. Numerous pontellid (blue) copepods were also observed to be very common in the samples. Hermes (1983) also reported a bloom of *Noctiluca* in Visayan Sea, specifically in the northern part of Guimaras Strait and Sorsogon Bay.

On 23 September, cases of paralytic shellfish poisoning were reported in Capiz, northern Panay, that were attributed to scallops (*Amusium pleuronectes*). Shellfish samples were collected from Northern Panay waters and analysis showed that the toxic dinoflagellate was present in the gut of mussels, oysters, and scallops, although in small numbers (with cell counts ranging from 1-20 *Pyrodinium* per individual shellfish). On 12 October, plankton samples were taken at 22 stations from Ayagao Bay, Sapian Bay, and Tinagong

Dagat. All samples taken from Tinagong Dagat were found negative for *Pyrodinium,* whereas those taken from Sapian Bay and Ayagao Bay showed very low levels (from one to seven *Pyrodinium* cells per litre of water). Shellfish collected during the survey were all found to be negative for *Pyrodinium.*

Aerial Survey

The aerial observations were made on the morning of 23 July between 08:00 and 09:00 hours. Figure 5(a) shows the locations of the various red-tide patches or visible blooms observed during the aerial survey. It should be noted that the visible blooms in intense orange-red patches cover practically the entire survey area but heavy concentrations were seen more conspicuously near the coastline.

Another aerial observaton was made on the morning of 30 July between 08:00 and 11:30 hours. Figure 5(b) shows the locations of the observed visible blooms that were generally noted to be very close to the coastline and more prominently near the mouths of river systems.

In Maqueda Bay and Villareal Bay, visible blooms were observed from the shoreline to the 3-fathom contour line. Intense orange-red patches could be seen along a 100-km stretch of the western Samar coastline from Bonoanan, Catbalogan, to Tinambacan. Visible blooms were observed along the entire coast of Daram Island (southern part of Samar Sea), particularly in Sumulit Bay, Cananayan Bay, Domiri Bay, Saa Bay, and around Bascal Island. Visible blooms were also observed along the coast of Carigara Bay, whereas no visible blooms were observed around Biliran Island, northwest of Leyte, and San Pedro Bay.

Observations from commercial flights in early August showed that the visible blooms were still present in Carigara Bay and eastern and central parts of Samar Sea (Hermes 1983). By mid-September, visible blooms had totally disappeared from the areas, as observed by R. Estudillo from commercial flights.

Spatial Distribution of the Dinoflagellate

The distribution of *Pyrodinium,* as observed from plankton collections, covers the coasts of Easten Visayas, provinces of northern Leyte (Carigara Bay), and western Samar (Maqueda Bay, Villareal Bay, Zummaraga Channel, and Samar Sea) northwestward to the coasts of Masbate and Sorsogon (Ticao Pass, Burias Pass, and Masbate Pass) (Fig. 2).

Generally, the dense population during the peak of the dinoflagellate blooms was observed in Maqueda Bay, Villareal Bay, Carigara Bay, and along the coast of western Samar from Catbalogan to Tinambacan. These are obviously the areas where visible blooms tend to concentrate (Fig. 5).

Phytoplankton hauls, to sample specific strata of some of these areas, were made with a 30-cm, 90 μm closing net, the only fine-mesh net available on board the research vessel. The locations of sampling stations are shown in Fig. 6. Three sets of data according to sampling depths were gathered and are presented in Table 1.

It was observed that the concentrations of *Pyrodinium* in the latter part of July varied from a minimum of 59 cells/L at 25-20 m to a maximum of 7134 cells/L at 5-0 m. As expected, a lowering of concentrations in deeper layers was observed; however, this is sometimes inconsistent, which may be due to the effects of diurnal variations in the behaviour of plankton. Generally, the dinoflagellate tends to concentrate in the coastal waters of Catabalogan, at the entrance of Maqueda Bay, and in the northernmost stations (stations 7 and 14). The minimum concentration of the dinoflagellate was observed in the northern part of the survey area and at the entrance of Carigara Bay (Fig. 7).

The concentrations of *Pyrodinium* in August along a transect from Catbalogan to Biliran Island ranged from a minimum of 637 cells/L north of Daram Island to a maximum of 138269 cells/L in the shallow waters off Catbalogan (Table 1). The concentration was observed to increase toward Biliran Island (Fig. 8).

The concentration of *Pyrodinium* at the station occupied during the 13 and 14 August survey was generally higher than those mentioned above, with counts ranging from a minimum of 2251 to a maximum of 123588 cells/L. This is not surprising considering that the sampling was carried out in the entire water column from 35 to 0 m, or from near the bottom to the surface. The highest concentrations were observed at the entrance to Maqueda Bay and between Biliran and Daram Islands and in Carigara Bay (Fig. 9).

The organism was also observed to be present in the plankton samples collected from Cebu City harbour, from the northernmost part of Camotes Sea, and from Sapian Bay and Ayagao Bay in northern Panay. Likewise, it was present in samples taken from the innermost part of Sorsogon Bay in Juban and the entrance of the bay in Magallanes, but absent in the plankton hauls from the bay proper. Counts of the dinoflagellate in all areas mentioned above were insignificant. The locations of these areas are shown in Fig. 2.

The observation that *Pyrodinium* is spreading in the tropical Indo-Pacific region may have some basis. It started during the massive red tides in

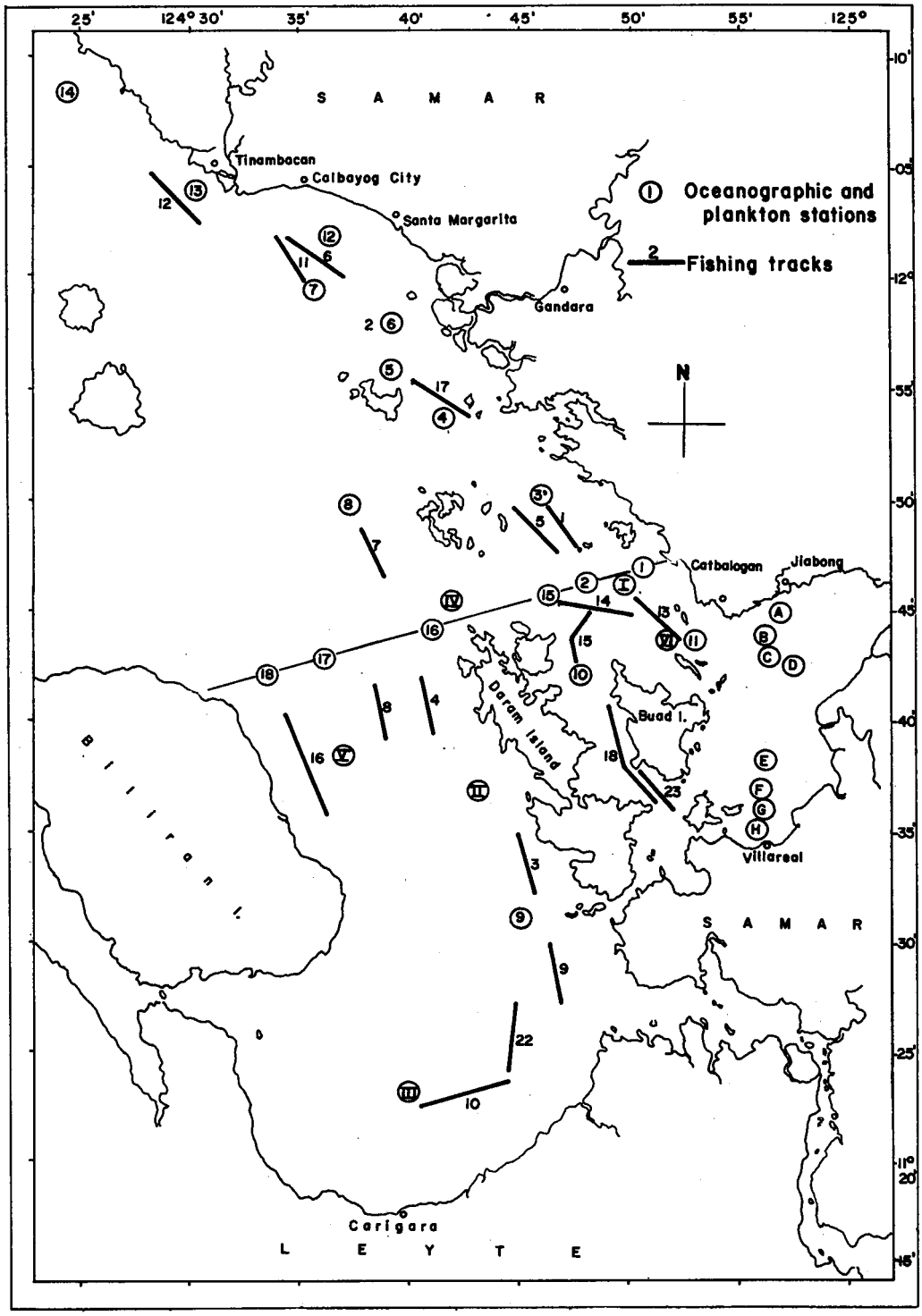

Fig. 6. Map showing oceanogrpahic stations and trawling tracks research vessel Researcher *in the coastal waters of western Samar and Carigara Bay.*

Table 1. Abundance of *Pyrodinium bahamense* var. *compressa* at different layers and different stations in the coastal waters of western Samar and Carigara Bay.

Date 1983	Station No.	Sonic depth (m)	Sampling layers (m)	Cell counts/L
24 July	I	12	5 — 0	5449
			10 — 5	4013
	2	27	5 — 0	6391
			15 — 10	3369
			25 — 20	1724
	3	15	5 — 0	2080
			15 — 10	1139
	4	18	5 — 0	1635
			15 — 10	3641
25 July	5	27	5 — 0	1932
			15 — 10	1734
			25 — 10	59
	6	11	5 — 0	297
			10 — 5	669
	7	26	5 — 0	7134
			15 — 10	3963
			25 — 20	4013
	8	59	5 — 0	149
			15 — 10	892
			25 — 20	186
	9	40	5 — 0	149
			15 — 10	1932
			25 — 20	59
27 July	10	30	5 — 0	342
			15 — 10	495
			25 — 20	803
	11	11	5 — 0	3418
			10 — 5	1783
1 August	14	68	5 — 0	7285
			15 — 10	892
			25 — 20	885
1 August	1	12	11 — 6	138269
	15	37	35 — 30	1019
	16	48	35 — 30	637
	17	65	35 — 30	892
	18	77	35 — 30	1465
13 August	I	28	25 — 0	11549
	II	46	35 — 0	2251
	III	46	35 — 0	19660
	IV	55	35 — 0	3800
	V	72	35 — 0	17197
14 August	VI	18	15 — 0	123588

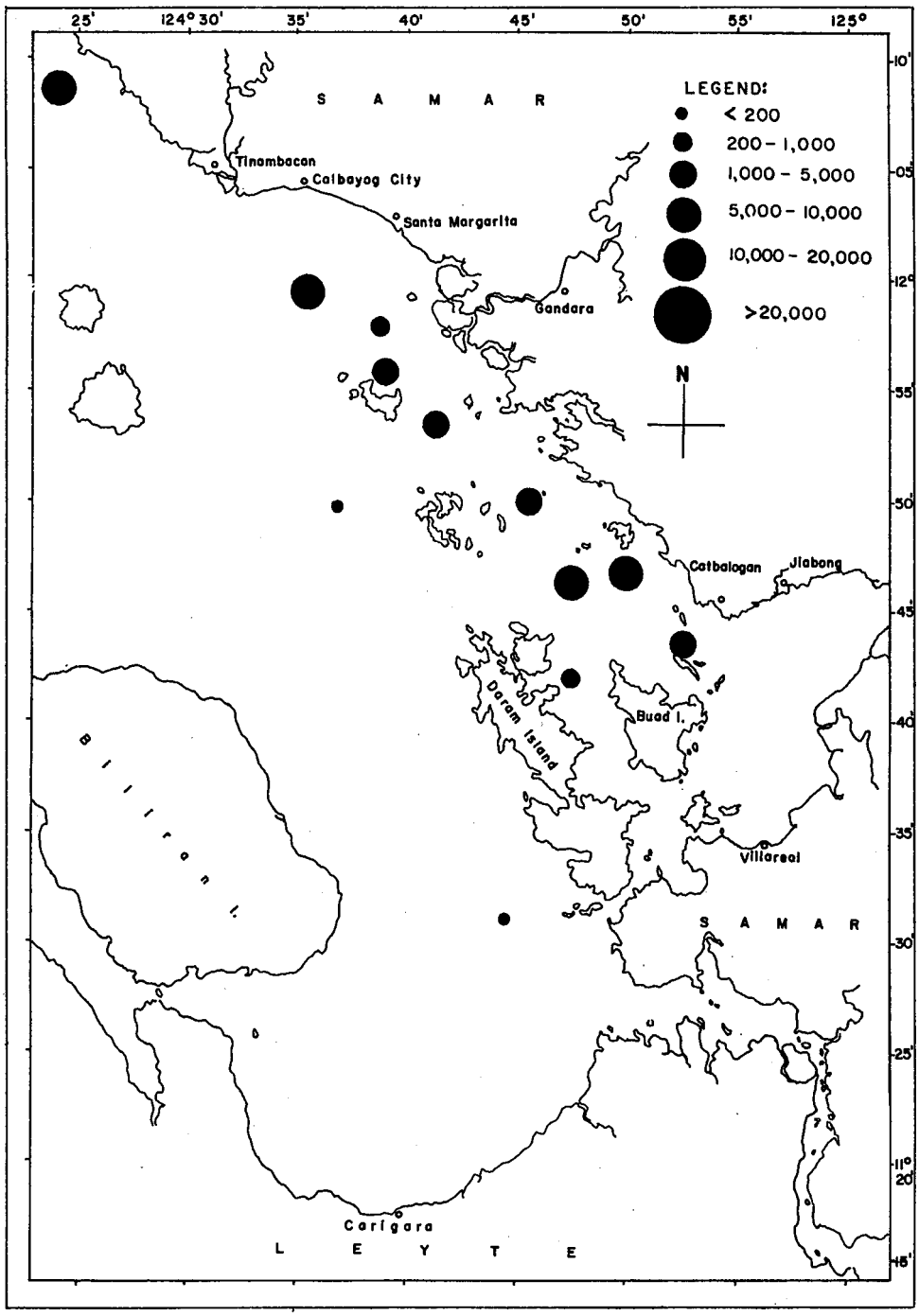

Fig. 7. Relative abundance of Pyrodinium bahamense *var.* compressa *in the coastal waters of western Samar collected by vertical haul from 5 — 0 m from 27 July — 1 August 1983. (Legend shows number of* Pyrodinium *cells per litre.)*

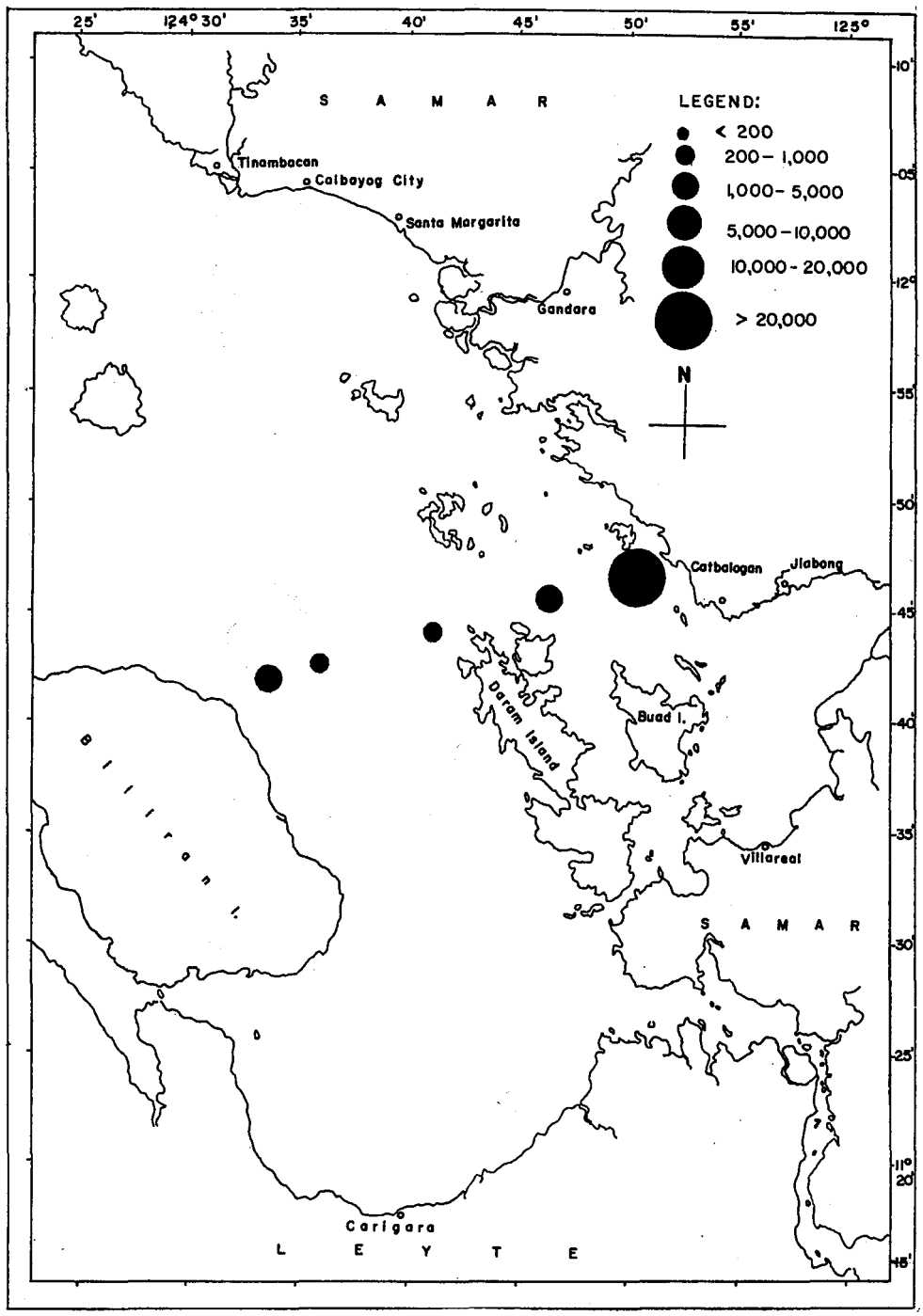

Fig. 8. Relative abundance of Pyrodinium bahamense *var.* compressa *along a transect from Catbalogan to Biliran Island collected by vertical haul from 35 — 30 m on 1 August 1983. (Legend shows number of* Pyrodinium *cells per litre.)*

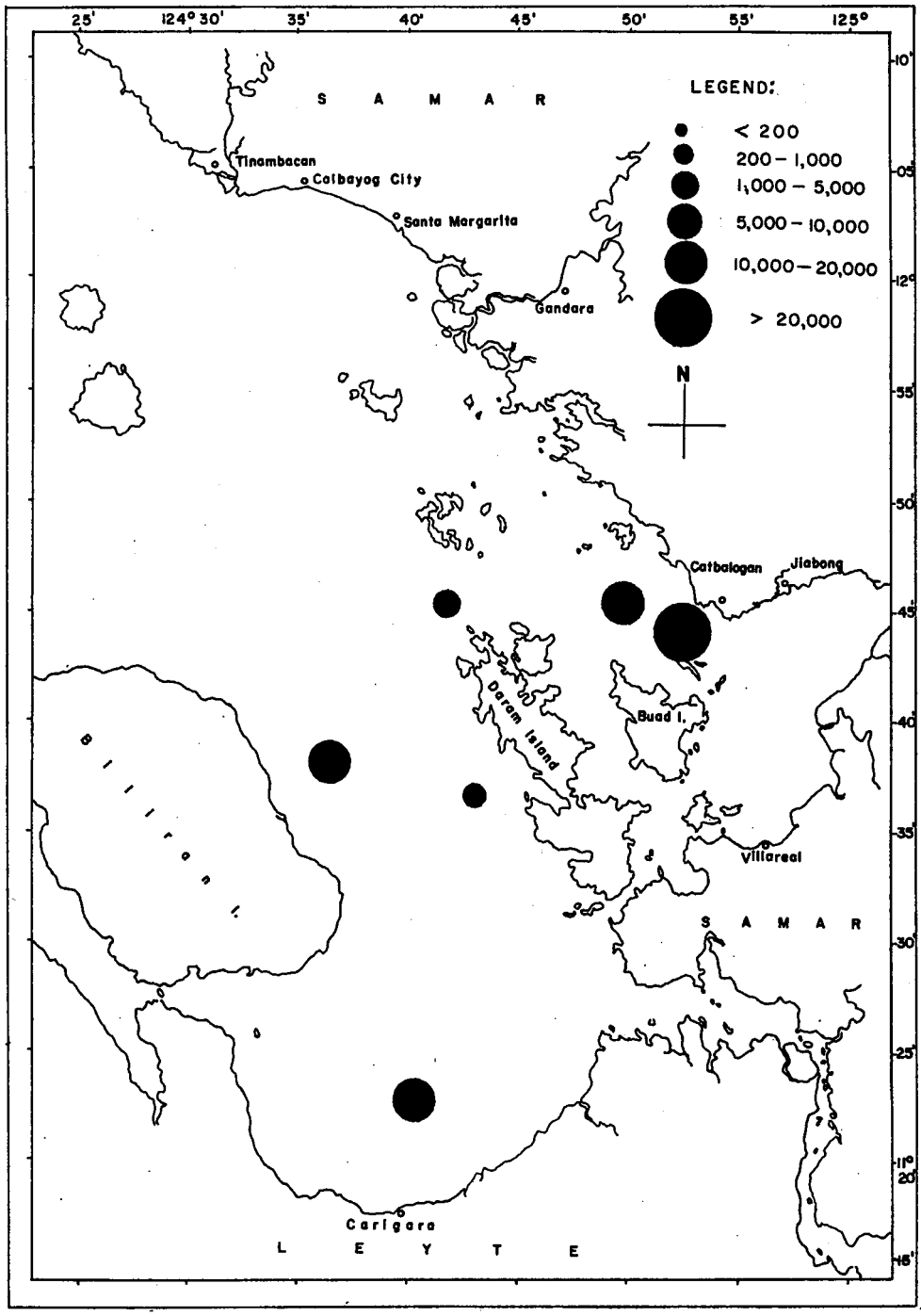

Fig. 9. Relative abundance of Pyrodinium bahamense *var.* compressa *in the coastal waters of western Samar and Carigara Bay collected by vertical haul from near the bottom to the surface (generaly from 35 — 0 m) on 13-14 August 1983. (Legend shows number of* Pyrodinium *cells per litre.)*

December 1975 — February 1976 in Papua New Guinea, which was followed in a matter of a month by the Brunei and Sabah outbreaks in March-May 1976. The Southern Equatorial Current easily accounts for the possible introduction of *Pyrodinium* into the area from Papua New Guinea (Maclean 1979). Similarly, this could also be the reason for the first occurrence of the organism in the Philippines. *Pyrodinium* was also found in Arumizu Bay in Palau Island (Western Pacific) (Harada et al. 1982) and there is a possibility that the organism was introduced into Philippine waters by the North Equatorial Current.

Physical Environment

Results of some of the oceanographic observations obtained during the period from 24 July — 4 August are presented in Table 2. The stations were established in areas where visible blooms were observed to be widespread. The locations of the sampling stations are shown in Fig. 6.

The observed surface water temperature ranged from 29.6-32.5°C. The bottom temperature ranged from 26.4-29.8°C.

The observed surface salinities ranged from 31.15-33.88 ppt, whereas the bottom salinities ranged from 33.73-35.28 ppt.

Both ranges described above are quite similar to those measured in mid-August, i.e., temperatures from 27.6-31.0°C and salinities from 31.9-34.9 ppt (Hermes 1983).

Water temperature and salinity values measured are within the range of the observed values for *Pyrodinium* blooms elsewhere, i.e., 24.4-31.9°C and 24.7-36.8 ppt in Papua New Guinea (Maclean 1977); 24.5-29.4°C and 24.3-32.08 ppt in Brunei (Beales 1976); 27.0-35.0°C and 30.0-36.0 ppt in Jamaica (Buchanan 1971), and 22.2-29.2°C and 30.5-36.5 ppt in Florida (Steidinger and Williams 1970).

Earlier studies of red tide showed that *Pyrodinium* can tolerate salinities as low as 14 ppt for a few days (Buchanan 1971). Laboratory experiments conducted in Papua New Guinea during the 1973 and 1974 blooms showed that the *Pyrodinium* band formed within the salinity range of 28.6 and 31.5 ppt, and can tolerate salinities as high as 40.7 ppt (Maclean 1977). *Pyrodinium* is atypical, having an optimum salinity of around 35.7 ppt (Wall and Dale 1969). This suggests that *Pyrodinium* favours high salinity, as noted above. The lower salinities and reverse temperature gradient associated with river runoff are merely indicators of the source of nutrients and not catalysts of the widespread blooms (Maclean 1977).

Transparency estimates as determined using a Secchi disc ranged from 1.5-12.5m, with exceptionally larger Secchi depths (14 m) at stations 14 (off Binalio Point, Tinambacan) and 18 (northeast of Biliran Island).

In the survey conducted by the University of the Philippines in the Visayas College of Fisheries (UPVCF) team in mid-August, the recorded transparency readings ranged from 5-11 m, which was within the range mentioned above. However, it was a marked decrease from the values observed in 1979 in Samar Sea, when Secchi depths were 15-20 m (Labao 1980).

The observed surface dissoved oxygen ranged from 3.19-5.40 mL/L, whereas the bottom dissolved oxygen ranged from 0.45-3.86 mL/L. The maximum dissolved oxygen values were observed at station 17 (southern Samar Sea). Both surface and bottom oxygen values were generally normal.

The observed prevailing winds at each station on any particular day, as determined by the vessel's instruments, were variable, with wind speeds ranging from 2-18 knots. Generally, the winds blow toward the southeasterly and southwesterly directions. The observations are similar to those recorded by the Catbalogan weather station in Samar. Five sampling stations (stations 1 and 15-18) were occupied on 4 August in a transect from Catbalogan to Biliran Island (Fig. 8) that showed the prevalence of light northeasterly winds (Table 2).

An analysis of wind data for possible correlation with the occurrence of the dinoflagellate bloom will be attempted in the near future. Initial studies showed that wind is at least as important as rainfall in promoting and sustaining *Pyrodinium* blooms (Maclean 1977).

Fishing and Examination of Gut Contents

A total of 49 fishing operations were conducted in areas outside Maqueda Bay (Samar Sea and nearby coast) during the monitoring period that yielded a total catch of 6637 kg of fish and invertebrates. Locations of fishing tracks are shown in Fig. 6. Fishing track nos. 19 and 20 were occupied in an area (Pambujan Bay, northern Samar) not affected by the red tide.

Table 4 shows the *Pyrodinium* concentrations inside the gut of fish and invertebrate samples taken from the trawl catch that were microscopically examined on board. A series of categories has been used and the total number of *Pyrodinium* cells in each category are as follows:

+= 1-20 *Pyrodinium* cells/fish or invertebrate
++= 21-100 *Pyrodinium* cells/fish or invertebrate
+++= 101-200 *Pyrodinium* cells/fish or invertebrate.

Table 2. Physicochemical data of the coastal waters of western Samar and Carigara Bay.

Date 1983	Station Number	Sampling Depth (m)	Transparency (m)	Wind Direction and Speed (knots)	Water Temperature (°C)	Salinity (ppt)	Dissolved Oxygen (mL/L)
24 July	1	0	3.6	NW (18)	29.9	31.80	3.58
		2			29.9	—	4.59
		4			30.0	—	3.02
		6			29.6	—	1.74
	2	0	6.0	SSE (10)	30.6	—	4.20
		2			30.1	—	4.00
		4			29.9	—	3.64
		10			29.8	—	2.69
		20			29.4	—	2.18
		25			29.1	—	1.85
	3	0	—	SSW (4)	30.0	33.17	4.31
		2			29.5	33.07	4.31
		4			30.0	33.44	4.20
		7			29.8	34.27	4.26
		10			29.8	35.10	3.76
	4	0	7.8	SE (3)	32.5	—	4.31
		2			32.3	—	4.31
		4			31.0	33.44	4.20
		6			28.5	33.44	3.81
		10			27.3	34.56	3.30
		15			26.5	34.27	3.02
25 July	5	0	5.0	SE (8)	29.6	33.17	3.70
		2			29.7	33.38	3.92
		4			29.7	33.17	3.92
		6			29.9	33.10	3.81
		10			29.5	—	3.02
		15			29.5	34.56	3.02
		20			29.7	34.56	3.64
		25			29.7	34.56	3.86
	6	0	2.5	NE (9)	29.9	30.68	3.75
		2			29.9	33.44	3.47
		4			29.9	33.73	3.14
		6			29.9	34.00	3.19
		10			29.6	34.00	2.86
	7	0	1.5	SE (4)	30.4	31.80	3.86
		2			30.3	32.61	4.87
		4			29.9	33.73	3.70
		6			29.7	33.73	3.75
		10			29.7	33.13	3.53
		15			29.6	33.13	3.86
		20			29.5	33.73	3.42
		25			29.5	33.73	3.42

Table 2. (continued)

Date 1983	Station Number	Sampling Depth (m)	Transparency (m)	Wind Direction and Speed (knots)	Water Temperature (°C)	Salinity (ppt)	Dissolved Oxygen (mL/L)
	8	0	8.0	SE (3)	30.0	—	3.53
		2			30.0	—	3.58
		4			29.9	—	3.70
		6			29.9	—	3.92
		10			29.7	—	4.14
		20			29.2	—	2.58
		30			28.9	—	2.46
		40			27.9	—	2.74
		50			27.6	—	1.90
		58			27.4	—	1.90
	9	0	—	SW (5)	29.8	33.44	3.42
		10			29.7	34.00	3.30
		20			29.4	34.83	2.86
		30			28.6	34.83	2.63
		38			28.3	34.83	2.35
27 July	10	0	9.1	SW (2)	29.7	30.96	3.86
		2			30.0	33.44	3.42
		4			29.9	34.00	3.58
		6			29.6	34.27	2.72
		10			29.9	34.27	3.42
		15			29.6	34.56	2.44
		20			29.0	—	1.74
		28			28.1	—	1.62
	11	0	6.0	NW (5)	30.0	30.41	4.26
		2			29.8	31.80	3.75
		4			29.9	34.00	—
		6			29.7	34.00	2.30
		10			29.6	—	1.62
1 August	12	—	9.0	WSW (1)	—	—	—
	13	—	—	W (4)	—	—	—
	14	—	14.0	NNE (4)	—	—	—
3 August	A	0	—	—	—	30.97	—
	B	0	—	—	—	31.09	—
	C	0	—	—	—	31.80	—
	D	0	—	—	—	32.07	—
	E	0	—	—	—	33.08	—
	F	0	—	—	—	32.79	—
	G	0	—	—	—	32.25	—
	H	0	—	—	—	31.15	—
4 August	1	0	6.1	NE (0)	30.2	33.88	3.19
		2			30.2	33.88	3.14
		4			30.1	34.44	1.76
		6			29.6	34.72	0.78
		10			29.5	34.16	0.45

Table 2. (continued)

Date 1983	Station Number	Sampling Depth (m)	Transparency (m)	Wind Direction and Speed (knots)	Water Temperature (°C)	Salinity (ppt)	Dissolved Oxygen (mL/L)
4 August	15	0	12.15	NE (1)	30.3	33.88	3.58
		2			30.2	33.60	3.64
		4			30.2	34.16	4.26
		6			29.8	34.16	3.25
		10			29.4	34.44	2.41
		15			29.3	34.72	1.90
		20			29.0	35.28	1.68
		30			28.7	35.28	1.40
		35			28.6	35.28	1.18
	16	0	9.0	NE (0)	30.7	33.88	4.09
		2			30.2	33.88	4.26
		4			30.2	33.88	5.04
		6			30.0	33.88	4.93
		10			29.5	34.72	3.47
		20			29.0	34.16	3.42
		30			29.1	35.28	2.07
		40			28.5	35.00	2.13
		45			28.1	35.00	2.30
	17	0	3.5	NE (2)	30.4	33.60	5.40
		2			30.1	33.88	5.21
		4			30.0	33.88	5.66
		6			29.9	34.72	4.59
		10			29.7	34.44	4.42
		20			29.3	35.00	4.03
		30			28.8	35.00	3.02
		40			28.0	35.28	2.30
		50			27.3	35.28	2.60
		63			26.7	—	2.63
	18	0	14.0	NE (3)	30.5	—	4.70
		2			33.3	—	4.42
		4			30.2	—	4.40
		6			29.9	—	4.26
		10			29.9	34.16	4.14
		20			29.2	34.72	3.92
		30			28.5	—	2.72
		40			27.8	—	2.97
		50			27.6	34.72	2.52
		60			27.3	34.44	2.46
		70			26.7	34.44	2.74
		75			26.4	34.72	2.52

++++= over 200 *Pyrodinium* cells/fish or invertebrate

The last category (i.e., over 200 cells/fish) extends to thousands or millions of cells per fish.

It can be seen from Table 4 that of the many varieties of food fish, the pelagic group (plankton-feeding fish), represented by five species, namely Indo-Pacific mackerel (*Rastrelliger brachysoma*), sardines (*Sardinella* spp.), Indian mackerel (*Rastrelliger kanagurta*), crevalle (*Selaroides* spp.), and anchovies (*Stolephorus* spp.) contained the most *Pyrodinium* cells. Hard-tail (*Megalaspis cordyla*), caught on two occasions, was also found to contain *Pyrodinium* cells.

Among the demersal fish species, slipmouths (*Leiognathus* spp.) contained the most *Pyrodinium*

and even as many as for some of the pelagic fish species. The other fish species occasionally found containing a few *Pyrodinium* organisms included the threadfin bream (*Nemipterus* spp.), goatfish (*Upenoides* spp.), and trigger fish (*Balistes* spp.). Whiting (*Sillago* spp.) and grouper (*Epinephelus* spp.), on one occasion, were also found to contain a relatively high number of *Pyrodinium*. Barracuda (*Sphyraena* sp.) was, on one occasion, found to contain over 200 *Pyrodinium* cells per fish.

Blue crabs (*Portunus* spp.), shrimps (*Penaeids*), and squids (*Loligo* spp.) rarely contained *Pyrodinium*. *Pyrodinium* cells were present in the gut of these edible invertebrates only during the peak of the bloom. The scallops (*Amusium pleuronectes*), which were caught only in some trawling tracks (track nos. 10, 15, and 16), were always found to contain *Pyrodinium*.

Fish and invertebrates caught by gill net, hook and line, baby trawl, and fish corral inside Maqueda Bay and Villareal Bay were sampled on several occasions and were also examined for the presence of the toxic dinoflagellate (Table 3). The fish containing the most *Pyrodinium* cells were also the

Table 3 Concentration of *Pyrodinium bahamense* var. *compressa* in the stomach of different species of fish and invertebrates caught by hook and line, gill net, baby trawl, and fish corral in Maqueda Bay from 27 July — 16 September 1983.

Month (1983)	July				August						September			
DATE	27	28	29	3	9	14	16	23	28	31	5	9	12	16
PELAGIC FISHES:														
Indo-Pacific mackerel (*Rastrelliger brachysoma*)	xxxx		xxx	xxxx		xxxx	xxx							
Sardines (*Sardinella* spp.)			xx	xx		x								
Moonfish (*Mene maculata*)														
Anchovies (*Stolephorus* sp.)														
Indian mackerel (*Rastrelliger kanagurta*)	xxxx	xxxx			xxxx									
Crevalle (*Selaroides* sp.)			xx		xx									
Frigate tuna (*Auxis* sp.)		x												
Hairtail (*Trichiurus lepturus*)														
Leather jacket (*Scomberoides* sp.)														
Round scad (*Decapterus* spp.)						xx								
Hardtail (*Megalaspis cordyla*)	xx													
Mullet (*Mugil* spp.)	xx					xxx								
DEMERSAL FISHES:														
Threadfin bream (*Nempiterus* spp.)		x												
Slipmouth (*Leiognathus* spp.)	xx		x		x									
Lizard fish (*Saurida* spp.)														
Goatfish (*Upeneus* spp.)				x										
Whitings (*Sillago* spp.)	xx			xx										
Croaker (*Sciaenidae*)														
Gerres (*Gerres* spp.)														
Therapon (*Therapon* spp.)	xx		x	x		x								
Trigger fish (*Balistes* sp.)						x								
Grunt (*Pomadasys* spp.)						x								
Grouper (*Epinephelus* spp.)														
Silverside (*Atherina* sp.)	xx			x										
INVERTEBRATES:														
Shrimps (*Peneaus* spp.)				x	xx									
Squid (*Loligo* spp.)														
Crab (*Portunus pelagicus*)														
Scallop (*Amusium pleuronectes*)			xxxx											

Notes: 1. x = 1 — 20
xx = 21 — 100
xxx = 101 — 200
xxxx = over 200
The four categories used represent the number of *Pyrodinium bahamense* var. *compressa* inside the stomach of fish and invertebrates.

2. All fishes caught by the bottom trawl from 28 August to 17 September 1983 were found to be negative for *Pyrodinium bahamense* var. *compressa*.

Table 4. Concentration of *Pyrodinium bahamense* var. *compressa* in the stomach of different

Month (1983)	July																				
Date	24	25		27	28			31			1			3		4	5		8		
Fishing Track Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17				
Re-Tracking																		1	17	10	4
PELAGIC FISHES:																					
Indo-Pacific mackerel (*Rastrelliger brachysoma*)	xxx	xxxx	xxxx	xx		xxx	xx	xx	x	x	x	xx	xxx	xxx	xxxx	xxxx	xxxx	xxxx	xx	x	x
Sardines (*Sardinella* spp.)	x	xx	xxx		xx	xx	xxx				x			xx			xx	x	x	x	
Moonfish (*Mene maculata*)	x																				
Anchovies (*Stolephorus* spp.)		x									x				x						
Indian mackerel (*Rastrelliger kanaguria*)																					x
Crevalle (*Selaroides* sp.)	xx	x											x								
Frigate tuna (*Auxis* spp.)																					
Hairtail (*Trichiurus lepturus*)	x																				
Shark (*Cancharliidae*)																					
Leather jacket (*Scomberoides* spp.)																					
Round scad (*Decapterus* spp.)																					
Hardtail (*Megalaspis cordyla*)																xx		xx			
DEMERSAL FISHES:																					
Threadfin bream (*Nemipterus* spp.)	x																				
Slipmouth (*Leiognathus* spp.)							xxxx									xxx	x				x
Lizard fish (*Saurida* sp.)																					
Flat fish (*Solea humilis*)																					
Goat fish (*Upeneus* spp.)	x	x											xx								
Whitings (*Sillago* spp.)	xxx																				
Croaker (*Scianidae*)	x																				
Flat fish (*Platycephalus* sp.)																					
Siganid (*Siganus* sp.)																					
Gerres (*Gerres* sp.)																			x		
Pomfret (*Apolectus*)																					
Barracuda (*Sphyraena* sp.)		x															xxxx				
Sea catfish (*Arius* sp.)																					
Threadfish (*Polyneumus* sp.)																					
Theraphon (*Theraphon* sp.)																		x			
Trigger fish (*Balistes* sp.)															x			x			
Ray (*Raja sp.*)																					
Grunt (*Pomadasys* sp.)																					
Eel (*Symbranchus* sp.)																					
Snapper (*Lutjanus* sp.)																x			x		x
Grouper (*Epinephelus* sp.)	xxx																				
Porgy (*Lethrinus* sp.)																					
Pomfret (*Stromateus* sp.)		x															x				
Glass fish (*Pentaprion longimanus*)		x																			
INVERTEBRATES:																					
Crabs (*Portunus pelagicus*)	xx																				
Shrimps (*Peneaus* sp.)	xxxx																				
Squid (*Loligo* sp.)	xxx																				
Scallop (*Amusium pleuronectes*)															xx	xxx				x	
Oyster (*Crassostrea* sp.)																					
Total Fishing Time (2,170 mins.)	30	32	42	38	30	30	30	26	27	60	58	56	65	77	30	60	48	24	54	55	47
Total Catch (6,637.24 kgs.)	26	9.5	107.7	31.63	97	129	14	27.5	33.9	88,85	122	123	67	228.05	55	341	83	0.71	17	211	262

NOTES:

1. x = 1 — 20
   xx = 21 — 100
   xxxx = 101 — 200
   xxxx = over 200

   The four categories used represent the number of *Pyrodinium bahamense* var. *compressa* inside the stomach of fish and invertebrates.

2. All fishes caught by the bottom trawl from 28 August to 17 September 1983 were found to be negative for *Pyrodinium bahamense* var. *compressa*.

pecies of fish and invertebrates caught by bottom trawl from 24 July – 17 September 1983.

August																														September	
10		11		13			14				16			18	19	21		22			23			26		27		28		31		6	10	17
							18							19	20	21								22				23						24
7	16	9		10	9	14			13	17	11	12					13	11	8	10	8			12	11		6	11	4	12			
M	XXX			XXX	XXX	XX	XX		XXX		X	XX					XX	XXXX						XXX									
		X			XX	X	X											X	XX					X									
				X						XX	X							X															
X				XXX												XXX			XXXX	X														
				X		X	X			XX	X																						
										X																							
		X				X	X			X	X																						
	XX			X						X	X	X														X							
	X																																
	XX	X																															
	XXX			XXX																XX													
60	67	115		65	60	61	60	30	15	60	60	30	32	30	60	60	30	30	30	30	35	30	21	60	15	30	30	15					
03	35	160		122	102.5	5.1	61	0.5	35	43.8	350	445	108.5	142	251	225	115	105	45	1.0	264	36	3.5	205	40	7.0	137	1214.5					

Table 5. Toxin bioassay results from mussel samples (extracted from the toxicity test reports of the Bureau of Research and Laboratories of the Ministry of Health, Metro Manila).

Date	Collection Site	% Mice Deaths					
		Death occurred in minutes	30 minutes to 2 hours	5 hours	17 hours	19 hours	24 hours
1983							
October	Jiabong	100	0	0	0	0	0
November	Jiabong	0	7	0	29*	0	0
8 December	Jiabong	0	0	6.6	0	0	15.9-32
		0	0	32.0**	0	0	89**
		0	0	77.7***	0	0	100***
1984							
4 January	Jiabong	0	0	0	0	90.66	78.0-100
11 January	Jiabong Mussel Farms						
	Jala	0	0	4.0	0	0	58.33
	Gran del Mar	0	0	6.6	0	0	18.57
	Green Ocean	0	0	12.0	0	0	72.72
	Vaquil	0	0	2.66	0	0	89.04
	San Pedro	0	0	4.0	0	0	84.72
	Holy Rosary	0	0	12.0	0	0	80.30
	Sto. Niño	0	0	13.3	0	0	96.92
	Maqueda Bay	0	0	12.0	0	0	87.87
23 January	Ayagao (Capiz)	0	0	0	0	0	0
	Sapian (Capiz)	0	0	0	0	0	0
5 February	Jiabong and Villaeral Mussel Farms						
	Gilbert Uy	0	0	0	0	0	4.0
	San Roque	0	0	0	0	0	2.6
	Amihanan	0	0	17.3	0	0	6.45
	Sta. Rosa I	0	0	16.0	0	0	20.6
	Sta. Rosa II	0	0	5.3	0	0	14.0
	Pacao	0	0	0	0	0	0
	Allied	0	0	0	0	0	0
	Bacarra	0	0	0	0	0	1.3
	Jia-An	0	0	6.6	0	0	15.7
	Cupido	0	0	2.6	0	0	15.7
	Vaquil	0	0	5.3	0	0	2.8

5 March	Jiabong and Villaeral Mussel Farms						
	Sto. Niño	0	0	0	0	0	0
	D' Pioneer	0	0	0	0	0	0
	Amihanan	0	0	0	0	0	0
	El Cupido	0	0	0	0	0	0
	Rabotaso	0	0	0	0	0	0
	Sta. Rosa	0	0	0	0	0	0
	Barsana	0	0	0	0	0	0
	Mahayag Achiever	0	0	0	0	0	0
	Gran del Mar	0	0	0	0	0	0
	Maqueda Bay	0	0	0	0	0	0
18 March	Jiabong and Villareal Bay Mussel Farms						
	Allied	0	0	0	0	0	0
	Amihanan	0	0	0	0	0	0
	Vaquil	0	0	0	0	0	0
	D'Pioneer	0	0	0	0	0	0
	Gran del Mar	0	0	0	0	0	0
	Maqueda Bay	0	0	0	0	0	0
	Pacao	0	0	0	0	0	0
	Sto. Niño	0	0	0	0	0	0
	Sta. Rosa	0	0	0	0	0	0
	San Rafael	0	0	0	0	0	0
	D' Alfa	0	0	0	0	0	0
	Efren	0	0	0	0	0	0
	D' Family	0	0	0	0	0	0
	Neptune	0	0	0	0	0	0
	Percy	0	0	0	0	0	0
	Tony	0	0	0	0	0	0
	Tulay	0	0	0	0	0	0
	Villahanon	0	0	0	0	0	0

0 — animal did not die
* — percentage of death from the remaining 186 mice which manifested illness by immobility (staying at one corner)
** — concentration of the inoculum doubled
*** — concentration of inoculum trebled

pelagic species. The Indo-Pacific mackerel still contained the most. The other food fish examined (both pelagic and demersal fish) were all found to contain low numbers of cells. Demersal species also contained *Pyrodinium* because Maqueda Bay and Villareal Bay are very shallow and *Pyrodinium* were observed to be present in the entire water column.

The scallops (*Amusium pleuronectes*), which were sampled only once in Maqueda Bay, were found to contain more than 200 *Pyrodinium* cells.

It can be seen from Table 3 that all fish caught in Maqueda bay and Villareal Bay within the period from 23 August — 16 September 1983 were found to be negative for *Pyrodinium*, which suggests that during this period the areas was already free from the toxic dinoflagellate.

It can also be seen from Table 4 that all fish caught by bottom trawl within the period from 28 August — 17 September 1983 were found to be negative for *Pyrodinium* and it can also be assumed that during this period the area had been cleared of the toxic dinoflagellate.

From the foregoing observations, it can be stated that Maqueda Bay and Villareal Bay appeared to have been cleared of the dinoflagellate first, as shown by the earlier disappearance of *Pyrodinium* in the gut of fish from these areas.

Unfortunately, no attempt was made to determine the toxicity of these contaminated seafoods due to a lack of facilities and expertise on board. However, the research vessel *Sardinella* collected fish samples in early August by miniature bottom trawl in Maqueda Bay and by purse seine in Samar Sea. The toxicity tests conducted at the UPVCF Department of Fish Processing Technology showed that fish samples from Samar Sea were found to be nontoxic (Hermes 1983).

Conversely, some fishes in Brunei, such as chub mackerels and sardines, were found to be toxic, with a toxin content ranging from 99-478 MU/100 g of whole fish. These species have also caused paralytic shellfish poisoning, but no fatalities were recorded (Beales 1976).

In the Philippines, there was no evidence of massive mortalities of fish and other aquatic animals during the red-tide period although there were some reports of a scattering of a few hundred smaller fish species along the beaches and in the harbour area during the early part of the event. Subsequent underwater observations using SCUBA revealed that no damage has been inflicted either to the fish or invertebrates, such as mussels and other shellfish, crustaceans, and corals.

Examination of the Gut Content of Mussels

During the early part of the monitoring program, a team of biologists from BFAR Central and Regional Offices was formed to concentrate on monitoring mussels in Maqueda Bay and Villareal Bay. The monitoring started on 23 August. At that time, these areas had already been cleared of the toxic dinoflagellate but monitoring continued on a biweekly basis (every Monday and Friday) by collecting samples from a number of mussel farms in the area. The gut of the mussels sampled was dissected and examined for the presence of the dinoflagellate. Counting and recording of the dinoflagellate in the gut of mussels was then carried out.

On 23 August, the mussel samples from Maqueda Bay area were found to be positive (101-200 *Pyrodinium* cells per shellfish) and those taken from Villareal Bay area were also positive (21-100 *Pyrodinium* cells). On 27 August, Maqueda Bay area became positive (21-100 *Pyrodinium* cells) and in Villareal Bay area the number of cells per shellfish was reduced (1-20 *Pyrodinium* cells). On 31 August, samples from Maqueda Bay area were positive (1-20 *Pyrodinium* cells) and samples from Villareal Bay become negative.

Some of the mussel samples taken from Maqueda Bay area still continued to be positive with *Pyrodinium* (1-10 cells) until 19 September.

The same results were obtained from samples collected on 23, 26, and 30 September on 3, 7, and 10 October. Even when mussels were still positive (1-20 *Pyrodinium* cells), BFAR technicians and some mussel farmers started eating mussels, but no paralytic shellfish poisoning or any ill effects were noted.

In spite of the above findings, the local ban on shellfish was extended because studies elsewhere showed that the toxins (or toxic chemical material) remained in the shellfish tissue for about 2 months after the complete disappearance of the causative dinoflagellate in the water. Previous studies reported that mussels accumulate toxins mainly in the hepatopancreas (the so-called digestive or dark gland).

Bioassay Test

The results of the toxin bioassays are shown in Table 5, as extracted from the toxicity test reports of the Bureau of Research and Laboratories of the Ministry of Health. It is difficult to interpret these data, however, because they only provide information on the presence or absence of the toxins. Use of the standard mouse bioassay is important, therefore, because it provides data on toxocity levels and allows comparisons to be made with data from other countries studying the same problem. It was observed in the test in October 1983 (Table 5) that the mussels were still highly toxic when 100% of the mice tested died within a few

minutes after injection of the mussel extract. In November, 7% of the mice died within 1 hour, whereas 186 mice (93%) manifested "illness" by immobility. Twenty-nine percent of this number died after 17 hours.

In December, 6.6% of deaths occurred 5 hours after injection. However, the percentage of deaths varied from 15.9-32% after 24 hours. When the concentration of the inoculum was doubled, 32% of the mice died in 5 hours and practically all of the remaining mice died after 24 hours. When the concentration was tripled, 77.7% of the mice died within 5 hours and no survivors were recorded after 24 hours.

The tests revealed that the concentration of the toxin diminished after December. However, the samples collected on 4 January 1984 showed 90% death within 19 hours and from 78-100% after 24 hours.

The collection and bioassay tests were continued and the toxins were still detected in the mussel samples. On 11 January, 2.66-13.3% of the deaths in mice occurred after 5 hours and 18.57-96.92% occurred after 24 hours. On 5 February, 0-17.3% of deaths in mice occurred after 5 hours and from 0-20.6% occurred after 24 hours. By 5 March, samples were found to be negative for the toxin and the ban on mussels was lifted on 15 March 1984.

Follow-up sampling and bioassay tests were conducted on 18 March that showed once again that the inoculum had no effect on the mice used.

The data from bioassay tests showed that green mussels or *Perna viridis* remained toxic for about 6.5 months after the complete disappearance of the toxic dinoflagellate in the water or about 5.5. months after the complete disappearance of the dinoflagellate in the gut of mussels.

Paralytic Shellfish Poisoning

Paralytic shellfish poisoning in the Philippines was probably caused by the same type of toxins involved in cases reported in other tropical and temperate countries, *Pyrodinium bahamense* var. *compressa* being the primary source of the toxins. Harada et al. (1982) identified the toxic substances of this species as gonyautoxin V, neosaxitoxin, saxitoxin, and two unknown toxins coded tentatively as PBT_1 and PBT_2.

During the outbreak of red tide in the Philippines, the symptoms manifested by the patients affected by PSP conformed well with those described in the works of Halstead (1965), Wills (1966), Worth et al. (1975), Beales (1976), and Hashimoto (1979). Generally, the victimes complained of a tingling sensation of the lips, tongue, mouth, face, and jaw within 30 min after the ingestion of the contaminated shellfish. The sensation progressed to the arms and legs with a feeling of lightness, numbness, and difficulty of movement and breathing. Practically all fatalities suffered nausea and vomiting, and death generally occurred within 17 hours after ingestion of the contaminated seafood.

Table 6 shows the number of reported illnesses and deaths caused by paralytic shellfish poisoning and Fig. 2 shows the areas where cases of poisoning have been reported. As mentioned earlier, some 278 cases with 21 deaths have been recorded. The actual number of victims may be higher because it is the usual practice in remote areas and neighbouring islands not to report incidences of poisoning.

The first deaths attributed to PSP, which occurred before the discovery of the red tide, occurred in the early morning of 21 June, involving a family of eight in Catbalogan, Samar. Two boys, aged 3 and 7 years, of the five children of the family died from respiratory paralysis 11 and 10 hours, respectively, after eating boiled mussels collected from Jiabong. Six other members of the family were also affected and sent to the hospital for treatment. The victims experienced vomiting and body weakness a few hours after supper. Twelve other families in the neighbourhood who also had mussels for their evening meal suffered the same symptoms experienced by the first affected family.

Between the latter part of June and first week of July, more and more cases of mussel poisoning were brought to the hospitals daily. During this period, three deaths attributed to mussel poisoning were reported in Tacloban City.

The incidence of paralytic shellfish poisoning increased after typhoon "Bebeng" hit Samar from 13-15 July. During the typhoon, about 100 pieces of bamboo poles used in mussel culture in Maqueda Bay and Villareal Bay were uprooted, and drifted onto the beaches of western and northern Samar, Sorsogon, and Masbate. Coastal residents who were not aware of the danger feasted on the mussels, which were still attached to the bamboo poles. As a result, as many as 30 poisoning victims were admitted to the hospitals in a single day in Catbalogan, resulting in the death of a 30-year old female from Catbalogan, Samar.

In San Antonio Island, northern Samar, 19 persons were reported to have suffered from paralytic shellfish poisoning following the ingestion of mussels collected from drifting bamboo poles. Of this number, only one person died.

In late July, a 6-year old girl from Barangay Quezon, Bulan, Sorsogon, died and 48 cases of paralytic shellfish poisoning were admitted to the hospital following the ingestion of the same shellfish species.

Table 6. Number of reported illness and deaths caused by paralytic shellfish poisoning (PSP) in the Philippines from 21 June — 23 September 1983.

Location	Number of Illness	Number of Deaths	Fatalities (Age)	Date 1983	Responsibile	Source/ Origin
Eastern Central Visayas						
Western Samar						
Catbalogan	95	5	Boy (3)	21 June	Green bay mussels (*Perna viridis*)	Maqueda Bay
			Boy (7)	21 June	Green bay mussels (*Perna viridis*)	Maqueda Bay
			Female Adult (33)	12 July	Green bay mussels (*Perna viridis*)	Maqueda Bay
			Female Adult (30)	16 July	Green bay mussels (*Perna viridis*)	Maqueda Bay
			Girl (8)	31 August	Giant Clam (*Tridacna* sp.)	Approach of Maqueda Bay
Villareal	19	0				
Barangay Borabod (Villareal)	7	2	Boy (3)	1 September	Scallop (*Amusium pleuronectes*)	Near Daram Island (Southern Samar Sea)
			Boy (11)	1 September	Scallop	Near Daram Island (Southern Samar Sea
Northern Leyte						
Tacloban	10	3	*	late June	Green bay mussel	Maqueda Bay
Palo	1	1	Girl (8)	12 July	Green bay mussel	Villareal Bay
Carigara	16	1	Female Adult (44)	8 Aug.	Squid (*Loligo* sp.)	Capoocan (Carigara Bay)
Northern Samar						
San Antonio Island	19	1	*	sometime between 17-23 July	Green bay mussel	Maqueda Bay
Catarman	9	0				
Western Central Visayas						
Northern Panay						
Barangay Barra, Capiz	7	1	Boy (5 1/2)	23 Sept.	Scallop	Olotayan Island (northern Panay)
Masbate						
Monreal, Ticao Island	25	2	Boy (4)	4 Aug.	Green bay mussel	Maqueda Bay
			Girl (8)	4 Aug.	Green bay mussels	Maqueda Bay
Southern Luzon						
Sorsogon						
Barangay Quezon, Bulan	49	1	Girl (6)	late July	Green bay mussel	Maqueda Bay
Juban	0	1	Girl (3)	10 Sept.	Black-lip pearl oyster (*Pinctada margaritifera*)	Innermost part of Sorsogon Bay
Camarines Norte						
Labo	0	2	*	early July	Green bay mussel	Maqueda Bay
Central Luzon						
Angeles City	0	1	*	early July	Green bay mussels	Maqueda Bay
Total reported cases of PSP: 278	257	21				

* No data available

In Monreal, Ticao Island, Masbate, 27 persons were admitted to the hospital and diagnosed as suffering from paralytic shellfish poisoning following the ingestion of mussels collected from drifting bamboo poles. Of this number, two children died.

On 31 August, an 8-year old girl from Barangay Mercedes, Catbalogan, Samar, died 9 hours after eating giant clams (*Tridacna* sp.) and on 1 September all members of a family of nine from Barangay Borabod, Villareal, were admitted to hospital with symptoms of paralytic shellfish poisoning. Two of the children (both boys), aged 3 and 11 years, who ate scallops (*Amusium pleuronectes*) caught in the waters north of Carigara Bay by baby trawlers died after 7 and 10 hours respectively.

On 10 September, a 3-year old girl from Juban, Sorsogon, died after eating black-lip pearl oysters (*Pinctada margaritifera*) collected from the innermost part of Sorsogon Bay and on 23 September at Barangay Barra, Capiz, in northern Panay, a family of eight were admitted to the hospital due to paralytic shellfish poisoning symptoms following the ingestion of scallops collected from the surrounding waters of Olotayan Island. One of the children (a boy), aged 5.5 years, died, whereas all other members of the family completely recovered after 30 hours.

It should be noted that the most toxic shellfish were the green bay mussels (*Perna viridis*), and to a lesser extent the scallops (*Amusium pleuronectes*). The juveniles of pearl oysters (*Pinctada margaritifera*) and giant clams (*Tridacna* sp.) were also implicated in some cases. It is interesting to note that a brachiopod (*Lingula anatina*), locally called "balay" and used as food in the Philippines, was reported to be toxic in the innermost part of Sorsogon Bay. Crustacean species such as crabs (*Portunus* spp.) and shrimps (*Penaeids*) have rarely been reported to be toxic. At least one fatal case had been attributed to the ingestion of squid (*Loligo* sp.).

Based on reported cases, the most hazardous shellfish in North America and Canada are mussels, clams, and scallops of various species, whereas clams (*Anadara maculosa*) and oysters (*Crassostrea echinata*) have been reported as the main source of paralytic shellfish poisoning in Papua New Guinea (Worth et al. 1975). In Sabah, most cases, involving seven fatalities, have also been attributed to clams (Beales 1976; Maclean 1979).

A number of people were admitted to hospital in Samar during the early part of July due to mild paralytic shellfish poisoning symptoms that were attributed to the ingestion of various species of fish. The food fish involved are mainly pelagic species such as mackerels (*Rastrelliger* spp.), sardines (*Sardinella* spp.), anchovies (*Stolephorus* spp.), roundscads (*Decapterus* spp.), mullets (*Mugil* sp.), crevalle (*Selaroides* sp.), whitings (*Sillago* spp.), milkfish (*Chanos chanos*), and slipmouth (*Leiognathus* spp.). Incidentally, these fish contained the most *Pyrodinium* cells (Tables 3 and 4). A number of illnesses due to the consumption of some of these fish species have also been reported in Papua New Guinea and Brunei (Beales 1976; Maclean 1979).

The above intoxication is due to the accumulation by fish of the dinoflagellate toxins, either directly or via the food chain. This intoxication is believed to occur as a result of the ingestion by man of the toxic contents of fish entrails or gills as it is a common practice in Samar and adjacent areas to cook and consume the fish, particularly those mentioned above, with the internal organs still intact. Usually, the fish are cooked with vinegar and in most of the reported cases the patients have eaten fish and shellfish cooked with vinegar. This observation conforms with the findings of Hashimoto (1979) that the toxin of dinoflagellates is stable in an acidic medium and unstable in an alkaline medium.

In one experiment, part of the 6637 kg of fish and invertebrates (including those found earlier to be most contaminated by the dinoflagellate) caught by the research vessel *Researcher* direct from the red tide areas (Table 4 and Fig. 6) were served as food for the 33 officers and crew of the vessel, 15 technical and support staff, 53 shipboard trainees, and 3 instructors from the Fishermen's Training Center. As expected, nobody suffered paralytic shellfish poisoning.

Based on the above findings, BFAR assured the consuming public that all kinds of fish are safe to eat provided that necessary precautions in their preparation are observed, i.e., the fish must be fresh and the gills, viscera, and contents of the abdominal cavity should be removed, and the fish washed thoroughly, preferably in running water.

Portions of the catch of the research vessel were distributed to the people of Catbalogan and Tacloban to allay fears among the general public that the fish were unsafe for human consumption. As a result of the experiment, a ban was not imposed on fish. Shipment of fresh and dried fish, crabs, and shrimps from the affected areas to Manila and adjacent areas was never stopped. Nevertheless, many of the people in the affected areas and neighbouring places refrained from eating fish for about 1 month. Fishing activities consequently dropped. It was only in early August when the people of Samar and Leyte started to eat fish again as a result of the campaign by BFAR,

Ministry of Health, and local government authorities to bring back the confidence of the consuming public with regard to eating fish.

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Detoxification of *Pyrodinium*-Generated Paralytic Shellfish Poisoning Toxin in *Perna viridis* from Western Samar, Philippines[1]

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Introduction

Until recently, the occurrence of "red tides" in the Philippine marine waters is almost unknown. If ever, the areas affected involved only small coves or, at most, a few hundred metres of shoreline.

In July-August 1983, a red tide occurred in Western Samar and Leyte, Philippines, the extent of which covered coastlines and lagoons approximating 300 km. The causative dinoflagellate, which previously had never been the cause of red tides in the country, fitted the description for *Pyrodinium bahamense* var. *compressa* (Steidinger et al. 1980) and is the same organism that caused several large red-tide outbreaks in Papua New Guinea (Maclean 1973, 1975a, b, 1977; Worth et al. 1975), Sabah, Brunei, and Palau (Maclean 1979; Beales 1976).

In the aftermath of its accompanying effects, i.e., paralytic shellfish poisoning (PSP), the occurrence left scores of townsfolk in the provinces of Western Samar and Leyte dead and more than 300 persons hospitalized after eating toxic mussels and fish and was responsible for the near collapse of the mussel *Perna viridis* Linnaeus industry in Western Samar and other regions of the Philippines. For the 8 months that a ban on the gathering, sale, and consumption of the Western Samar mussel was in effect, the crop, worth ₱30 million (US$2.2 million), remained at sea, untapped for food.

A combination of PSP toxins had been found in a variety of shellfish that were able to absorb *P. bahamense* var. *compressa* in Palau (Harada et al. 1982). The shellfish, namely *Spondylus butleri, Tridacna corcea,* and *Septifer bilocularis,* contained 62-97% saxitoxin and lower amounts of a novel toxin coded PBT_1. Also found were neosaxitoxin (neoSTX), gonyautoxins III and V (GTX_3, GTX_5), and PBT_2, the latter being found only in the dinoflagellate cells. If true to form, Western Samar mussels would contain the same toxins, if not more, but in varying proportions.

There is clearly a need for the development of acceptable detoxification procedures to counteract the unusually prolonged retention time of neurotoxins in mussels. These procedures must assure modest gains by the farmer, but more importantly, the health and safety of the consuming

[1]SEAFDEC contribution no. 144.

public. It is with this in mind that research efforts were directed at probable solutions.

This paper will report on the results of detoxification producedures using ozone, chlorine, and PVP-iodine (polyvinylpyrrorlidone-iodide-iodine). Earlier, ozone was shown to be effective in detoxifying toxins from *Mya arenaria* exposed to *Gonyaulax tamarensis* (Dawson et al. 1976) and toxins from *Gymnodinium breve* (Blogoslawski et al. 1975; Thurberg 1975).

Materials and Methods

Packing and Transport of Mussels

Approximately 50 kg of 8-month old *Perna viridis* was collected from mussel farms in Maqueda Bay, Jiabong, Western Samar, Philippines, on 10 August 1983 — a few days after the height of a red-tide episode that took place from 23 July — 7 August 1983. The mussels were packed in plastic bags and placed in styrofoam boxes. The temperature inside the boxes was maintained at 22-25° C using crushed ice. The boxes were transported to Tacloban and then flown to Iloilo where studies were carried out in the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC).

Acclimation and Detoxification

The mussels were acclimated by floating the bags on 32 ppt seawater at a temperature of 26-28° C for 30 min. The samples were then taken out of the bags, distributed randomly on plastic trays, and placed in 4600-L fibreglass tanks for detoxification. The system consisted of a simple modification of the setup used by Blogoslawski et al. (1979).

Three chemicals were used: (1) chlorine, (2) ozone, and (3) PVP-iodide-idoine complex. To the first treatment, calcium hypochlorite was aded to the water to a concentration of 0.5 ppm. In the second treatment, ozone was generated from a Sander ozonizer (Erwin Sander Elektro-apparatebau, Eltze/Peine Am Osterberg, West Germany) unit at the rate of 25 mg 0_3/ hour. In the third treatment, polyvinylpyrrorlidone-iodide-iodine complex (Actomar^R K-30, Ciba-Geigy Ltd., Basle, Switzerland)[2] was added to produce a final concentration of 2 ppm. The tanks were maintained for 24 hours, after which the water in each setup was replaced with fresh solutions having the same amount/discharge rate of each chemical as stipulated earlier.

[2]Reference to a true name does not necessarily mean endorsement of the product.

For the control treatment, a lot of undeclustered shells were placed in a fourth tank and supplied daily with a mixture of *Dunaliella salina* and *Tetraselmis chuii*. As with the three chemical treatments, water replacement and feed renewal were carried out on a 24-hour basis.

Extraction of Toxin and Quantification

The standard mouse bioassay as advocated by APHA (1970) was modified for use in this study. Four or five pieces of mussel were declustered from each lot, washed with water, and opened by cutting the adductor muscles. The meat was then removed with a minium of disturbance, rinsed with water and collected in a beaker, transferred onto wire sieves, drained for 5 min, and homogenized in a blender.

A 100-g lot of homogenized flesh was mixed with 100 mL 0.1*N* HCl and boiled slowly for 5 min. The mixture was cooled and the pH adjusted to between 2 and 4 by adding 0.5*N* HCl or 0.1*N* NaOH. The volume was adjusted to exactly 200 mL by adding distilled water and the extract was mixed thoroughly. The extract was centrifuged for 5 min. The supernatant was taken for injection into experimental mice. Extractions were carried out after 3, 5, 6, 7, 8, and 15 days.

For quantifying the toxins, mice weighing 17-21 g were used. Orienting titrations were conducted with each extract by injecting a 1.0 mL aliquot into a mouse intraperitoneally. The time of death, estimated from the exact moment of injection to the last obvious yawning pant, was determined to the nearest 5 sec. Whenever possible, the time of death was adjusted to between 5 and 7 min by diluting the extract with distilled water. In all final injections, three mice were used.

The average time of death for each extract was computed and the corresponding mouse equivalent (ME) was drawn from Sommer's table (Halstead 1965). The value was multiplied by the degree of dilution; by 200, representing the total amount of extract; and the correction factor for the weight of the mouse. The resulting value represents the toxin content of the sample in mouse units (MU)/ 100 g mussel meat.

Results

The average toxicity of the mussels collected a full week after the height of the red tide and prior to experimental use was 1165 MU/ 100 g (range 945-1310 MU/ 100 g). On the basis of the equivalence of 1 MU to 0.183 μg of saxitoxin, the toxicity was 213 μg/ 100 g, almost triple the threshold set by the United States Food and Drug Administration for

closure of shellfish beds (i.e., 80 μg/100 g).

Figure 1 shows the changes in toxicity of the mussels using four methods of detoxification. Ozone and PVP-iodide-iodine appear to effectively lower toxicity between the 6th and 7th days; in fact, to a value lower than the threshold level. The steep reduction in toxicity during this period was, to a certain extent, observed in those mussels continuously fed with the phytoplankton *Dunaliella salina* and *Tetraselmis chuii.* This trend did not continue, however, as indicated by a gradual insignificant decrease from the 8th day onward. The toxicity on the 8th day was calculated to be 67 μg/100 g and on the 15th day 44 μg/100 g, just barely lower than the threshold level. Chlorine was not as effective a detoxifier as the other treatments. By interpolation from the curve in Fig. 1, it would take 14 days to lower the toxicity to the safe level using chlorine.

At the concentrations the three chemicals were administered in the experiment, the mussels did not seem to have undergone any metabolic stress, as indicated by 100% opening up and unabated pumping activity. Similar studies on surf clams *Spisula solidissima* exposed to doses of ozone between 4.5 and 7.5 ppm for 2 weeks led to considerable stress (Blogoslawski et al. 1979), a condition that was improved by lowering the ozone levels to 0.7-1.2 ppm.

Discussion

The toxicity of the mussel *Perna viridis,* which accumulated toxins from a heavy bloom of *Pyrodinium bahamense* var. *compressa,* in Western Samar was considerably elevated. By standard mouse assay a full week after the red-tide bloom, the mussels had an average toxin content of 213 μg/100 g.

The values obtained were comparable to those of shellfish also made toxic by *P. bahamense* elsewhere in the Western Pacific and a variety of shellfish made toxic by *Protogonyaulax tamarensis* in Japan. For example, the butter clam *Spondylus butleri* collected from Palau in 1981 had a peak toxicity of 1100 MU/100 g (Harada et al. 1982).

When our extracts were injected into mice undiluted, killing times were, on average, between 2.5 and 3.5 min, similar with most results obtained by Maclean (1975b), using the shellfish *Spondylus,*

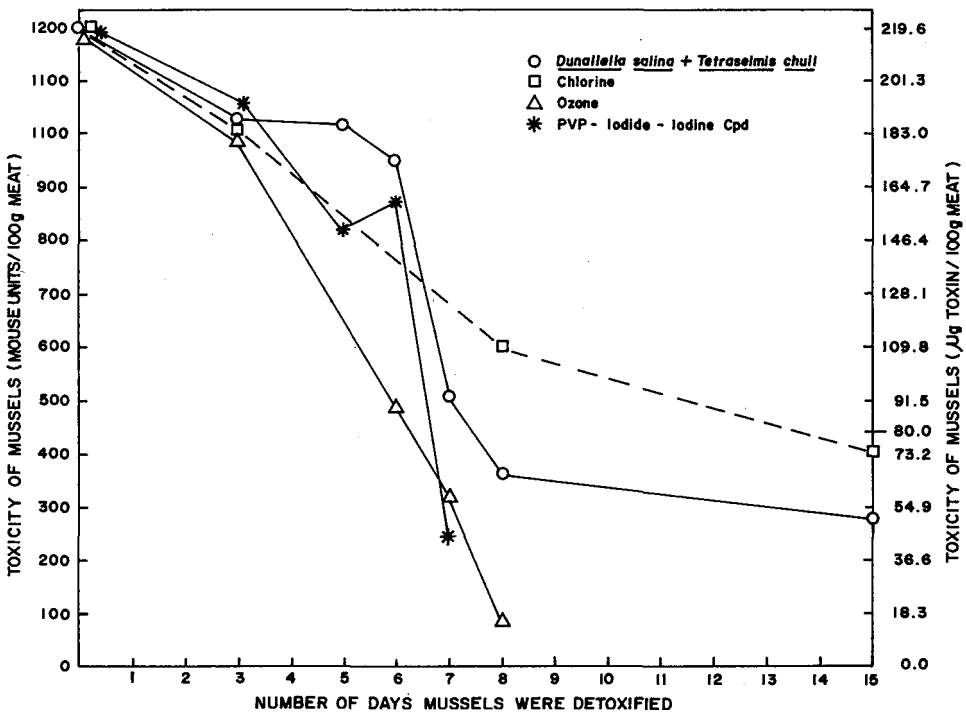

Fig. 1. Changes in the toxicity of mussel Perna viridis *L. from Maqueda Bay, Western Samar, after detoxification.*

Chama, Ostrea trapezina, and *Barbatia parvillosa* collected from Papua New Guinea. Based on his data, the shortest killing time was 1 min 30 sec. This should have given a toxicity of between 3130 and 3470 MU/100 g.

The potency of toxins from a variety of shellfish that assimilated from *P. tamarensis* during a red tide in Japan varied. The highest toxicity recorded in the scallop *Patinopecten yessoensis* from Ofunato Bay was 3500 MU/100 g in 1981, taking only digestive glands (Maruyama et al. 1983), and 1700 MU/100 g with pooled organs in 1979 (Oshima et al. 1982).

Although the characteristics of PSP due to *P. bahamense* have been fully documented (Maclean 1973, 1975b, 1977; Beales 1976; Worth et al. 1975) and its toxin components fractionated and characterized (Harada et al. 1982), no study has dealt with the detoxification or inactivation of these components.

Detoxification at the concentrations used was able to reduce the toxicity by one-half in 6 days and to a value lower than the threshold level between the 7th and 8th days. Although a shorter period should have been targeted, such as the 48-hour time constraint for shellfish depuration, the detoxification period might have been shorter had there been a flow-through system instead of the one at hand. As emphasized earlier, the water in all four tanks was static and was changed only once every 24 hours.

Although there have been a handful of similar studies on the effective inactivation by ozone of PSP toxins in mussels, oysters, and other shellfish that were absorbed from *Gonyaulax tamarensis, G. catenella,* and *Gymnodinium breve* blooms (Thurberg 1975; Dawson et al. 1976; Blogoslawski and Stewart 1978; Blogoslawski et al. 1979), none have dealt with *Pyrodinium* PSP.

Mya arenaria, which readily and rapidly assimilates PSP from *G. tamarensis* when subjected to flowing water with either high and low doses, lost a considerable amount of toxin within 24 hours (Blogoslawski et al. 1979). In both doses, the toxins after 48 hours were so low that the shells were considered safe for human consumption, whereas the controls did not come near the threshold level even after 72 hours. When the initial level was high (775 μg/100 g), however, reductions after 48 hours were 44.4 and 23.7%, respectively, and 10.6% in the controls. The above was also found true in our experiments.

A number of workers used isolated and semi-purified toxins by bubbling ozone. *G. breve* toxins reacted at 20-110 mL/min and were progressively inactivated (Blogoslawski et al. 1975, 1978), but not the untreated extracts, which brought total mortality to mice in 7-10 min and the killifish *Fundulus heroclitus* in 15-30 min (Blogoslawski et al. 1975). PSP toxins from *Mytilus edulis* and *Modiolus demissus* exposed for 5 min at 110 L/min were completely inactivated at pH 7.8 but not at pH 3.8, an indication that toxins are stable at low pH or even become much more potent. It is important to note that many of the people who died in Western Samar and Leyte ate mussels or noneviscerated fish boiled in vinegar or mussels simply broiled and dipped in vinegar.

The use of PVP-iodide-iodine is still not widespread, although there are many studies showing its ability to act against a wide variety of microorganisms (Casagrande 1978). At SEAFDEC, PVP-iodide-iodine has been used extensively to depurate heavily contaminated oysters, *Crassostrea iredalei.* At 2.0 mg/L, it has the same effect as chlorine at 0.5 ppm in purging the coliforms from oyster guts.

Chlorine is not suitable for detoxification work as suggested by our data. It would take 14 days to bring down the toxicity to slightly lower than the safe level. In studies with *G. catenella* toxins, sodium hypochlorite neutralized the toxin over long periods (Chin 1970). Likewise, it lowers flavour quality and decreases acceptability despite the fact that it is cost effective.

The setup fed with *Dunaliella salina* and *Tetraselmis chuii* was originally intended to indicate and approximate the reduction of toxicity in the field where there is no further absorption of toxin from the seawater and live cells or cysts from the mud. The data indicated that reduction was rather slow as on the 15th day the toxicity had not gone below acceptable levels. It should be noted that the ban on shellfish gathering in Maqueda Bay was finally lifted during the first week of March, 8 months after it was established. In the field, mussels are continually supplied live *Pyrodinium* cells, or in cases of slight turbulence generated by winds, with cysts that may be toxic as well (Dale et al. 1978).

There are not many data on the persistence of PSP toxins generated by *P. bahamense* var. *compressa.* Worth et al. (1975) observed that *Crassostrea echinata* was toxic for 15 weeks after the complete disappearance of the dinoflagellate from Papua New Guinea waters, a finding repeatedly demonstrated by northern-hemisphere researchers (Prakash et al. 1971; Quayle 1969). The decrease in toxicity proceeded at different rates in different shells.

PSP toxins produced by *Pyrodinium bahamense* var. *compressa* are as problematic as those produced by *Protogonyaulax catenella, P.*

acatenella, and *P. tamarensis* in Japan, United States, and Canada, in terms of the time needed for the toxins to be eliminated. In Japan, *Mytilus edulis* kept in a laboratory tank lost about half of the toxin within 1 week (Yasumoto et al. 1979).

With *Patinopecten yessoensis,* toxicity declined rapidly during 3 days from 1700 MU to 200 MU, went up to 520 MU for unknown reasons, and then declined gradually thereafter until it levelled off at 100 MU after 5 months (Oshima et al. 1982). *Mytilus edulis* PSP had a shorter period for toxin reduction: peak toxicity was observed on 15 June and no toxicity was demonstrable 6 weeks later when *P. tamarensis* had disappeared (Oshima et al. 1982).

Upward inflection from a lower level may mean the existence of bioconversion or toxin transformation to another form, as confirmed in Bay of Fundy scallops *Placopecten magellanicus* (Hsu et al. 1979; Shimizu and Yoshioka 1981). The methods we used did not fulfill the requirement for replications for the samples to be thoroughly declustered and randomized prior to the start of the detoxification run, so our results should be interpreted as preliminary.

Ozone gas and PVP-iodide-iodine may effectively inactivate PSP toxins from *P. viridis* without affecting the pumping ability. It is to be noted that recent collaborative work in Canada by White and Blogoslawski has shown no effect of ozone on depuration of toxins from clams (A. White, personal communication, 1984). Similar negative results have been found in Japan.

Due to public health and safety considerations, and everybody's desire for the mussel industry to flourish, work on ozone and PVP should be intensified with the purpose of finding the effective time and dosage for total inactivation of the toxins. In view of toxin interconversion, which accounts for increased toxicity scores, more work needs to be conducted. To obtain definite results, more replications must be set up.

The complexitiy of ozone's chemistry has led many to investigate its effects. When added to seawater, it reacts with free bromide ions to form hypobromite ions and hypobromous acid. Blogoslawski et al. (1979) believed that hypobromous acid, in addition to dissolved ozone itself, was responsible for inactivating PSP. There is, however, a paucity of information on PVP-iodide-iodine.

Ozone is now widely used to depurate coliform-loaded oysters the world over. The establishment of an ozone depuration/detoxification station in Maqueda Bay, which henceforth will be subject to *Pyrodinium* blooms, must be considered to routinely purify mussels and inactivate PSP should any toxic red-tide episode arise.

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Status of Shellfish Toxicity in Singapore

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Introduction

No incidence of shellfish toxicity related to red tide has been recorded in Singapore to date. Toxic dinoflagellates, fatal to humans, have not been detected in Singapore's waters. Only one species of dinoflagellates, *Cochlodinium catenatum,* has been found to be dominant in some of the phytoplankton blooms reported. This species has been confirmed to be nontoxic to humans by mouse bioassay using the Association of Analytical Chemists' method.

The occurrence of blooms locally has been found to be associated with either high water temperature (>30°C) or low salinity (<28 ppt) or both, and peak densities coincided with neap tides when mixing of offshore and coastal waters was minimal. The bloom is usually patchy and is concentrated mainly in the upper 2-m layer of the water.

Toxicity to fish and mammals has not been detected or recorded. Nontoxic problems, caused by the bloom, for fish include asphyxiation, oxygen supersaturation at the surface (≈ 10 ppm oxygen), and high pH (>9.0). Farmed fish that are affected are mainly the fast-swimming pelagic ones, such as snappers (*Lutjanus* spp.), yellowfin jack (*Caranx ignobilis*), black pomfret (*Formio niger*), and white pomfret (*Pampus chinensis*).

The Primary Production Department, with the assistance of the Pharmacology Department, monitors the waters and mussels in the fish farming areas along Johore Strait (waterway separating Singapore from West Malaysia) for paralytic shellfish poisoning (PSP) on a fortnightly basis. Results from all tests have so far been negative. Depuration studies, using a UV-sterilizing system, has been carried out on green mussels (*Perna viridis*) with the sanitation aspect in view rather than shellfish toxicity.

Shellfish Poisoning in Association with the Occurrence of Potentially Toxic Dinoflagellates in the Gulf of Thailand

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Introduction

Paralytic shellfish poisoning in tropical regions was reported in the Indo-Pacific region by Maclean (1975a, b, 1977, 1979) and Worth et al. (1975). The last recorded deaths resulting from the consumption of shellfish in Papua New Guinea occurred in 1972 and 1973. Similar occurrences in Brunei's coastal waters and in neighbouring Sabah took place in 1976 (Beales 1976, 1982). In the Philippines, several persons died after consuming mussels in June 1983 (Hermes and Villoso 1983). The causative organism was *Pyrodinium bahamense* var. *compressa* (Böhm) (Steidinger et al. 1980), which was confirmed by Harada et al. (1982) to contain paralytic shellfish poisons at a high level of 1.5×10^{-4} MU/cell. Chemical studies were undertaken by Yasumoto et al. (1984) to identify the toxins in this dinoflagellate and infected bivalves at Koror, Palau Islands; they reported the presence of gonyautoxin V and VI, neosaxitoxin, saxitoxin, and decarbamoyl-saxitoxin.

Red tides in Thai coastal waters have been documented back to 1957 (Charernphol 1957; Suvapepun 1982; Suvapepun et al. 1984). Until recently, there have been no previous medical records of shellfish poisoning in the Gulf of Thailand. The first case of patients suffering from neurological symptoms involved 63 people, one of whom died, in the area of the mouth of the Pranburi River on the western coast of the Gulf of Thailand. The toxification of bivalves in the area was also observed by the Division of Food Analysis, Department of Medical Science, Ministry of Public Health. It was reported that some samples of *Perna viridis* possessed up to 714 MU/g paralytic shellfish toxins.

In response to this recognized PSP in Thai waters, this study has been carried out since May 1983 to detect the causative organisms and to osberve the occurrence of the toxic bloom and red tides in the toxic shellfish bed.

Materials and Methods

Phytoplankton was collected from the area in the Pranburi River where poison shellfish were observed. Five stations were sampled in adjacent areas from the mouth of the river upstream to approximately 2 km (at the incident area). Five-litre water samples were collected at three depths: subsurface, mid-depth, and near bottom. Cell counts were estimated by concentrating seawater to 5 mL by filtering through a 20-μm silk cloth; then 1 mL of the concentrate was counted under a microscope using both fresh and preserved samples. The stomach contents of the mussel *Perna viridis* were collected from the affected area and were also examined. Weekly samples were obtained from 16 May 1983; after June 1983, samples were collected on a monthly basis until August 1984.

Results and Discussions

The species composition of the plankton sampled during the incident of PSP is presented in Table 1. The plankton community was dominated by Cyanobacteria and diatoms, of which *Skeletonema costatum*, *Thalassiosira* spp., *Chaetoceros* spp., and *Cyclotella* were the major species. Dinoflagellates formed a significant constituent of the population. Their appearance, however, was uncommon to the plankton

Table 1. Species composition of phytoplankton collected on 16 May 1983 from the mouth of the Pranburi River (listed in order of dominance by number).

Bacillariophyceae
Skeletonema costatum
Chaetoceros pseudocurvisetus
Cyclotella sp.
Thalassiosira spp.
Chaetoceros spp.
Bacteriastrum spp.
Nitzschia closterium
Rhizosolenia spp.
Pleurosigma spp.
Hemiaulus sinensis
Dinophyceae
Protoperidinium quinquecorne
Prorocentrum micans
Peridinium spp.
Dinophysis caudata
Dinophysis sp.
Cyanophyceae
Cyanobacteria

Table 2. Rainfall data for May 1983, Pranburi River.

Date	Quantity (mm)	Date	Quantity (mm)
1	0.0	16	0.0
2	0.0	17	0.0
3	0.0	18	0.0
4	0.0	19	0.0
5	0.0	20	0.0
6	0.0	21	0.0
7	0.0	22	15.0
8	15.7	23	Trace
9	10.1	24	Trace
10	2.0	25	5.6
11	Trace	26	14.3
12	0.0	27	3.5
13	3.6	28	Trace
14	0.0	29	0.5
15	0.0	30	0.6

Source: Environmental Health Department, unpublished data.

Table 3. Concentration of pollutants (kg/month) in Pranburi River in 1983.

Month	Total nitrogen	Phosphate	Lead
March	3732	—	544
April	3655	233	1166
May	12312	518	1814
June	6091	907	1685
July	5668	778	778
August	1089	3110	1011
September	2566	1555	—

Source: Environmental Health Department, unpublished data.

Table 4. Salinity (ppt) of water at incident area.

	Surface	Bottom
16 May 1983	6	—
March 1984	5.98	6
April 1984	12.62	13.23
May 1984	8.35	9.03
June 1984	13.93	15.99
August 1984	19.95	20.49

communities in the estuarine waters of the Gulf of Thailand. The most abundant species was *Protoperidinium quinquecorne,* at a density of 40000 cells/L. Other species that were found in large numbers included *Prorocentrum micans, Peridinium* spp., and *Dinophysis* spp. These species, however, are not toxic. Small numbers of *Protogonyaulax* sp. appeared in the samples during the PSP occurrence, but were quite rare after May 1983, until August when cell numbers increased again but did not bloom. The appearance of *Protogonyaulax* in August 1984 did not affect the shellfish as the level of toxins in shellfish collected from Pranburi River were not above acceptable levels. The analysis of stomach contents of *Perna viridis* collected from the infected area did not contain any suspected causative organisms; only a few cells of diatoms remained.

According to rainfall data collected about 5 km from the incident area in May 1983 (Table 2), heavy rainfall was recorded on 8 May, i.e., 2 days before the red tide appeared in the mouth of the Pranburi River. The occurrence of the red tide after the heavy rain may be related to substances in land runoff. This was supported by total nitrogen data (Table 3), which showed that the concentration in May was very high.

The salinity at the incident area during the peak of the PSP occurrence was 6 ppt surface salinity. Between March and August 1984, the surface salinity ranged from 5.98-19.95 ppt and bottom salinity from 9.03-20.49 ppt (Table 4). Depth of the water in the area was 4 m.

Because *Protogonyaulax* sp. was found at the mouth of the Pranburi River during the PSP incident, this organism may have been involved in the toxification of shellfish there even though present in extremely small numbers . Onoue et al. (1980) reported that, in concentrations of as few as

10 cells/L, *P. catenella* affected oysters in Senzaki Bay significantly.

It is difficult to draw a conclusion regarding the identification of the causative organisms; this must await further studies.

Thanks are extended to the staffs of the Estuarine Fisheries Division and Marine Fisheries Laboratory for their help in the collection of samples, to Dr. Hidesaki Takano of Tokai Regional Fisheries Research Laboratory, and Dr. Yasuwo Fukuyo of the Laboratory of Fisheries Oceanography, Faculty of Agriculture, University of Tokyo, for identifying the species of dinoflagellate. The Department of Environmental Health and Department of Medical Science are also acknowledged for providing unpublished PSP and environmental data.

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Red Tide and Paralytic Shellfish Poisoning Phenomena in Thailand

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Introduction

Red tide seems to be a seasonal phenomenon that occurs from the end of January to August. This phenomenon, which occurs particularly in the inner part of the Gulf of Thailand where four major rivers, Bang Pakong, Chao Phraya, Tha Chin, and Mae Klong open into the sea, has been observed during the past 30 years.

In 1957, Charenpol reported on its impact on the fisheries industry. Since then, the red-tide phenomenon has been observed more frequenty. *Ceratium, Rhizosolenia, Chaetoceros, Skeletonema, Nitzchia, Thalassionema, Pleurosigma,* and *Trichodesmium* were recorded as the major species of phytoplankton usually found during the algal bloom (Paphavasit and Tamiyavanich 1984). Certain species were reported to be associated with estuarine areas of the Chao Phraya and Bang Pakong rivers. They included *Bacteriastrum, Triceratium, Coscinodiscus, Cyclotella, Hemidiscus, Thalassiotrix,* and *Surirella.*

Between 12 and 21 February 1981, an extensive red tide was observed at the Tha Chin estuary. Chernbamroong and Tharnbupha (1981), from the Department of Fisheries, reported that the bloom was dominated by the dinoflagellates *Noctiluca miliaris* and *Ceratium furca.*

Suvapepun (1982) reported that during the blooms in 1981-1982 the colour of the red tide, which was yellowish-green, was from *Trichodesmium,* whereas the gelatinous white colour was from the combination of *Coscinodiscus, Rhizosolenia,* and *Hemidiscus,* which also gave rise to a strong fishy odour. *Noctiluca,* the bloom of which is found seasonally along the coast during February and March, caused the green colour.

Suvapepun et al. (1984) stated that *Trichodesmium erythraeum* was the major species of algal bloom in 1983 along the east coast of the inner gulf from Chonburi to Chantaburi; it lasted from May to June. When washed ashore, it resulted in a very bad smell. This bloom caused extensive damage to fish farms in Rayong and Chonburi. At the same time, *Trichodesmium erythraeum* also bloomed along the west coast of the inner gulf from the mouth of the Mae Klong River to Petchaburi.

A Case Study of Shellfish Toxicity in Thailand

From 12-16 May 1983, the same time when an extensive bloom of *Trichodesmium erythraeum* was reported in the gulf, a case of shellfish poisoning was reported at Pranburi, Prachuabkirikan Province on the west coast of the gulf. When the green mussel, *Perna viridis,* collected locally from the natural population within Pranburi River, was consumed by the local people, they showed symptoms of paralytic shellfish poisoning (PSP), such as numbness of the mouth, hands, and feet; disruption of breathing; and vomitting. Thirty-four people were rushed to the hospital, fourteen were hospitalized, and a 3-year old girl died (Subcommittee on the Water Quality and the Quality of Living Resources in Thai Waters 1983).

Investigations of that case were carried out by various groups, such as the Department of Fisheries, Department of Public Health, and Department of Marine Science of Chulalongkorn University. During preliminary investigations, phytoplankton samples were collected along with environmental parameters and it was found that the algal bloom consisted of a combination of various species of diatoms, dinoflagellates, and Cyanobacteria. Dinoflagellates of several species were found to be more abundant than usual. *Protoperidinium quinquecorne, Prorocentrum micans,* two species of *Peridinium,* and three species of *Gonyaulax* were reported, with maximum

densities of *Protoperidinium quinquecorne* and Cyanobacteria. The water in Pranburi River had a high BOD value, with the maximum being 5.8 mg/L (Subcommittee on the Water Quality and the Quality of Living Resources in Thai Water 1983). Details of phytoplankton composition and environmental quality can be found in Suvapepun (1984).

The Study of Toxicity

The green mussel was collected from the area and it was found that the concentration of water-soluble paralytic shellfish poison was about 100 times higher than the permissible standard of 4 MU/g as reported by the Environmental Health Division, Department of Health. Saitanu and Tamiyavanich (1984) reported the maximum toxicity of PSP as 12057 μg/100 g, which was quite high.

Investigation of the Source of Toxicity

The Department of Marine Science, Chulalongkorn University, became involved in the investigation immediately after the Pranburi incident was reported. After more than 1 year of investigation, and in cooperation of Japanese experts Drs Yasuwo Fukuyo and Takashi Ishimaru, from the University of Tokyo, and Dr. Masaaki Kodama, from Kitasato University, the results point out that this was truly a case of PSP. The study of the toxin from the green mussels collected showed that it was a gonyautoxin. Investigations are now going on to confirm whether *Protogonyaulax* sp., which was found although not in very high concentrations during the incident, could be the source of this biotoxin; and the specific name of this organism is being looked into. It is suspected to be *Protogonyaulax* sp., which occurs naturally in the inner gulf in low concentrations, because *P. tamarensis* was reported to cause toxicity elsewhere. The physical appearance of the *Protogonyaulax* sp. collected is very similar to *P. tamarensis*, although there are some structural differences and *P. tamarensis* is a cold-water species. As of now, pure cultures of *Protogonyaulax* sp. are being studied.

It should also to be noted that Pranburi, where this case of PSP was reported, it not a location for mussel culture. Local people gather naturally grown mussels for their own consumption. Therefore, this case should not be confused with commercially farmed mussels in the market.

It should be mentioned that, along with studies on PSP, investigations are also under way on diarrhoeal shellfish poisoning (DSP). The organism believed to be the source of DSP, *Dinophysis caudata,* is also found in the inner gulf.

This paper has discussed the first occurence of biotoxin in relation to algal blooms in Thailand. However, Piyakarnchana and Tamiyavanich (1979) have emphasised that the red-tide phenomenon could become more serious because of increasing pollution from rivers. Because mariculture has become widespread along estaurine areas of the inner gulf, it is important that more attention be given to this problem.

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Indo-Pacific Toxic Red-Tide Occurrences, 1972-1984[1]

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Introduction

The scattered literature on red tides in this region was first assembled in 1979 (Maclean 1979). At the time, only one dinoflagellate species was found to be responsible, *Pyrodinium bahamense* var. *compressa.*

The first recorded toxic blooms of this species occurred in 1972 in Port Moresby, Papua New Guinea (PNG), when three deaths from paralytic shellfish poisoning (PSP) occurred. It was found that red tides were probably regular seasonal events in various parts of PNG.

In 1976, *P. bahamense* blooms caused seven deaths in Sabah, and numerous hospitalizations there and in Brunei. Red tides were sighted in Sabah in 1977 and toxic clams discovered but there were no cases of PSP.

Given the huge and remote areas involved, 200 km of coastline in PNG and 300 km in Borneo, it was difficult to blame pollution and Maclean (1979) posed the question — was the red tide spreading? Since his report, toxic red tides do appear to have spread, but the evidence is still ambiguous.

Pyrodinium Red Tides Since 1976

Three years passed between the 1977 Borneo red tides and the next toxic blooms. From April 1980, *P. bahamense* var. *compressa* caused two deaths and a number of illnesses from PSP in Sabah over a 3-month period; blooms lasted until June 1980. Blooms were observed there again during December 1980 and January 1981. No further red tides have been observed in Sabah, yet, in three incidents over the 4 months from November 1983 to March 1984, a total of 11 children have been killed by PSP and 14 persons hospitalized.

The Philippine Islands experienced their first toxic red tides during June to September 1983, when 21 deaths and about 250 notified illnesses were reported from *P. bahamense* var. *compressa* blooms in the Samar Sea. Recently, evidence has accumulated to indicate that *Pyrodinium* blooms may be very widespread throughout the region. For example, certain crabs in Fiji have been found to contain PBT, or *Pyrodinium bahamense* toxin; there are annual red tides in Tumon Bay, Guam, and in nearby Palau, *Pyrodinium* blooms may be a permanent feature of Arumizu Bay; a dinoflagellate bloom was observed in Asau Bay, Western Samoa; shellfish poisoning resembling PSP has been confirmed in the Solomon Islands; and there may be annual red tides in Nanumea Lagoon in Tuvalu. The geographic proximity and similarity in environment — tropical, with little pollution, probably high water salinity, fringing coral reefs — indicate that all these events and probably many more as yet undescribed blooms and PSP outbreaks are due to *Pyrodinium bahamense* var. *compressa.*

Other Toxic Blooms

At the northern end of the South China Sea, Hong Kong has witnessed rapidly increasing numbers of red tides. Apart from a typhoon-induced bloom in June 1971 causing mild toxicity in shellfish, only six more non-toxic blooms were recorded in the 1970's. Since 1980, however, over 40 red-tide occurences were recorded up to the end of 1983. Only one toxic red tide so far has been recorded to be associated with fish kills. The causative organism was a *Gymnodium* sp. and the bloom occured in October 1983. No marine food poisoning has been reported.

The Gulf of Thailand has probably been host to red tides for many years, but they were first reported in 1967, as coastal aquaculture was developing in

[1]ICLARM contribution No. 216.

the gulf. No mortalities of fish or humans were reported until the period May-July 1983, when there were 60 serious illnesses and one death from PSP from a river flowing into the gulf. A number of dinoflagellate species were blooming during this period, although the major one may be *Protogonyaulax tamarensis* (or a new *Protogonyaulax* species). *P. tamarensis* causes toxic blooms in Japan. Meanwhile, *Pyrodinium bahamense* var. *compressa* has been found on the Andaman Sea coast of Thailand.

In the western Indo-Pacific, India experienced its first (recorded) PSP problem in August 1981, when there were three deaths and 82 serious illnesses reported from eating toxic bivalves in Tamil Nadu, on the east coast of peninsular India. One death from PSP and seven illnesses were reported on the west coast in April 1983. The dinoflagellates responsible are not known.

Indonesia has no fully documented records of red tides. However, northern Java has many coastal industrial centres and blooms, especially *Noctiluca,* were observed in samples taken between 1978 and 1981. In November 1983, there were two separate instances of PSP from eating clupeoid fish (*Sardinella* and *Selaroides*), which claimed four lives and hospitalized 191 others in Wulanggitang, East Flores.

In Australia, there are records of severe, toxic red tides in Sydney Harbour since the last century; toxic shellfish on the coast of New South Wales in 1935; and fish kills associated with "water discolourations" in Port Phillip Bay, Victoria, in 1950 and 1959 (and probably more recently). Even New Zealand has begun to experience dinoflagellate bloom problems: a recent export shipment of green mussels was found to contain significant amounts of DSP when examined on arrival in Japan.

A summary of these phenomena is contained in Annex A and illustrated in Fig. 1. Bibliographic sources are given in Annex A and complete references are given in Annex B.

Discussions

As more exploratory surveys are made, the incidence of red tides and PSP probably will be found to encompass the entire Indo-Pacific region.

Pyrodinium bahamense var. *compressa* appears to be capable of blooming in a variety of remote tropical situations and cannot be associated with pollution. In answer to the previously posed question, the increase in reported *Pyrodinium* red tides can only be associated with (1) natural phenomena and (2) a growing awareness of their effects, leading to more conscientious reporting.

The non-*Pyrodinium* toxic red tides, fish kills, and PSP, share somewhat different environmental situations. They are associated with urban or industrial situations and not with coral reefs. Also, they consist of several or many dinoflagellate species, either together or in succession.

It is becoming clear that there are two discrete situations in the region that require separate consideration — the industrial areas, where increased pollution is almost surely to blame for increased PSP-related problems, and the "rural" (especially coral reef) areas, where there appears to be no unnatural cause.

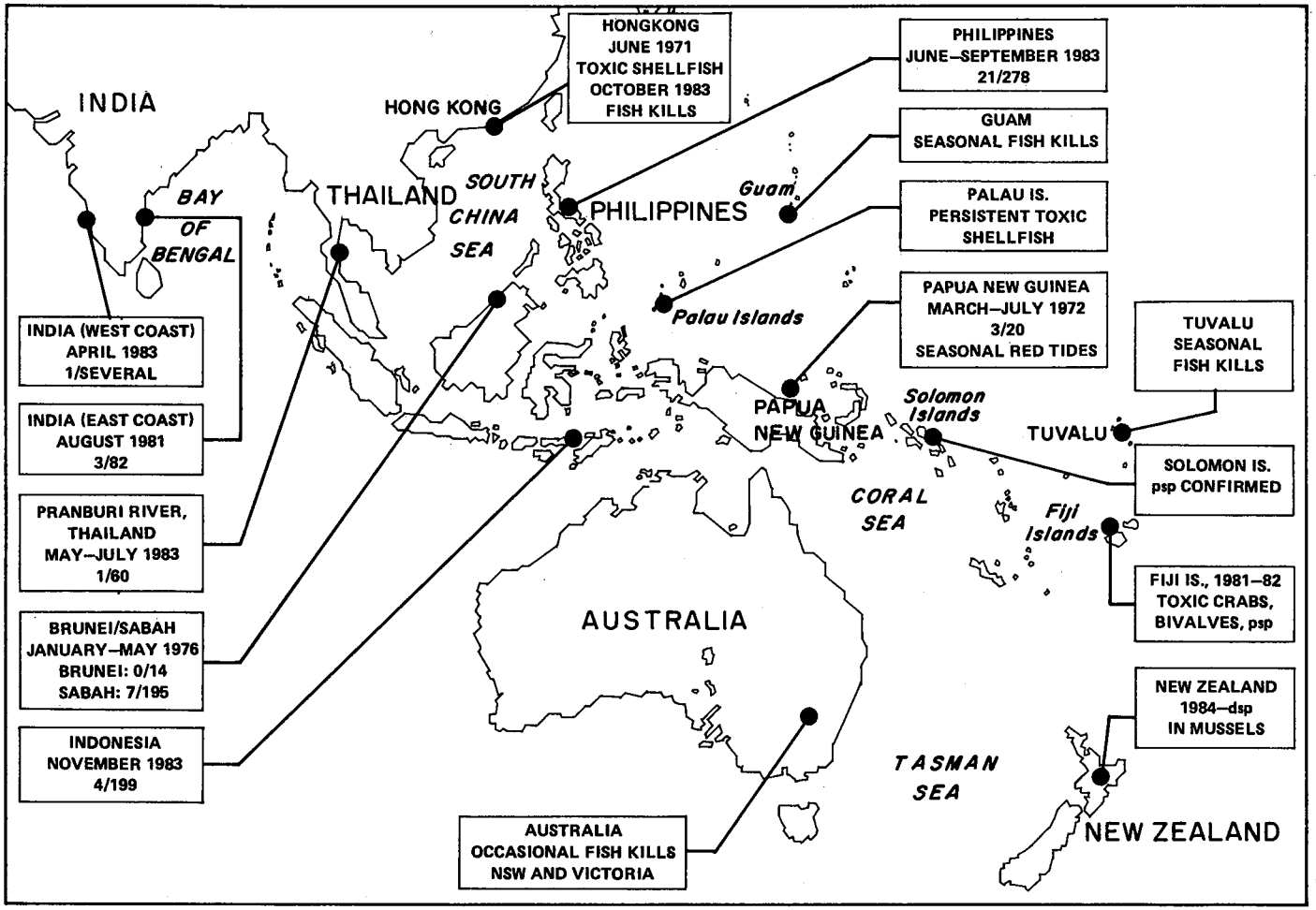

Fig. 1. Sites of Indo-Pacific red tides and paralytic shellfish poisonings. Numbers and dates in boxes refer to number of deaths/illnesses, and time of first reported incidents.

Annex A. Summary of Indo-Pacific Toxic Red-Tide Occurrences.

Country	Date	Report	Source
Australia	Irregular?	Since 1865, red water discolourations and mass oyster mortality in Sydney Harbour. Occasional blooms of *Goniaulax spinifera* and *Gymnodinium* sp.	Maclean (1979)
	1891	Extensive red tides, fish kills in Sydney Harbour by *Glenodinium rubrum* and *Glenodinium spirale.*	
	1935	Toxic mussels, south coast New South Wales.	Le Messurier (1935)
	Feb-May 1950	In Port Phillip Bay, Victoria, massive kills of bottom fish, molluscs, and crustaceans attributed to *Gymnodinium* sp. bloom.	Maclean (1979)
	March 1951	Blooms of an unarmoured dinoflagellate, Port Phillip Bay.	
	March 1959	Heavy fish kills associated with brown water discolouration in Port Phillip Bay.	
	Annual	*Peridinium* sp? seasonal blooms in Swan River, Perth.	
	Annual	Blooms in Sydney Harbour.	
Brunei	March-May 1976	First reported blooms of *Pyrodinium bahamense* var. *compressa* in Brunei; 14 nonfatal cases from eating *Rastrelliger* and *scads (Selar)* — both planktivorous fish. Whole fish and bivalves found toxic.	Beales (1976)
	1 May 1980	Red tide sighted off Brunei. People warned against eating shellfish	Straits Times (1 May 1980)
Fiji	?	Shellfish poisoning resembling paralytic shellfish poisoning has been found, attributed to the arc shell *Anadara antiquata.*	Yasumoto et al. (1984)
	1981-1982	Two of five species of xanthid crabs from Suva barrier reef and samples from Okinawa contained paralytic shellfish toxins, including PBT (*Pyrodinium bahamense* toxin) previously only found in *Pyrodinium* and *Pyrodinium*-infested bivalves.	Raj et al. (1983)
Guam	April, annually	Red water, sometimes large fish kills by *Gymnodinium* sp. in Tumon Bay, known as Father Sanvitores' Blood.	Tsuda, personal communication
Hong Kong	June 1971	Red tide of *Noctiluca scintillans* Macartney) in southern waters over 3 days following a typhoon. Caused by wind only, because normally a summer resident in Hong Kong.	Fung and Trott (1973)
	1980-1982	Five outbreaks with three fish kills increasing during 1981 and 1982, and more species identified. *Noctiluca, Prorocentrum, Gymnodinium,* and *Gonyaulax* are the most significant species, the latter two of which are potentially toxic. Since 1980, the dinoflagellate species that occur in Tolo Harbour have increased in variety. Most recently recorded is a bloom of *Exuviella* sp., which may be taxonomically identical to *Chatonella* sp., the most troublesome red tide organism in Japanese waters.	Lam (1984)

Annex A. (continued)

Country	Date	Report	Source
	1983	During the period September-December 1983, the following observed blooms and effects were reported, mostly in Tolo Harbour: 19-20 Sept., *Ceratium furca;* 18 Sept.-17 October (at least), *Gymnodinium* sp. (*breve?*), with kills of wild and cultured fish; 14 Dec., *Prorocentrum* spp., 12-16 Dec. *Gymnodinium* sp. and *Noctiluca;* 28 Dec. *Gymnodinium* sp? (not the same as the toxic species in October).	Phillips, personal communication
		There have been at least 12 red tides (up to August) in 1983. More than 20 since 1979. Red tides at the mouth of the Pearl River in China are affecting Hong Kong fish stocks.	South China Morning Post (25 Aug. 1983)
	1984	The frequency of red tides in Hong Kong waters has increased dramatically in the last few years. After the 1971 red tide, no serious incidence up to 1980. Since then many, mostly localized, occurrences especially in Tolo Harbour. There are now 13 bloom species present; only one toxic so far.	Morton, personal communication; Lam (1984)
India	August 1981	In Tamil Nadu, three children died, 82 others had neurotoxic symptoms. *Meretrix casta* clam was responsible. Toxicity assays made.	Davy and Graham (1982 quoting Bhat 1981)
	Annual?	*Noctiluca miliaris* formed a dense bloom in May 1977 following the decay of the *Trichodesmium* bloom off Goa.	Devassy et al. (1979)
	April 1983	One PSP death; several hospitalized from clams in Kumble estuary, Mangalore. *Meretrix casta* responsible. Variety of bivalves found highly toxic.	Karunasagar et al. (1984)
Indonesia	November 1983	Two incidents in Wulanggitang, East Flores of PSP from clupeiod fish *Sardinella* and *Selaroides;* four dead, 191 hospitalized	Adnan (this volume)
New Zealand	1984	Diarrhetic shellfish poison found in shipment of green mussels to Japan.	F.H. Chang, personal communication
Palau	Continuous?	Arumizu Bay, Koror, may have high levels of *P. bahamense* var. *compressa* year round as toxic shellfish were found in May and December. Red tide observed once. Identity of toxins discovered in dinoflagellate and shellfish.	Harada et al. (1982)
Papua New Guinea	Annual	Red tides of *Pyrodinium bahamense* var. *compressa,* periodic in various sites. Many incidences of paralytic shellfish poisoning. Toxin found in range of bivalves.	Maclean (1973, 1975)
	September 1969 Dec. 1975- Feb.1976	*Gonyaulax polygramma* bloom near Madang. Vast red tide near Madang, probably *Trichodesmium,* but two illnesses from eating planktivorous fish reported.	Maclean (1973)
Philippines	Pre-1908	Rusty, bioluminescent red tides seasonal in Manila Bay; January-March 1908; adverse effects on marine life, attributed to *Peridinium.*	Smith (1908)

Annex A. (continued)

Country	Date	Report	Source
	1976	The marine red tides from Sabah entered the Tawi-Tawi Island group. Dead fish observed; no fatalities.	Tamesis (1976)
	June-Sept. 1983	Vast red tides through Samar Sea caused by *Pyrodinium bahamense* var. *compressa*. Several hundred affected, officially about 20 dead, mostly from eating fish, *Rastrelliger, Sardinella,* and mullets — one instance milkfish (*Chanos chanos*).	Hermes (1983) Hermes and Villoso (in press)
	Annual March-May	Red tide like blooms of *Peridinium* cf. *quinquecorne* Abe in Maribago Bay, Cebu. Only in bright sunlight, otherwise sessile, attaches to substrate.	Horstmann (1980)
Sabah	Jan.-March	A total of seven children died, another 195 ill in three incidents of shellfish poisoning near Kota Kinabalu and in Brunei Bay.	Roy (1977)
		Pyrodinium bahamense var. *compressa* responsible. Red tides and dead fish prevalent. Blooms went to a depth of 8 m; extremely low oxygen tension especially below 10 m, H_2S smell below 10 m with large numbers of decomposing organisms; thermocline 5-6 m deep. All invertebrates (including corals) and most fish killed at some reefs.	Wood, personal communication
		Red tide disappeared at the end of April, toxins negligible by mid-June.	Snell (1977)
	Mar. 1977	Red tide sighted. *Meretrix* spp. clams very toxic	
	April-May 1980	Red tide noted end April in Brunei Bay. On 17 May, two children died and about 30 others affected — all Vietnamese refugees on an island set aside for them.	Sabah Department of Fisheries (1980)
	19 June 1980	Red tide again in Brunei Bay.	Ting Thieng Ming, personal communication
	30 December 1980	Red tide in Brunei Bay.	
	28 January 1981	Brunei Bay red tide still present.	
	November 1983	Four children died and five hospitalized from PSP.	
	7 January 1984	Two children died, six given emergency treatment for PSP.	
	15 March 1984	Five children died, three hospitalized. Bivalves associated with the November 1983-March 1984 deaths found highly toxic. No red tides seen since 1981.	
Samoa (Western)	June 1983	Dinoflagellate bloom at experimental mussel farm, Asau Bay. No ill effects on those eating raw mussels.	Bell et al. (1983)
Solomon Islands	?	Shellfish poisoning resembling paralytic shellfish poisoning have been confirmed.	Yasumoto et al. (1984)
Thailand	Annual	Red tides more common from late January to August. Common in the inner sector of the Gulf of of Thailand.	Piyakarnchana et al. (1984)

Annex A. (continued)

Country	Date	Report	Source
	May 1983	Red tide along Chonburi to Chanthaburi province coastlines. Bad odour and caused fish kills in nearby fish farms.	
	May-July 1983	PSP outbreak in Prachuab Kirikhan province. 60 severe cases, 1 death. First reported occurrence in Thailand. *Protogonyaulax, Gymnodinium,* and *Peridinium* in blooms, but *Noctiluca miliaris* dominant in estuaries.	National Research Council of Thailand and Srinakharinwirot University, Bangsaen (1984)
	September 1983	*Ceratium furca* red blooms in the inner Gulf of Thailand.	
	December 1983	*Dinophysis caudata* caused red blooms in the inner gulf along the northern coastline. Some children ill from bivalves (not PSP, however).	
		P. bahamense var. *compressa* in plankton in Andaman Sea near Phuket.	F.J.R. Taylor, personal communication.
Tuvalu	Annual?	Periodic red tides in Nanumea lagoon cause fish die-offs.	Uwate et al. (1984 quoting Fisheries Development Limited 1976)

Annex B. A Bibliography on Toxic Red Tides and Shellfish Poisoning Related to the Indo-Pacific Region[1].

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ICLARM

Introduction

References to toxic red tides in the Indo-Pacific mainly concern the dinoflagellate *Pyrodinium bahamense* var. *compressa*. The literature is small because the organism was not discovered in the region until 1972. It is appropriate to compare this variety with the other variety of the species in the Red Sea and central America, *P. bahamense* var. *bahamense;* a separate reference list on this variety is provided.

Even more recently, the causative agent of the ciguatoxin-type poisons was found to be a benthic dinoflagellate, *Gambierdiscus toxicus*. Elements of this family of poisons are found in the Indo-Pacific in shellfish-poison type dinoflagellates, crustaceans, molluscs, and fish. A selection of references on this subject is also provided.

Finally, new — for the region — red tide dinoflagellates are implicated in recent poisonings in the Gulf of Thailand. This bibliography will be enlarged to include relevant references when the species concerned have been identified.

(1) Indo-Pacific toxic red tides

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Resource Papers

Shellfish Poisoning in Japanese Waters

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Historical and Present Status of Shellfish Poisoning

Paralytic shellfish poisoning (PSP) was first confirmed in Japan in 1948. Since then, 90 people have been affected and 3 deaths have been reported.

The causative species have been identified as *Protogonyaulax tamarensis* and *P. catenella.* In 1977-1978, shellfish poisonings became a problem in northern Japan. Initially, the problem involved scallops, but the areas of poisoning are continuing to expand from northern to southern waters and the number of affected species is also increasing.

Along with a rapid increase of the culture of shellfish, administrative measures had to be set up by prefectural governments to regulate the harvest and shipping of shellfish.

In 1978, the Fisheries Agency, Ministry of Agriculture, Forestry and Fisheries, started monitoring the culture of shellfish and regulating shipping and set up values of toxification. In 1980, the Ministry of Welfare established laws to control shellfish poisoning following the measures used by the Fisheries Agency. Although the regional monitoring network has been functioning since 1981 in Northern waters and is administered by the Fisheries Agency, it was expended in 1984 to cover the whole of Japan.

With respect to diarrhetic shellfish poisoning (DSP), the first confirmed case was reported in 1977 in northern Japan. The causative species were identified as *Dinophysis fortii* and *D. acuminata.* Toxicological analyses were carried out and the nature of the toxins was identified. The areas of DSP poisoning and the number of species affected have been expanding ever since.

Figure 1 shows the expansion of areas of shellfish poisoning in recent years. Between 1978 and 1982, the number of areas affected by PSP increased from 2 to 10 and the number of shellfish species affected increased from 3 to 8. For DSP, the number of areas affected increased from 3 to 20 and the number of species affected increased from 3 to 10.

Shellfish production has continued to expand over the years. In the coastal waters of Japan, total production reached 680,000 tons in 1982, which amounts to 21% of coastal fisheries production. The increase in production is especially noticeable with respect to scallops and oysters.

Monitoring System for Shellfish Poisoning

Figure 2 is an example of the shellfish monitoring and investigation systems in Seto Inland Sea, western Japan. The systems are being conducted in five different regions around Japan. About 150 scientists, plus 200 administrative staff, are engaged at present. The budget of the Fisheries Agency reached ¥0.5 billion, which was also used for information exchange and investigation of red tides involving several noxious species without toxins (Fig. 23).

Present Guidelines for Monitoring Shellfish Poisoning

The amount of toxins in shellfish, occurrence of causative plankton species, and environmental factors concerned should be measured at an appropriate interval. Through these operations, the distribution of affected shellfish and degree of toxification will be clarified. An effective monitoring system could thus be established to prevent damage to human health and fisheries activities in inshore waters.

(1) The sampling station should be established to take into account the area of shellfish production

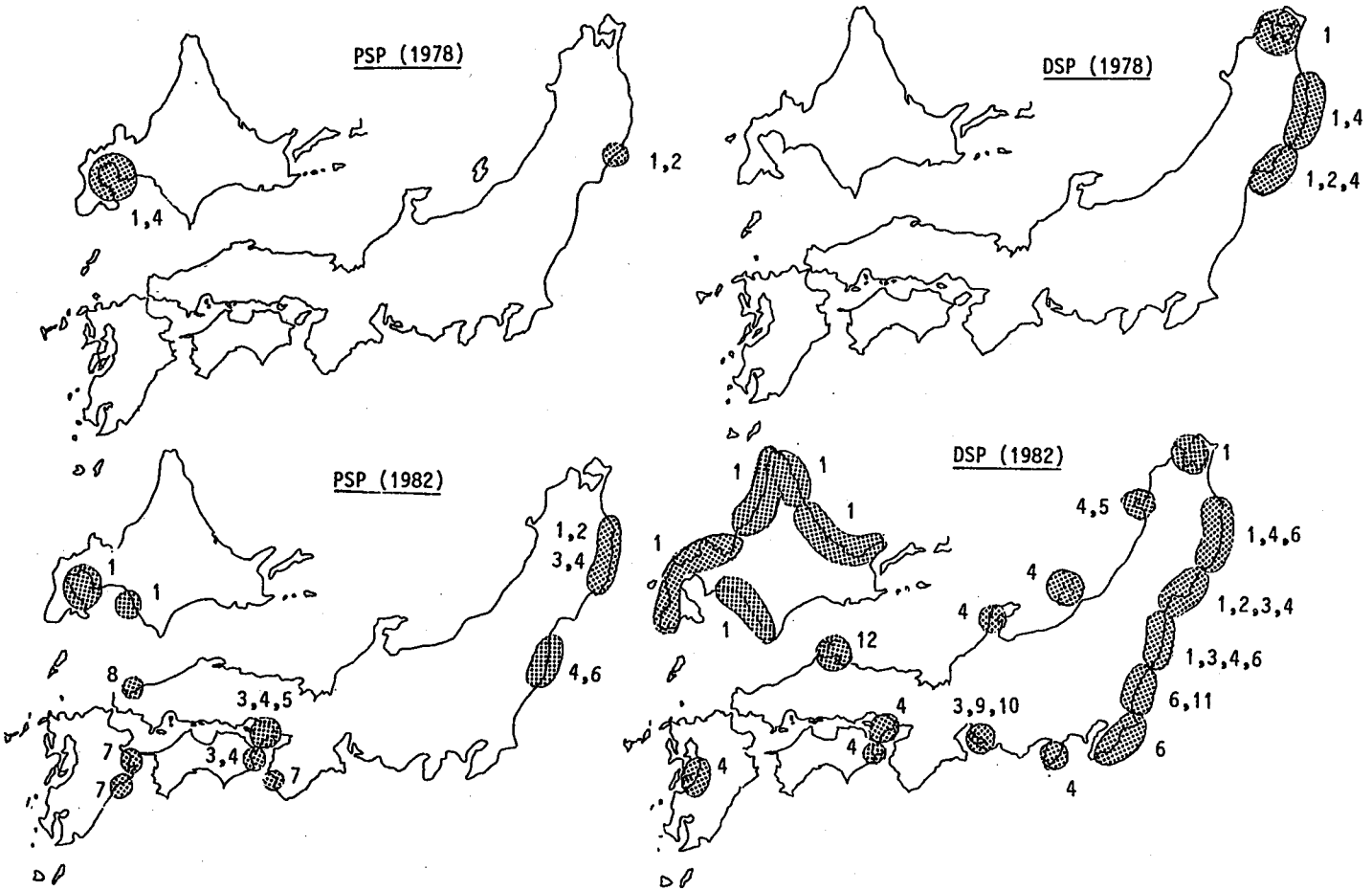

Fig. 1. Expansion of areas affected by shellfish poisoning.

1. *Patinopecten* (M.) *yessoensis*
2. *Chlamys* (A.) *f. nipponensis*
3. *Ruditapes philippinarum*
4. *Mytilus edulis*
5. *Mytilus coruscus*
6. *Gomphina* (M.) *melanaegis*
7. *Chlamys* (M.) *nobilis*
8. *Crasostrea gigas*
9. *Fulvia mutica*
10. *Mactra* (M.) *chinensis*
11. *Meretrix lamarchii*
12. *Pecten* (N.) *albicans*

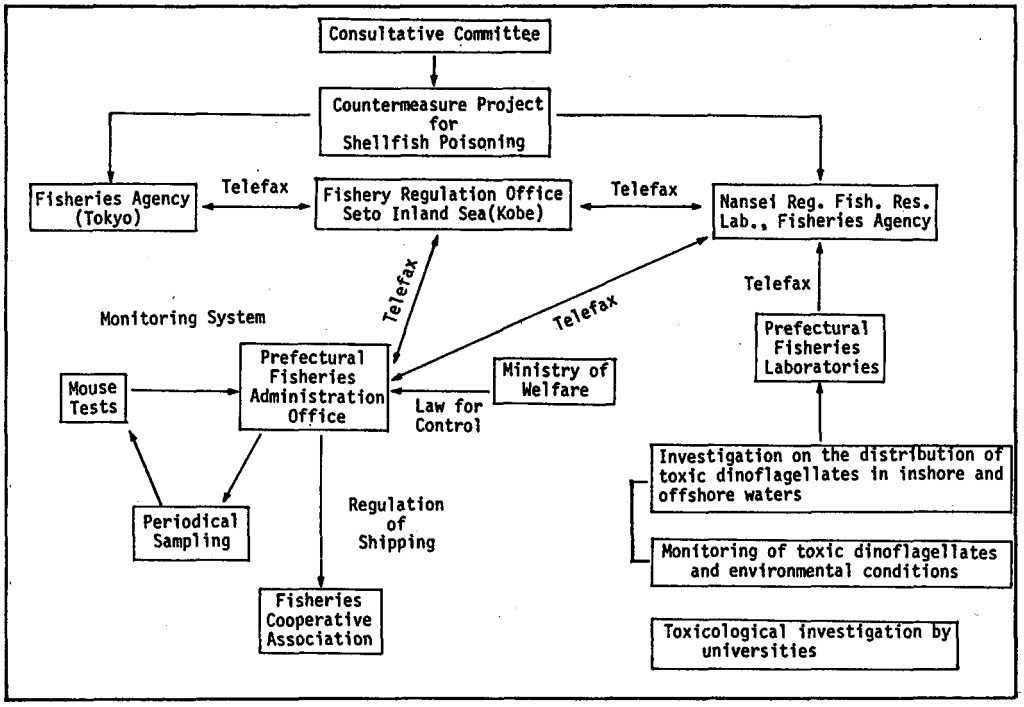

Fig. 2. Shellfish poisoning monitoring and investigation systems in Seto Inland Sea.

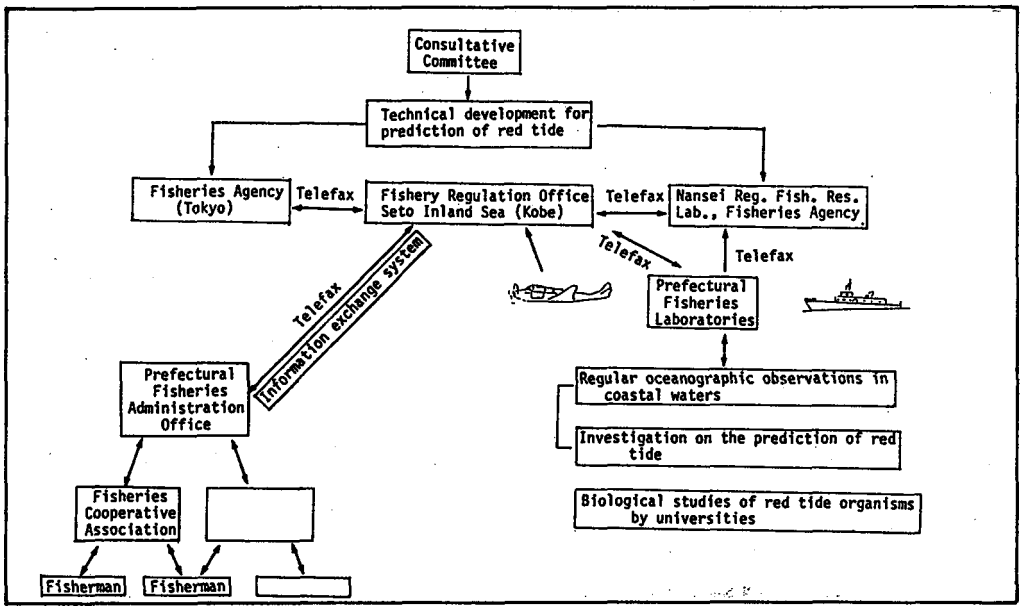

Fig. 3. Information exchange and red-tide investigation systems in Seto Inland Sea.

and distribution of toxic organisms. The period of sampling has to be selected depending on the characteristics of poisoning.

(2) Items of investigation and method of analysis:

(a) *Examination of shellfish toxicity:* Toxicity tests for PSP and DSP are necessary for each species of shellfish.

(i) Care should be taken to ensure that representative specimens are collected to reflect the areal distribution of the particular shellfish population.

(ii) Sampling is preferably carried out directly by the staff on board research ships belonging to the fisheries laboratory. When collections are made using commercial boats, the position of the sampling station, depth, and water temperature should be measured and recorded by the official staff on board.

(iii) More than 300 g of meat is needed to make up one sample. Preparation of samples is again preferably carried out by the official staff. After measuring length, height, weight with shell, and weight without shell, the samples should be put into polyethylene bags, chilled with dry ice or ice as soon as possible, and sent to the designated laboratory. Detailed plans for shipping procedures should have been discussed earlier.

(iv) Toxicity measurements (mouse tests) are conducted, for the most part, using the mid-gut gland. The value is then converted into the amount per edible portion. When examination of the gut is not feasible because of the size of the species of shellfish, examination can be carried out using the edible portion or whole body.

(b) *Examination of plankton:*

(i) At the same time as the shellfish sampling is being carried out, an appropriate volume of water should be collected for plankton surveys.

(ii) Acetic acid with formaldehyde solution (glacial acetic acid: formaldehyde = 1: 1) is an effective fixative. Add the original solution to seawater samples to attain 5% of the total volume. For the concentrated sample stock, however, addition of 20% is required.

(iii) Seawater samples can be concentrated using the settling method. By several repetitions, the final amount could be reduced down to 10 mL; 1 mL for each microscopic counting is appropriate. The amount of seawater and volume of sample for final enumeration have to be determined based on the cell concentration in the field of survey.

(3) Reporting the results of the examination:

(a) Soon after the analyses have been carried out, data sheets should be prepared. These data sheets should be sent to the related organizations as quickly as possible, regardless of the positive or negative results. Because reports of the toxicity tests need to be prepared quickly, there is no need to wait until the completion of the plankton enumeration.

(b) Regulation of the management of these data is necessary, especially for public announcements.

Main Research Themes for Clarifying Shellfish Poisoning

To prevent or reduce the damage caused by shellfish poisoning, the following investigations are being carried out at the same time as monitoring operations.

(1) Analyses of the mechanisms of toxification:

(a) *Life history and ecology of toxic dinoflagellates:* The ecology of toxic dinoflagellates, e.g., distribution, seasonal fluctuation of vegetative cells, distribution and germination of cysts, transportation, and accumulation, is an important subject to investigate. The study of the life history of causative species is also useful.

(b) *Observation of the environmental conditions:* Measurements and analyses of related environmental conditions, e.g., weather, sea condition, transparency, water temperature, salinity, chlorophyll-*a*, and major nutrients, are useful tools to understanding the total scope of poisoning.

(c) *Feeding of shellfish and detoxification:* Rates of ingestion of toxic dinoflagellates by shellfish and the accumulation of toxins are measured through laboratory experiments together with observations in the natural environment. It is also important to develop techniques of detoxification.

(d) *Development of techniques to predict the occurrence of shellfish poisoning:* To prevent and reduce the damage caused by the poisoning of shellfish or fish, appropriate techniques to predict the appearance of toxic dinoflagellates, as well as the toxin accumulation in the shellfish, are being developed through the analysis of environmental and biological factors using computer systems. A biological or ecological model is also effective for short-period forecasting of the blooming of particular species. From an ecological point of view, research on zooplankton needs to be carried out as well because the grazing pressure of zooplankton has a close relationship with the production of phytoplankton.

(2) Toxicological studies: A biochemical approach is also effective in promoting measures to reduce damages from the poisoning of shellfish. It would be useful to develop a suitable technique of detoxification for humans as well as for shellfish.

Measurement of toxicity by chemical methods to replace the mouse tests will, hopefully, be developed in future studies.

Summary

Under the supervision of the Fisheries Agency, each prefectual government has the responsibility of carrying out monitoring programs. Although some problems exist, monitoring operations have been proceeding successfully. Investigations concerning various aspects of shellfish poisoning are being conducted by 6 national institutions, 15 universities, and about 30 fishereis laboratories. Because shellfish poisoning is a kind of natural phenomenon, the only defense is the establishment of precise monitoring techniques to reduce damages. Efforts to improve monitoring, however, need to be carried out through fundamental research.

A Summary of Paralytic Shellfish Poisoning in Canada

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History

Paralytic shellfish poisoning (PSP) occurs along both the east and west coasts of Canada. The first published report occurred in 1793 in British Columbia when some of the crew members of Captain George Vancouver's expedition suffered from PSP after eating contaminated mussels. Because of taboos against eating shellfish among certain coastal tribes of North American Indians, it is likely that cases of PSP extend back even earlier than this, however. In total, there have been more than 300 documented cases of PSP in Canada since 1793, resulting in about 35 deaths. Many instances of PSP have probably gone unreported, especially among native peoples in remote areas.

Geographical Distribution

Toxic dinoflagellate blooms and contaminated shellfish are distributed as shown in Fig. 1. It is considered that shellfish can become contaminated along the entire coastline of British Columbia (west coast), although certain areas are inaccessible for sampling. On the east coast, toxic dinoflagellate blooms contaminate shellfish along the St. Lawrence River Estuary and throughout the central and southern Bay of Fundy. In 1982, the first occurrence of PSP (from mussels pickled in vinegar) was reported in Newfoundland, Canada's easternmost province. Because shellfish were found to be toxic in several separate locations in Newfoundland, it may be that the toxic dinoflagellate is endemic to the region, but only occasionally experiences conditions conducive to bloom formation.

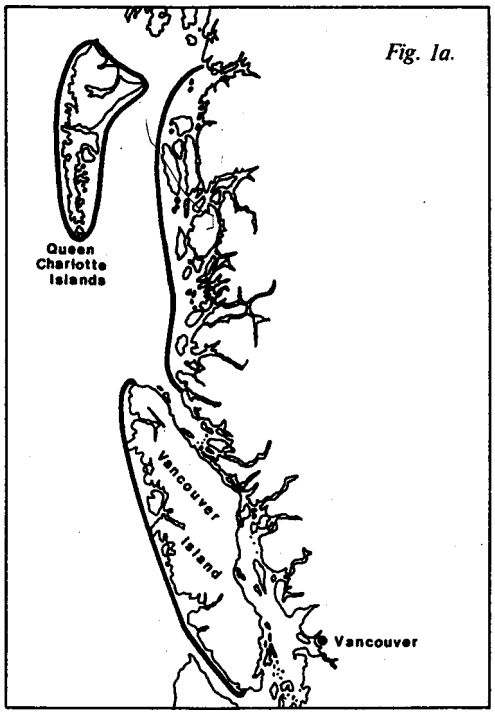
Causative Dinoflagellates

The dinoflagellates responsible for PSP in British Columbia are *Gonyaulax (= Protogonyaulax) catenella* and *G. acatenella.* In eastern Canada, the culprit is *G. excavata* (= *G. tamarensis* var. *excavata*). Several of the toxins from the west and east coast dinoflagellates are identifical to those found in *Pyrodinium bahamese* var. *compressa* in Palau. Thus, the PSP situation in Canada and the results of investigations on the dinoflagellates, shellfish, and finfish involved has applicability to the emergent PSP problem in Southeast Asia.

Shellfish Contamination

A number of Canadian species of filter-feeding shellfish accumulate paralytic shellfish toxins and pose the risk of PSP to vertebrate consumers. Carnivorous shellfish also become toxic, acquiring the toxins secondarily from their filter-feeding prey. Along the west coast PSP is often associated with the consumption of butter clams (*Saxidomus giganteus*) and blue mussels (*Mytilus edulis*). In eastern Canada, PSP is most often associated with consumption of soft-shell clams (*Mya arenaria*), blue mussels, and rough whelks (*Buccinum undatum*), the latter of which is a popular food item in the Province of Quebec.

It should be noted that sea scallops (*Placopecten magellanicus*) in the Bay of Fundy accumulate extremely high amounts of toxins. Fortunately, the part of the organism that is eaten in this area, i.e., the adductor muscle, remains poison-free. Sometimes, scallop viscera become most toxic during the winter, when *Gonyaulax* blooms do not occur, pointing to the probable acquisition of toxins



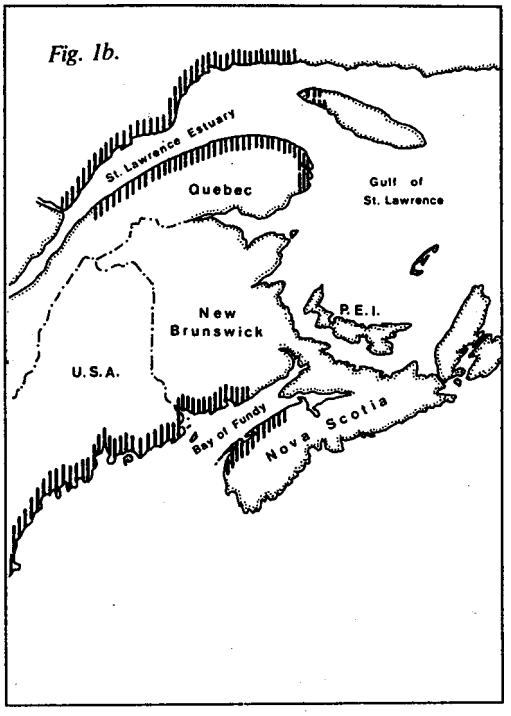


Fig. 1. The locations along the Canadian coasts of blooms of toxic dinoflagellates and of shellfish which accumulate the paralytic toxins. In British Columbia (Fig. 1a), the areas closed permanently to harvesting of certain shellfish are indicated. On the east coast (Fig. 1b), toxic shellfish occur in the southern Bay of Fundy and St. Lawrence River estuary (hatched areas). The first case of PSP in Newfoundland (off the map to the east) was reported in 1982.

through ingestion of overwintering *Gonyaulax* cysts.

Toxic Bloom Patterns

On the west coast of Canada, toxic *Gonyaulax* blooms may occur at any time between April and November. Most filter-feeding shellfish that become toxic during the blooms lose the toxins within several weeks to a few months. The butter clam, however, stores the toxins (in its siphon) for long periods and commonly remains toxic during the winter.

Gonyaulax blooms occur annually on the east coast of Canada some time between June and September. Blooms generally last for 3 or 4 weeks, during which time shellfish become toxic. Until recently, shellfish usually cleansed themselves of toxins by September or October and were then safe for harvesting. Over the past 5 years or so, however, shellfish in many prime digging areas in the Bay of Fundy have remained toxic year round, consistent with a trend of intensification of *Gonyaulax* blooms in the Bay of Fundy since about the mid-1970s. The reason for the intensification is unknown, but it is unlikely that pollution is involved because of the small amount of industrial and municipal input into the bay and the tremendous tidal mixing action in the area.

It should be stressed that the occurrence of a visible red tide is not necessary for the contamination of shellfish with dangerous levels of dinoflagellate toxins. In fact, red tides caused by *Gonyaulax* species are unusual in Canadian waters, yet recurring *Gonyaulax* blooms contaminate shellfish annually.

Toxicity Monitoring

The monitoring system for paralytic shellfish toxins in Canada is quite simple and effective. It was developed by Canadian scientists in the mid-1940s

and has been employed, with little change, to the present. The safety threshold was chosen to be 80 μg of toxins/ 100 g meat. This figure has, over the years, become the international standard.

In brief, shellfish samples are collected by fisheries officers at least weekly during the potential danger period, paying special attention to key stations that are recognized through experience to precede adjacent areas in terms of the timing of the annual rise in toxicity. Samples are taken to the regional fisheries inspection laboratory (Department of Fisheries and Oceans) for extraction according to the AOAC method (Appendix 4). Extracts prepared by regional laboratories on both coasts are sent by air express to a central mouse-bioassay facility in Ottawa, which is part of the Department of Health and Welfare. Toxicity results are telephoned to the regional laboratories where the decision is made to close or open areas to shellfish harvesting. The process requires 1.5-2 days from the time of sampling. If the toxicity score exceeds 80 μg/ 100 g meat, the entire shellfish area is posted with warning signs (Fig. 2) and the area

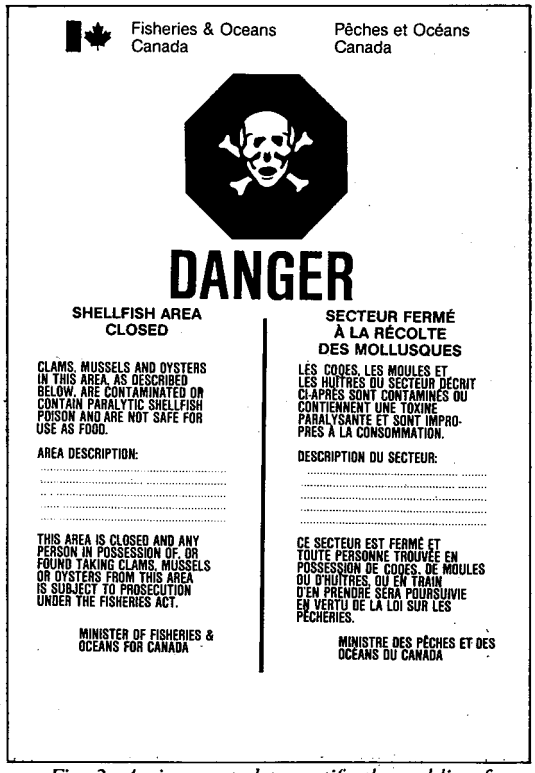


Fig. 2. A sign posted to notify the public of closure of shellfish area because the shellfish contain paralytic toxins from red-tide microorganisms.

becomes officially closed to harvesting. Regional laboratories also have the capability to conduct mouse bioassays, but do so only for checking commercial shipments, distributors, restaurants, etc., and for research purposes.

In the Bay of Fundy, blue mussels become so highly toxic and are so rarely eaten by residents that a permanent ban on their harvesting has been enacted. In British Columbia, to cope with the problem of remote and inaccessible shellfish areas, permanent closures of vast stretches of coastline to the harvesting of shellfish are in effect.

Impact on Shellfish Industry

For many years, the shellfish industry in eastern Canada has operated with harvesting bans in effect during the summer months. Shellfish digging and distribution were geared to these summer closures. In recent years, however, continuous closure of many productive shellfish areas has been necessary and has resulted in considerable economic loss to the region through unemployment of shellfishermen and a shortage of shellfish supply.

The situation on the west coast is somewhat different. Permanent closures of vast shellfish areas have been in effect there for many years. The economic consequence is the loss of these potential resources.

Detoxification

Attempts at detoxifying shellfish contaminated with paralytic shellfish toxins have been made on several occasions over the years. Methods have included transplantation to waters free of the toxic dinoflagellates, and various temperature, salinity, and chemical treatments. So far, an economically feasible method has not yet been developed.

Several years ago, reports in the literature suggested that there may be some promise in using ozone to remove low to moderate amounts of toxins from shellfish. However, my laboratory has recently completed a rigorous series of studies on the effects of ozone on the detoxification of soft-shell clams and has not found any evidence of increased detoxification using ozone treatments.

Fish

Toxic *Gonyaulax* blooms can affect fish as well as shellfish resources. Herring kills in the Bay of Fundy in 1976 and 1979 were caused by *Gonyaulax* toxins, with toxin transfer through the food web. These events sparked a research program in my laboratory, the results of which may be summarized into the following general conclusions: (1) during

toxic *Gonyaulax* blooms, marine zooplankton can accumulate the toxins, (2) the toxins can be transferred through the food web to fish, resulting in fish kills, (3) marine fish, in general, are sensitive to the toxins, in fact as sensitive as warm-blooded vertebrates, (4) since fish larvae are also sensitive to the toxins, recurrent *Gonyaulax* blooms may influence year-class strength of certain stocks, (5) fish, unlike shellfish, are not able to accumulate the toxins in their flesh, and (6) the toxins may be present in fish viscera and PSP may result if this material is eaten with little or no processing.

Current Research

Research in Canada on toxic dinoflagellates and shellfish toxicity is currently limited to two laboratories — Dr. F.J.R. Taylor's laboratory at the University of British Columbia (west coast) and my laboratory in St. Andrews, New Brunswick (east coast). The emphasis in Dr. Taylor's laboratory is on systematics and taxonomy, whereas the emphasis in my laboratory is on physiology and ecology. Current research focal points in Canada are the following: (1) taxonomy, systematics, and evolution, (2) physiology and ecology, using cultures in the laboratory, (3) investigations on bloom dynamics and controlling mechanisms, particularly long-term environmental cycles, (4) distribution of dinoflagellates relative to hydrographic conditions, (5) the role of overwintering cysts in bloom initiation, and (6) means of predicting dinoflagellate blooms, shellfish toxicity, and fish kills.

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Dinoflagellate species of importance. (The photographs and descriptions are provided through courtesy of Dr. Y. Fukuyo.)

Pyrodinum bahamense PLATE var. *compressa* (BÖHM) STEIDINGER, TESTER et TAYLOR.

Cell is polygonal or rounded and compressed anterio-posteriorly. At apex a low broad apical boss with truncated end is obvious. At antapex of non-catenate cell or the most posterior cell of a chain, a long spine continuing from sulcal left list is developed. Cingulum is equatrial with wide lists, and displaced about one cingular width. Ventral area is indented deeply, widened posteriorly and enclosed by wide list coming out from both right and left margins. On the surface of cell conspicuous ridges run, showing thecal plate configuration. The thecal plates are thick, and have many trichocyst pores. Cells usually form a chain of two to eight cells and sometimes more than thirty cells during the period of rapid growth. Cell color is yellow-brown. The length varies from 30 to 47 μm, and the width from 38 to 53 μm. The dorso-ventral dimension is slightly less than the width.

The thecal plate formula is Po, Pc, 3', 7", 6c, 6s, 6‴, 1p, 1⁗. Some taxonomists consider that the first precingular plate is one of the member of apical plate series, and epithecal plate formula should be expressed as Po, Pc, 4', 6". Apical pore plate Po is ovoidal and has a large apical pore, which is usually covered with apical closing platelet Pc. At the center of the Po plate, a small anterior attachment pore is found clearly. The fourth apical plate has a ventral pore near the margin between the first precingular plate. In hypotheca large sulcal posterior plate occupied at the center of the hypotheca, and near the margin between the fifth postcingular plate.

Pyrodinium bahamense var. *compressa* sometimes blooms to make a red tide associated with the infestation of marine organisms by paralytic shellfish toxins.

A species *Pyrodinum bahamense* contains two varieties: var. *compressa* and var. *bahamense*. The var. *bahamense* appears mainly in the tropical Atlantic Ocean, and no infestation of marine organisms has been reported. Morphologically the var. *compressa* differs from the var. *bahamense* in some characters. The var. *compressa* has a low, broader apical boss without a prominent apical spine and is more compressed anterio-posteriorly. The var. *compressa* forms a chains of over thirty cells. These differences were thouroughly studied by Steidinger et al. (1980).

Steidinger et al. (1980) reported a resting cyst of the var. *compressa* similar to that of the var. *bahamense*, which was observed by Wall and Dale (1968) in detail through a single cyst incubation experiment. The resting cyst of the var. *bahamense* is spherical and possesses around 60 radiating, randomly arranged spines.

Useful scientific papers on the taxonomy of *Pyrodinium bahamense:*

Plate, L. 1906. *Pyrodinium bahamense* n. g., n. sp. die Leucht-Peridinee des "Feuersees" von Nassau, Bahamas. Archiv fuer Protistenkunde und Protozen-algen-tilze, 7; 411-429.

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Steidinger, K.A., Tester L.S. and Taylor F.J.R. 1980. A redescription of *Pyrodinium bahamense* var. *compressa* (Böhm) stat. nov. from Pacific red tides. Phycologia 19; 329-337.

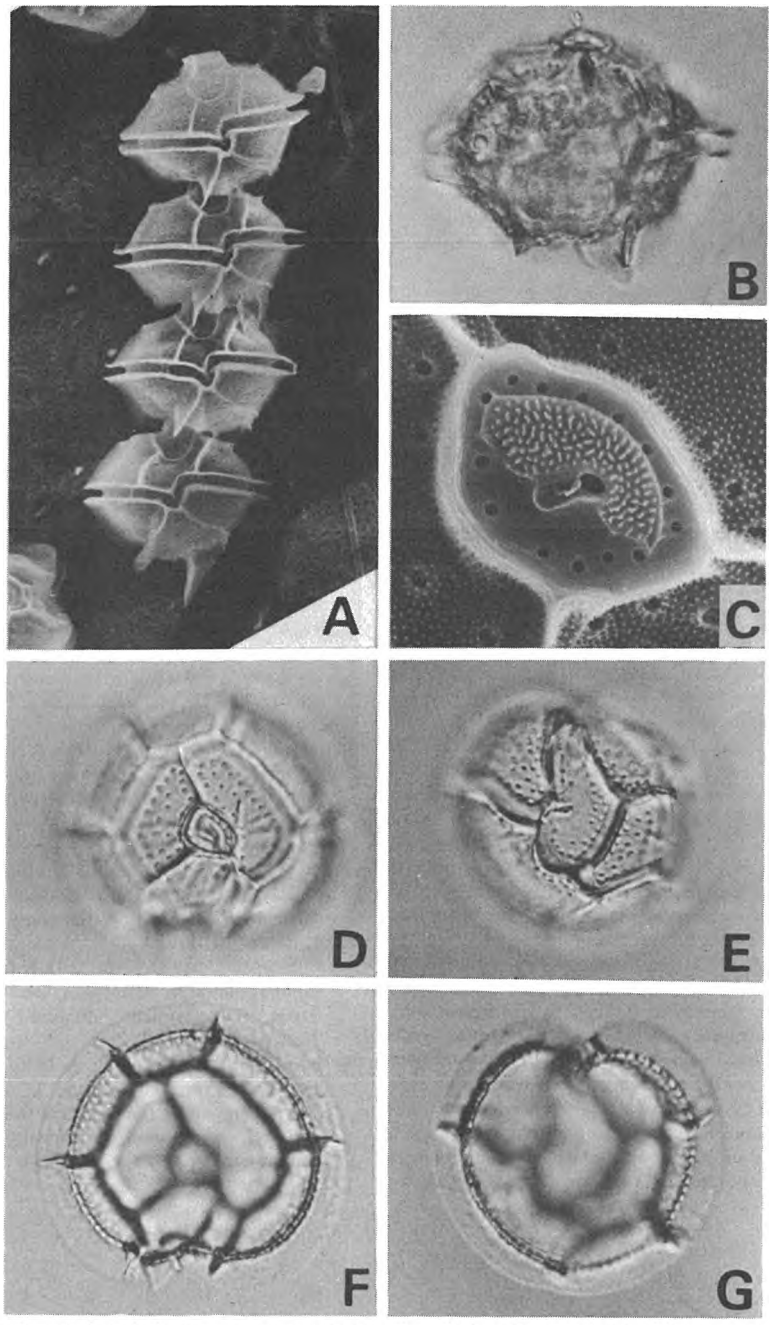

Fig. 1. Pyrodinium bahamense *var.* compressa. A *a chain of four cells;* B. *oblique-ventral view;* C. *apical pore plate;* D,F. *epithecal plates;* E.G. *hypothecal plates.*

Protogonyaulax catenella (Whedon et Kofoid) Taylor

Cell is rounded. The length is usually less than the width, but sometimes variations occur. Epitheca and hypotheca are nearly equal in altitude. Both shoulders of epitheca are convex. Shape of hypotheca is asymmetric, height of the right half is shorter than that of the left. At apex and antapex neither boss nor spine exists. Antapex is slightly concave. Cingulum concaves deeply, and is equatrial, descending one cingular width, and has low ridges without lists at both upper and lower margins. Ventral area is impressed and widened posteriorly one and a half times. At both right and left margins it has wing-like sulcal lists, which look like spines when observed ventro-apically.

Plate formula is Po, Pc, 4', 6", 6c, 8s, 5‴, 2⁗. Thecal plates are so thin that it is quite difficult to observe their pattern without dissection and staining. The plates are covered with delicate outer thecal membrane. The apical pore plate Po is triangular, wide dorsally, and has a fishhook-shaped apical pore which is partly closed by an apical closing platelet Pc. An ellipsoidal anterior attachment pore is clear when cells grow rapidly and form a chain after asexual binary fission, and is sealed up when cells swim individually.

The ventral pore is absent on the suture between the first and fourth apicals. In the sulcus eight small platelets are found. A large round posterior attachment pore is located in the right half of the sulcal posterior plate. The cingulum is composed of six plates nearly equal in size.

Cells commonly make a chain of two or four cells after asexual binary fission. A eight-cell chain is formed by newly hatched cells from a resting cyst. A chain of more than sixteen cells is seldom found in natural population when they grow rapidly. The length and width varied from 21 to 48 μm and 23 to 48 μ respectively. The cell of length 29-32 μm and width 31-33 μ is the most common. The ratio of length to width is from 0.65 to 1.14. The ratio varies greatly from one isolate to another, but within an isolate it is usually uniform.

A vegetable cell of *P. catenella* closely resembles that of *P. tamarensis* in size, shape and thecal plate configuration. It is quite difficult to distinguish them without dissection of thecal plates. The most useful distinction is the presence or absence of the ventral pore: *P. tamarensis* has the pore while *P. catenella* has no pore.

A resting cyst is ellipsoidal with rounded ends. Cell wall is thick, and covered with an amorphous transparent mucilagenous substance with which many fine detrital particles incorporate. Cell contents are pale or colorless granules, light-brown globules and one or two red pigmented bodies. The central body is 38 to 56 μm in length and 23 to 32 μm in width.

Protogonyaulax catenella and *P. tamarensis* have a resting cyst of the same morphology. For identification, it is necessary to incubate a single cyst and to observe characteristics of cell germinated from it, since the cyst is simple in shape and has no character specific to each species.

Useful scientific papers on the taxonomy of *Protogonyaulax catenella:*

Dale, B. 1977. Cysts of the toxic red-tide dinoflagellate *Gonyaulax excavata* (Braarud) Balech from Oslofjorden, Norway. Sarsia 63, 29-34.

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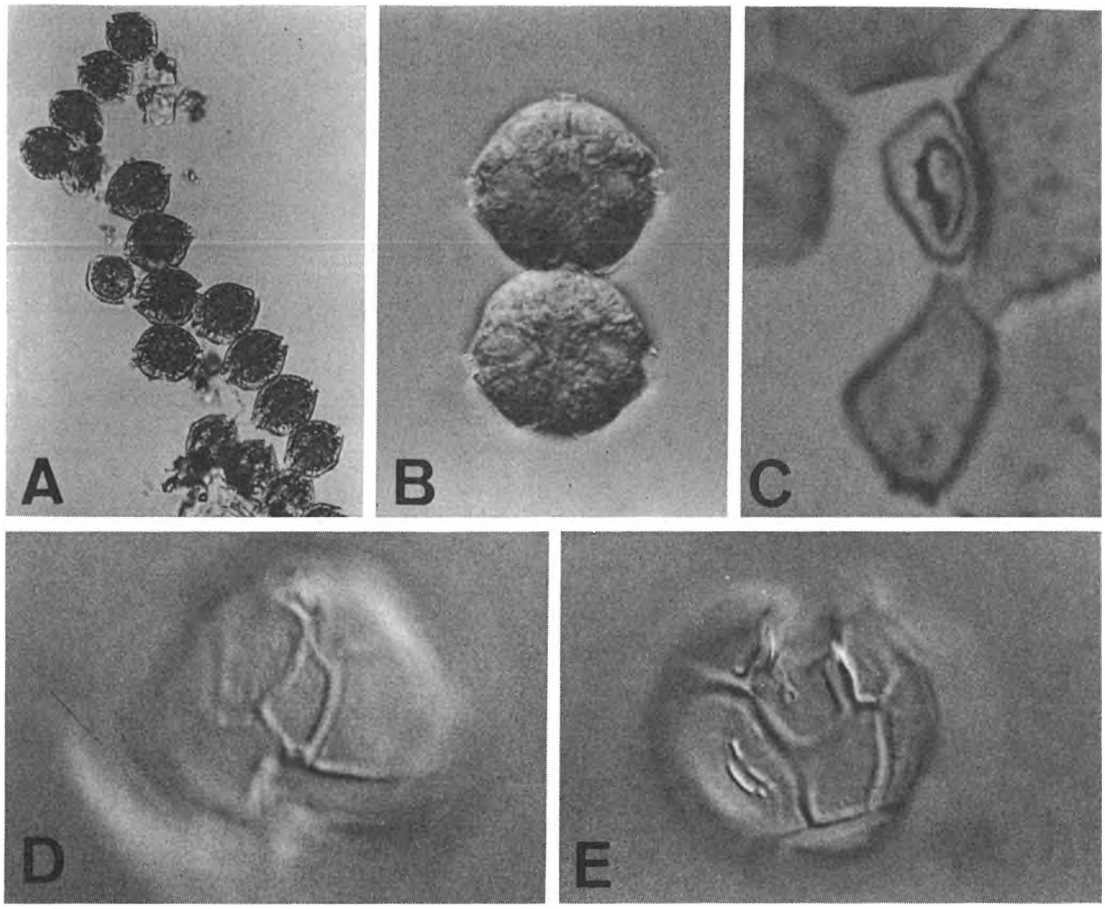
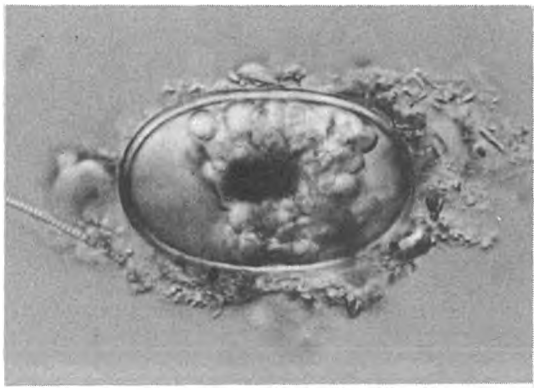
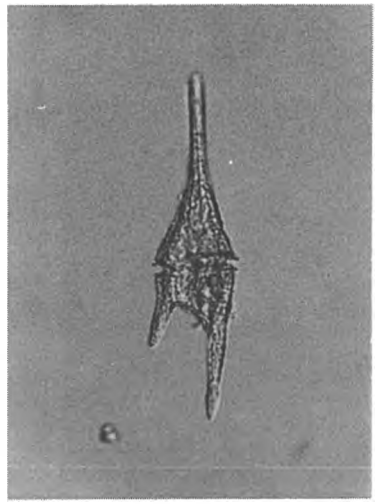


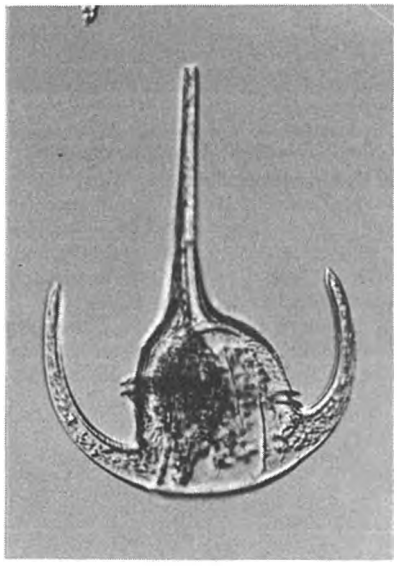
Fig. 2. Protogonyaulax catenella. A. *chain forming cells;* B. *a chain of two cells;* C. *apical pore plate and the first apical plate;* D. *ventral view of epitheca;* E. *hypothecal plates.*



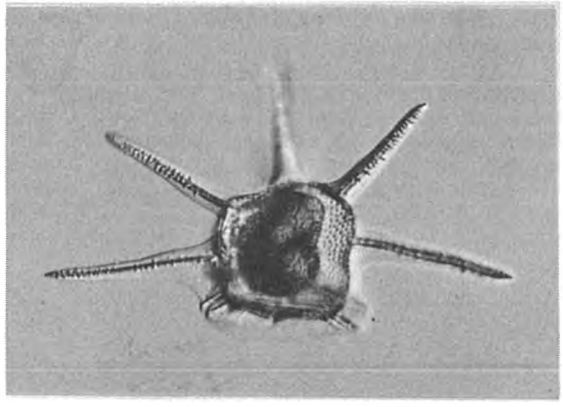
Cyst of Protogonyaulax



Ceratium furca

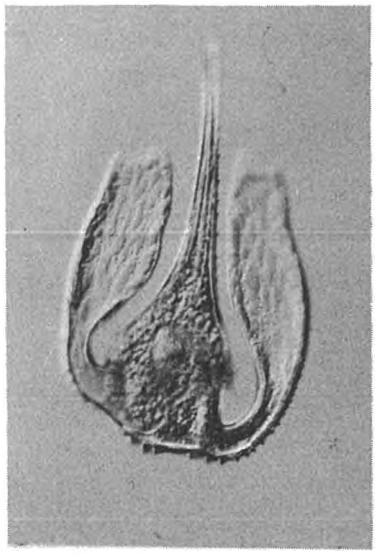


Ceratium breve

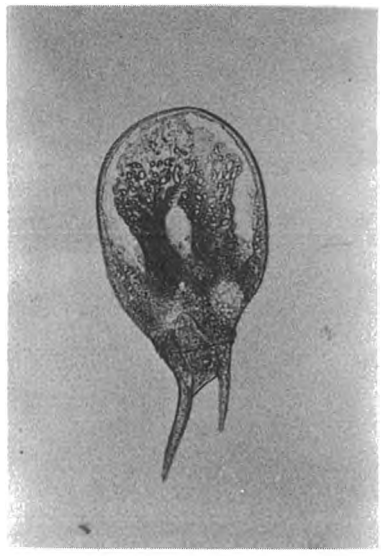


Ceratocorys horrida

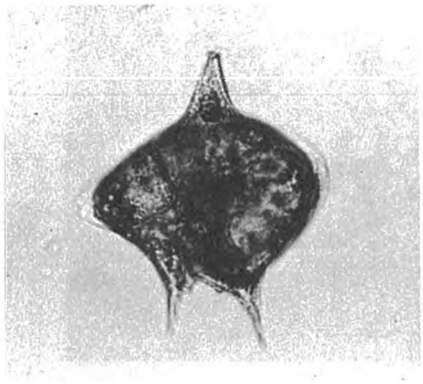
Fig. 3. Photographs of other species (provided through courtesy of Dr. Y. Fukuyo).



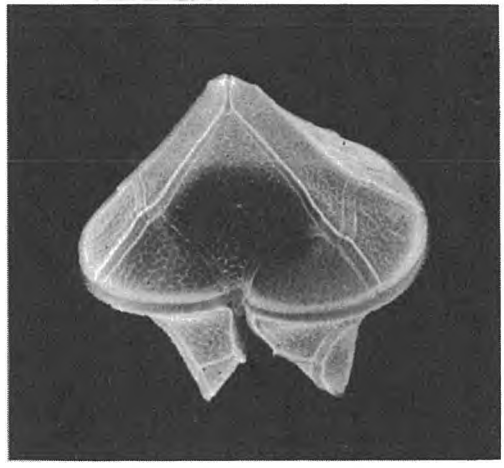
Ceratium platycorne



Ceratium gravidum

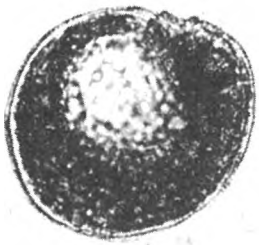


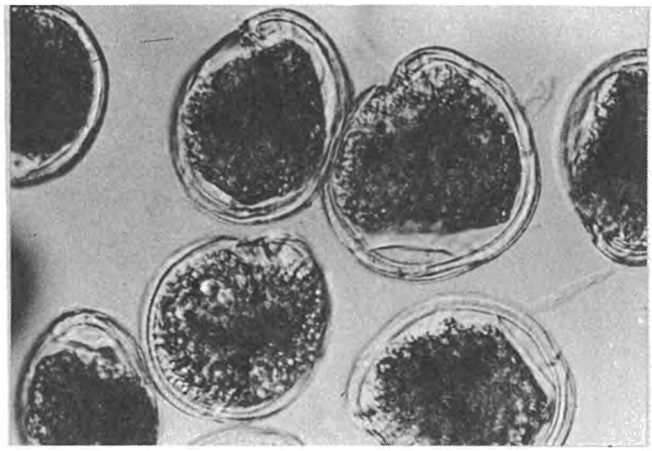
Protoperidinium depressum



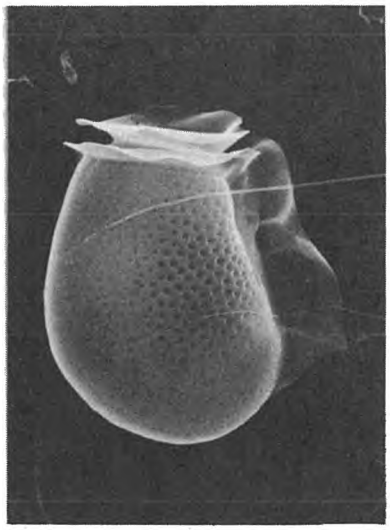
Protoperidinium conicum

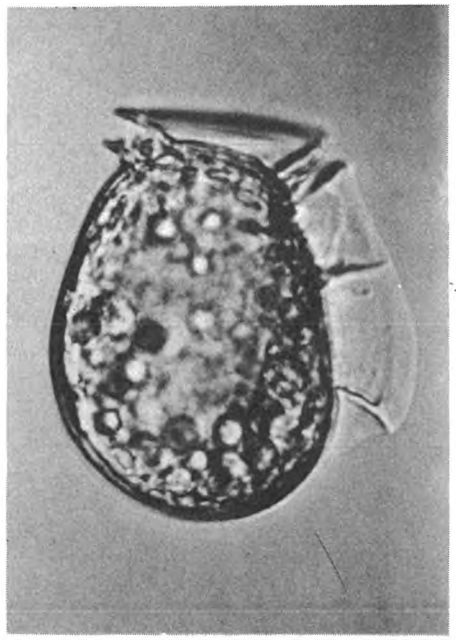
Fig. 3 (continued)





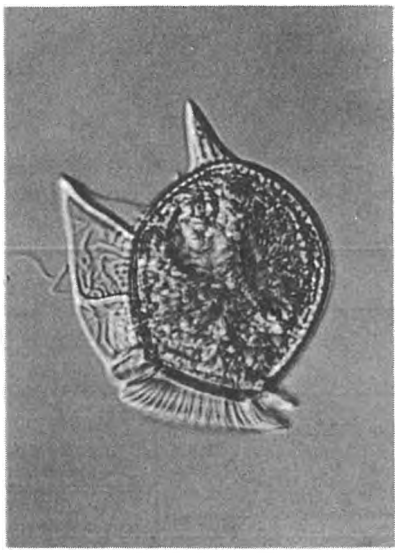
Gambierdisus toxicus



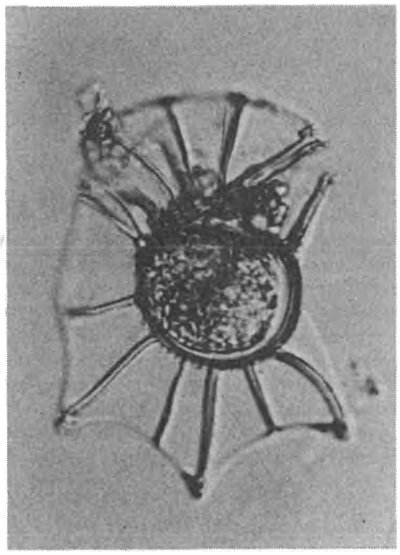


Dinophysis fortii

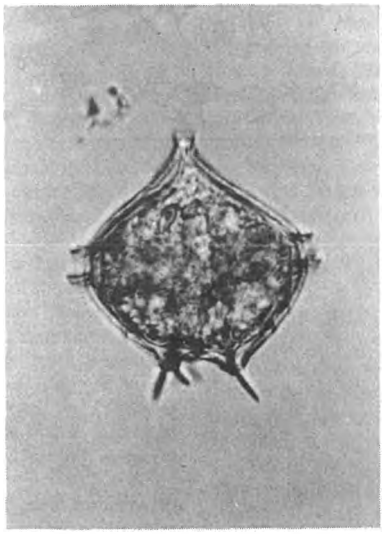
Fig. 3 (continued)



Dinophysis hastata



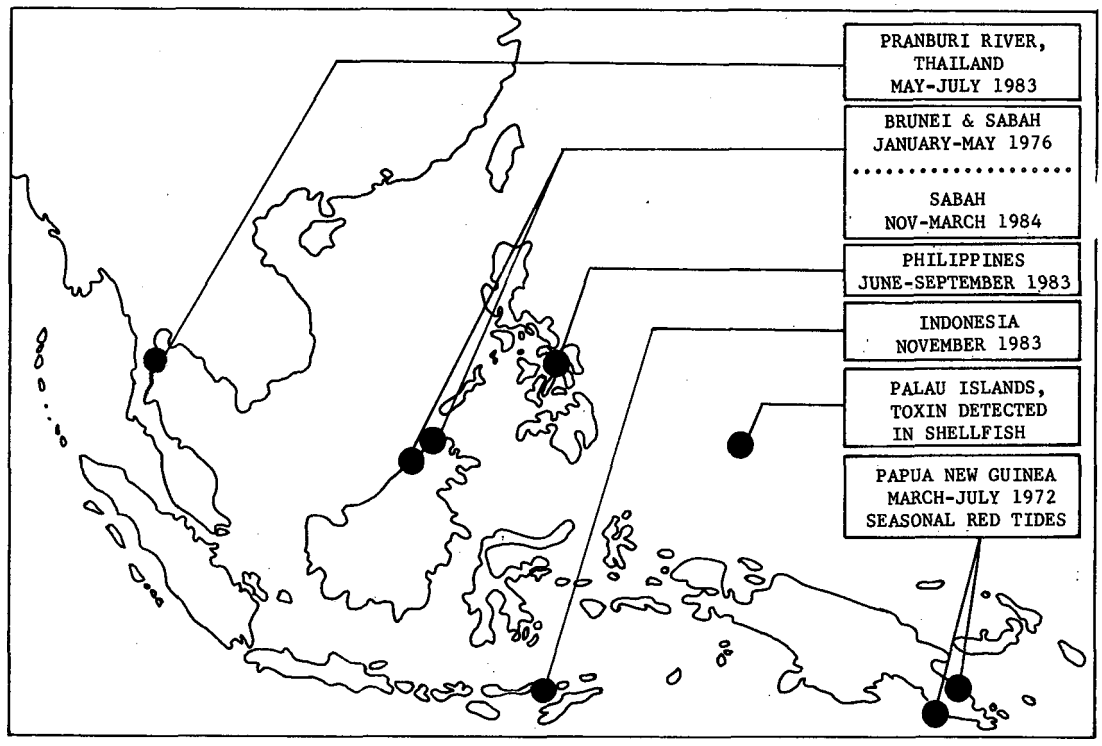
Ornithocercus thumii



Protoperidinium pellucidum

Fig. 3 (continued)

Location of PSP Outbreaks in Southeast Asia



Standard Mouse Bioassay for Paralytic Shellfish Toxins

The following standard method has been copied with permission of the Official Methods of Analysis, 1980. 13th ed. William Horwitz, Ed., Association of Official Analytical Chemists, Washington, DC, USA, pages 287, 298-299.

Paralytic Shellfish Poison Biological Method

(*Caution:* Use rubber gloves when handling materials which may contain paralytic shellfish poison).

Materials

(a) Paralytic shellfish poison standard solution — 100 μg/mL. Available from Division of Microbiology, Food and Drug Administration, 1090 Tusculum Ave, Cincinnati, OH 45226, as acidified 20% alcohol solution. Standard is stable indefinitely in cool place.

(b) Paralytic shellfish poison working standard solution — 1 μg/mL. Dilute 1 mL standard solution to 100 mL with distilled water. Solution is stable several weeks at 3-4°C.

(c) Mice — Healthy mice, 19-21 g, from stock colony used for routine assays. If <19 g or >21 g, apply correction factor to obtain true death time (see Sommer's Table). Do not use mice weighing 23g and do not re-use mice.

Standardization of Bioassay

Dilute 10 mL aliquots of 1 μg/mL standard solution with 10, 15, 20, 25, and 30 mL water, respectively, until intraperitoneal time of 5-7 min. pH of dilutions should be 2-4 and must not be >4.5. Test additional dilutions in 1 mL increments of water, eg, if 10 mL diluted with 25 mL water kills mice in 5-7 min, test solutions diluted 10 + 24 and 10 + 26.

Inject group of 10 mice with each of 2 or preferably 3 dilutions that fall within median death time of 5-7 min. Give 1 mL dose to each mouse by intraperitoneal injection and determine death time as time elapsed from completion of injection to last gasping breath of mouse.

Repeat assay 1 or 2 days later, using dilutions prpeared above which differed by 1 mL increments of water. Then repeat entire test, starting with testing of dilutions prepared from newly prepared working standard solution.

Calculate median death time for each group of 10 mice used on each dilution. If all groups of 10 mice injected with any 1 dilution gave median death time <5 or >7 min, disregard results from this dilution in subsequent calculations. On the other hand, if any groups of 10 mice injected with 1 dilution gave median death time falling between 5 and 7 min, include all groups of 10 mice used on that dilution, even though some of median death times may be <5 or >7 min. From median death time for each group of 10 mice in each of selected dilutions, determine number of mouse units/mL from Sommer's Table. Divide calculated ug poison/1 mL by mouse units/1 mL to obtain conversion factor (CF value) expressing ug poison equivalent to 1 mouse unit. Calculate average of individual CF values, and use this average value as reference point to check routine assays. Individual CF values may vary significantly within laboratory if techniques and mice are not rigidly controlled. This situation will require continued use of working standard or secondary standard, depending on volume of assay work performed.

Use of Standard with Routine Assays of Shellfish

Check CF value periodically as follows: If shellfish products are assayed less than once a week, determine CF value on each day assays are performed by injecting 5 mice with appropriate dilution of working standard. If assays are made on several days during week, only 1 check need be made each week on dilution of standard such that median death time falls within 5-7 min. CPF value thus determined should agree with average CF avalue within ±20%. If outside this range, complete group of 10 mice by adding 5 mice to the 5 mice already injected, and inject second group of 10 mice with same dilution of standard. Pool the average CF value determined for second group with that of first group. Take resulting value as new CF value. Variation of <20% represents significant change in response of mice to poison, or in technique of assay. Changes of this type require change in CF value.

Repeated checks of CF value ordinarily produce consistent results within ±20%. If wider variations are found frequently, the possibility of uncontrolled or unrecognized variables in method should be investigated before proceeding with routine assays.

Preparation of Sample

(a) Clams, oysters, and mussels. —Thoroughly clean outside of shellfish with fresh water. Open by cutting adductor muscles. Rinse inside with fresh water to remove sand or other foreign material. Remove meat from shell by separating adductor muscles and tissue connecting at hinge. Do not use heat or anesthetics before opening shell, and do not cut or damage body of mollusk at this state. Collect about 100-150 g meats in glazed dish. As soon as possible, transfer meats to No. 10 sieve without layering, and let drain 5 min. Pick out pieces of shell and discard drainings. Grind in household-type grinder with 1/8-1/4" (3-6mm) holes or in blender until homogeneous.

Sommer's Table

Death time: mouse unit relations for paralytic shellfish poison (acid)

Death Time[a]	Mouse Units	Death Time[a]	Mouse Units
1:00	100	5:00	1.92
10	66.2	05	1.89
15	38.3	10	1.86
20	26.4	15	1.83
25	20.7	20	1.80
30	16.5	30	1.74
35	13.9	40	1.69
40	11.9	45	1.67
45	10.4	50	1.64
50	9.33		
55	8.42	6:00	1.60
		15	1.54
2:00	7.67	30	1.48
05	7.04	45	1.43
10	6.52		
15	6.06	7:00	1.39
20	5.66	15	1.35
25	5.32	30	1.31
30	5.00	45	1.28
35	4.73		
40	4.48	8:00	1.25
45	4.26	15	1.22
50	4.06	30	1.20
55	3.88	45	1.18
3:00	3.70	9:00	1.16
05	3.57	30	1.13
10	3.43		
15	3.31	10:00	1.11
20	3.19	30	1.09
25	3.08		
30	2.98	11:00	1.075
35	2.88	30	1.06
40	2.79		
45	2.71	12:00	1.05
50	2.63		
55	2.56	13	1.03
		14	1.015
4:00	2.50	15	1.000
05	2.44	16	0.99
10	2.38	17	0.98
15	2.32	18	0.972
20	2.26	19	0.965
25	2.21	20	0.96
30	2.16	21	0.954
35	2.12	22	0.948
40	2.08	23	0.942
45	2.04	24	0.937
50	2.00	25	0.934
55	1.96	30	0.917
		40	0.898
		60	0.875

[a]Minutes: Seconds.

Correction table for weight of mice

Wt of Mice, g	Mouse Units
10	0.50
10.5	0.53
11	0.56
11.5	0.59
12	0.62
12.5	0.65
13	0.675
13.5	0.70
14	0.73
14.5	0.76
15	0.785
15.5	0.81
16	0.84
16.5	0.86
17	0.88
17.5	0.905
18	0.93
18.5	0.95
19	0.97
19.5	0.985
20	1.000
20.5	1.015
21	1.03
21.5	1.04
22	1.05
22.5	1.06
23	1.07

(b) Scallops. — Separate edible portion (adductor muscle) and apply test to this portion alone. Drain and grind as in (a).

(c) Canned shellfish. — Place entire contents of can (meat and liquid) in blender and blend until homogeneous or grind 3 times through meat chopper. For large cans, drain meat 2 min on No. 8-12 sieve and collect all liquid. Determine weight of meat and volume of liquid. Recombine portion of each in proportionate amounts. Blend recombined portions in blender (or grind) until homogeneous.

Extraction

Weigh 100 g well mixed material into tared beaker. Add 100 mL 0.1N HCl, stir thoroughly and check pH. (pH should be <4.0, preferably about 3.0. If nececessary, adjust pH as indicated below.) Heat mixture, boil quently 5 min, and let cool to room temperature. Adjust cooled mixture to pH 2.0-4.0 (never >4.5) as detected by *BHD Universal Indicator, phenol blue, Congo red paper,* or pH meter. To lower pH, add 5*N* HCl dropwise with stirring; to raise pH, add 0.1*N* NaOH dropwise with constant stirring to prevent local alkalinization and consequent destruction of poison. Transfer mixture to graduated cylinder and dilute to 200 mL.

Return mixture to beaker, stir to homogeneity, and let settle until portion of supernate is translucent and can be decanted free of solid particles large enough to block 26-gauge hypodermic needle. If necessary, centrifuge mixture or supernate 5 min at 3000 rpm or filter through paper. Only enough liquid to perform bioassay is necessary.

Mouse Test

Intraperitoneally inoculate each test mouse with 1 mL acid extract. Note time of inoculation and observe mice carefully for time of death as indicated by last gasping breath. Record death time from stopwatch or clock with sweep second hand. One mouse may be used for initial determination, but 2 or 3 are preferred. If death time or median death time of several mice is <5 min, make dilution to obtain death times of 5-7 min. If death time of 1 or 2 mice injected with undiluted sample is >7 min, a total of ≥mice must be innoculated to establish toxicity of sample. If large dilutions are necessary, adjust pH of dilution by dropwise addition of dilute HCl (0.1 or 0.01 *N*) to pH 2.0-4.0 (never >4.5). Inoculate 3 mice with dilution that gives death times of 5-7 min.

Calculation of Toxicity

Determine median death times of mice, including survivors, and from Sommer's Table determine corresponding number of mouse units. If test animals weigh <19 g or >21 g, make correction for each mouse by multiplying mouse units corresponding to death time for that mouse by weight correction factor for that mouse from Sommer's Table; then determine median mouse unit for group. (Consider death time of survivors as >60 min or equivalent to <0.875 mouse unit in calculating median). Convert mouse units to μg poison/mL by multiplying by CF value.

μg Poison/100 g meat
= (μg/mL) x dilution factor x 200.

Consider any value >80 μg/100 g as hazardous and unsafe for human consumption.

Source of Standard Saxitoxin Solution

Standard saxitoxin solution may be obtained upon written request from the following:

Mr James Gilchrist
Food and Drug Administration
Public Health Service,
1090 Tusculum Ave,
Cincinnati, Ohio 45226
U S A

Each vial contains 3 mL of purified saxitoxin at 100 μg/mL in 20% ethanol in water at pH 3.0. Keep vials well sealed in a cool place (refrigerator), but do not freeze. Solution will maintain constant activity for years. For preparing dilutions, do *not* pipette by mouth. Use of protective gloves is recommended.

Symptoms and Treatment of PSP

Symptoms

A good account of the symptoms associated with PSP is given by Halstead (1965):

Paralytic shellfish poisoning may be diagnosed readily by the presence of pathognomonic symptoms which usually manifest themselves within 30 minutes. Initially there is a tingling or burning sensation of the lips, gums, tongue and face, with gradual progression to the neck, arms, fingertips, legs and toes. The paresthesia later changes to numbness, so that voluntary movements are made with difficulty. In severe cases ataxia and general motor incoordination are accompanied in most instances by a peculiar feeling of lightness, 'as though one were floating in air.' Constrictive sensations of the throat, incoherence of speech, and aphonia are prominent symptoms in severe cases. Weakness, dizziness, malaise, prostration, headache, salivation, rapid pulse, intense thirst, dysphagia, perspiration, anuria, and myalgia may be present. Gastrointestinal symptoms of nausea, vomiting, diarrhoea, and abdominal pain are less common. As a rule, the reflexes are not affected. Pupillary changes are variable, and there may be an impairment of vision or even temporary blindness. Mental symptoms vary, but most victims are calm and conscious of their condition throughout their illness. Occasionally, patients complain that their teeth feel 'loose or set on edge.' Muscular twitchings and convulsions are rare.

Prakash et al. (1971) distinguish the following sequence of poisoning:

Tingling sensation or numbness around lips, gradually spreading to face and neck. Prickly sensation in fingertips and toes. Headache, dizziness, nausea.	MILD
Incoherent speech. Progression of prickly sensation to arms and legs. Stiffness and noncoordination of limbs. General weakness and feeling of lightness. Slight respiratory difficulty. Rapid pulse.	SEVERE
Muscular paralysis. Pronounced respiratory difficulty. Choking sensation.	EXTREME

Many factors affect symptoms and severity of poisoning. In fatal cases death is caused by respiratory paralysis and cardiovascular collapse, usually within 12 hr of consumption of toxic shellfish.

Treatment

Prakash et al. (1971) state:

Although much is known about how the poison behaves in the body, no one has discovered an antidote for it. Many have tried and are still trying. Until one is discovered, the best protection against PSP is public education on what areas produce toxic shellfish, what species and what parts of their bodies are hazardous, how cooking affects toxicity, and where, and where not to seek advice on use of shellfish.

According to Halstead (1965):

The treatment of paralytic shellfish poisoning is largely symptomatic. The poison has no specific antidote. Apomorphine is more effective than lavage in removing pieces of shellfish from the stomach. Since mussel poison is readily absorbed on charcoal, Lloyd's reagent and similar absorbents may be tried. Alkaline fluids are of value since the toxin is unstable in that medium. Diuresis may be instituted with 5 percent ammonium chloride.

Artificial respiration is an important adjunct and should be instituted promptly if there is any evidence of respiratory embarrassment. Experimentally this technique has been used with good results and is recommended (Sapeika, 1953; Murtha, 1960). Primary shock may be present and require attention.

Drug therapy has varying degrees of success. The anticurare drugs, such as neostigmine, epinephrine, ephedrine, and DMPP (1,1-dimethyl-4-phenylpiprazinium iodide) are also recommended (Murtha, 1960). To counteract the acetylcholine esterase-like inhibitory effect, Pepler and Loubser (1960) advised using oximes, such as pyridinealdoxime methiodide. Digitalis and alcohol are not recommended.

Prakash et al. (1971) consider that the first treatment should be the emptying of the stomach of toxic materials as quickly as possible. They also mention that no treatment should be given which might increase posion absorption by the digestive system, such as alcohol. Finally, they suggest that copious amounts of warm water may be beneficial by serving as an emetic, as well as inducing urination.

Halstead, B.W. 1965. Poisonous and venomous marine animals of the World. Vol. 1 US Government Printing Office, Washington, DC, USA, 157-270.

Prakash, A., J.C. Medcof, and A.D. Tennant. 1971. Paralytic shellfish poisoning in eastern Canada. Bulletin of the Fisheries Research Board of Canada, No. 177.

Toxic Red Tides and Shellfish Toxicity in Southeast Asia

The occurence of toxic red tides and paralytic shellfish poisoning (PSP) have become more frequent in Southeast Asian waters in recent years. A consultative meeting was organised by the Southeast Asian Fisheries Development Center (SEAFDEC) and the International Development Research Centre (IDRC) of Canada on 11-14 September 1984 to review the status of shellfish toxicity in Southeast Asia and discuss ways and means for its improved study and control. It was attended by 28 officials and researchers from Brunei Darussalam, Canada, Indonesia, Japan, Malaysia, Philippines, Singapore, and Thailand, including participants from the International Center for Living Aquatic Resource Management, IDRC and SEAFDEC.

This volume resulted from the meeting and provides, for the first time, a review of past problems and current research on the subject. It includes edited papers, recommendations and conclusions of the meeting.

The papers describe the background related to PSP and red tide in the region as well as the measures taken to protect consumers. Also included are resource papers outlining conditions in Canada and Japan and the protective measures which have been adopted by the two countries.

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