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.

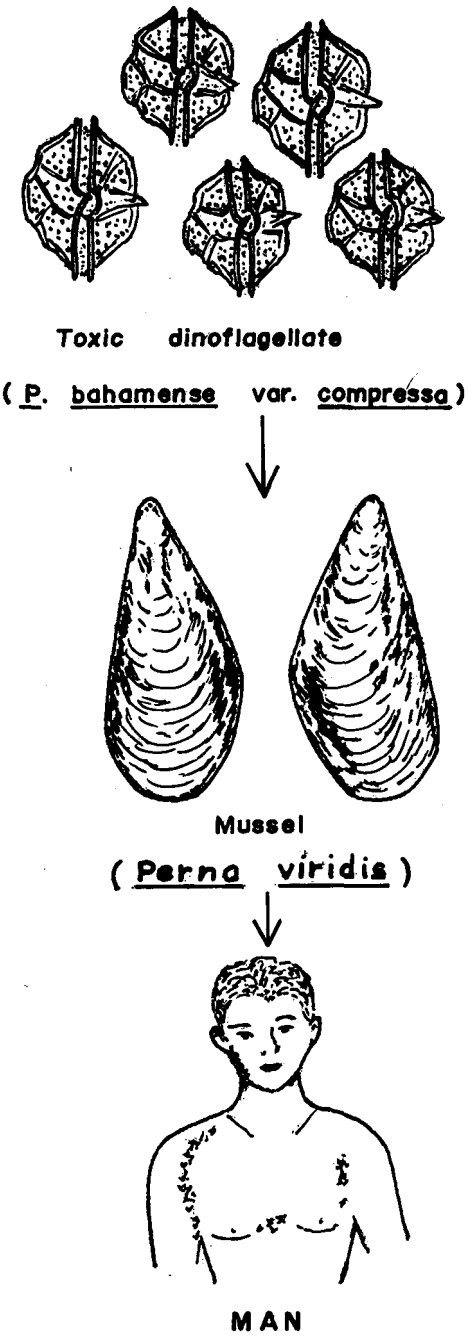

Fig. 1. Pathway of human intoxication through ingestion of toxic dinoflagellate.

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),

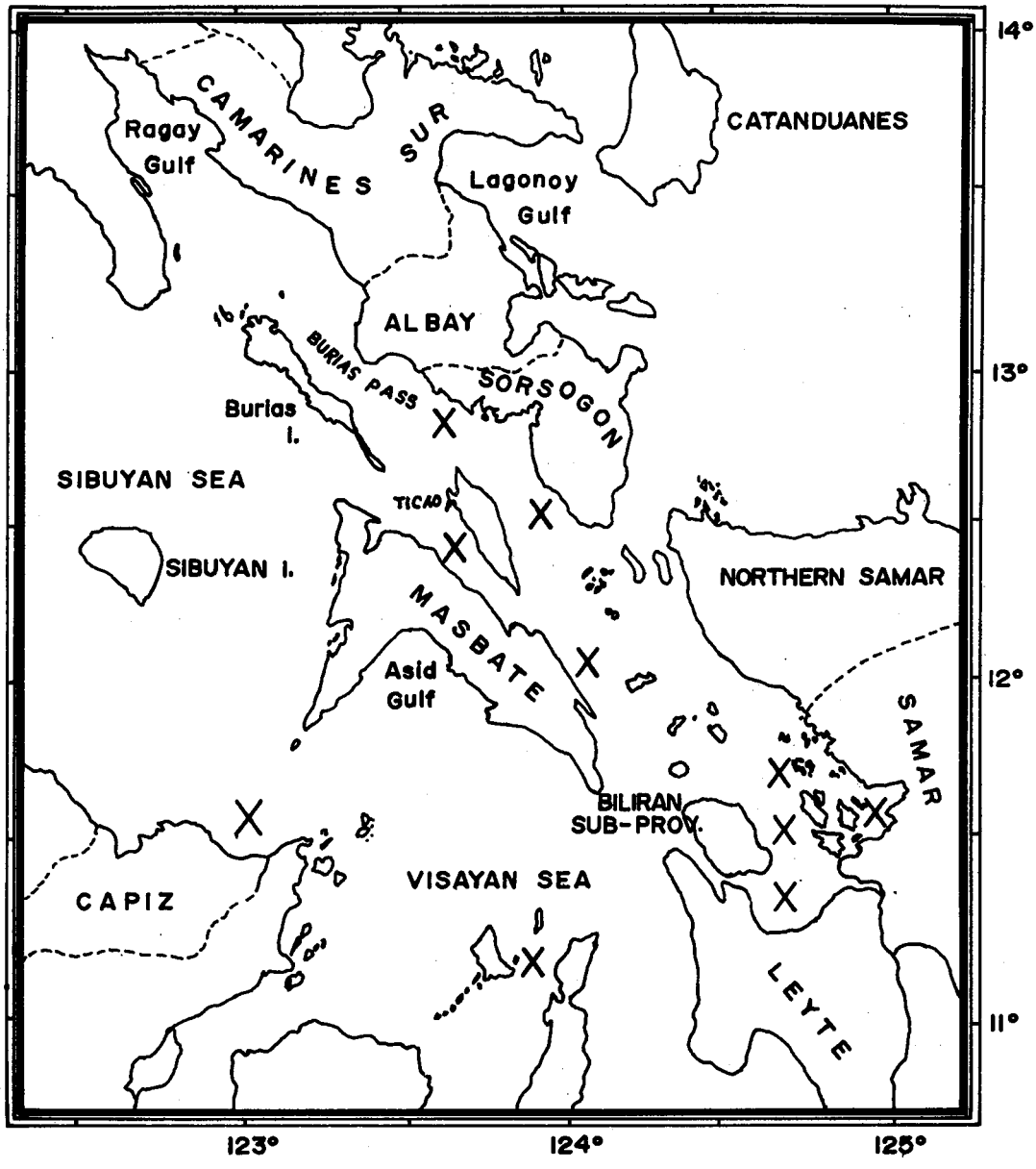

Fig. 2. Eastern central Philippines, areas affected by the red tide/ PSP (X).

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,

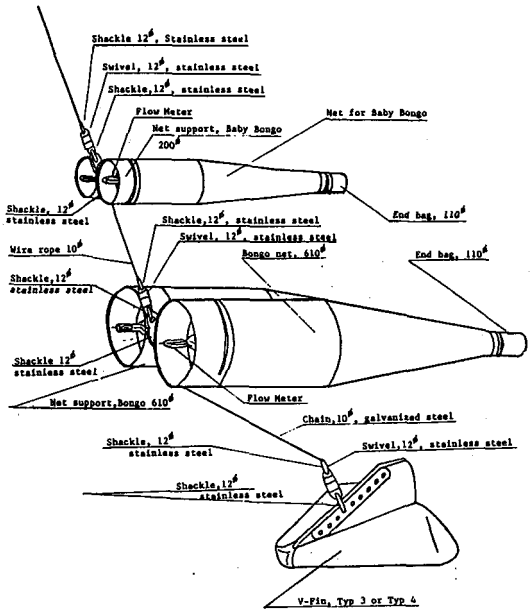

Fig. 3. Bongo nets for plankton collection (reproduced from Alu-Bau GMBH)

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

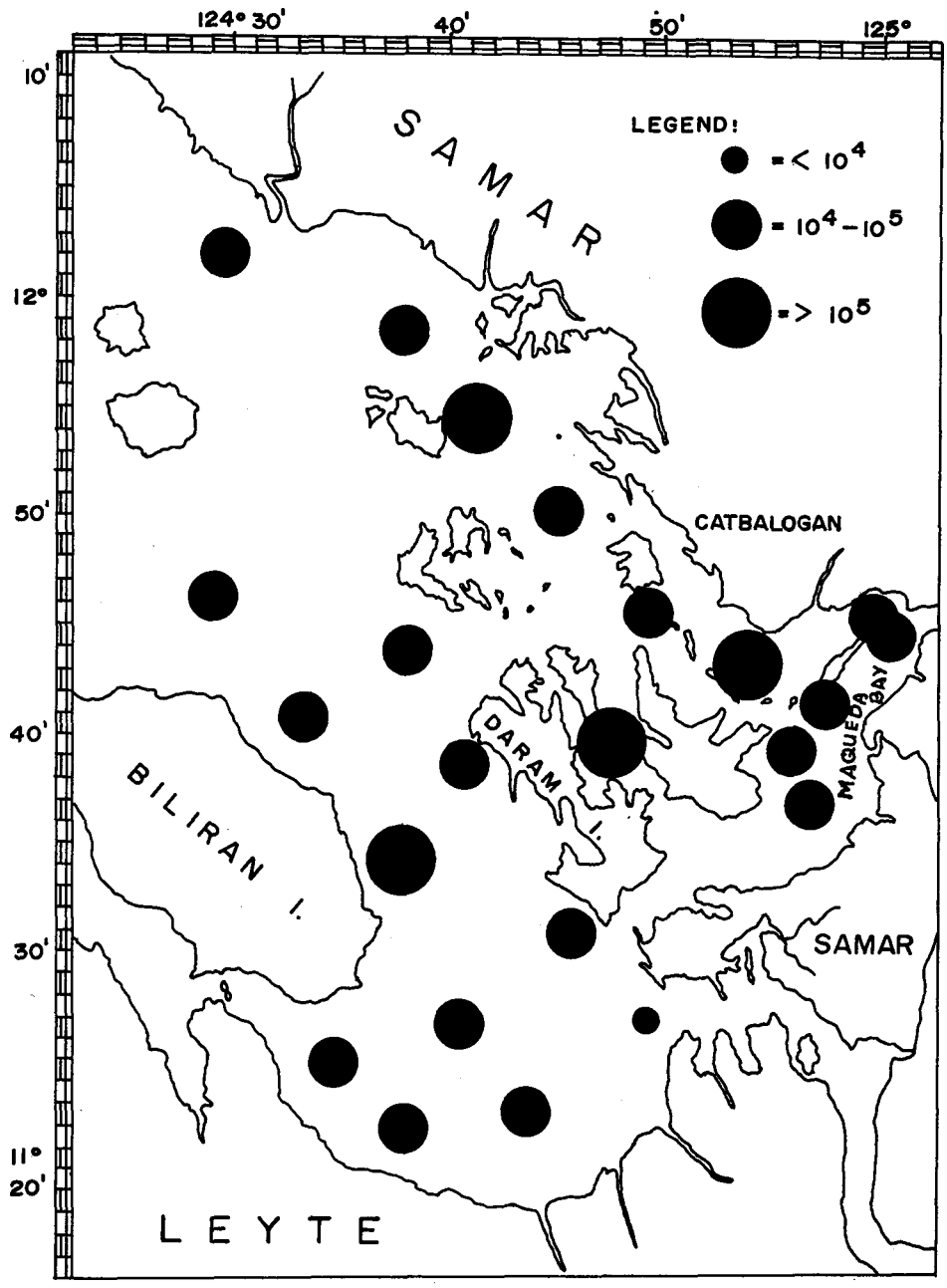

Fig. 4. P. bahamense *surface (0-1 m) densities (cells/ L) in Samar Sea, Carigara and Maqueda bays; based on Nansen Water Bottle samples.*

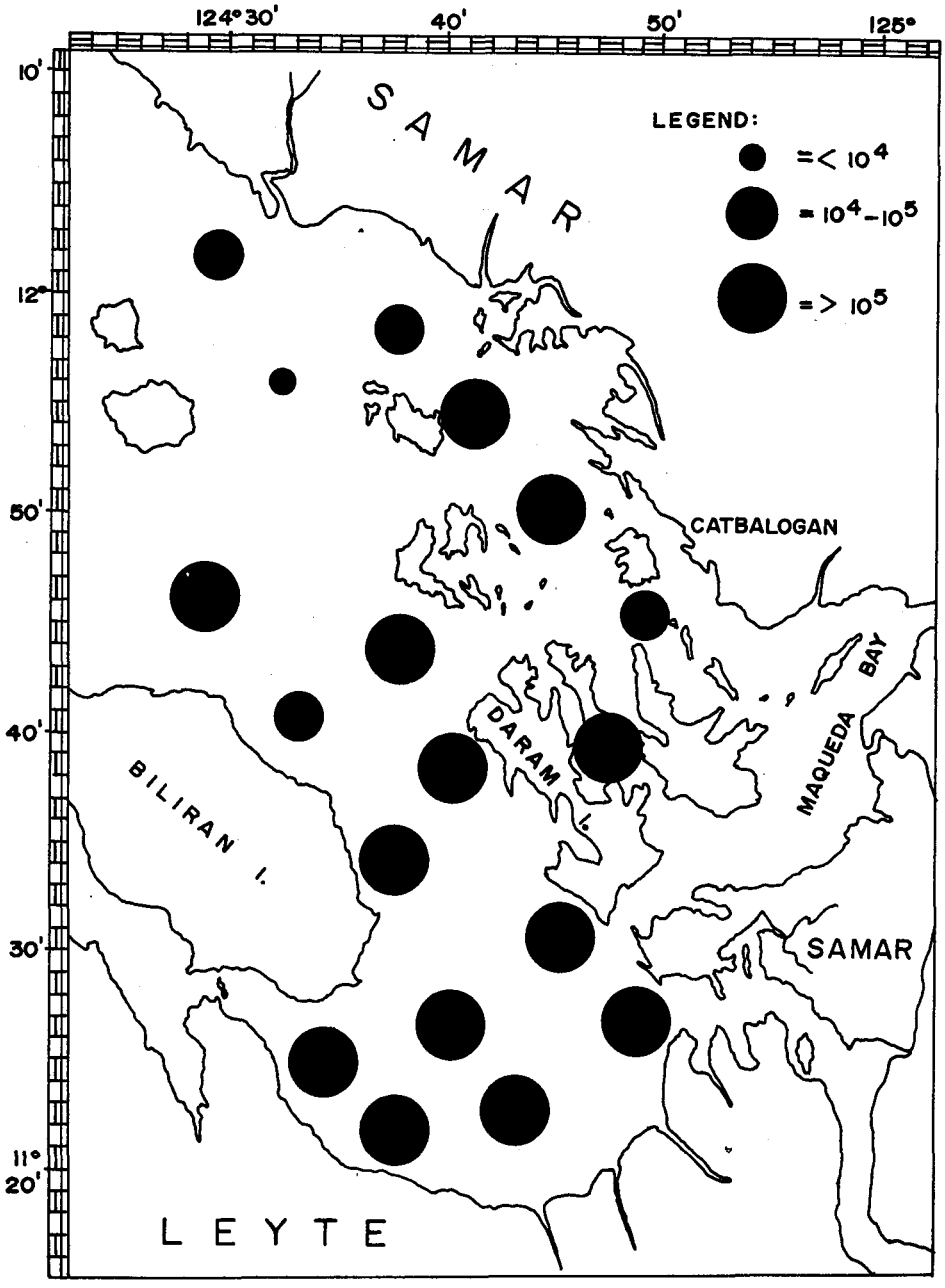

Fig. 5. P. bahamense *densities (cells/L) in Samar Sea and Carigara Bay based on double oblique tows with a 20-cm bongo sampler (0.047 mm).*

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

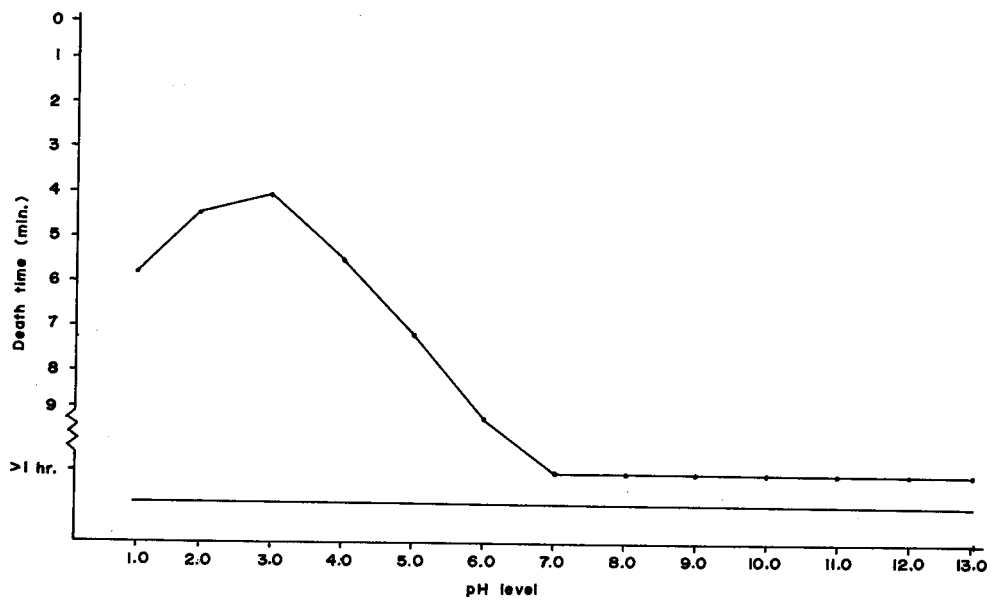

Fig. 6. Effect of pH on the toxicity of crude Perna viridis *extract.*

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.

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