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## SHELLFISH AQUACULTURE AND PARALYTIC SHELLFISH POISONING

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### ABSTRACT

The history and current status of paralytic shellfish poisoning problems in shellfish aquaculture are presented. The characteristics of the newly discovered toxins from economically important shellfish were introduced. It was confirmed that scallops have the ability to convert gonyautoxins to saxitoxin in the sequence: gonyautoxin-I → gonyautoxin-II → neosaxitoxin → saxitoxin. The scallop adductor muscle has the ability to detoxify; the PSP Alaska butter clam has also the ability to convert gonyautoxins to saxitoxin and store it in the siphon. Precautions considered to be necessary for shellfish aquaculture in connection with PSP are discussed.

Accumulation of noxious substances, both natural and synthetic, in fish and shellfish, transported through the food chain have been a subject of great public concern. Such substances are normally so minute in quantity that their existence can be recognized only due to the recent development of analytical methods and increased understanding of molecular toxicology. In a few cases, however, such metabolite transfer from organism to organism have been known to us because of the drastic biological activity of the involved compounds. One noted example is the shellfish toxins.

Paralytic shellfish poisoning is one of the most drastic forms of food poisonings which has been known from the ancient time. People empirically knew that shellfish in certain areas can become toxic in certain times of the year. Sommer and Meyer (1) for the first time showed the relationship between the toxicity of shellfish and a dinoflagellate in the Californian waters. The organism was named Gonyaulax catenella. Some years later, the similar observation was made with the PSP on the Canadian Atlantic coast. Their causative organism was identified as Gonyaulax tamarensis (2). The toxic principle responsible for the poisoning was determined to be a small molecular nitrogenous compound, saxitoxin. The name came from Alaskan butter clam, Saxidomus giganteus (3) from which the toxin was originally isolated. In certain Alaskan areas this normally non-toxic clam contains a considerable amount of toxins in its siphon. Schantz and his co-workers identified the same toxin in the Californian mussel, Mytilus californica, and the dinoflagellate, G. catenella (4). Since then, it was generally regarded that the PSP was caused by saxitoxin.

However, the recent studies mostly done by our group at the University of Rhode Island have revealed that in many cases toxic shellfish contain multiple toxins, and that saxitoxin represents only a small portion of the

of the total toxicity (5). This is true with all the toxic shellfish obtained from various parts of the world except Alaska butter clam in the Southeast Alaska (Table 1). The toxin components in the causative organisms also follow the same trend, and thus several toxins were discovered in the intact cells (6).

The chemical structures of these newly discovered toxins are all closely related to saxitoxin. Gonyautoxin-II, which is the major toxin in many samples, was assigned the structure of 11 $\alpha$ -hydroxysaxitoxin by our group and gonyautoxin-III as its 11 $\beta$ -isomer (7). Later it was found out that both compounds actually exist in their sulfate forms (8). Neosaxitoxin, which was first discovered in Alaska butter clam, was given the structure of 1-hydroxysaxitoxin based upon chemical and spectroscopic evidence (9). Gonyautoxin-I has recently been assigned the structure of 11 $\alpha$ -dihydroxynesosaxitoxin sulfate (10). Gonyautoxin-I and gonyautoxin-IV form an equilibrium mixture, in which the former is the predominant component indicating gonyautoxin-IV is the more sterically hindered 11 $\beta$ -isomer.

The pharmacological action of all the isolated toxins seems to be similar to that of saxitoxin; the blocking of sodium ion influx through excitable membranes. The manifestation of the poisoning is usually very fast and the direct cause of deaths is respiratory paralysis. The toxin is heat-stable at a lower pH and cannot be destroyed by heating. The canning process destroys only a part of the toxicity. The toxin is easily destroyed by oxidation at a high pH range, which is not applicable in the ordinary food processing.

In the past, the occurrence of PSP was considered to be limited to specific areas, e.g., Alaska, Northern California, etc. However, increasing numbers of incidents have recently been reported around the world. The socio-economical impact of PSP is now becoming a serious problem.

In September, 1972, a large scale red tide took place in New England coast north of Cape Ann, where PSP was not a serious problem before. The toxicity of shellfish in the area reached a very high level. Fortunately, the incident was discovered at a very early stage, but over thirty people were poisoned and some seriously. The most serious impact of this red tide was an economic one. The collection and transportation of shellfish was banned immediately. The bad publicity created by television, radio and newspapers implanted unjustifiable fear of seafoods in general among the public. People started to shun even fish and lobsters which are absolutely safe. One time the price of fish dropped to one-third of the ordinary prices at the Boston fish market. Sea food restaurants lost customers and tourism dwindled. It is difficult to estimate the total monetary loss in this incident, but it certainly reached the order of million dollars. The President declared the area as a federal disaster area.

In 1976 there were 268 PSP incidents widely scattered in western Europe. The poisonings were traced to mussels originated from Spain. Analysis of a sample confiscated by Swiss Federal Government showed the presence of gonyautoxins, neosaxitoxin and saxitoxin, a typical profile found in other PSP samples (11). The toxic mussel was cultured on the

TABLE 1. Toxin Contents in Various Shellfish Samples.

Toxic Shellfish	Causative Organism*	Collection Site	Purified Toxins <sup>†</sup>									
			STX	GTX <sub>1</sub>	GTX <sub>4</sub>	GTX <sub>2</sub>	GTX <sub>3</sub>	GTX <sub>5</sub>	neoSTX	GTX <sub>6</sub>	GTX <sub>7</sub>	
<u>Mya arenaria</u>	<u>G. tamarensis</u>	Essex, MA	++	++	+	+++	+	+				+
<u>Mya arenaria</u>	<u>G. tamarensis</u>	Hampton, MA	++	±		+++	+	+				+
<u>Mytilus edulis</u>	<u>G. catenella</u>	Oase Bay, Mie, Japan	+	+++	+	++	+	+	++			
<u>Tapes japonica</u>	<u>G. catenella</u>	Oase Bay, Mie, Japan	+	+++	+	++	+	+	++			+
<u>Mytilus edulis</u>	<u>Gonyaulax</u> sp.	Vigo, Spain	+++	+	+	++	+	+	++			+
<u>Mytilus edulis</u>	<u>G. catenella</u> ?	Haines, AK	+++	++	++	++	++	+	+			+
<u>Mytilus edulis</u>	<u>G. catenella</u> ?	Elfin Cove, AK	+	+++	+	+	++	+	+			+
<u>Saxidomus giganteus</u>	?	Porpoise Island, AK	+++									+
<u>Placcopecten magellanicus</u>	<u>G. tamarensis</u>	Bay of Fundy, N.S., Canada	+	++	+	++	++	+	+	++	++	+
<u>Mytilus</u> spp.	<u>G. catenella</u>	Inland Sea, Japan	+			+++	++	++	++			++

\* Tentative assignment: all Gonyaulax spp.

† See Figure 3: STX = saxitoxin; GTX, etc. = Gonyautoxins I etc.; neoSTX = neosaxitoxin

Northwest coast of Spain and was shipped to various parts of Europe in large quantities. Due to the insufficient international communication, the identification of the causative organism was not accomplished, but the toxin profile clearly indicated that a *Gonyaulax* species was involved.

In the northern part of Japan, the aquaculture of the scallop, *Patinopecten yezoensis*, is a very profitable enterprise. It started just some ten years ago and quickly spread to many parts of Hokkaido, Aomori Prefecture, and Iwate Prefecture. A tremendous amount of capital was invested for the hanging type culture gears. Usually several baskets are hung by a line attached to a raft or a line spun between buoys. The manipulation of the hanging lines are often done by a crane placed in a boat.

Since a few years ago, the large scale cultures at such places as Ofunato Bay or Funkawan Bay started to be plagued by PSP. The problem is most serious at Funkawan Bay in Hokkaido, where the loss due to the PSP amounted to \$10 million in 1978.

The toxicity level of scallop at Funkawan Bay reached 300 mouse units/1 g hepatopancreas in June, 1978, and did not go down until October (12). This year the matter is worse; they had to halt the shipment completely, which amounts to about 70% of the total Japanese scallop production. Japanese government set a tentative guideline of 400 mouse units per 100 g meat for the legal limit. The U.S. and Canadian governments set the limit, 400 mouse units or 80 µg saxitoxin dihydrochloride equivalent per one hundred gram meat. The World Health Organization recommendation also follows the U.S. guideline. The problem with the Japanese case is rather unique because Japanese people consume the whole scallop rather than only the adductor muscle. In a Canadian study it was reported that the adductor muscle part of sea scallop, *Placopecten magellanicus*, is devoid of toxicity. The toxicity is mostly localized in the hepatopancreas, and partly at the mantle, the foot, etc. The discarded part of *P. magellanicus* at Bay of Fundy indeed showed a very high toxicity, while the adductor muscle part was safely shipped for consumption (13). In this respect, our recent study revealed a very interesting phenomenon.

When the scallop toxin mixture was incubated with a homogenate of the adductor muscle in a buffer solution at pH 5.8, a substantial loss of toxicity was observed. This ability to inactivate the toxins is apparently limited to the adductor muscle. When the toxin mixture was incubated with the remaining parts of the scallop, there was no change of the total toxicity, but there were significant changes in the toxin composition (Figure 1). The changes indicate that there is the biotransformation of toxins in the shellfish body, which is summarized in Figure 2. All the toxins are eventually reduced to saxitoxin which involves the reductive removal of a sulfate group and a hydroxyl moiety. The similar transformation was also confirmed in Alaska butter clam, which may explain the accumulation of saxitoxin in the siphon (Table 2).

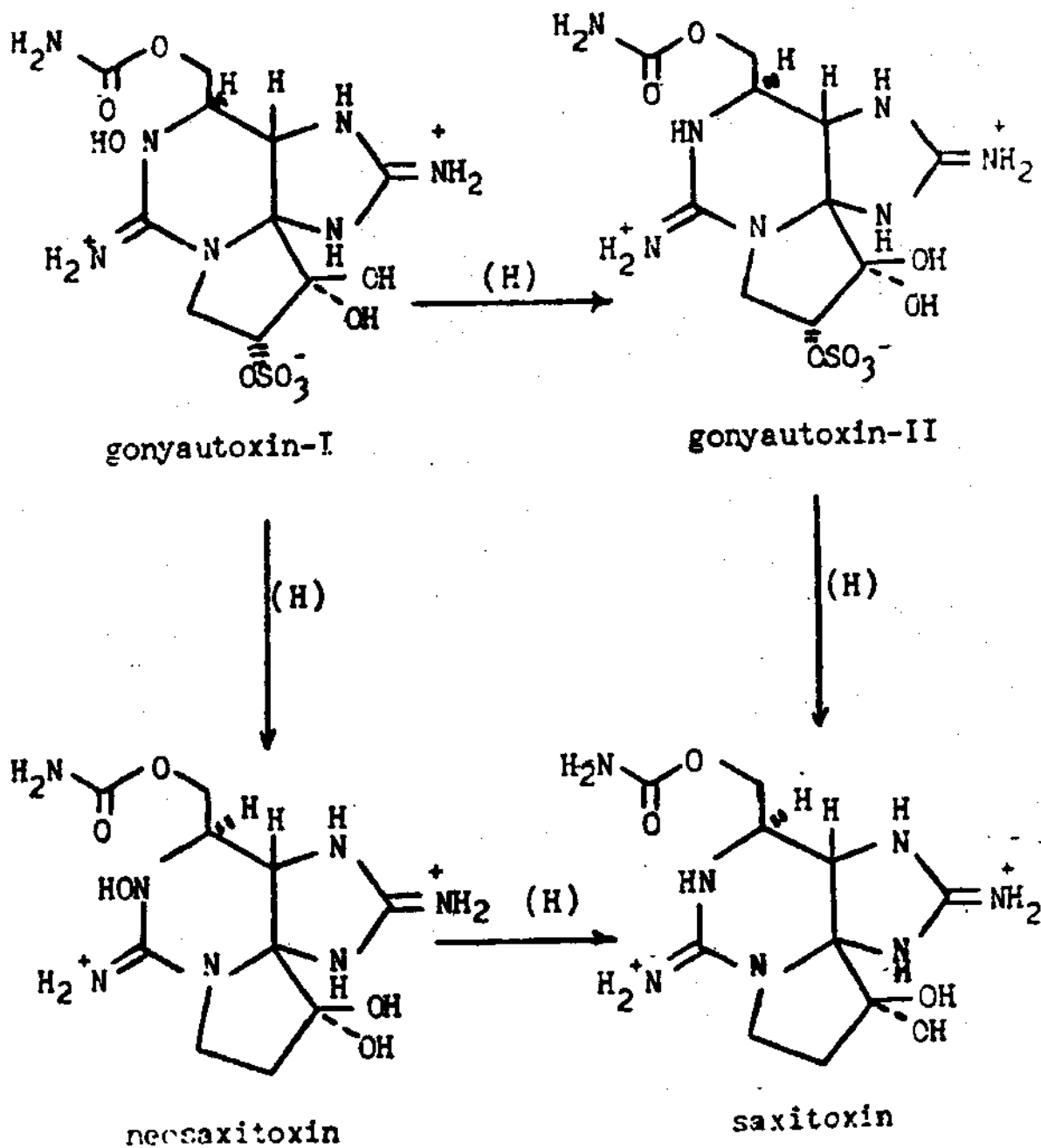


Figure 1. Toxin profiles in the toxin extracts incubated with scallop *Placopecten magellanicus* (a) gill, (b) foot, (c) visceral mass and (d) adductor muscle in acetate buffer (pH 5.9) for 3 days. STX: saxitoxin; GTX: gonyautoxin; neoSTX: neosaxitoxin. Chromatographic pattern on a Bio-Rex 70 column.

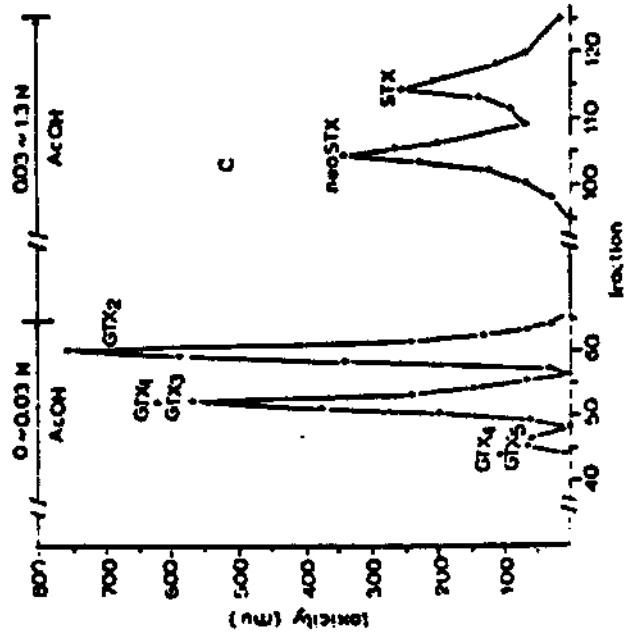
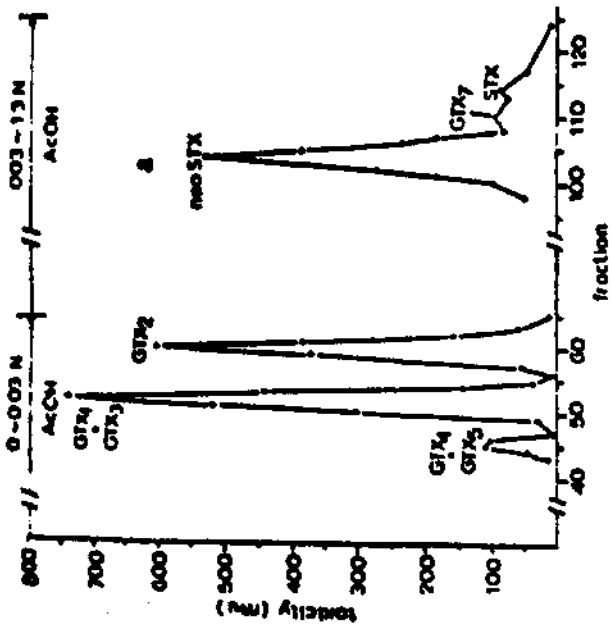
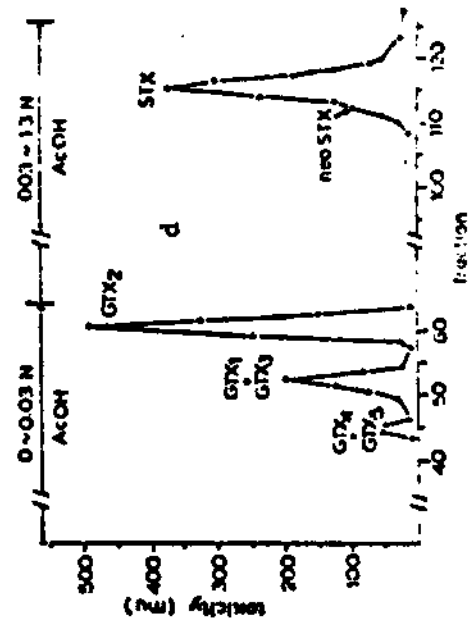
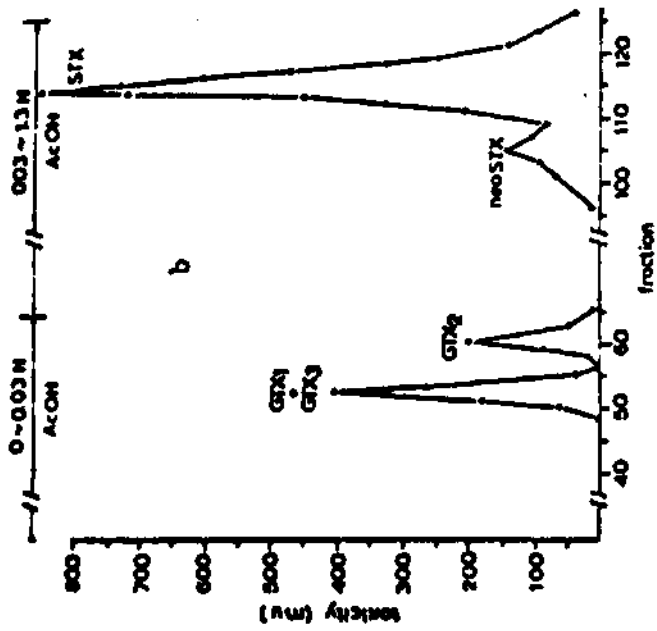


Figure 2. Transformation of paralytic shellfish poisons in the body of the sea scallop *Placopecten magellanicus*.

TABLE 2. Toxin Contents in Toxic Mussels and Butter Clams from Puget Sound, Washington.

Toxins	<u>Mytilus</u> sp. (whole meat%)	<u>Saxidomus giganteus</u> (whole meat %)	<u>S. giganteus</u> (siphon %)
GTX <sub>1</sub>	54.0	31.7	0
GTX <sub>2</sub>	11.3	21.4	0
GTX <sub>3</sub>	3.4	6.4	0
GTX <sub>4+5</sub>	25.2	12.2	0
neoSTX	6.2	6.7	0
STX	0	20.9	>95

The ability of the scallop siphon to detoxify the PSP toxins puts scallops at a uniquely advantageous position in aquaculture. The adductor muscle, which is only commercially valuable part in this country, is free of the toxins regardless of the toxicity of the remaining parts. Other bivalves such as mussels, oysters and clams, whose whole bodies are to be consumed, always face unpredictable risks. One incident of PSP could finish a well-established operation and wipe out the total investment overnight. Predictability of PSP occurrence--if one can predict the potentiality of an area for PSP occurrence or forecast the bloom of toxic dinoflagellates--will tremendously diminish the risk shellfish growers now are facing. In order to discuss this subject, it is necessary to explain the mechanism of the toxin accumulation in shellfish and the life cycle of the causative organisms.

Basically, filter-feeders like bivalves pick up the toxic dinoflagellates and the toxins in the cells accumulate in the hepatopancreas. It was generally assumed that visible blooms (red tides) are necessary to make the shellfish toxic. The accumulated toxins will gradually dissipate if there is no more new intake of the toxins. In reality, however, there are cases shellfish maintain the toxicity in the absence of any visible blooms. The extremely toxic deep-sea scallop in the Bay of Fundy is found in the depth of more than 100 m, where there is no possibility of existence of the photosynthetic motile dinoflagellate. A plausible explanation is that the shellfish collects the toxins from the cysts form of the dinoflagellate which is proved to be highly toxic (14). The cyst is considered to play a key role in the reoccurrence of blooms. Once the motile organisms exhaust nutrients or other conditions become inadequate, they become dormant cysts and stay in the sediments until the condition becomes appropriate for the next bloom. Theoretically, the examination of sediment for such cysts can determine the potentiality of particular area for toxic dinoflagellate blooms. We have recently analyzed three different strains of Gonyaulax spp. hatched from cysts obtained from proximate but different locations in Massachusetts. Although they are morphologically very close, their toxin productivity is quite different (Table 3) (15).

TABLE 3. Toxin Profile in Gonyaulax Organisms of Different Origins.

Organisms	STX	GTX <sub>1</sub>	Purified Toxins					neoSTX	Toxicity
			GTX <sub>2</sub>	GTX <sub>3</sub>	GTX <sub>4</sub>	GTX <sub>5</sub>			
<u>G. tamarensis</u> <sup>1</sup>	++	++	+++	+	+	+++	+++	1 mu/10 <sup>4</sup> cells	
<u>G. tamarensis</u> <sup>2</sup>	+	++	++	++	+	+	+	1 mu/5x10 <sup>4</sup> cells	
<u>G. tamarensis</u> <sup>3</sup>	±	++	++	++	+	+	+	1 mu/10 <sup>5</sup> cells	
<u>G. catenella</u> <sup>4</sup>	+	+++	++	+	+	++		1 mu/4x10 <sup>4</sup> cells	
<u>G. catenella</u> <sup>5</sup>	++	++	++	++	+	+	++	1 mu/10 <sup>5</sup> cells	
<u>G. catenella</u> <sup>6</sup>				(+)				1 mu/10 <sup>6</sup> cells	

<sup>1</sup>Ipswich, MA; <sup>2</sup>Mill Pond, Cape Cod, MA; <sup>3</sup>Perch Pond, Cape Cod, MA;

<sup>4</sup>Oase Bay, Japan; <sup>5</sup>Sequin Bay, WA; <sup>6</sup>LaJolla, CA.

This finding led us to assume that each confined area has a dominant indigenous strain of Gonyaulax organisms whose toxicity varies from non-toxic to strongly toxic. They recycle by the dormant cyst-motile form mechanism. In a closed area, the bloom will be confined, and the toxicity of the shellfish there will be determined by the toxicity of the native strain and its population. In areas whose indigenous Gonyaulax spp. are nontoxic, shellfish remain nontoxic despite high counts of organisms in the water. It is advisable to avoid locations where toxic Gonyaulax cysts are found in the sediment for shellfish aquaculture.

Constant monitoring of toxicity should be a required practice in shellfish aquaculture. The U.S. legal limit of 80 µg saxitoxin equivalent/100 g shellfish meat was set after a number of safety considerations. One of them is that once the toxicity reaches that level, it increases very rapidly. Another consideration is that even in close proximity, the toxicity varies tremendously.

The toxicity levels vary also according to depth. Despite the generally accepted concept that the organisms in bloom (red tide) exist near the surface of water, it was pointed out that the largest population was found at a certain temperature layer or at the interface of different water layers. Nishihama (16), working at Uchiura Bay in Hokkaido, found that the toxic Gonyaulax population slowly migrates from surface to depth as the water layer interface moves deeper. Accordingly, scallops in the upper (-10M) baskets reached the maximum toxicity in late May, but those in the lower (-25M) baskets on the same line reached the maximum toxicity in the middle of July. Since most shellfish aquacultures are done by hanging methods, this remarkable vertical variation in toxicity is very significant. Enough precaution should be taken in sampling toxicity test specimens.

Toxicity variation among different kinds of shellfish--not all shellfish accumulate the PSP toxins in the same manner even if they are placed at a same location and feed on an identical plankton mixture. It has been said for a long time that oysters have always lower levels of toxicity compared to other shellfish. This makes oysters a rather advantageous aquaculture product as far as PSP is concerned. The reason is not clear, but probably oysters have the ability to degrade the toxins. As mentioned earlier, sea scallops (probably other scallops too) have ability to transform some of the toxins. Especially the adductor muscle, with its enzyme system to detoxify PSP, remains nontoxic unless the muscles are contaminated in the shucking process. Mussels, Mytilus sp., are used as the test specimen in the PSP monitoring program. They are very fast in accumulating toxins. On the other hand, they also depurate very rapidly. So it is quite possible that even after the toxicity in mussels go below the danger level, still other shellfish in the area maintain a high level of toxicity. As a rule, if the causative organism disappears, the toxicity in mussels will dissipate in a few weeks. Alaska butter clam, however, converts the toxins to saxitoxin and accumulate in the siphon tissues where the toxin stays for a length of time. For that reason, Alaska butter clam may not be suitable for aquaculture in areas where PSP danger is eminent. It is still under investigation if this is true with other types of clams.

#### CONCLUSION

With increasing numbers of people who want to venture in shellfish aquaculture, the paralytic shellfish poisoning problems should be given the most serious consideration. In a culture of high density, one small scale PSP incident will suffice to wipe out the total investment. It is highly desirable to investigate the potentiality of a designated area for the presence or absence of toxic dinoflagellate cysts before any decision is made. It is also highly desirable to choose an area where no previous incident of PSP was observed. Cysts can be easily transported with shellfish, therefore, the seed should be brought in only from safe areas. Once the organisms are brought in, they very likely stay there permanently by the cyst-motile life cycle. Constant toxicity monitoring is important after the shellfish start to go into the market. Since it is not easy to run the mouse assay individually, the establishment of one assay center in the country will be a good solution.

Selection of appropriate shellfish will also bring more security to the venture. The scallop aquaculture, for example, is free of PSP threat as long as only the adductor muscle is the commercial product.

There is no means to prevent selectively the growth of the toxic dinoflagellates. Actually dinoflagellates along with diatoms are important food of shellfish. Complete elimination of such organisms from the water also means the starvation of shellfish. Despite the general belief, a visible bloom is not a requirement to have toxic shellfish; several hundred cells per liter are enough to make shellfish toxic.

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