Pure & Appl. Chem., Vol. 61, No. 1, pp. 7–18, 1989.
Printed in Great Britain.
© 1989 IUPAC

Recent studies on paralytic shellfish poison in Japan

Kanehisa Hashimoto and Tamao Noguchi

Laboratory of Marine Biochemistry, Faculty of Agriculture, University of Tokyo, Tokyo 113, Japan

Abstract - In Japan, the occurrence of Protogonyaulax catenella red tide, along with paralytic shellfish poison (PSP)-infestation of bivalves, was for the first time confirmed in 1975, at Owase Bay, Mie Prefecture. Since then, extensive studies have been carried out on various aspects of PSP such as: search for and identification of responsible plankton species, surveillance for PSP-infested areas and bivalves, isolation and characterization of PSP components, development of chemical assay methods, ichthyotoxicity of PSPs, and search for other PSP-bearing organisms. An attempt is made to overview the PSP studies in Japan, focusing on recent findings.

INTRODUCTION

Paralytic shellfish poison (PSP) is one of the most notorious marine toxins known. PSP, once ingested by humans, evokes paralysis and other symptoms, with frequent death (ref. 1). It is estimated that the number of victims of paralytic shellfish (PS) poisoning worldwide between 1972–1983 exceeded 900, including 40 deaths.

Sommer et al. (ref. 2) elucidated the course of events leading to PS poisoning: Protogonyaulax (formerly Gonyaulax) catenella produces PSP, and grows up to a high density under favorable environmental conditions; the organism then infests bivalves which in turn may poison humans when ingested. Later, P. tamarensis, Pyrodinium bahamense, Pyr. bahamense var. compressa, Aphanizomenon flos-aquae, etc., were added to the list of PSP-producers (TABLE 1). Schantz et al. (ref. 3) isolated the first PSP toxin from the Alaska butter clam Saxidomus giganteus and it was designated saxitoxin (STX) later. Subsequent studies showed that PSP is not composed of STX alone, but of more than ten components whose structures are closely related to each other. Some components, such as STX and gonyautoxin (GTX)-2, have specific toxicities comparable to that of tetrodotoxin (TTX). The minimum

TABLE 1. PSP-producing plankton

Species	Area	PSP components
Protogonyaulax catenella	Pacific coasts - California, British Columbia, Alaska, Japan, Venezuela, Chile	GTXs, STXs
P. tamarensis	North Atlantic coasts - New England, Canada; North Sea coasts - England, Denmark, W. Germany, Holland, Norway; Japan	GTXs, STXs
P. acatenella	British Columbia	unknown
P. phoneus	North Sea	unknown
P. cohorticula	Gulf of Thai	GTXs
Pyrodinium bahamense	Brunei, Papua New Guinea	unknown
Pyr. bahamense var. compressa	Palau Is.	STXs, GTXs
Gymnodinium catenatum	Tasman Sea, Japan	PXs, GTXs
Cochlodinium sp.	Japan	Zn-bound PXs
Aphanizomenon flos-aquae	New England (lakes)	STXs

GTXs = gonyautoxins; STXs = saxitoxins; PXs = protogonyautoxins.

lethal dose (MLD) of PSP in humans is estimated to be 3,000 MU* (ref. 4) based mainly on fatal cases induced by this toxin. PSP is roughly three times more toxic than TTX whose MLD in humans is considered to be 10,000 MU**.

In Japan, there occurred three suspected PS poisoning incidents during 1948-1962. In January 1975, a P. catenella red tide was observed for the first time in Japan at Owase Bay, Mie Prefecture. On this occasion, PSP was detected both in the responsible plankton and in some infested bivalves inhabiting the same bay (ref. 4). Since that time, PSP, along with the responsible plankton, has been extensively studied in Japan. Recent studies on this marine toxin in Japan are overviewed here, focusing on several aspects.

PARALYTIC SHELLFISH POISONING, ALONG WITH THE RESPONSIBLE PLANKTON

As TABLE 2 shows, a total of seven PS poisoning incidents have so far been recorded in Japan, including three suspected ones which occurred before 1975 when PSP was first identified in the Owase Bay incident. In January 1979, 16 patients exhibited the symptoms specific to PS poisoning, after eating oysters collected from Senzaki Bay, Yamaguchi Prefecture. Onoue et al. (ref. 5) demonstrated that PSP was responsible. In April of the same year, cultured scallops became highly toxic with PSP in Funka Bay, Hokkaido, and hence were prohibited from being marketed. At that time, several people gathered and ate the mussels attached to the scallop-culturing rafts, and three were poisoned, with one death resulting. In May 1982, two inhabitants in Ofunato, Iwate Prefecture, were lightly poisoned due to ingestion of the protochordate, ascidian Holocynthia roretzi which is also a plankton feeder. The responsible toxin was confirmed to be PSP (ref. 6). In the early summer of 1987, several persons were poisoned by ingesting short-necked clams collected from Yamakawa Bay, Kagoshima Prefecture. The responsible toxin was demonstrated to be PSP (ref. 7).

TABLE 2. Incidents of paralytic shellfish poisoning in Japan

Date	Causative shellfish	Location of collection	Number of patients (Deaths)	PSP compo-nents
July 1948	Short-necked clam, Tapes japonica	Toyohashi, Aichi Pref.	12 (1)	?
May 1961	Scallop, Chlamys nipponensis akazara	Ofunato Bay, Iwate Pref.	20 (1)	?
Feb. 1962	Oyster, Crassostrea gigas	Miyazu Bay, Kyoto	42 (0)	?
Jan. 1979	Oyster, C. gigas	Senzaki Bay, Yamaguchi Pref.	16 (0)	PXs, GTXs, STXs
Apr. 1979	Mussel, Mytilus edulis	Funka Bay, Hokkaido	3 (1)	-
May 1982	Ascidian, Holocynthia roretzi	Ofunato Bay, Iwate Pref.	2 (0)	GTXs, STXs
June 1987	Short-necked clam, T. japonica	Yamakawa Bay, Kagoshima Pref.	2 (0)	GTXs

GTXs = gonyautoxins; STXs = saxitoxins; PXs = protogonyautoxins.

In Japan, a total of about 100 patients, including three deaths, due to ingesting PSP-infested or suspected shellfishes have so far been reported. In addition, more than 29 patients, including 15 deaths, due to ingestion of PSP-bearing crabs have been reported, as

*,**One mouse unit (MU) of PSP and TTX is defined as the amount of each toxin which can kill a 20 g ddY strain male mouse in 15 and 30 min, respectively, after intraperitoneal administration.

described below. According to the recent food-hygienic statistics, 30,000-50,000 patients, including 10-20 deaths, due to food poisoning are recorded each year in Japan. Even though the proportion of PS poisoning in all food poisoning incidents is not very large, even after the crab poisoning incidents are added to the PS poisoning, the economic damage which fishermen and other workers of associated industries suffer from PSP toxification of bivalves is very serious.

In Japan, P. catenella was first found as a PSP-producer in Owase Bay. Subsequent surveys showed that this plankton species is widely distributed from the northernmost (Hokkaido) to southernmost (Kyushu) part of Japan, and often forms a red tide, infesting bivalves inhabiting those regions. P. tamarensis was first detected as a responsible plankton when bivalves were toxified in Ofunato Bay, Iwate Prefecture, and was later detected in other areas such as Seto Inland Sea. Unlike P. catenella, this species never forms a red tide, but often toxifies scallop and other bivalves to an extremely high level (ref. 8). Gymnodinium catenatum, which was recently recognized as a new PSP-producer in Australia (ref. 9), was also found and confirmed as a PSP-producing plankton when oysters became highly toxic in Senzaki Bay, Yamaguchi Prefecture during December 1986-January 1987 (ref. 10 & 11). A fourth plankton species, Cochlodinium sp., has very recently been found to occur in Yatsushiro Sea (ref. 12). This species may be distributed in other areas of Japan and also in other countries.

Subsequent to the Owase Bay incident, scallops became highly toxic in 1978 at Funka Bay, Hokkaido, where there had been no previous records of PSP toxification of bivalves. This led to the establishment of a nationwide monitoring system for PSP toxification of bivalves. Since then, much more information on PSP-infested bivalves and associated phenomena became available. From Hokkaido to Kyushu, bivalves are toxified, depending upon locality, season, and year (Fig. 1). Red tide formation is not always a prerequisite for the toxification of bivalves. A P. catenella red tide occurred in Kitaura Bay, Miyazaki Prefecture in 1982. At that time, the red tide not only infested bivalves, but also caused a mass mortality of cultured yellowtail Seriola quinqueradiata in the same bay. Toxification of bivalves has also been reported from East Hokkaido, the coasts of Ibaraki Prefecture, Tanabe Bay (Wakayama Prefecture), Tsushima Islands (Nagasaki Prefecture), and Yamakawa Bay (Kagoshima Prefecture).

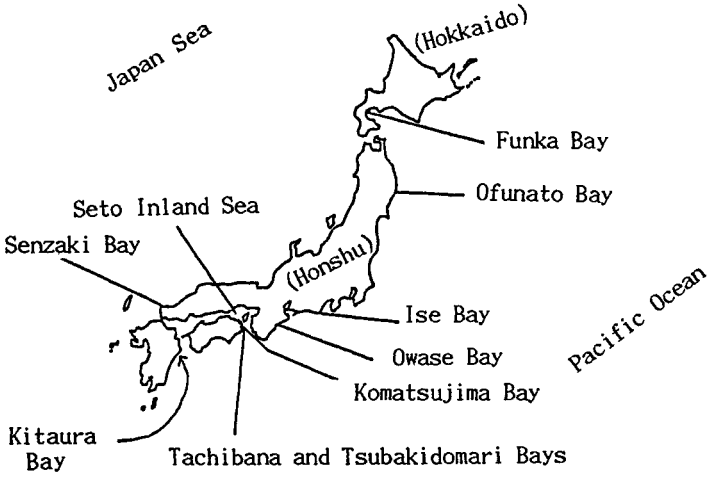


Fig. 1. Map showing main PSP-infested areas of Japan.

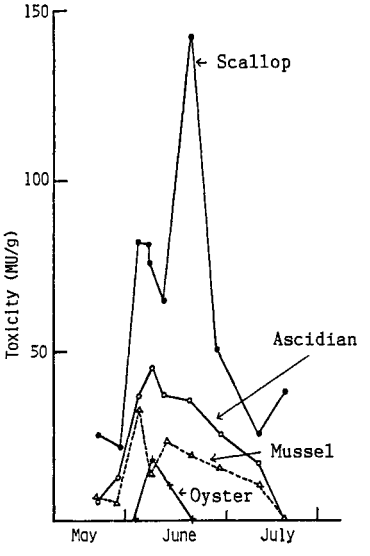


Fig. 2. Seasonal variations in PSP toxicity of some species of organisms inhabiting Ofunato Bay (ref. 13).

Various species of bivalves are toxified: scallop Patinopecten yessoensis, oyster Crassostrea gigas, mussel Mytilus edulis, "akazara" scallop Chlamys nipponensis akazara, "hiogi" scallop C. nobilis, short-necked clam Tapes japonica, etc. In addition, the protochordate, ascidian Holocynthia roretzi is sometimes toxified. Figure 2 shows the seasonal variations in PSP toxicity of several bivalves which were placed in essentially the same environments, indicating widely different patterns of toxification (ref. 13). The scallop became toxic most quickly and remained fairly toxic for a long period, while the oyster was toxified most slowly, reflecting their differences in accumulation and metabolism of PSP.

TABLE 3 shows the anatomical distribution of PSP toxicity in scallops collected from Ofunato Bay. Scallop specimens were excised into tissues immediately after collection, and kept frozen until assay (ref. 8). The adductor muscle was nontoxic to weakly toxic (3-13 MU/g) even in the specimens whose digestive glands were extremely toxic (6,500-11,000 MU/g). The adductor muscle was not toxic at all in the specimens whose digestive glands showed toxicity scores of about 1,000 MU/g. In this connection, a freezing/thawing-associated transfer of PSP from highly to lowly toxic tissues was noticed.

TABLE 3. Toxicity of the adductor muscle, digestive gland, and other parts in PSP-infested fresh unfrozen scallop (ref. 8)

(MU/g)

Date of collection	Adductor muscle*1 (A)	(B)	(C)	Digestive gland	Combined other parts
May 29, 1982	3-13	-	-	6,500-11,000	111-237
July 6, 1982	-	-	N.D.*2	1,400- 2,900	43-132
July 20, 1982	-	N.D.	N.D.	1,100- 1,700	33- 55
Sep. 10, 1982	N.D.	N.D.	N.D.	860	43
Sep. 24, 1982	N.D.	N.D.	N.D.	970	-
Oct. 8, 1982	N.D.	-	N.D.	1,000	-
Oct. 23, 1982	N.D.	N.D.	N.D.	640	-
Nov. 13, 1982	N.D.	-	-	520	-

*1(A): Non-treated.
(B): Visceral impurities wiped off with tissue paper.
(C): Washed with sea water lightly.
*2Less than 2 MU/g.

In the case of the ascidian as well, the hepatopancreas was much more toxic than other tissues (ref. 6). In the ascidian-responsible poisoning incident mentioned previously, the victims ingested the whole edible part including the hepatopancreas.

Government quarantine limits to protect against PSP toxification by bivalves have been set in Japan. In USA and Canada, the quarantine limit of PSP in bivalves for home consumption has been set at 80 μg (as STX)/100g edible part. In Japan, on the other hand, the quarantine limit is set at 4 MU/g edible part, a value which is essentially the same as that in USA and Canada. The quarantine limit for PSP in bivalves for canning is set at 500 MU/g digestive gland (almost equivalent to 100 MU/g edible part), based on the results of model experiments (ref. 14 & 15). Other quarantine limits are set for bivalves to be processed in other ways. Since these quarantine limits were set, almost no PS poisoning has occurred as far as bivalves or their products marketed through authorized routes are concerned.

CHEMICAL ASPECTS OF PARALYTIC SHELLFISH POISONING

Schantz et al. (ref. 3) isolated the first toxin STX from the PSP-infested bivalve *Saxidomus giganteus*. They subsequently isolated this toxin from the responsible plankton *P. catenella* (ref. 16), and elucidated its structure in 1975 (ref. 17). In Japan, Kawashiro et al. (ref. 18) detected a toxin other than STX in toxic akazara scallop *Chlamys nipponensis akazara* collected from Ofunato Bay, and designated it "chlamytoxin", but no further studies were made. Evans (ref. 19) also found unknown toxins in toxic mussel. In 1975, Shimizu et al. (ref. 20 & 21) found STX and several unknown toxins in a toxic bivalve *Mya arenaria* collected from New England and also in the responsible plankton *P. tamarensis*. When subjected to column chromatography on Bio-Gel P-2 and Bio-Rex 70 (H^+), each source gave rise to several toxins, such as gonyautoxins 1-8 (GTX_{1-8}) and neoSTX, in addition to STX.

Oshima et al. (ref. 22) collected *P. tamarensis* strains and scallops from several areas and in various years, and analyzed for PSP composition (Fig. 3). *P. tamarensis* to some extent differed in toxin composition depending upon the area and year of collection. The scallop specimens showed a toxin composition clearly different from those of the responsible plankton, suggesting an *in vivo* conversion between PSP components. Ueda et al. (ref. 23) also described that the toxin composition of Ofunato scallops widely differed depending upon the year of collection. Both research groups demonstrated that the Ofunato scallops contained a fairly large amount of unidentified toxins, in addition to known toxins.

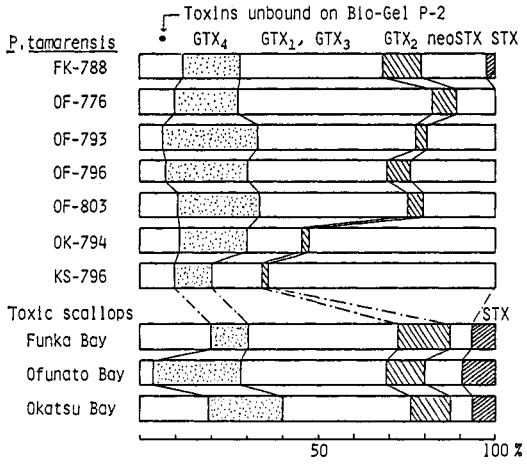
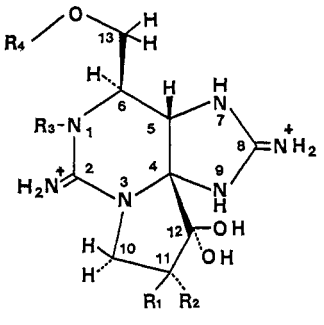


Fig. 3. Toxin composition of Protogonyaulax tamarensis isolates and toxic scallops of northern Japan (ref. 22).

Hall et al. (ref. 24) isolated four unknown PSP components from Protogonyaulax sp., and designated them B_1, B_2, C_1 and C_2. These toxins were easily converted into known toxins under acidic conditions. Noguchi et al. (ref. 25 & 26) also isolated or detected four low-toxicity components from both the toxic Senzaki Bay oyster and the responsible plankton, and named them protogonyautoxins 1-4 (PX_{1-4}). It was found later that Hall's C_1 and C_2 corresponded to PX_1 and PX_2, respectively, and that C_2 (or PX_2) corresponded to GTX_8 and C_1 (or PX_1) to the epimer of GTX_8, as described below. The toxic ascidian responsible for the poisoning incident in Ofunato showed a PSP composition featuring large amounts of the low-toxicity components such as PX_1 and PX_2 (ref. 6).

The structure of GTX_5 was elucidated by Nishio et al. (ref. 27). This toxin is of low toxicity, with a specific toxicity of 280 MU/mg. It gave rise to STX when heated in dilute HCl, increasing the toxicity sharply. The toxin contained one mol SO_3^-/mol, and showed an NMR spectrum which was indistinguishable from that of STX except for a chemical shift of H (13). Based on these and other data, they concluded the structure of GTX_5 to be carbamoyl-N-sulfo-STX (Fig. 4). Harada et al. (ref. 28) isolated GTX_5 and GTX_6 from Pyr. bahamense var. compressa, and elucidated their structures to be carbamoyl-N-sulfo-STX and -neoSTX, respectively, by essentially the same techniques. The specific toxicity of GTX_6 was 290 MU/mg, but is sharply increased when it was converted into neoSTX on acid hydrolysis. Almost simultaneously, Koehn et al. (ref. 29) isolated both components from a Protogonyaulax plankton and clarified their structures. GTX_5 and GTX_6 corresponded to Hall's B_1 and B_2, respectively.

Kobayashi and Shimizu (ref. 30) isolated GTX_8 from a culture of P. tamarensis (Ipswich strain) and analyzed the structure by means of ^{1}H-NMR and ^{13}C-NMR spectrometry. They



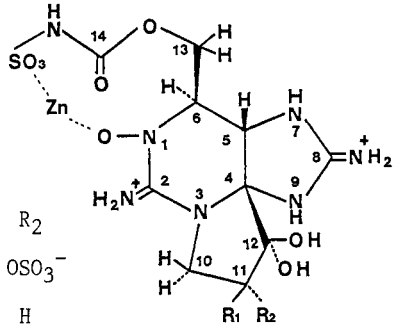
	R_1	R_2	R_3	R_4	Sp. toxicity (MU/mg)
STX	H	H	H	$CONH_2$	5,500
neoSTX	H	H	OH	$CONH_2$	3,900
GTX_1	H	OSO_3^-	OH	$CONH_2$	5,000
GTX_2	H	OSO_3^-	H	$CONH_2$	4,200
GTX_3	OSO_3^-	H	H	$CONH_2$	5,600
GTX_4	OSO_3^-	H	OH	$CONH_2$	1,600
GTX_5 (B_1)	H	H	H	$CONHSO_3^-$	280
GTX_6 (B_2)	H	H	OH	$CONHSO_3^-$	290
PX_1 (C_1, epi-GTX_8)	H	OSO_3^-	H	$CONHSO_3^-$	30 ~ 40
PX_2 (C_2, GTX_8)	OSO_3^-	H	H	$CONHSO_3^-$	300 ~ 600
PX_3 (C_3)	H	OSO_3^-	OH	$CONHSO_3^-$	--
PX_4 (C_4)	OSO_3^-	H	OH	$CONHSO_3^-$	--
decarbamoyl STX	H	H	H	H	4,200

Fig. 4. Structures and specific toxicities of PSPs.

proposed the structure of carbamoyl-N-sulfo-11β-hydroxy-STX sulfate. In the same year, Wichmann et al. (ref. 31) isolated and crystallized two low-toxicity components (C_1 and C_2) and demonstrated the identity of C_2 and C_1 with GTX_8 and its 11-α epimer, respectively, mainly by X-ray diffraction and NMR analyses (Fig. 4). Onoue et al. (ref. 32) isolated and identified the two new toxins designated PX_1 and PX_2 from the Senzaki oyster and the responsible plankton P. catenella. Based on elemental analysis, ^1H- and ^{13}C-NMR spectrometry, they concluded that PX_1 and PX_2 corresponded to Hall's C_1 and C_2, respectively. Specific toxicities of PX_1 and PX_2 were 30-40 MU/mg and 300-600 MU/mg, respectively, but were sharply increased when treated with dilute HCl, since these components were converted into GTX_2 and GTX_3, respectively.

Regarding PX_3 and PX_4 (or Hall's C_3 and C_4), Noguchi et al. (ref. 26) detected two unknown spots in an electropherogram of a Senzaki oyster extract, and assumed a priori their presence. On acid hydrolysis, the two suspected spots disappeared and instead gave rise to GTX_4 and GTX_1, respectively. From these results, they proposed carbamoyl-N-sulfo-11α-hydroxy-neoSTX sulfate and its 11β-epimer, as the structures of PX_3 and PX_4, respectively (Fig. 4). Subsequently, Hall et al. (ref. 33) succeeded in the isolation of both these components. Harada et al. (ref. 34) isolated an unknown PSP component from Pyr. bahamense var. compressa and infested bivalves, and identified it as decarbamoyl STX (Fig. 4). Nagashima et al. (ref. 35) found that this PSP component accounted for most of the toxicity of the toxic digestive gland of "hiogi" scallop Chlamys nobilis. This toxin was demonstrated to be derived from the PXs enzymatically (ref. 36).

Shimizu et al. (ref. 20 & 37) have isolated and elucidated the structures of other PSP components. Figure 4 collectively shows the structures of all these PSP components, along with their specific toxicities. They reported the specific toxicity of neoSTX to be 2,000 MU/mg. Daigo et al. (ref. 38) isolated this toxin from a toxic crab and determined the toxicity to be 3,900 MU/mg, as cited here. Very recently, Onoue and Nozawa (ref. 39) isolated three unknown low-toxicity PSP components from a toxic dinoflagellate Cochlodinium sp. They examined the structures of the two major components, concluding one to be a Zn complex of PX_3 and the other to be that of PX_4 (Fig. 5).



	R_1	R_2
I_b	H	OSO_3^-
I_c	OSO_3^-	H

Fig. 5. Proposed structures of toxins I_b and I_c of Cochlodinium sp. (ref. 39).

ASSAY METHODS FOR PARALYTIC SHELLFISH POISON

In Japan, PSP is assayed by the official method using mice. It requires ddY strain male mice weighing 18-20 g, but not any special instrumentation. This method is simple and convenient but not so accurate, and can not give any information on toxin composition, nor distinguish PSP from other neurotoxins such as TTX. In addition, animal rights activists all over the world are strongly opposed to bioassay using even mice. TLC and electrophoresis are of course useful means for PSP detection, but not suitable for PSP determination.

With this background, attempts have been made to develop analytical methods using HPLC in Japan as well as some other countries. Onoue et al. (ref. 40) proposed an HPLC method in which PSP components were separated by ion exchange column chromatography, and were spectrofluorometrically detected by means of o-phthalaldehyde (OPA). The lower limit of detection was 0.1-1 nM depending upon the PSP component. This method can assay for PSP and TTX simultaneously, but has some disadvantages: e.g., OPA reacts with α-amino groups of contaminating substances such as free amino acids, making it sometimes difficult to detect PSP peaks in the elution pattern. Oshima et al. (ref. 41) developed another HPLC method for PSP analysis in which t-butylhydroperoxide was used as the fluorogenic agent. Recently, Nagashima et al. (ref. 42 & 43) have developed an ion-pairing reverse phase HPLC method in which periodate and other compounds are used as the fluorogenic agents (Fig. 6). By this method, all components can be determined satisfactorily (Fig. 7).

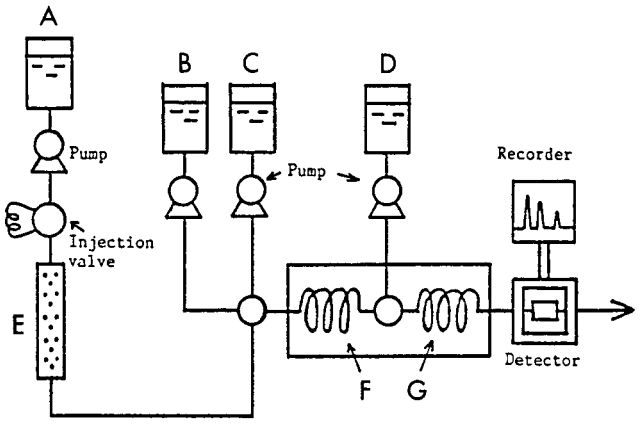


Fig. 6. Schematic diagram of HPLC system for PSP analysis (ref. 42 & 43).
A, mobile phase: 0.05 M phosphate buffer (pH 7.0) containing 2 mM heptanesulfonic acid and methanol (99:1) for GTXs and (75:25) for STXs.
B-D, reagents for detection: B, 0.05 M periodic acid; C, 0.2 N KOH plus 1 M ammonium formate in 50% formamide; D, 1% chloroacetaldehyde in 1 M citrate buffer (pH 4.0).
E, silica ODS column (300 x 6 mm).
F and **G**, reaction coils: 7 m x 3 mm and 10 m x 3 mm, respectively.

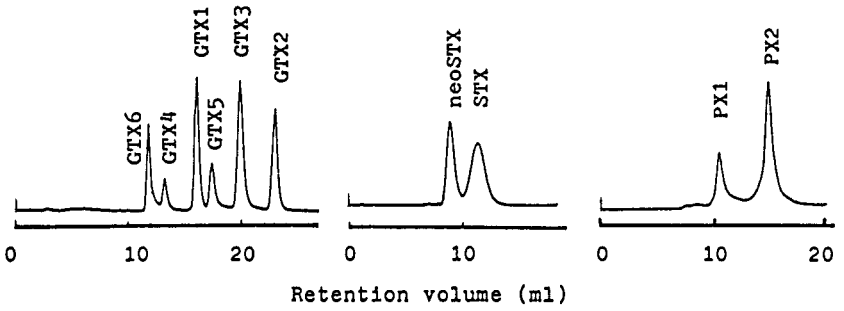


Fig. 7. HPLC of standard mixtures of GTXs (left), STXs (center) and PXs (right)(ref. 43).

ICHTHYOTOXICITY

PS poisoning in humans, resulting in frequent deaths, has a long history. Sea birds are also known to be casualties from ingesting toxic bivalves. White (ref. 44-46) claimed that zooplankton-vectored PSP also intoxicated adult herring in Fundy Bay, Canada. He also observed that most of the herring specimens were killed when PSP was injected into the stomach at a level of 100-200 MU/20g body weight.

In Japan, many cultured yellowtails were killed in Kitaura Bay, Miyazaki Prefecture, in 1982 when a P. catenella red tide occurred. Reports of other fish mortality incidents associated with PSP have been rare. One of the reasons why such kinds of fish mortality reports have been so rare in Japan, may, at least partly, be explained by the possibility that fish killed in the larval stage have been overlooked. As another reason, it may be pointed out that fish generally are highly resistible to PSP administered orally. TABLE 4 shows MLD values for PSP in marine and freshwater fishes (ref. 47). When given PSP intraperitoneally, most fishes showed MLD values below about 10 MU/20 g body weight, with somewhat higher values for pufferfish and goby. In contrast, all the fishes tested showed a much high resistibility to PSP when administered orally. All of the fish species tested showed MLD values over 100 MU/20 g, with the highest value of >1,700 MU/20 g for tigerfish. The mechanism involved in such a high oral PSP resistibility remains to be elucidated.

TABLE 4. Minimum lethal doses (LD_{99}) of PSP in marine and freshwater fishes administered intraperitoneally and orally (ref. 47)

Fish		LD_{99}(MU/20 g body weight)	
		On i.p. administ.	On oral administ.
Marine fishes:			
Anchovy	Engraulis japonica	2-4	-
Horse mackerel	Trachurus japonicus	1.5-2	180-200
Mackerel	Pneumatophorus japonicus	1.5-2	125-190
Girella	Girella punctata	1-2	-
Japanese parrotfish	Oplegnathus fasciatus	1	-
Mullet	Mugil cephalus	1-2	-
Tigerfish	Therapon jarbua	1-2	>1,700
Filefish	Stephanolepis cirrhifer	2-2.5	640-700
Flatfish	Limanda herzensteini	-	540-600
Goby	Acanthogobius flavimanus	9-17	-
Puffer	Fugu niphobles	14-29	-
Freshwater fishes:			
Chum salmon	Oncorhynchus keta	<4	-
Silver salmon	O. kisutch	7-12	-
Carp	Cyprinus carpio	2-5	120
Rainbow trout	Salmo gairdneri	1-8	320-400
Tilapia	Tilapia nilotica	2-5	>400

PARALYTIC SHELLFISH POISON-BEARING CRABS

PSP has also been detected in several other organisms listed in TABLE 5, in addition to the above-mentioned plankton and infested bivalves. Among these, xanthid crabs inhabiting the tropical islands of the Pacific, possess PSP at a high level (ref. 4). Pufferfish of course contain TTX as the major toxin, but also contain PSP as trace components (ref. 48). The horseshoe crab (ref. 49) and a calcareous red alga Jania sp. (ref. 50) also contain low levels of PSP.

In the Southwestern Islands of Japan, at least 29 people were poisoned and at least 15 died due to ingestion of xanthid crabs during 1909-1988. Figure 8 shows a leaflet which is distributed in these areas to alert visitors. Noguchi et al. (ref. 51) isolated a toxin from the toxic crab, and identified it as STX. The toxicity levels of xanthid crab species are generally high, with the highest score of 16,500 MU/g being reported. Since the MLD of PSP in humans is estimated to be 3,000 MU, only one gram of such a toxic crab specimen could kill at least five persons.

TABLE 5. Additional PSP-bearing organisms reported to date

Species	Area	PSPs contained as:
Xanthid crab species: Zosimus aeneus, Atergatis floridus, Platypodia granulosa	Ryukyu & Amami Is.	Major components
A. floridus	Pac. coasts of Honshu	Minor components
Pufferfish	-----	Minor components
Horseshoe crab Carcinoscorpius rotundicauda	Thailand	
Calcareous alga Jania sp.	Ryukyu Is.	

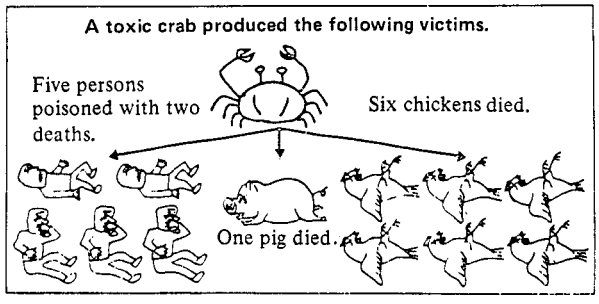


Fig. 8. Illustration of a case of crab poisoning.

Koyama et al. (ref. 52 & 53) re-examined the toxin composition of two toxic species, Zosimus aeneus and Atergatis floridus, and for the first time detected neoSTX, along with small amounts of GTXs. Toxicity ratios of STX, neoSTX and GTXs were 55-65:30-40:5 for Z. aeneus, and 55:45:trace for A. floridus. In another xanthid crab, Platypodia granulosa, neoSTX was barely detected, but instead an unidentified toxin was found which accounted for about 30% the total toxicity. Yasumoto et al. (ref. 54) obtained similar results with xanthid crab species. Oshima et al. (ref. 55) identified decarbamoyl STX in Z. aeneus collected from the tropics. The origin of PSP in these crabs remains to be solved, since known PSP-producing plankton species have not been detected in areas inhabited by toxic crabs.

As to the physiological function of PSP in crabs, Noguchi et al. (ref. 56) found that Z. aeneus and A. floridus released PSP from the exoskeleton on light wiping with gauze ("handling stimulus"). TABLE 6 shows an example of such data. Some specimens of Z. aeneus released up to 800 MU of toxin on a single handling stimulus, whereas nontoxic crab species released none. When given handling stimuli once a day for several weeks, Z. aeneus specimens released a decreasing amount of toxin day by day, but showed increased toxin release again when rested for a week.

TABLE 6. Details of the crab specimens used, along with the average amount of toxin released per "handling stimulus" (ref. 56)

Species	Place of catch	No. of specimens tested (Period of test, days*1)	No. of specimens which released toxin	Average amount of toxin released per stimulus (MU)			
				5	5-10	11-100	101-
Zosimus aeneus	Cebu Is., Philippines	5 (11)	5	4	0	1	0
		12 (56)	12	2	2	7	1
		10 (35)	10	0	0	5	5
	Ishigaki Is., Okinawa Pref.	7 (11)	6	2	1	3	0
	Kuroshima Is., Okinawa Pref.	6 (11)	5	2	2	1	0
Atergatis floridus	Ishigaki Is., Okinawa Pref.	6 (11)	6	0	2	4	0
	Miura Pen., Kanagawa Pref.	5 (11)	4	3	0	1	0
Platypodia granulosa	Ishigaki Is., Okinawa Pref.	1 (11)	1	0	1	0	0
Leptodius exaratus	Miura Pen., Kanagawa Pref.	2 (23)	0	0	0	0	0
Gaillardiellus orientalis	Miura Pen., Kanagawa Pref.	2 (23)	0	0	0	0	0
Pachygrapsus crassipes	Miura Pen., Kanagawa Pref.	2 (23)	0	0	0	0	0

*1 "Handling stimuli" were given once every two days for a total of 11 days, or after one-week rest, were given again in the same manner for a longer period up to 56 days.

Fish generally are sensitive to neurotoxins such as PSP and TTX. This, along with the above observations, suggests that PSP acts as a defense agent in toxic crab species. In this connection, toxic crab species show a much higher resistibility to PSP than do nontoxic crab species (TABLE 7). When a toxin solution was injected through a joint of the leg, specimens of nontoxic crab species were instantly killed at 1-10 MU/20 g body weight, whereas those of the toxic species survived even at as high a dosage as up to 5,000 MU/20 g (ref. 57). This suggested that toxic crab species are endowed with a unique physiological or neuro-physiological system, making it possible for them to accumulate large amounts of PSP. The mechanism involved, however, remains to be elucidated.

TABLE 7. Resistibility of a toxic crab (A. floridus) and five nontoxic crabs against PSP (ref. 57)

Species	Sex*1	Place of catch	Body Weight (g)	Dose (MU/20 g)	Time required until death (min)*2
Atergatis floridus	M	Arazaki, Kanagawa Pref.	3	10,000 (crab PSP)	60
	M	Kominato, Chiba Pref.	9	10,000 (")	60
	M	"	6	10,000 (")	inst. death
	M	Arazaki,	7	10,000 (scallop PSP)	25
	F	"	14	10,000 (")	20
	M	"	11	10,000 (")	1440 (24 h)
	F	"	7	5,000 (")	(-)
	F	"	5	5,000 (")	(-)
	M	"	10	2,000 (")	(-)
	M	"	33	2,000 (")	(-)
	M	"	20	1,000 (")	(-)
	M	"	20	1,000 (")	(-)
	M	"	20	1,000 (")	(-)
	F	"	15	1,000 (")	(-)
Pilumnopeus indica	M	"	2	2 (")	inst. death
	M	"	2	2 (")	inst. death
Pachygrapsus crassipes	F	"	7	5 (")	0.7
	M	"	5	0.8(")	0.9
	F	"	5	0.8(")	1.1
Leptodius exaratus	M	"	6.5	5 (")	3.1
	F	"	3	3 (")	(-)
	M	"	5	2 (")	(-)
Eriphia laevimona	F	"	30	10 (")	inst. death
	M	"	37	9 (")	inst. death
Gaillardiellus orientalis	M	"	6	5 (")	0.5

*1M: male, F: female.
*2(-): survival.

PARALYTIC SHELLFISH POISON-PRODUCING BACTERIA

It has often been reported that the toxicity level of a bivalve is not always parallel to the density of the responsible PSP-producing plankton in surrounding waters. In this connection, the amount of PSP in plankton cells also differs widely depending upon the plankton strain and culture conditions, and may differ even between cells of the same clone (ref. 58).

Recently, some marine toxins such as TTX and neosurugatoxin have been demonstrated to be produced by marine bacteria (ref. 59-62). This, along with the above-mentioned phenomena, suggests a possible involvement of marine bacteria. Kodama et al. (ref. 63) recently have reported the production of STX by a bacterium which was isolated from the nuclei of P. tamarensis cells (Fig. 9).

Noguchi et al. (ref. 64) screened for PSP-producing bacteria in PSP-infested oysters collected from Senzaki Bay. The dominant strains of bacteria were isolated and cultured in ORI medium (ref. 65). Harvested cells of each bacterial strain were ultrasonicated and the extracts were examined for toxicity by the mouse bioassay method. The extract of a strain (L-2) isolated from the gill was toxic, though weakly. The toxin productivity of this strain depended to some extent upon culture conditions (TABLE 8). The PSP fraction was prepared from each of the cell extracts, and subjected to HPLC analysis by Nagashima's method (ref. 42). Three peaks appeared which agreed with $PX_{1,2}$, GTX_5 and neoSTX in retention time. These results essentially support Kodama's observation. However, further studies seem necessary to substantiate PSP-producing bacteria.

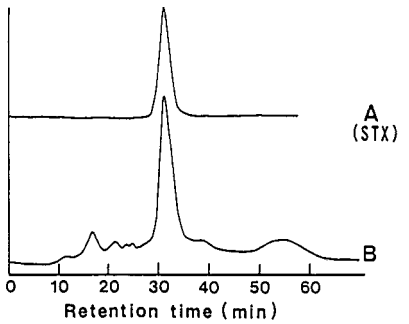


Fig. 9. Identification of a bacterial toxin as saxitoxin by HPLC-fluorometric analysis (ref. 63). **A**, saxitoxin standard (0.2 MU); **B**, bacterial toxin (0.3 MU).

TABLE 8. Toxin production by bacterium L-2 strain under various culture conditions (500 ml-flask)(ref. 64)

Culture conditions	Wet weight of cells (g)	Total toxicity (MU)	MU/g
2 x ORI (pH 6.4)	2.14	4.6	2.2
" (pH 5.4)	2.19	3.3	1.5
" (pH 7.6)	2.02	3.6	1.8
" (0.02% NaCl, pH 7.6)	1.91	<1	---

CONCLUSION

PSP has been posing an increasing food-hygienic problem on a worldwide scale. In 1983, for example, a large scale red tide with Pyr. bahamense as the dominant species spread over Southeast Asia, resulting in many poisoning incidents in the Philippines and Thailand (ref. 66). In 1986, PSP poisoning incidents were reported for the first time in Taiwan (ref. 67) and Korea (ref. 68). Much information is now available on PSP, but there still remain many problems to be solved, e.g., the identification of other PSP-producing plankton, the true origin of PSP, metabolic fate of PSP components, especially of low-toxicity components, and the development of antidotes to PSP. It is eagerly hoped that these and other PSP-associated problems will be solved by international collaboration by scientists in various fields.

Acknowledgement The authors express their heartfelt thanks to Professor Miyoshi Ikawa, University of New Hampshire, U.S.A., for fruitful discussions and assistance with the manuscript.

REFERENCES

1. B.W. Halstead, Poisonous and Venomous Marine Animals of the World, 1168 pp., Darwin, New Jersey (1988).
2. H. Sommer, W.F. Whedon, C.A. Kofoid and R. Stohler, Arch. Pathol., 24, 537-559 (1937).
3. E.J. Schantz, J.D. Mold, D.W. Stanger, J. Shavel, F.J. Riel, J.P. Bowden, J.M. Lynch, R.S. Wyler, B. Riegel and H. Sommer, J. Am. Chem. Soc., 79, 5230-5235 (1957).
4. Y. Hashimoto, Marine Toxins and Other Bioactive Marine Metabolites, 369 pp., Japan Scientific Societies Press, Tokyo (1979).
5. Y. Onoue, T. Noguchi and K. Hashimoto, Bull. Japan. Soc. Sci. Fish., 46, 1031-1034 (1980).
6. Y. Nagashima, T. Noguchi, J. Maruyama, S. Kamimura and K. Hashimoto, Bull. Japan. Soc. Sci. Fish., 50, 331-334 (1984).
7. Y. Onoue and K. Nozawa, Bull. Japan. Soc. Sci. Fish. (in press).
8. T. Noguchi, Y. Nagashima, J. Maruyama, S. Kamimura and K. Hashimoto, Bull. Japan. Soc. Sci. Fish., 50, 517-520 (1984).
9. Y. Oshima, M. Hasegawa, T. Yasumoto, G. Hallegraeff and S. Blackburn, Toxicon, 25, 1105-1112 (1987).
10. T. Ikeda, S. Matsuno, S. Sato, T. Ogata, M. Kodama and Y. Fukuyo, Proceedings of the International Symposium on Red Tides, Elsevier (in press).
11. T. Noguchi, S. Chen, O. Arakawa, T. Ikeda and K. Hashimoto, Abstracts of the Annual Meeting, p. 79, Japan. Soc. Sci. Fish., Tokyo (1988).
12. Y. Onoue, Reports of a Grant-in-Aid for Scientific Research, 33 pp., Ministry of Education, Science and Culture, Japan (1987).
13. Y. Oshima, T. Yasumoto, M. Kodama, T. Ogata, Y. Fukuyo and F. Matsuura, Bull. Japan. Soc. Sci. Fish., 48, 525-530 (1982).
14. T. Noguchi, Y. Ueda, Y. Onoue, M. Kono, K. Koyama, K. Hashimoto, Y. Seno and S. Mishima, Bull. Japan. Soc. Sci. Fish., 46, 1273-1277 (1980).
15. T. Noguchi, Y. Ueda, Y. Onoue, M. Kono, K. Koyama, K. Hashimoto, Y. Seno and S. Mishima, Bull. Japan. Soc. Sci. Fish., 46, 1339-1344 (1980).
16. E.J. Schantz, J.M. Lynch, G. Vayvada, K. Matsumoto and H. Rapoport, Biochemistry, 5, 1191-1195 (1966).
17. E.J. Schantz, V.E. Ghazarossian, H.K. Schnoes, F.M. Strong, J.P. Springer, J.O. Pezzanite and J. Clardy, J. Am. Chem. Soc., 97, 1238-1239 (1975).
18. I. Kawashiro, H. Tanabe, A. Ishii and T. Kondo, J. Food Hyg. Soc. Japan, 3, 273-277 (1962).
19. M.H. Evans, Br. J. Pharmacol., 48, 847-865 (1970).
20. Y. Shimizu, M. Alam. Y. Oshima and W.E. Fallon, Biochem. Biophys. Res. Commun., 66, 731-737 (1975).
21. Y. Shimizu, M. Alam and W.E. Fallon, Proceedings of the First International Conference on Toxic Dinoflagellate Blooms, p. 275-285, The Massachusetts Science and Technology Foundation, Wakefield (1975).

22. Y. Oshima, T. Hayakawa, M. Hashimoto, U. Kotaki and T. Yasumoto, Bull. Japan. Soc. Sci. Fish., 48, 851-854 (1982).
23. Y. Ueda, T. Noguchi, Y. Onoue, K. Koyama, M. Kono and K. Hashimoto, Bull. Japan. Soc. Sci. Fish., 48, 455-458 (1982).
24. S. Hall, P.B. Reichardt and R.A. Neve', Biochem, Biophys. Res. Commun., 97, 649-653 (1980).
25. Y. Onoue, T. Noguchi, J. Maruyama, K. Hashimoto and T. Ikeda, Bull. Japan. Soc. Sci. Fish., 47, 1643 (1981).
26. T. Noguchi, Y. Onoue, J. Maruyama, K. Hashimoto, S. Nishio and T. Ikeda, Bull. Japan. Soc. Sci. Fish., 49, 1931 (1983).
27. S. Nishio, T. Noguchi, Y. Onoue, J. Maruyama, K. Hashimoto and H. Seto, Bull. Japan. Soc. Sci. Fish., 48, 959-965 (1982).
28. T. Harada, Y. Oshima and T. Yasumoto, Agric. Biol. Chem., 46, 1861-1864 (1982).
29. F.E. Koehn, S. Hall, C.F. Wichmann, H.K. Schnoes and P.B. Reichardt, Tetrahed. Lett., 23, 2247-2248 (1982).
30. M. Kobayashi and Y. Shimizu, J. Chem. Soc., Chem. Commun., 827-828 (1981).
31. C.F. Wichmann, W.P. Niemczura, H.K. Schnoes, S. Hall, P.B. Reichardt and S.D. Darling, J. Am. Chem. Soc., 103, 6977-6978 (1981).
32. Y. Onoue, T. Noguchi, J. Maruyama, K. Hashimoto and H. Seto, J. Agric. Food Chem., 31, 420-423 (1983).
33. S. Hall, S.D. Darling, G.L. Boyer, P.B. Reichardt and H.-W. Liu, Tetrahed. Lett., 25, 3537-3538 (1984).
34. T. Harada, Y. Oshima and T. Yasumoto, Agric. Biol. Chem., 47, 191-193 (1983).
35. Y. Nagashima, Y. Sato, T. Noguchi, Y. Fuchi, K. Hayashi and K. Hashimoto, Marine Biology, 98, 243-246 (1988).
36. T. Noguchi, S. Chen, O. Arakawa and K. Hashimoto, Mycotoxins and Phycotoxins 88, Elsevier (in press).
37. Y. Shimizu, Kagaku-to-Seibutsu, 18, 792-799 (1980).
38. K. Daigo, A. Uzu, O. Arakawa, T. Noguchi, H. Seto and K. Hashimoto, Bull. Japan. Soc. Sci. Fish., 51, 309-313 (1985).
39. Y. Onoue and K. Nozawa, Mycotoxins and Phycotoxins 88, Elsevier (in press).
40. Y. Onoue, T. Noguchi, Y. Nagashima, K. Hashimoto, M. Ito and K. Tsukada, J. Chromatogr., 257, 373-379 (1983).
41. Y. Oshima, M. Machida, K. Sasaki, Y. Tamaoki and T. Yasumoto, Agric. Biol. Chem., 48, 1707-1711 (1984).
42. Y. Nagashima, J. Maruyama, T. Noguchi and K. Hashimoto, Bull. Japan. Soc. Sci. Fish., 53, 819-823 (1987).
43. Y. Nagashima, T. Noguchi and K. Hashimoto, Mycotoxins and Phycotoxins 88, Elsevier (in press).
44. A.W. White, J. Fish. Res. Bd. Can., 34, 2421-2424 (1977).
45. A.W. White, Toxic Dinoflagellate Blooms, p. 381-384, Elsevier, North Holland, New York, Amsterdam and Oxford (1979).
46. A.W. White, Can. J. Fish. Aquat. Sci., 37, 2262-2265 (1980).
47. T. Saito, T. Noguchi, T. Takeuchi, S. Kamimura and K. Hashimoto, Bull. Japan. Soc. Sci. Fish., 51, 257-260 (1985).
48. M. Kodama, T. Ogata, T. Noguchi, J. Maruyama and K. Hashimoto, Toxicon, 21, 897-900 (1983).
49. N. Fusetani, H. Endo, K. Hashimoto and M. Kodama, Toxicon, Suppl. 3, 165-168 (1982).
50. Y. Kotaki, M. Tajiri, Y. Oshima and T. Yasumoto, Bull. Japan. Soc. Sci. Fish., 49, 283-286 (1983).
51. T. Noguchi, S., Konosu and Y. Hashimoto, Toxicon, 7, 325-326 (1969).
52. K. Koyama, T. Noguchi, Y. Ueda and K. Hashimoto, Bull. Japan. Soc. Sci. Fish., 47, 965 (1981).
53. T. Noguchi, K. Koyama, A. Uzu and K. Hashimoto, Bull. Japan. Soc. Sci. Fish., 49, 1883-1886 (1983).
54. T. Yasumoto, Y. Oshima and T. Konta, Bull. Japan. Soc. Sci. Fish., 49, 957-960 (1981).
55. Y. Oshima, Y. Kotaki, T. Harada and T. Yasumoto, Seafood Toxins (ed., by E.P. Ragelis), ACS Symp. Ser. No. 262, pp. 161-170, American Chemical Society, Washington, D.C., (1984).
56. T. Noguchi, K. Daigo, O. Arakawa and K. Hashimoto, Toxic Dinoflagellates, p. 293-298, Elsevier, New York·Amsterdam·Oxford (1985).
57. K. Koyama, T. Noguchi, A. Uzu and K. Hashimoto, Bull. Japan. Soc. Sci. Fish., 49, 485-489 (1983).
58. T. Ogata, M. Kodama and T. Ishimaru, Toxicon, 25, 923-928 (1987).
59. T. Noguchi, J.K. Jeon, O. Arakawa, H. Sugita, Y. Deguchi, Y. Shida and K. Hashimoto, J. Biochem., 99, 311-314 (1986).
60. T. Yasumoto, D. Yasumura, M. Yotsu, T. Michishita, A. Endo and Y. Kotaki, Agric. Biol. Chem., 50, 793-795 (1986).
61. U. Simidu, T. Noguchi, D.F. Hwang, Y. Shida and K. Hashimoto, Appl. Environm. Microbiol., 53, 1714-1715 (1987).
62. T. Kosuge, K. Tsuji, K. Hirai and T. Fukuyama, Chem. Pharm. Bull., 33, 3059-3061 (1985).
63. M. Kodama, T. Ogata and S. Sato, Agric. Biol. Chem., 52, 1075-1077 (1988).
64. T. Noguchi, S. Chen, O. Arakawa, T. Ikeda, U. Simidu and K. Hashimoto, Proceedings of the International Symposium on Mycotoxins and Phycotoxins, Aug. 16-19, 1988, Tokyo, Japanese Association of Mycotoxicology, Tokyo (in press).
65. U. Simidu and K. Tsukamoto, Appl. Environm. Microbiol., 50, 781-790 (1985).
66. A.W. White, M. Anraku and K.K. Hooi, ed., Toxic Red Tides and Shellfish Toxicity in Southeast Asia, 133 pp., Southeast Asia Fish. Developm. Center, Singapore, and Internat. Developm. Res. Center, Ottawa (1984).
67. D.F. Hwang, T. Noguchi, Y. Nagashima, I-C. Liao and K. Hashimoto, Bull. Japan. Soc. Sci. Fish., 53, 623-626 (1987).
68. O. Arakawa, T. Noguchi, D.F. Hwang, D.S. Chang, J.K. Jeon and K. Hashimoto, Proceedings of the International Symposium on Red Tides, Elsevier (in press).