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PARALYTIC SHELLFISH POISONING IN SOUTHERN CHINA

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D. M. Anderson, D. M. Kulis, Y.-Z. Qi, L. Zheng, S. Lu and Y.-T. Lin. Paralytic shellfish poisoning in southern China. *Toxicon* **34,** 579–590, 1996.—The rapidly expanding mariculture and commercial region along the southern coast of China has experienced sporadic outbreaks of paralytic shellfish poisoning for nearly 30 years, yet virtually nothing is known of the nature of that toxicity or of the causative organisms. This study presents the first direct comparisons of the high performance liquid chromatography toxin composition profiles of shellfish implicated in paralytic shellfish poisoning outbreaks in Daya Bay with *Alexandrium tamarense* cultures established from those waters. The three cultures that were analyzed produced an unusually high proportion of the low potency *N*-sulfocarbamoyl toxins C1 and C2 (nearly 90% of the total), and only trace quantities of the other saxitoxin derivatives. Total toxicity was thus very low with mild acid extraction, ranging between 7.2 and 12.7 fmole cell^{-1}, or 0.7–0.9 pg saxitoxin equiv. cell^{-1}. Following acid hydrolysis using the standard AOAC extraction method, the dominant toxins in the cultures were gonyautoxins 2 and 3 and decarbamoyl gonyautoxins 2 and 3. Total potency increased fourfold to 2.6–3.4 pg saxitoxin equiv. cell^{-1} following acid hydrolysis. These cultures are thus at the low end of the range of toxicities recorded for members of the *A. tamarense* species complex. Two scallop samples and one mussel sample collected from Daya Bay during paralytic shellfish poisoning episodes in 1990 and 1991 were also analyzed following the AOAC extraction procedure. The toxin profiles were similar for the three shellfish samples, in that the same suite of toxins were present in each, but the relative proportion of those toxins varied. The dominant toxins were gonyautoxins 2 and 3 and toxins C1–C4. Total toxicity was 336 and 654 μg saxitoxin per 100 g meat for the scallop samples, and 723 for the mussels. Toxins C3,4 were present in the shellfish at up to 22 mole%, but were not detected in cultures, even when mild acid was used for extraction. Despite the otherwise similar nature of the culture versus the shellfish toxin signatures, the presence of C3,4 indicates that another strain or species of *Alexandrium*, or possibly a paralytic shellfish poisoning-producing species of another genus was responsible for the 1990 and 1991 paralytic shellfish poisoning outbreaks in Daya Bay. Since the cultures analyzed were of low intrinsic toxicity, *A.*

tamarense may be more widespread along the south coast of China than is suggested by the sporadic pattern of past paralytic shellfish poisoning outbreaks. Blooms with high cell densities are required to generate sufficient toxin to be dangerous. The alarming increase in algal blooms in Chinese waters due to persistent and growing pollution may make these low toxicity populations more problematic in the future.

INTRODUCTION

The nature and extent of paralytic shellfish poisoning (PSP) outbreaks in China are not well known, in large part due to the lack of analytical facilities for detecting those toxins and the absence of a systematic and comprehensive monitoring program for shellfish or plankton. Nevertheless, it is clear that PSP outbreaks have occcurred on several occasions in the past along the coasts of the South and East China seas and continue to occur to this day.

The first published records of human illness from PSP are from Zhejiang Province, where 40 separate episodes resulted in 23 fatalities and 423 nonfatal intoxications between 1967 and 1979 (Fu *et al.*, 1982). The vector for toxicity was the marine snail *Nassarius succinstus*. No causative algal species was identified, but mouse bioassays of toxin extracts were consistent with the presence of PSP toxins. Toxicity ranged from 344 to 3572 μg saxitoxin (STX) equivalents per 100 g snail meat. Other snail species (e.g. *Cerithidea cingulata*, *Bullacta exarata*) and the shellfish *Tellina tridescus* collected at the same time were nontoxic.

Paralytic shellfish poisoning was reported in Dongshan (the southern portion of Fujian Province) in December, 1986 resulting in one fatality and 136 hospitalizations. The vector implicated in these intoxications was the clam *Ruditapes phillipenensis* (Lin *et al.*, 1988). No toxicity testing was conducted, but the symtoms observed were consistent with PSP. An unidentified *Gymnodinium* species was thought to have caused the toxicity. A year later, in November 1989, four fishermen ate the snail *N. succinstus* collected from Fuding in the northern part of Fujian Province and reported symptoms of PSP (Qiu, 1990).

More recent PSP episodes have occurred in Guandong Province, and again in Fujian. Five persons became ill in February 1989 after eating the clam *Pinna pectinata* (Jian and Deng, 1991) which was found to contain up to 600 μg STX equivalents per 100 g shellfish meat. The illnesses occurred in Huizhou, but the shellfish probably originated from Daya Bay (Fig. 1). In March, 1991 there were two human fatalities and several hospitalizations following consumption of the mussel *Perna viridis* collected from Daya Bay (Lin *et al.*, 1994). A survey of Guandong Province from 1990 to 1992 found PSP toxins in 33 edible marine organisms, with toxicity ranging up to 1000 μg STX equivalents per 100 g meat (Lin *et al.*, 1994). In June, 1994 PSP recurred in Zhejiang Province and caused five illnesses and one death following consumption of the snail *Nassarius*.

Despite these events and the clear potential for toxicity in a variety of seafood products, shellfish monitoring is minimal along the Chinese coast. It thus seems likely that the above episodes underestimate the true extent of the PSP problem. These outbreaks should be viewed in the context of China's expanding 'red tide' problem, which has worsened considerably over the last two decades (Qi *et al.*, 1993). From a few isolated algal bloom reports in the early 1970s, the problem has progressed to the stage where where 40–50 red tides are now reported every year. Only a few of these are toxic, but the trend is ominous

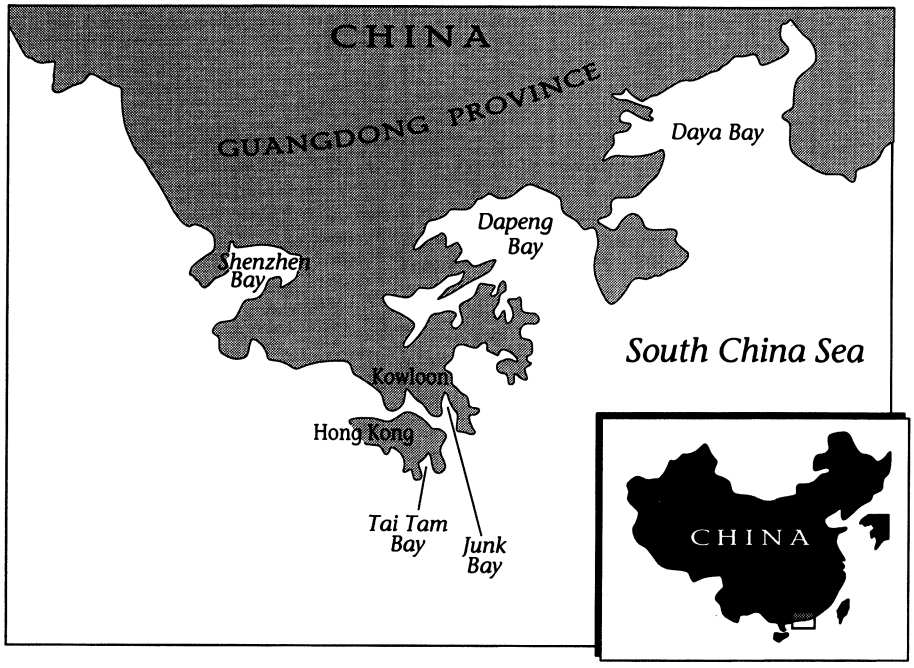

Fig. 1. Map of southern China showing Guandong Province and locations of Dapeng and Daya Bays.

and seems linked to increases in pollution in China's coastal waters over the same interval. The combination of pollution, which is expected to continue increasing well into the next century, a rapid expansion in shellfish aquaculture, and the documented presence of PSP-causing algae in Chinese coastal waters suggests that shellfish toxicity will continue to be a major problem for many years.

Here we present the first high performance liquid chromatography (HPLC) analyses of algal cultures and shellfish extracts from Southern China. These data are the first in a series of studies to characterize the organisms causing PSP in China and the patterns of toxicity in the many seafood species that are vectors for those toxins.

MATERIALS AND METHODS

Cultures

Cultures of *Alexandrium tamarense* strains were established from germinated cysts isolated from Daya Bay sediments (Fig. 1). Species identities were confirmed by E. Balech (personal communication). Clonal strains, ATCI01-1, ATCIO2-1, and ATCIO3-1 were produced by re-isolating individual cells from these original cultures. All were maintained in f/2 medium (Guillard and Ryther, 1962), modified by adding H_2SeO_3 and reducing the concentration of $CuSO_4$ to a final concentration of 10^{-8} M. Cultures used for toxin analysis were inoculated into duplicate 25 ml volumes of medium made with 0.2 μm filtered Vineyard Sound seawater (31 practical salinity units, p.s.u.). These were incubated at 20°C on a 14:10 hr light:dark cycle (*c.* 250 μE m^{-2} sec^{-1}) irradiance provided by cool white fluorescent bulbs. Growth was monitored on a Turner Designs model 10 fluorometer.

Shellfish

Three shellfish samples were collected from Daya Bay at times when PSP toxicity was suspected. Scallops (*Chlamys nibilis*) were collected on 4 May 1990 and 17 April 1991 (hereafter termed scallop samples 1 and 2,

respectively), and mussels (*Mytilus smaragdinus*) on 17 April 1991 (mussel 1). Entire animals were extracted for scallop sample 2 and mussel sample 1, but only the hepatopancreas was analyzed in scallop sample 1.

Toxin extraction and analysis

Toxin extracts of the clonal cultures were obtained as described in Anderson *et al.* (1994), with the following modifications. Approximately 250 μl of 0.5 M acetic acid or 0.2 N HCl was added to an equal volume of cell pellet plus seawater (final concentrations 0.25 and 0.1 N, respectively). Each sample was then sonified for 3 min at a setting of 6 A (Branson sonicator, model 975) in an ice bath. When hydrolysis was desired, the HCl extracts were heated in a boiling water bath for 5 min once the sample temperature reached 97°C. All culture extracts were frozen at − 20°C until analysis, then thawed, mixed, re-centrifuged as above, and filtered through 0.45 μm syringe filters (Anderson *et al.*, 1994).

For the shellfish, 100 g of meat from each sample were blended for 2 min with 100 ml of 0.1 N HCl according to the standard Association of Official Analytical Chemists (AOAC, 1980) method. The mixture was centrifuged and the supernatants refrigerated until analysis. Prior to analysis, the samples passed through a 0.45 μM cellulose acetate centrifugal filter and the filtrate was purified with a Millipore Sep-Pak light C18 cartridge. As discussed in the text, analyses with HPLC indicated that this initial extraction at the time of the outbreaks was not sufficient to hydrolyze all of the sulfamate toxins to their carbamate derivatives, so the shellfish extracts were re-hydrolyzed by mixing each extract 1:1 with 0.2 N HCl and heating in a water bath as described above.

Extracts of cultures and shellfish were analyzed for the STXs by HPLC using the three step isocratic elution method of Oshima *et al.* (1989), with the following modifications. For the C1–C4 toxins, column temperature was maintained at 30°C and the post column reaction temperature at 45°C to aid in separation of the toxins and to achieve greater sensitivity for toxins C1 and C2. The mobile phase used for the elution of toxins C1–C4 was adjusted to pH 5.8 with 0.05 M acetic acid. Gonyautoxins (GTX) were analyzed with a column temperature of 23°C and post column reaction temperature of 35°C. By lowering the post column reaction temperature, the fluorescence of GTX1, GTX4, and GTX6 are enhanced. Column and post column reaction temperatures were maintained at 24°C and 50°C, respectively, for the STX group. Neosaxitoxin fluorescence response was increased by lowering the post column temperature from the recommended 60°C to 50°C. The column eluate was mixed with 7 mM periodic acid in 80 mM sodium phosphate buffer. The HPLC system consisted of a Waters 600E multi-solvent delivery system connected to a Waters Wisp 700 autosampler and Shimadzu RF-535 fluorescence detector. A Beckman C8 Ultrasphere Octyl 5 μm analytical colmn (25 mm × 46 mm) and a Brownlee HPLC New Guard Column were used for the separation of the toxins. External standard solutions generously supplied by Y. Oshima were run prior to sample analysis and after every fourth sample. Toxin composition profiles were determined from analyses of two separate samples. GTX1,4 = gonyautoxins 1 and 4; GTX 2,3 = gonyautoxins 2 and 3; GTX5 = gonyautoxin 5 (or B1; Hall, 1982); GTX6 = gonyautoxin 6 (or B2); C1,2 = toxins C1 and C2; C3,C4 = toxins C3 and C4; dc = decarbamoyl. The terms GTX1,4, GTX2,3, C1,C2 and C3,C4 are used to represent the pooled concentrations of two toxins to account for possible epimerization. Toxicities (in STX equivalents $cell^{-1}$) were calculated from the molar composition data using individual potencies (Oshima *et al.*, 1992). The specific toxicities of the individual toxins (μg STX equiv. μmol^{-1}) were as follows: C1, 3.45; C2, 55.0; C3, 7.6; C4, 32.9; GTX1, 567.6; GTX2, 205.2; GTX3, 364.3; GTX4, 414.7; GTX5, 36.8; dcGTX2, 371.9; dcGTX3, 430.6; NEO, 527.9; dcSTX, 293.0; STX, 571.1.

RESULTS

Shellfish

The toxin compositions of the three shellfish extracts is detailed in Table 1 and summarized in Fig. 2A and B. The toxin profiles were similar for the three shellfish samples, in that the same suite of toxins were present in each. The relative proportion of those toxins varied, however. For example, scallop sample 1 contained 36.4 mole% GTX2,3, whereas scallop sample 2, collected in the same location almost 1 year later, contained only 8.8%. On the other hand, the former sample contained less C1,2, C3,4, dcGTX3, dcSTX and STX than the latter. Mussel sample 1 contained predominantly GTX2,3 at 56.7 mole%, exceeding the high levels found in scallop 1 for which this was also the dominant toxin derivative. The mussel sample contained very little C3,4 compared to the two scallop samples. Following re-hydrolysis of these shellfish samples, no C toxins were detected, and toxins GTX1–4 increased proportionally (data not shown).

When the molar concentrations of the toxins in the shellfish are converted to STX equivalents, scallop samples 1 and 2 contained 335 and 654 μg STX per 100 g meat, and

Table 1. HPLC analysis of Chinese shellfish extracts

Sample	Toxin															
	C1	C2	C3	C4	GTX1	GTX2	GTX3	GTX4	GTX5	GTX6	dcGTX2	dcGTX3	NEO	dcSTX	STX	Total
Scallop 1																
Average μM concentration	0.51	0.12	1.11	0.18	0.43	1.80	0.63	0.26	UD	UD	0.56	0.30	0.19	0.25	0.33	6.68
	(0.02)	(0.00)	(0.26)	(0.04)	(0.02)	(0.03)	(0.00)	(0.02)			(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.31)
μg STX/100 g shellfish meat	0.35	1.35	1.68	1.16	49.33	73.88	45.90	21.94	UD	UD	41.88	26.01	19.85	14.83	37.58	335.72
	(0.01)	(0.04)	(0.39)	(0.26)	(1.87)	(1.42)	(0.22)	(1.29)			(0.45)	(0.52)	(0.00)	(0.35)	(0.11)	(2.31)
Average mole%	7.56	1.84	16.58	2.64	6.51	26.97	9.44	3.96	0.00	0.00	8.43	4.52	2.82	3.79	4.93	100.00
	(0.07)	(0.15)	(3.08)	(0.48)	(0.06)	(1.78)	(0.49)	(0.05)			(0.30)	(0.30)	(0.13)	(0.09)	(0.25)	
Average combined epimer pair %*	9.41		19.22		10.47	36.41			0.00	0.00	8.43	4.52	2.82	3.79	4.93	100.00
	(0.11)		(1.78)		(0.05)	(1.13)					(0.30)	(0.30)	(0.13)	(0.09)	(0.25)	
Scallop 2																
Average μM concentration	1.61	0.71	2.83	0.13	0.10	0.64	0.54	0.60	UD	UD	1.01	1.27	0.37	2.25	1.38	13.44
	(0.45)	(0.16)	(0.06)	(0.05)	(0.02)	(0.00)	(0.00)	(0.03)			(0.02)	(0.06)	(0.00)	(0.07)	(0.01)	(0.64)
μg STX/100 g shellfish meat	1.11	7.84	4.30	0.87	11.86	26.08	39.67	49.60	UD	UD	75.39	109.19	38.85	131.71	157.56	654.03
	(0.31)	(1.73)	(0.08)	(0.36)	(1.76)	(0.23)	(0.26)	(2.57)			(1.82)	(5.08)	(0.21)	(3.90)	(1.66)	(4.48)
Average mole%	11.97	5.30	21.05	0.98	0.78	4.73	4.05	4.45	0.00	0.00	7.54	9.43	2.74	16.72	10.26	100.00
	(2.76)	(0.92)	(0.60)	(0.45)	(0.08)	(0.27)	(0.22)	(0.02)			(0.54)	(0.89)	(0.12)	(0.31)	(0.38)	
Average combined epimer pair %*	17.28		22.04		5.23	8.78			0.00	0.00	7.54	9.43	2.74	16.72	10.26	100.00
	(1.84)		(0.52)		(0.05)	(0.24)					(0.54)	(0.89)	(0.12)	(0.31)	(0.38)	
Mussel 1																
Average μM concentration	1.63	0.33	0.33	UD	0.82	5.78	1.87	0.25	UD	UD	0.66	0.33	0.13	0.31	1.06	13.50
	(0.08)	(0.01)	(0.09)		(0.02)	(0.19)	(0.07)	(0.05)			(0.03)	(0.01)	(0.00)	(0.00)	(0.01)	(0.08)
μg STX/100 g shellfish meat	1.13	3.59	0.50	UD	93.55	237.06	136.55	20.57	UD	UD	49.09	28.46	14.04	18.14	120.79	723.46
	(0.05)	(0.12)	(0.14)		(1.93)	(7.69)	(4.88)	(3.82)			(2.16)	(1.16)	(0.63)	(0.09)	(1.43)	(10.78)
Average mole%	12.08	2.42	2.44	0.00	6.10	42.79	13.88	1.84	0.00	0.00	4.89	2.45	0.99	2.29	7.83	100.00
	(0.65)	(0.06)	(0.70)		(0.16)	(1.14)	(0.41)	(0.35)			(0.19)	(0.09)	(0.05)	(0.02)	(0.05)	
Average combined epimer pair %*	14.50		2.44	0.00	7.94	56.67			0.00	0.00	4.89	2.45	0.99	2.29	7.83	100.00
	(0.36)		(0.35)		(0.26)	(0.78)					(0.19)	(0.09)	(0.05)	(0.02)	(0.05)	

Figures in parentheses = S.D.; UD, undetectable.

*Epimer pair toxins are: C1 + C2, C3 + C4, GTX1 + GTX4 and GTX2 + GTX3.

mussel sample 1 had 723. [These levels represent the toxicity after the inital extraction using the AOAC (1980) method at the time of the PSP outbreak.] In scallop sample 1, 36% of this total toxicity was due to GTX2,3, and 20% to dcGTX2,3. In scallop 2, STX accounted for 24% of the potency, dcSTX for 20%, GTX2,3 and dcGTX2,3 for 10 and 28%, respectively. For the mussel, 52% of the toxicity was as GTX2,3, 11% as dcGTX2,3, and 17% as STX.

Cultures

Cultures of all three Daya Bay isolates of *A. tamarense* extracted with mild (0.25 N) acetic acid (HOAc) contained the same relative proportions of the different STXs. The HPLC analyses of each strain are presented separately in Table 2, but for simplicity in comparisons with shellfish (Fig. 2A), the toxin composition data from the three strains were also averaged to give one 'culture' value. The dominant toxins in the cultures were clearly C1,2, representing approx. 90 mole%. Toxins NEO, GTX5, GTX1,4, GTX2,3, and dcGTX3 were also present, but each at < 5 mole%. The total toxin content of the isolates ranged between 7.2 and 12.7 fmole cell^{-1}, or 0.7–0.9 pg STX equiv. cell^{-1} based on the potencies of the individual toxins.

When the culture extracts were treated with hot HCl in a manner equivalent to the standard AOAC (1980) method for shellfish extracts, the toxin composition profiles changed considerably, due to hydrolysis of the low potency sulfamate toxins to carbamates (Table 3). The dominant toxins in the hydrolyzed samples were GTX2,3 at 64–85 mole%, followed by dcGTX3 and STX, which ranged from 3–18 and 5–12 mole%, respectively. The averages of these analyses are plotted in Fig. 2B to show the relative abundance of the different toxins in hydrolyzed culture extracts. As expected, the strong acid extraction did not significantly alter the total number of moles of toxin in each extract (Table 2 and Table 3), but did change the toxin content or potency, which increased approx. fourfold to 2.6–3.4 pg STX equiv. cell^{-1}.

DISCUSSION

Toxin composition comparisons

Here we present the first HPLC analyses of extracts from toxic shellfish and cultures of the suspected causative organisms from Daya Bay, an important aquaculture site in Guandong Province in southern China. PSP toxins were present in the cultures of *A. tamarense* established from cysts collected within the bay, and in the shellfish collected when human illnesses occurred there in 1990 and 1991.

The three cultures that were analyzed produced an unusually high proportion of the low potency *N*-sulfocarbamoyl toxins C1 and C2 (nearly 90% of the total), and only trace quantities of the other saxitoxin derivatives. When hydrolyzed, the dominant toxins in the cultures were GTX2,3 and decarbamoyl GTX2,3. Some clear similarities were noted when these hydrolyzed culture extracts were compared to the shellfish extracts. For example, the most abundant toxins in the shellfish and the hydrolyzed culture extracts were GTX2,3 for all but scallop sample 2, which had proportionally more of the C toxins, presumably due to incomplete hydrolysis. All contained low but detectable amounts of STX, dcSTX, NEO, dcGTX2,3, and GTX1,4. There were, however, significant differences between the toxin profiles of the shellfish and the cultures. Toxins C1,2 and C3,4 comprised up to 40% of the three shellfish extracts, but no C toxins were detected in the hydrolyzed culture

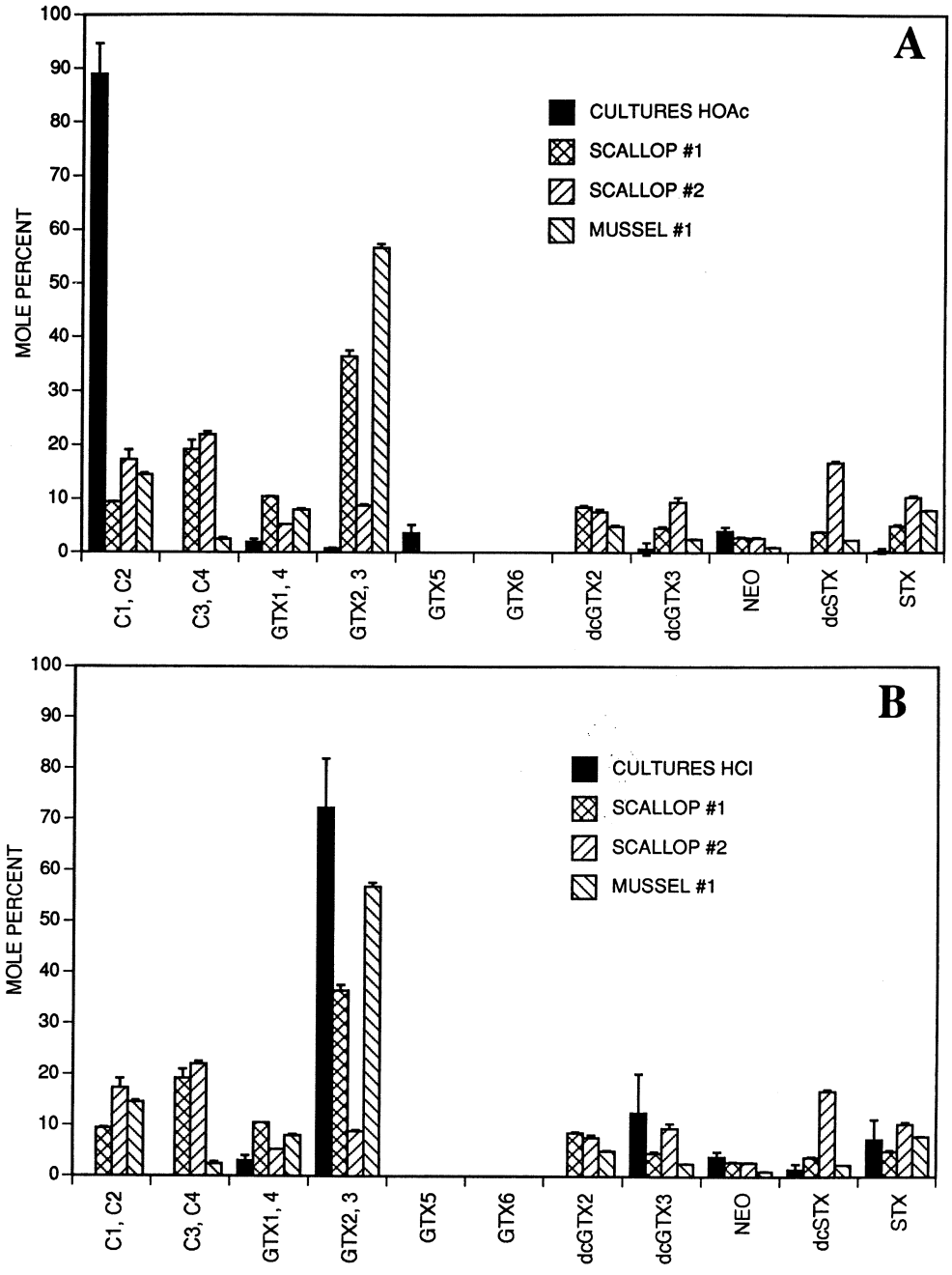

Fig. 2. Comparison of the toxin composition of shellfish and cultures of *A. tamarense* from Daya Bay. (A) Cultures (average of three strains) extracted in mild 0.5 N HOAc (final concentration 0.25 N), compared to scallop and mussel extracts using the AOAC (1980) method in hot 0.1 N HCl (final concentration 0.05 N). (B) Cultures (average of three strains) extracted in hot 0.2 N HCl (final concentration 0.1 N) to mimic the AOAC (1980) procedure, compared to scallop and mussel extracts using the same methods.

Table 2. HPLC analysis of Chinese *Alexandrium* extracts

Sample		Toxin															
		C1	C2	C3	C4	GTX1	GTX2	GTX3	GTX4	GTX5	GTX6	dcGTX2	dcGTX3	NEO	dcSTX	STX	Total
Culture:	ATCI01-1, acetic acid extract																
	Average fmol cell^{-1}	1.13	10.40	UD	UD	UD	UD	0.06	0.24	0.46	UD	UD	UD	0.42	UD	UD	12.72
	(Std. Dev.)	(0.51)	(1.43)					(0.00)	(0.04)	(0.02)				(0.10)			(2.10)
	Average fg STX cell^{-1}	3.90	571.58	UD	UD	UD	UD	22.37	100.07	16.98	UD	UD	UD	222.82	UD	UD	937.73
	(Std. Dev.)	(1.78)	(78.82)					(0.94)	(15.22)	(0.61)				(52.72)			(150.08)
	Average mole%	8.46	82.16	0.00	0.00	0.00	0.00	0.49	1.90	3.71	0.00	0.00	0.00	3.28	0.00	0.00	100.00
	(Std. Dev.)	(3.75)	(3.28)					(0.09)	(0.04)	(0.68)				(0.34)			
	Average combined epimer pair %*	90.62		0.00		1.90	0.49			3.71	0.00	0.00	0.00	3.28	0.00	0.00	100.00
	(Std. Dev.)	(3.51)				(0.02)	(0.04)			(0.68)				(0.34)			
Culture:	ATCI02-1, acetic acid extract																
	Average fmole cell^{-1}	0.50	6.84	UD	UD	UD	UD	0.05	0.11	0.42	UD	UD	UD	0.36	UD	0.07	8.34
	(Std. Dev.)	(0.06)	(0.57)					(0.00)	(0.00)	(0.02)				(0.09)		(0.01)	(0.76)
	Average fg STX cell^{-1}	1.73	376.00	UD	UD	UD	UD	18.38	45.13	15.43	UD	UD	UD	187.71	UD	38.66	683.04
	(Std. Dev.)	(0.20)	(31.47)					(0.98)	(0.23)	(0.67)				(48.18)		(7.74)	(89.45)
	Average mole%	6.01	82.02	0.00	0.00	0.00	0.00	0.61	1.31	5.05	0.00	0.00	0.00	4.20	0.00	0.80	100.00
	(Std. Dev.)	(0.19)	(0.80)					(0.03)	(0.16)	(0.34)				(1.01)		(0.13)	
	Average combined epimer pair %*	88.03		0.00		1.31	0.61			5.05	0.00	0.00	0.00	4.20	0.00	0.80	100.00
	(Std. Dev.)	(0.50)				(0.08)	(0.02)			(0.34)				(1.01)		(0.13)	
Culture:	ATCI03-1, acetic acid extract																
	Average fmole cell^{-1}	UD	6.36	UD	UD	UD	UD	0.07	0.18	0.13	UD	UD	0.13	0.32	UD	UD	7.20
	(Std. Dev.)		(0.97)					(0.00)	(0.01)	(0.07)			(0.05)	(0.00)			(0.87)
	Average fg STX cell^{-1}	UD	349.76	UD	UD	UD	UD	25.35	75.81	4.77	UD	UD	56.49	171.01	UD	UD	683.19
	(Std. Dev.)		(53.42)					(3.54)	(4.26)	(2.74)			(22.34)	(3.73)			(39.87)
	Average mole%	0.00	88.03	0.00	0.00	0.00	0.00	0.96	2.56	1.96	0.00	0.00	1.94	4.55	0.00	0.00	100.00
	(Std. Dev.)		(4.00)					(0.03)	(0.24)	(1.80)			(1.35)	(0.64)			
	Average combined epimer pair %*	88.03		0.00		2.56	0.96			1.96	0.00	0.00	1.94	4.55	0.00	0.00	100.00
	(Stc. Dev.)	(2.00)				(0.12)	(0.01)			(1.80)			(1.35)	(0.64)			
Culture:	Average, acetic acid extracts																
	Average fmole cell^{-1}	0.54	7.87	UD	UD	UD	UD	0.06	0.18	0.34	UD	UD	0.04	0.37	UD	0.02	9.42
	(Std. Dev.)	(0.55)	(2.09)					(0.00)	(0.06)	(0.15)			(0.07)	(0.09)		(0.03)	(2.75)
	Average fg STX cell^{-1}	1.88	432.44	UD	UD	UD	UD	22.03	73.67	12.40	UD	UD	18.83	193.84	UD	12.89	767.98
	(Std. Dev.)	(1.90)	(114.66)					(3.60)	(24.26)	(5.68)			(29.59)	(46.59)		(18.76)	(158.47)
	Average mole%	4.82	84.07	0.00	0.00	0.00	0.00	0.69	1.93	3.57	0.00	0.00	0.65	4.01	0.00	0.27	100.00
	(Std. Dev.)	(3.87)	(3.52)					(0.20)	(0.52)	(1.50)			(1.07)	(0.74)		(0.38)	
	Average combined epimer pair %*	88.89		0.00		1.93	0.69			3.57	0.00	0.00	0.65	4.01	0.00	0.27	100.00
	(Std. Dev.)	(5.73)				(0.57)	(0.22)			(1.64)			(1.17)	(0.81)		(0.42)	

Figures in parentheses = S.D.; UD, undetectable.
*Epimer pair toxins are: C1 + C2, C3 + C4, GTX1 + GTX4 and GTX2 + GTX3.

Table 3. HPLC analysis of Chinese *Alexandrium* extracts

Sample		Toxin															
		C1	C2	C3	C4	GTX1	GTX2	GTX3	GTX4	GTX5	GTX6	dcGTX2	dcGTX3	NEO	dcSTX	STX	Total
Culture:	ATCI01-1, hydrochloric acid extract																
	Average fmole cell^{-1}	UD	UD	UD	UD	0.12	0.40	7.25	0.17	UD	UD	UD	0.21	0.46	UD	0.40	8.99
	(Std. Dev.)					(0.04)	(0.00)	(1.26)	(0.04)				(0.05)	(0.06)		(0.04)	(1.40)
	Average fg STX cell^{-1}	UD	UD	UD	UD	65.29	81.10	2640.32	68.49	UD	UD	UD	89.84	242.66	UD	228.15	3415.85
	(Std. Dev.)					(25.34)	(0.07)	(457.93)	(15.14)				(19.89)	(33.83)		(23.00)	(535.29)
	Average mole%	0.00	0.00	0.00	0.00	1.23	4.51	80.38	1.82	0.00	0.00	0.00	2.46	5.13	0.00	4.48	100.00
	(Std. Dev.)					(0.43)	(0.99)	(2.12)	(0.18)				(1.27)	(0.12)		(0.35)	
	Average combined epimer pair %*	0.00		0.00		3.05	84.88			0.00	0.00	0.00	2.46	5.13	0.00	4.48	100.00
	(Std. Dev.)					(0.30)	(1.56)						(1.27)	(0.12)		(0.35)	
Culture:	ATCI02-1, hydrochloric acid extract																
	Average fmole cell^{-1}	UD	UD	UD	UD	UD	0.21	3.99	0.18	UD	UD	UD	1.11	0.21	0.10	0.81	6.61
	(Std. Dev.)						(0.02)	(0.19)	(0.05)				(0.02)	(0.00)	(0.01)	(0.04)	(0.12)
	Average fg STX cell^{-1}	UD	UD	UD	UD	UD	42.72	1455.14	75.26	UD	UD	UD	478.29	109.47	29.78	460.76	2651.42
	(Std. Dev.)						(4.00)	(68.49)	(21.71)				(9.24)	(0.65)	(3.58)	(21.46)	(52.07)
	Average mole%	0.00	0.00	0.00	0.00	UD	3.16	60.39	2.76	0.00	0.00	0.00	16.82	3.14	1.54	12.20	100.00
	(Std. Dev.)						(0.50)	(2.45)	(1.19)				(0.90)	(0.06)	(0.30)	(0.49)	
	Average combined epimer pair %*	0.00		0.00		2.76	63.55			0.00	0.00	0.00	16.82	3.14	1.54	12.20	100.00
	(Std. Dev.)					(0.60)	(1.48)						(0.90)	(0.06)	(0.30)	(0.49)	
Culture:	ATCI03-1, hydrochloric acid extract																
	Average fmole cell^{-1}	UD	UD	UD	UD	UD	0.27	4.34	0.21	UD	UD	UD	1.21	0.23	0.17	0.33	6.77
	(Std. Dev.)						(0.01)	(0.16)	(0.00)				(0.01)	(0.03)	(0.00)	(0.05)	(0.14)
	Average fg STX cell^{-1}	UD	UD	UD	UD	UD	55.36	1582.47	88.57	UD	UD	UD	520.16	119.91	50.72	188.41	2605.60
	(Std. Dev.)						(3.05)	(56.97)	(2.30)				(4.75)	(16.87)	(1.69)	(30.01)	(41.51)
	Average mole%	0.00	0.00	0.00	0.00	UD	3.99	64.18	3.16	0.00	0.00	0.00	17.87	3.35	2.56	4.89	100.00
	(Std. Dev.)						(0.19)	(1.39)	(0.21)				(0.75)	(0.57)	(0.05)	(1.24)	
	Average combined epimer pair %*	0.00		0.00		3.16	68.17			0.00	0.00	0.00	17.87	3.35	2.56	4.89	100.00
	(Std. Dev.)					(0.10)	(0.79)						(0.75)	(0.57)	(0.05)	(1.24)	
Culture:	Average hydrochloric acid extracts																
	Average fmole cell^{-1}	UD	UD	UD	UD	0.04	0.29	5.20	0.19	UD	UD	UD	0.84	0.30	0.09	0.51	7.46
	(Std. Dev.)					(0.06)	(0.08)	(1.63)	(0.04)				(0.45)	(0.12)	(0.07)	(0.21)	(1.36)
	Average fg STX cell^{-1}	UD	UD	UD	UD	21.76	59.72	1892.64	77.44	UD	UD	UD	362.76	157.35	26.83	292.44	2890.96
	(Std. Dev.)					(34.08)	(16.23)	(595.61)	(17.46)				(194.17)	(64.30)	(20.93)	(122.71)	(484.87)
	Average mole%	0.00	0.00	0.00	0.00	0.41	3.88	68.32	2.58	0.00	0.00	0.00	12.38	3.87	1.37	7.19	100.00
	(Std. Dev.)					(0.61)	(0.72)	(8.79)	(0.75)				(7.06)	(0.92)	(1.06)	(3.59)	
	Average combined epimer pair %*	0.00		0.00		2.99	72.20			0.00	0.00	0.00	12.38	3.87	1.37	7.19	100.00
	(Std. Dev.)					(1.06)	(9.66)						(7.74)	(1.01)	(1.16)	(3.93)	

Figures in parentheses = S.D.; UD, undetectable.
*Epimer pair toxins are: C1 + C2, C3 + C4, GTX1 + GTX4 and GTX2 + GTX3.

extracts. Clearly, hydrolysis of the toxins in the shellfish samples was not complete following the AOAC extraction procedure conducted at the time of the outbreak. If hydrolysis had been carried to completion, the C toxins would not have been detected and GTX1–4 content would have been proportionally higher, exactly as occurred with the cultures and with the re-hydrolyzed shellfish extracts.

Given these similarities in toxin composition, it might appear that the *A. tamarense* cultures isolated from Daya Bay are representative of the algal populations that caused the PSP toxicity in 1990 and 1991. However, toxins C3,4 were present in the shellfish extracts at up to 22 mole%, but were not detected in culture extracts, even when mild, 0.25M acetic acid was used (Table 2). Numerous biochemical pathways have been proposed to explain differences in PSP toxin composition between shellfish and the dinoflagellates they ingest (e.g. Cembella *et al.*, 1993; Lee *et al.*, 1992), but the production of C toxins from other derivatives has not been reported. We conclude that another strain or species of *Alexandrium*, or possibly a PSP-producing species of another genus (such as *Gymnodinium catenatum* or *Pyrodinium bahamense*) was responsible for the 1990 and 1991 PSP outbreaks in Daya Bay. To our knowledge, however, *P. bahamense* has never been reported in Chinese coastal waters, but it is a common and significant source of PSP in the nearby Phillipines (Gonzales, 1989). Likewise, *G. catenatum* motile cells have been reported in Manila Bay (Y. Fukuyo, personal communication), and empty cysts of that species have been recovered from Daya Bay (Qi *et al.*, submitted). These may, however, be relic cysts eroded from ancient deposits, as the distinctive *G. catenatum* cells and chains have not been observed in plankton samples from the area. The simplest explanation for the discrepancy in toxin composition profiles is that a strain of *A. tamarense* occurs within Daya Bay that produces C3 and C4 toxins. Establishment of new *Alexandrium* cultures from the area and characterization of their toxicity should help to resolve this uncertainty.

Toxin content

The cultures of *A. tamarense* analyzed in this study contained between 7.2 and 12.7 fmole cell^{-1}, or 0.7–0.9 pg STX equiv. cell^{-1} based on the potencies of the individual toxins (Table 2). When the culture extracts were hydrolyzed, the toxin content or potency increased fourfold to 2.6–3.4 pg STX equiv. cell^{-1} (Table 3). Known as "Proctor enhancement" (Proctor *et al.*, 1975), this increase in toxicity is due to the hydrolysis of *N*-sulfocarbamoyl toxins (sulfamates) to their carbamate analogues. Even with this increase, the toxicity of the Chinese isolates are at the low end of the range of toxicities recorded for cultures of the *A. tamarense* group (*A. tamarense*, *A. excatatum*, *A. catenella*, and *A. fundyense*) from other regions of the world. A useful summary by Cembella *et al.* (1988) reports values of 20–32 pg STX equiv. cell^{-1} for the most potent isolates from this group found along the northeastern coasts of the United States and Canada. Values of 1–4 pg STX equiv. cell^{-1} were reported for some low toxicity isolates, such as those from southern New England where PSP outbreaks are infrequent (Maranda *et al.*, 1985).

In the same way that blooms of the low toxicity *Alexandrium* populations in southern New England must reach high cell densities in order to generate dangerous and noticeable levels of toxicity in shellfish (e.g. Anderson *et al.*, 1994), Chinese *A. tamarense* populations must necessarily be dense to cause significant problems in Daya Bay. In those waters, at least an order of magnitude more cells must be present to produce the same amount of toxin as blooms of the most toxic *Alexandrium* isolates on the list compiled by Cembella *et al.* (1988). If the isolates analyzed in this study are representative of Chinese *A.*

tamarense populations, it is likely that low density blooms of this species could occur along the Chinese coast, but not be detected due to the low intrinsic toxicity of the isolates and the lack of routine plankton monitoring programs. This inference is supported by a recent survey of dinoflagellate cysts in coastal sediments of the South and East China Seas, in which *A. tamarense* was found in eight relatively contiguous locations from the mouth of the Pearl River through Guandong and Fujian Provinces (Qi *et al.*, submitted). This distribution covers the region in which PSP events have been recorded (Lin *et al.*, 1994), but the cyst distribution for *A. tamarense* is more continuous than the patchy pattern of PSP outbreaks would suggest.

SUMMARY

This study provides the first direct comparison of toxin composition profiles of shellfish implicated in PSP outbreaks in southern China and cultures of *A. tamarense* established from the same waters. Although there were clear similarities between the profiles of the shellfish and the cultures, one particular difference—the presence of toxins C3,4 in the shellfish but not the algae—argues that another species or strain was responsible for the 1990 and 1991 outbreaks of PSP. The cultures analyzed are of low intrinsic toxicity due to their high content of low potency *N*-sulfocarbamoyl saxitoxin derivatives. Therefore, *A. tamarense* may be more widespread than is suggested by the sporadic pattern of reported PSP outbreaks along the south coast of China. The increasing pollution of Chinese coastal waters (Qi *et al.*, 1993) may make these low toxicity populations more problematic in the future, as a direct correlation between pollution and red tides has been reported in several areas of the world (Lam *et al.*, 1987; Murakawa, 1987). Finally, the potency of the toxins from these *A. tamarense* isolates varies dramatically with the degree of hydrolysis. Shellfish extractions utilizing the standard AOAC procedure did not completely hydrolyze toxins C1–C4 to their more potent GTX analogues, and thus there was undetected, 'cryptic' toxicity that could represent a public health threat. Care must be taken in the future to adjust the pH, time and temperature of the extraction procedure to maximize interconversions and ensure that the most conservative measurement of toxicity is obtained in monitoring programs for that region of China.

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