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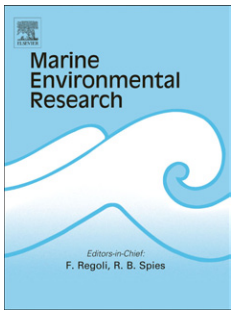


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# Source and profile of paralytic shellfish poisoning toxins in shellfish in Daya Bay, South China Sea

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**Abstract**

Changes in cell density and cyst flux of *Alexandrium tamarense*, paralytic shellfish poisoning (PSP) toxin contents in shellfishes, and environmental parameters were measured in two stations in Daya Bay, South China Sea from March 2005 to July 2006. Vegetative cells of *A. tamarense* occurred sporadically; however, they presented abundantly during the winter months. Meanwhile, cyst flux reached its maximum level just following the peak abundance of motile cells. The PSP contents in shellfish were generally low, but higher in winter with the maximum of 14 015 μg STX equiv./kg. The majority of toxins were found in digestive glands, with a maximum of 66 227 μg STX equiv./kg. There were significant positive relationships between toxin level and vegetative cell density and cyst flux. This indicates that vegetative cells and cysts of *Alexandriu*m significantly influenced PSP level, and could be an important source of PSP toxins in shellfish during winter.

Key words: Paralytic shellfish poisoning (PSP) toxins; *Alexandrium tamarense*; Cyst; Phytoplankton; Algal toxins; Toxicity; South China Sea

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## 1. Introduction

Paralytic shellfish poisoning (PSP) toxins are biotoxins produced by micro-phytoplankton that can accumulate in filter-feeding shellfish (Shumway, 1990; Hallegraeff, 1995). Subsequent ingestion of contaminated shellfish by humans can result in serious illness or even death. The incidence of human poisoning has increased markedly since the early 1970s (Hallegraeff, 1995). Species within the dinoflagellate genus *Alexandrium*, such as *A. tamarense* and *A. catenella*, have been known as important producers of PSP toxins. Resting cysts of these toxic dinoflagellates have been shown to contain toxins (Bravo et al., 1998; Persson et al., 2006) and sometimes have been reported to contain six (Oshima et al., 1992) to ten folds (Yentsch and Mague, 1979) more toxin than vegetative cells. Dinoflagellate cysts are one of the food sources of bivalves (Tsujino and Uchida, 2004; Persson et al., 2006). Many studies have suggested that consumption of cysts may be the direct cause of toxicity in bivalves, especially during the winter months (Shumway et al., 1988; Schwinghamer et al., 1994; Anderson, 1997; Bravo et al., 1998).

Daya Bay is a site of recurrent PSP episodes (Anderson et al., 1996; Lin et al., 1999) with the highest PSP toxin levels among the Chinese coastal areas (Lin et al., 1999; Jiang et al., 2000a). Human mortalities and hospitalizations have occurred from time to time in nearby areas following consumption of toxic mussels. Five persons were poisoned due to consumption of the contaminated shellfish in 1989 (Jian et al., 1991), and three persons died after shellfish consumption in 1993 (Lin et al., 1999). Bivalves collected from this sea area had PSP toxin concentrations exceeding the regulatory limit of 800 μg STX equiv./kg, and a maximum concentration of 10 000 μg STX equiv./kg was reported (Jiang et al., 2000a; Yang et al., 2005). The highest contents of PSP toxins were detected during the winter months (Jiang et al., 2000a). Despite the long-known occurrence of PSP toxins, PSP causative species, such as *Alexandrium* spp. and *Gymnodinium catenatum*, were seldom recorded in phytoplankton survey (Zhou et al., 1998; Liu et al., 2006). However, abundant *Alexandrium* cyst were recorded in surface sediments from this area, and it was suggested that high PSP toxin levels in bivalves during the winter months were probably due to ingestion of cysts of

*Alexandrium* (Wang et al., 2004). Therefore, we set up sediment traps at two stations of Daya Bay, bred shellfish synchronously at the same areas, and conducted phytoplankton surveys. For phytoplankton community and its relationships with environmental parameters, see Wang et al. (2009). In this study, we analyzed the annual data of *Alexandrium* vegetative cell and its cyst, and the PSP concentrations in shellfish. The purpose is to investigate the seasonal variation in the population size of *Alexandrium* (cell density and cyst flux) and their relationship with PSP levels in shellfish.

## 2 Materials and Methods

### *2.1 Site description*

Daya Bay is located in the southeast part of the South China Sea (22°30´-22°50´N, 114°30´-114°50´E). It is a semi-enclosed shallow embayment with depth ranging from 6 m to 15 m. It covers an area of 650 $km^2$ and is separated into several small sub-basins. The bay has a mild and wet subtropical climate with an annual mean air temperature of 22 °C. The coldest months are January and February, with a monthly mean air temperature of about 15 °C, and the hottest months are July and August, with a monthly mean air temperature around 28 °C. Salinity usually lies between 22 and 33 with little fluctuation except during the typhoon season from July to September. The Daya Bay Nuclear Power Station (DNPS), the first nuclear power station in China, has been operating since February 1994, and it discharges cooling water at the rate of $2.9\times10^7$ $m^3$/year (Liu et al., 2006).

Sampling stations (Fig. 1) were located in two cultural areas of the bay. St. 1 was located at 22°33′25.7″N, 114°30′54.1″E, where *Pteria* spp. were bred in suspended cages. St. 2 was at a fish cage area (22°33′51.7″N, 114°31′20.2″E), which was closer to the shore and close to the residential area.

### *2.2 Nutrients and hydrographic parameters*

Temperature (T), Salinity (S) and dissolved oxygen (DO) were recorded *in situ* with an YSI meter (YSI 85, YSI Incorporated, USA) once every three days. Transparency and nutrients were measured every two weeks. Transparency was measured with a Secchi disk.

Water samples for nutrient determination were collected in the subsurface water column (0.5 m), immediately filtered through glass fibre filters (Whatman GF/F, porosity=0.7 μm), and placed in a freezer. Nutrients, including dissolved inorganic nitrogen (DIN, the sum of ammonium, nitrite and nitrate), dissolved inorganic phosphorus (DIP, orthophosphate) and dissolved silicon (DSi, silicate), were analysed following standard methods of State Oceanic China (SOC, 1991) within 24 hours.

*2.3 Phytoplankton sampling and observation*

Water samples of 1000 mL were collected in the subsurface water column once every three days for phytoplankton observation and fixed immediately with neutralized formalin to a final concentration of 4%. The fixed water samples were finally concentrated to 20 mL by sedimentation. Phytoplankton species were counted from aliquots of 0.5-1.0 mL sub-samples using a microscope (Leica DMIRB) at magnifications of 100x to 600x. The phytoplankton abundance was presented as cell numbers per millilitre (cells/mL).

The thecal plate morphology of *Alexandrium* cells was examined using phase contrast and UV epifluorescence microscopy after calcofluor staining (Fritz and Triemer, 1985) under a Leica DMIRB microscope. The thecal plate tabulation and the shapes of plates and apical pore complex proved that the dominant *Alexandrium* species was *A. tamarense*.

*2.4 Sediment collection and cyst observation*

The sediment trap was set to collect settling materials from March 24, 2005 to June 10, 2006. The trap consisted of a cylindrical PVC collector (height 35cm, diameter 7cm, height/diameter ratio 5), flexible tube holder, subsurface buoy, surface buoy marker, and anchor. The trap was moored at 1.0 m from the sea bottom (depth at the deployment location was about 6 m).

Trap samples were collected every two weeks. The settled material was suspended in 2000 mL seawater, and the suspension was divided into two 1000 mL aliquots, one of which was fixed with 4% buffered formalin and subsequently concentrated to 50 mL by sedimentation.

Sediment samples were gently sonicated for 30 seconds. The sonicated material was then

sieved through 125 μm and 20 μm metallic screens to remove larger particles and detritus, and the slurry remaining on the 20 μm screen was washed into a beaker and collected into a plastic tube to a final sample volume of 10 mL with filtered seawater. For observation, 0.1 to 0.5 mL aliquots of sample were placed on a 1 mL counting chamber and diluted with appropriate distilled water.

Observation was under an inverted light microscope (Leica DMIRB). Only living cysts that had not germinated were counted. At least three observations were conducted for each sample until 200 dinoflagellate cysts were observed. Mean daily cyst flux (cysts/cm$^2$ d) was calculated. Four seasons during the survey were defined as: spring, March to May; summer, June to August; autumn, September to November; and winter, December to next February.

*2.5 Shellfish breeding and PSP analysis*

*2.5.1 Shellfish breeding and sampling*

At the beginning of the survey on March 24, 2005, 600 scallops (*Chlamys nobilis*) were bought from the local shellfish farm. Half of the scallops were placed in lantern nets and hung from long lines 3 m below the surface in each of the two stations. Green mussels (*Perna viridis*) were collected from fishing crossrails and ropes and shellfish cages. About 10 scallops (mean shell length of 9.48±0.44cm) and 20 green mussels (mean shell length of 9.19±0.95cm) were collected in each station for PSP toxin determination every two weeks. The shells were dissected immediately after being washed with a brush and rinsed with DI water. Whole soft tissues were removed from the shells and washed with DI water gently. Digestive glands were carefully removed from the rest of tissues. Both the digestive glands and other tissues were weighed and placed in plastic bags separately and stored in a freezer. During the transportation to the laboratory (within 4 h), the tissue samples were stored at -20 °C.

*2.5.2 Mouse bioassay*

As the toxicities of most samples were below detection level, the digestive glands were treated and analysed first in order to screen out any contaminated samples. If the digestive glands were toxic to test mice, the remaining tissues of the same sample then treated and assayed. Sample treatment and mouse bioassay were performed according to the standard

AOAC procedure (Association of Official Analytical Chemists, AOAC, 1990) with the exception that samples sizes were smaller (about 20 g digestive gland homogenate, equal volume of 0.1N HCl and digestive gland homogenate). Mouse bioassays were conducted using ICR strains, 19–21 g male mice. The mice (3-6 mice per extract) were observed for 1 h, and the mean survival time was used to calculate the toxicity. When the extracts were too toxic, it was necessary to dilute the shellfish extract until the survival time was between 5 and 7 min. Bioassay results were calculated using a computer-based version of Sommer's table and correction table for mouse weight (Sommer and Meyer, 1937). The mouse bioassay was standardized using pure saxitoxin purchased from the National Research Council of Canada, Institute for Marine Biosciences (Halifax, Canada). The toxicity of the sample equals to the sum of toxicities of the digestive glands and the other tissues per kg total tissue (μg STX equiv./kg), and the toxicity of the digestive gland was expressed as μg STX equiv. per kg digestive gland.

*2.5.3 HPLC analyses*

Four digestive gland samples with high toxicity in mouse bioassay were selected for HPLC analysis (Table 1). Each sample was divided into two sub-samples. One sub-sample was extracted in 0.1 N HCl according to the AOAC extraction procedure as described in 2.5.2, and another was extracted in 1% (v/v) acetic acid solution according to Lawrence et al. (2004). Toxin contents and profiles were determined using the post-column oxidation method (Oshima, 1995) modified by Anderson et al. (1996). The HPLC system used was an Agilent Series 1100, fitted with an autosampler and fluorescence detector (Agilent, USA). Three separate isocratic elutions were employed to separate different sets of PSP toxins at a flow rate of 0.8 mL/min: (1) 2 mM tetrabutyl ammonium phosphate solution adjusted to pH 6.0 with acetic acid for C toxins; (2) 2 mM 1- heptanesulphonic acid in 10 mM ammonium phosphate buffer (pH 7.1) for GTX and dcGTX group; and (3) 2 mM 1-heptanesulphonic acid in 30 mM ammonium phosphate buffer (pH 7.1): acetonitrile (100:5) for the STX group. Toxin standards, GTX1-5, neoSTX, STX, dcSTX, dcGTX2/3 and C1, C2, were purchased from the National Research Council of Canada, Institute for Marine Biosciences (Halifax,

Canada). The standards solutions were diluted with 0.03 N acetic acid as required. PSP toxin profiles were calculated from the percentages of individual toxin concentrations of the total PSP toxin concentrations for each sample (mol %). The relative toxicities (μg STX equiv./kg) were calculated based on the specific toxicities given by Oshima (1995).

## 3 Results

### *3.1 Environmental conditions*

Water temperature ranged from 14.0 to 32.8 °C, with the lowest recorded from December to early March and the highest from July to November. Salinity was relatively stable with an annual mean value of 29.3, except for the rainy season from April to May and from July to September.

Nutrient concentrations showed similar seasonal changes at the two stations. Concentrations of DIN ranged from 2.04 μmol/L to 14.94 μmol/L, and showed an annual cycle characterized by higher values during spring and summer and lower values during late autumn and winter. Concentrations of DIP remained consistently low, and ranged from 0.044 μmol/L to 1.67 μmol/L. DSi concentrations were remarkably high during the flood season between July and September, and low in winter and the early spring dry season. Annual mean values in DIN and DIP showed no significant differences between the two stations; however, DSi level was obviously higher at St. 1 than St. 2, with mean concentrations of 17.8 μmol/L and 12.2 μmol/L, respectively.

### *3.2 Abundance of* A. tamarense *vegetative cells*

The vegetative cells of *A. tamarense* occurred sporadically in the water column and showed a regular seasonal cycle. Vegetative cells only occurred during spring (March to May) and winter (November to February) (Fig. 2). The abundance during spring was low, ranging from 0.2 to 1.4 cells/mL. The vegetative cells were not detected during summer and autumn (Fig. 2) when water temperature exceeded 25 °C. Cell numbers increased at the end of November with decreasing water temperature and peaked in January and February, when water temperatures were around 20 °C. Cell numbers were markedly higher in St. 1 than in St.

2, and the maximum cell number of 603 cells/mL was observed in St. 1 on February 19, 2006. Maximum number coincided with low water temperature and low nutrient concentrations during the winter months.

The phytoplankton assemblages were dominated by diatoms, which constituted 93.2% of total phytoplankton population. Dinoflagellates occurred in low numbers with a mean abundance of 9.4 cells/mL, and represented only 1.2% of total abundance. The abundance of total phytoplankton was 3 920 cells/mL when the maximum cell number of *A. tamarense* occurred. The most dominant species was *Skeletonema costatum* at that time, with a cell density of 3 230 cells/mL and proportion of 82.5%. *A. tamarense* accounted for 98.3% of dinoflagellate, but only 15.3% of total phytoplankton.

*3.3 Cyst flux of A. tamarense*

Cysts of *A. tamarense* occurred frequently in the sediment traps, but in low numbers (mostly <5 cysts/cm$^2$ d). Cysts appeared to have a seasonal cycle similar to that of the vegetative population and occurred more abundantly during spring and winter (Fig. 2, Fig. 3). High cyst fluxes were observed approximately two weeks to one month after the vegetative cell peaks. For example at St. 1, there was a significant increase in cyst flux in January 2006 just following vegetative cell peaks in December and January, and the maximum flux of 42.6 cysts/cm$^2$ d was observed on February 11-26, 2006, right after the maximum number of vegetative cells. Meanwhile, a strong positive correlation existed between cyst flux and the cumulative number of vegetative cell (Cysts = $6.64 \times 10^{-5} \times$ Cells + 0.77, $r$= 0.995, n = 27, $p$<0.0001).

*3.4 PSP toxins in shellfish*

The extracts of shellfish tissues in the present study killed mice with typical symptoms associated with PSP toxins. Seasonal variations in toxin concentration are illustrated in Fig. 4. The PSP toxins in the two stations were generally below the regulatory limit, except for the winter months. The concentrations of PSP toxins were lower in spring and early summer, and PSP toxins were not detected between August and October. Toxin concentrations increased significantly in January, and a maximum level of 14 015 μg STX equiv./kg was recorded in *P.*

*viridis* samples collected from St. 1 on January 3, 2006 (Fig. 4). The majority of toxins were detected in digestive glands, with the maximum of 66 227 μg STX equiv./kg in digestive glands from *C. nobilis* collected at St. 1 on January 3, 2006 (Fig. 5). Toxins in other tissues were very low to undetectable except for five samples collected in January 2006, with a maximum level of 392 μg STX equiv./kg. The results showed that the hepatopancreatic tissues from both *P. viridis* and *C. nobilis* were unsafe for human consumption during the winter months, while the tail muscle would be safe.

Toxin levels in digestive glands from *C. nobilis* (mean value of 3 373 μg STX equiv./kg) were significantly higher than those from *P. viridis* (mean value of 1 531 μg STX equiv./kg). However, the contribution of digestive gland to total tissues is lower in *C. nobilis* than in *P. viridis*. Therefore, toxin concentrations in total tissues of the two shellfish were similar, with mean values of 399 and 394 μg STX equiv./kg in *C. nobilis* and *P. viridis*, respectively.

The sharp increase in concentrations of PSP toxins in January was coincident with the occurrence of *Alexandrium* population (both vegetative cells and cysts), with peak concentrations or abundances between January and March. PSP concentrations were clearly higher in St. 1 than in St. 2, and the averages in digestive glands and total tissues were 3 796 and 632 μg STX equiv./kg in St. 1 and 1 107 and 161 μg STX equiv./kg in St. 2, respectively.

*3.5 PSP toxin profiles*

Fig. 6 illustrates the toxin profiles in four digestive gland samples treated by HCl and acetic acid, respectively. Of the 12 components analyzed in this study, GTX5 and dcGTX2 were not detected in any of the samples, and only trace amounts of decarbamoyl toxins were detected with a mean proportion of 2.2%. The PSP toxin profiles in HCl extracts were rich in carbamate toxins (mean 63.7% of the total) and dominated by GTX2 (mean 35.1%), GTX3 (mean 26.2%) and GTX4 (mean 15.7%). C toxins accounted for a medium amount of the total (around 0-12% for C1, 1–7% for C2). In addition to these components, a trace amount of decarbamoyl toxins (mean 3.3%) was detected. In contrast, in acetic acid extracts, large amounts of C toxins were detected instead, and C1 (around 38-68% of the total, mean 53.6%) was the most abundant. On the other hand, concentrations of GTX toxins dropped

significantly and accounted for 4-31% of the total, with an average of 17.8%. The relative concentrations of STX toxins (including STX, dcSTX and neoSTX) in acetic acid extracts (mean 22.1%) were similar to those in HCl extracts (mean 18.4%); however, percentages of STX increased from 6.7% in HCl extracts to 13.9% in acetic acid extracts on average.

Though samples collected from St. 1 on 3 January 2006 were most toxic during study period (Fig. 4, 5), the toxin profiles (sample 1 and 1') showed less toxic GTX2 and C1 (Fig. 6). Other less toxic samples (samples 2-4, and 2'-4') had higher proportions of more toxic components such as STX, neoSTX and GTX4. However, toxin concentrations on a molar basis (μmol/kg) in sample 1 and 1' were 5-8-fold greater than those in other samples (Table 1).

Toxin concentrations on a molar basis (μmol/kg) were comparable between any two parallel samples treated by HCl or acetic acid (Table 1). The toxicities in HCl extracts approximately coincided with those analyzed by mouse assay. However, samples treated by acetic acid showed only one-third to two-thirds of those treated by HCl, especially in samples with rich contents of C1 toxin (Table. 1, Fig. 6).

## 4 Discussion

High abundance of *A. tamarense* vegetative cells was observed in this study, with a maximum number of 603 cells/mL. In previous phytoplankton studies, vegetative cells of *Alexandrium* were seldom observed in the water column during seasonal routine observation due to the long interval of sampling (Liu et al., 2006). However, peak abundance of *A. tamarense* was captured in this survey by highly frequent sampling (once every three days).

*Alexandrium tamarense* showed a high dispersal of temperature/cell density couples (Fig. 7). It never occurred at temperatures greater than 30 °C, and peak abundances occurred when temperature lies between 16 and 20 °C. *A. tamarense* is a stenohaline alga (ca. 15-25 °C) with optimal growth at 17-22 °C (Yan et al., 2002). The blooms of *Alexandrium* usually occurred in temperatures between 13 and 18 °C (Giacobbe et al., 1996; Gracéc et al., 2004). After the operation of DNPS, the annual mean water temperature increased by 0.4-1.1 °C (Tang et al.,

2003), and the variation in temperature was more significant in winter months, increasing from 16.04 °C to 18.03 °C (Wang et al., 2008), which seem to favor the growth of *Alexandrium*. Meanwhile during this period, nutrient loadings had increased significantly in the bay (Wang et al., 2008), facilitating the rapid growth of phytoplankton, especially the small diatoms with high growth rates (Liu et al., 2006; Wang et al., 2009). The phytoplankton abundances peaked in spring and summer during the early 1990s (Zhou et al., 1998), but frequent winter peaks have been observed since the late 1990s (Liu et al., 2006; Wang et al., 2009). Generally, Daya Bay is characterized by high levels and rapid compensation of nutrients, however, temporary shortage of nutrients occurs when the diatom bloom exhausts the nutrients, particularly during the dry low-nutrient winter season (Wang et al., 2009). Dinoflagellates such as *Alexandrium* are more competitive in nutrient depletion conditions due to its higher nutrient affinities and capability to utilize organic nutrients (Collos et al., 2007). As a result of higher winter temperatures and increased nutrient loading, *Alexandrium* has occurred more frequently and abundantly during winter months, which contributes to the high PSP concentrations in shellfish.

Cyst fluxes were recorded during summer and autumn (June to October), when no vegetative cells of *A. tamarense* were observed in the water column. The absence of vegetative cells in the subsurface water column during the observation does not necessarily indicate their absence in the entire water column. More likely, the water sample collection did not catch *A. tamarense* cell patches due to random sampling. In addition, this period is also the typhoon season in South China Sea. Strong storms may cause a high resuspension rate, which could result in redeposition of bottom cysts into sediment traps.

Similar to the total phytoplankton abundance, cell density and cyst flux of *A. tamarense* were clearly higher in St. 1, located in a shellfishery area, than those in St. 2, located in a fish cage area. This resulted in higher PSP levels in shellfish collected from St. 1. The low primary production in the fishery area was observed in another study in this area as well (Huang et al., 2005). Meanwhile, higher concentrations of *Alexandrium* cyst were recorded in surface sediments from the shellfishery area in the same sea area (Wang et al., 2004). This

phenomenon may be interpreted as compensatory growth of phytoplankton against predatory pressure by shellfish.

Daya Bay has been reported to have the highest PSP concentrations among the Chinese coastal areas (Lin et al., 1999; Yang et al., 2005). Jiang et al. (2000a) found that the highest concentration of PSP toxins occurred in January during their survey conducted between 1997 and 1999. However, *Alexandrium* was seldom observed in previous phytoplankton surveys (Liu et al., 2006). Our results showed that high cell numbers and cyst fluxes of *Alexandrium* coincided with high PSP levels in shellfish. In addition, content of PSP toxins showed a strong positive relationship with the accumulative abundances of vegetative cells and cysts [$\ln(PSP) = 0.41 \times \ln(Cells) + 2.34$, $r = 0.647$, n = 27, $p = 0.002$; $\ln(PSP) = 0.37 \times Cysts + 5.22$, $r = 0.373$, n = 27, $p = 0.04$]. These results suggest that vegetative cells and cysts of *Alexandrium* influenced significantly the PSP levels, and could have contributed to high PSP levels in shellfish during winter.

However, toxin levels were not always consistent with concentrations of vegetative cells and cysts. The inconsistence of PSP contamination in shellfish and concentration of *Alexandrium* cells and cysts might be explained by the variability of *Alexandrium* toxicity, which depends on environmental conditions, among species and strains (Anderson, 1990). Toxicities in *Alexandrium* vegetative cells and cysts seem to be species and strain specific. The average toxicity of *A. tamarense* vegetative cells is approximately 16 pg STX equiv./cell (Persson et al., 2006). Oshima et al. (1992) reported as much as 269 pg STX equiv./cyst in *A. tamarense*, and Bricelj and Shumway (1998) reported 325 pg STX equiv./cell of an *Alexandrium* sp. Beppu et al. (2008) measured the toxicities of six trains of *Alexandrium* from the coastal water of Hiroshima Prefecture, Japan, and found that variation in the toxin level was varied about 30 fold among strains (from 3.7 to $111.5\times10^{-6}$ MU/cell), or 10 fold even between *A. tamarense* strains isolated from the same sea area in the same month of different years.

The toxin profiles of HCl treated samples were similar to those reported by Jiang et al. (2000b) in the same sea area, in which GTX toxins were found to be the main component.

GTX toxins were dominant in contaminated shellfish as determined by the AOAC HCl extraction method in other geographic regions (Lassus et al., 1989; Ichimi et al., 2001; Abouabdellah et al., 2008). Toxin profiles of acetic acid extracts showed good similarity with those of *Alexandrium* strains isolated from the nearby sea areas (Jiang et al., 2000b; Wang et al., 2006), which were predominated by C1/C2 toxins and had only trace amounts of STX toxins. The results suggested PSP toxins in filter-shellfish detected in this study were possibly produced by a local strain. It is well known that the AOAC extraction procedure used in the sample preparation converts the sulphocarbamoyl toxins (C1–4, B1, B2) into corresponding carbamates (Laycock et al., 1995; Anderson et al., 1996), i.e., C3,4 to GTX1,4 and C1,2 to GTX2,3 (Turrell et al., 2007). Our results also showed evident toxin conversion through the HCl procedure, as displayed by the remarkable increase of GTX2, 3 and sharp decrease of C1, and meanwhile a comparatively stable overall toxin concentrations on molar basis (mol/kg). The carbamates are considerably more toxic than the corresponding sulphocarbamoyls, and, consequently, changes in toxin profile after HCl treatment will increase the toxicity of the shellfish extract (Kao, 1993). In our study, toxicities of HCl extracts were 1.5-3.5-fold higher than those of corresponding acetic acid extracts. Despite the increase toxicity through HCl procedure, we suggest using HCl extracts in the mouse bioassay. This procedure better protects human health by considering the potential conversion of C toxins into more poisonous GTX toxins in human body.

## 5 Conclusion

Our one-year observation found that high PSP toxin levels in shellfish coincided with peak abundances of cell and cyst of *A. tamarense* during winter months, though the toxin levels were generally below the regulatory limit for most seasons of the year. The significant, positive relationship between toxin level, and vegetative cell and cyst abundance reveals possible toxin source from *Alexandrium* thriving during winter months. More specifically, the majority of the toxins were accumulated in the digestive glands.

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**Tables**

Table 1 PSP toxin contents in digestive gland samples by HPLC analysis and mouse bioassay using the AOAC HCl procedure and acetic acid extraction procedure, respectively

| | 1 | 1’ | 2 | 2’ | 3 | 3’ | 4 | 4’ |
|---|---|---|---|---|---|---|---|---|
| Toxin concentration by HPLC (μmol/kg) | 191.5 | 173.4 | 18.6 | 15.3 | 23.5 | 18.6 | 22.8 | 24.4 |
| Toxicity by HPLC analysis (μg STX equiv./kg) | 41 584 | 11 993 | 4 477 | 1 842 | 5 377 | 3 424 | 6 135 | 2 832 |
| Toxicity by mouse bioassay (μg STX equiv./kg) | 51 565 | - | 6 636 | - | 6 780 | - | 5 551 | - |

1 and 1’: *P. viridis* samples from St. 1 on January 3, 2006, 2 and 2’: *P. viridis* samples from St. 1 on January 22, 2006, 3 and 3’: *P. viridis* samples from St. 1 on February 26, 2006, 4 and 4’: *C. nobilis* samples from St. 1 on March 16, 2006. Samples 1-4 were treated by HCl, samples 1’-4’ were treated by acetic acid. “-” indicates without bioassay data.

**Figure captions**

Fig. 1 Sampling stations in Daya Bay, South China Sea. Also shown is the location of the Daya Bay Nuclear Power Station (DNPS). St. 1 was located at a shellfish cultural area, and St. 2 was at a fish cage area close to a residential area.

Fig. 2 Seasonal changes in cell density of *Alexandrium tamarense*

Fig. 3 Seasonal changes in cyst flux of *Alexandrium tamarense*

Fig. 4 Concentrations of PSP toxins in shellfish

Fig. 5 Concentrations of PSP toxins in digestive glands of shellfish

Fig. 6 PSP toxin profiles in digestive glands from shellfish samples. 1 and 1': *P. viridis* samples from St. 1 on January 3, 2006, 2 and 2': *P. viridis* samples from St. 1 on January 22, 2006, 3 and 3': *P. viridis* samples from St. 1 on February 26, 2006, 4 and 4': *C. nobilis* samples from St. 1 on March 16, 2006. Samples 1-4 were treated by HCl, samples 1'-4' were treated by acetic acid.

Fig. 7 Relationship between cell density of *Alexandrium tamarense* and water temperature.

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**Title:** Source and profile of paralytic shellfish poisoning toxins in shellfish in Daya Bay, South China Sea

**Highlights**

> Annual survey on *Alexandrium* population and PSP was conducted. > *Alexandrium* cells and cysts occurred abundantly during the winter months. > PSP contents in shellfish were generally low, but high in winter. > *Alexandrium* thriving during winter months could be possible toxin source in shellfish.

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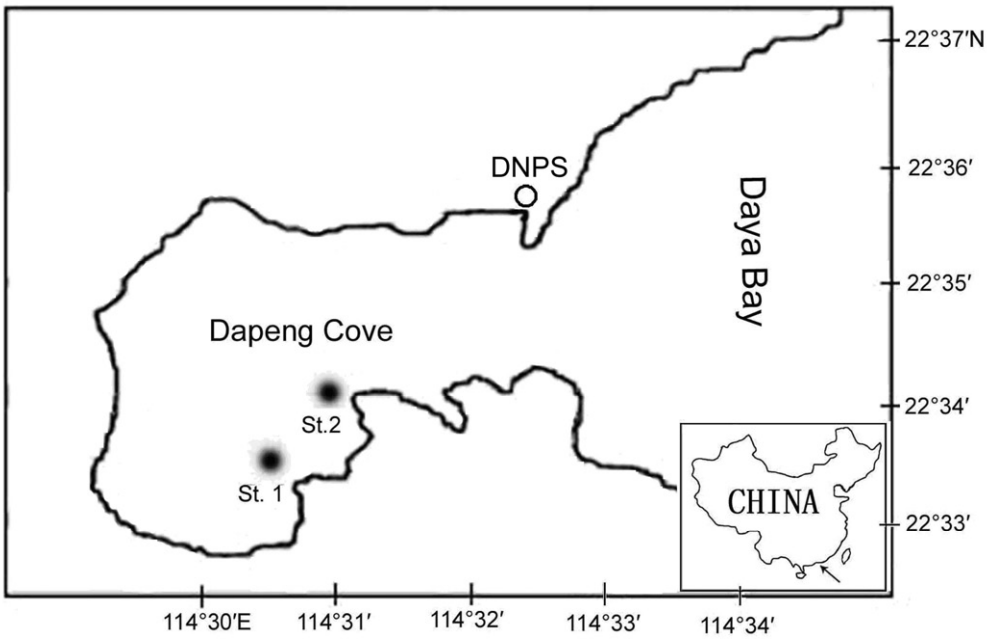


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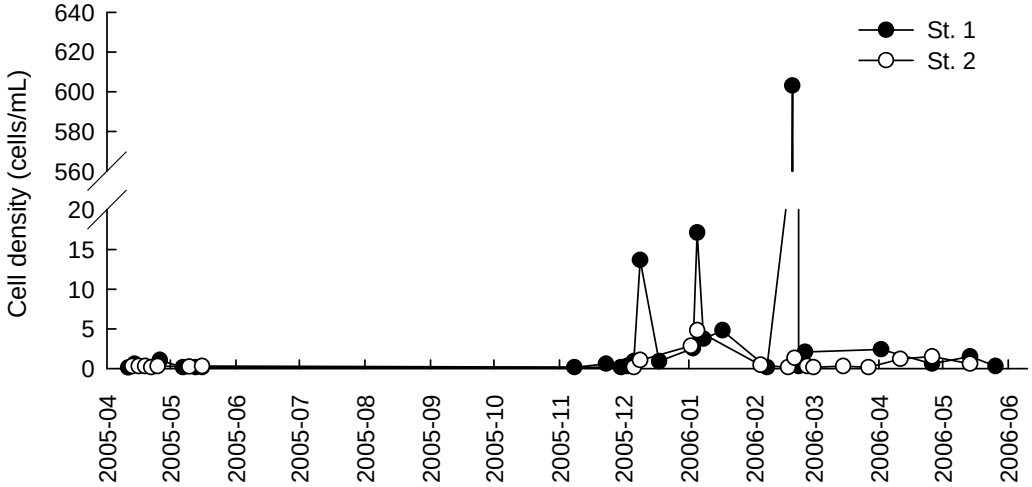


Fig. 2 Seasonal changes in cell density of *Alexandrium tamarense*

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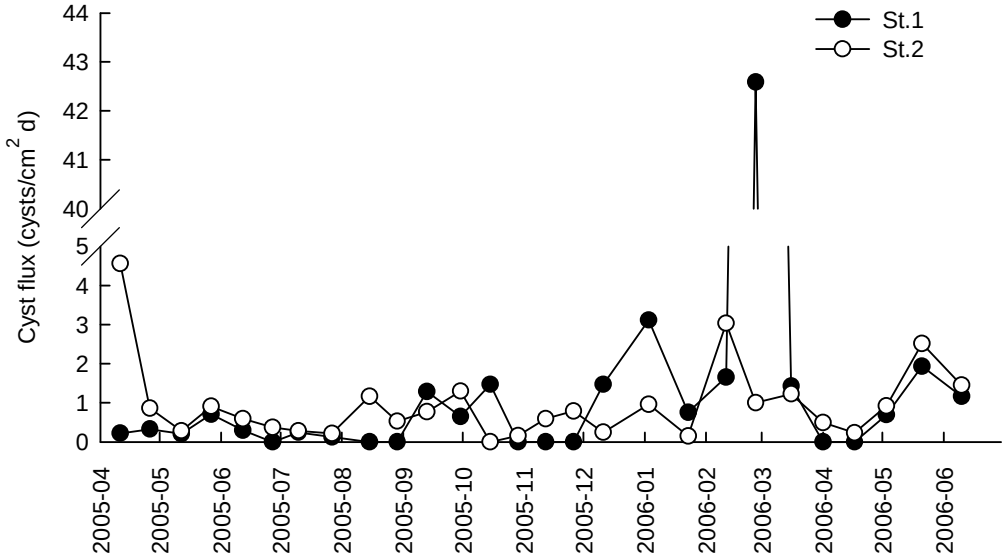


Fig. 3 Seasonal changes in cyst flux of *Alexandrium tamarense*

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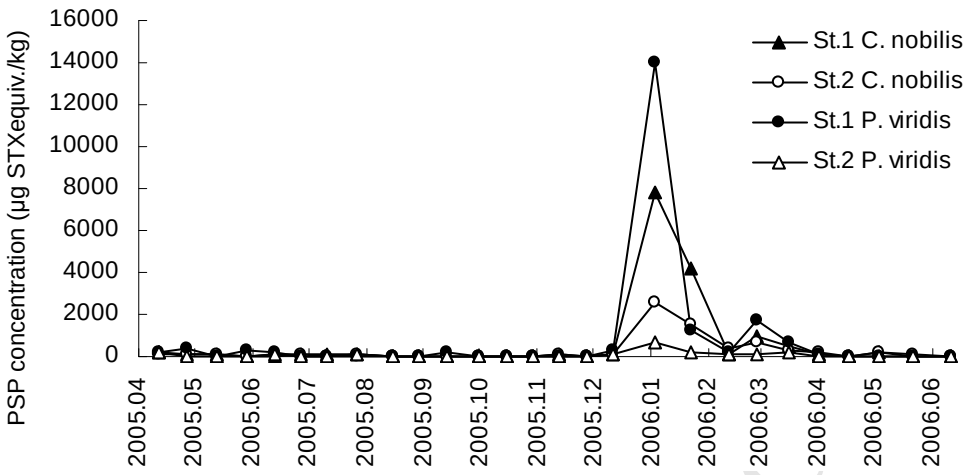


Fig. 4 Concentrations of PSP toxins in shellfish

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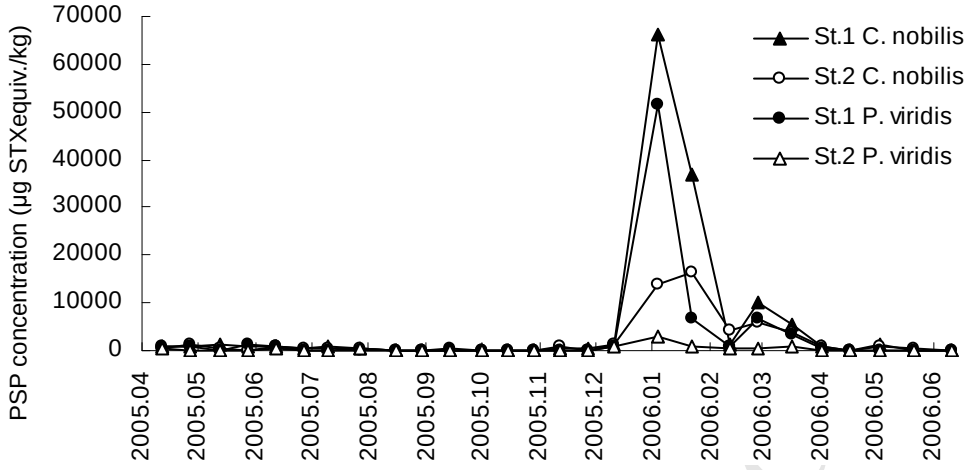


Fig. 5 Concentrations of PSP toxins in digestive glands of shellfish

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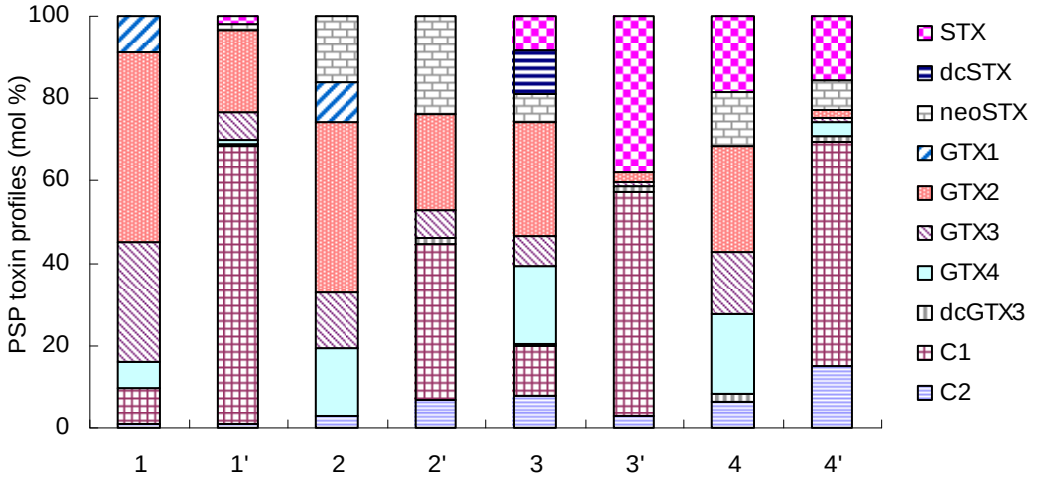


Fig. 6 PSP toxin profiles in digestive glands from shellfish samples. 1 and 1': *P. viridis* samples from St. 1 on January 3, 2006, 2 and 2': *P. viridis* samples from St. 1 on January 22, 2006, 3 and 3': *P. viridis* samples from St. 1 on February 26, 2006, 4 and 4': *C. nobilis* samples from St. 1 on March 16, 2006. Samples 1-4 were treated by HCl, samples 1'-4' were treated by acetic acid.

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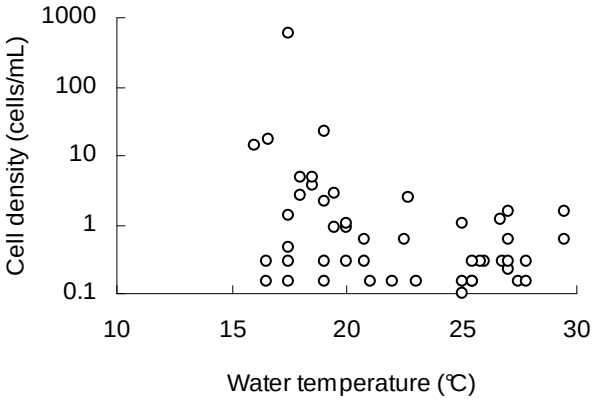


Fig. 7 Relationship between cell density of *Alexandrium tamarense* and water temperature.

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