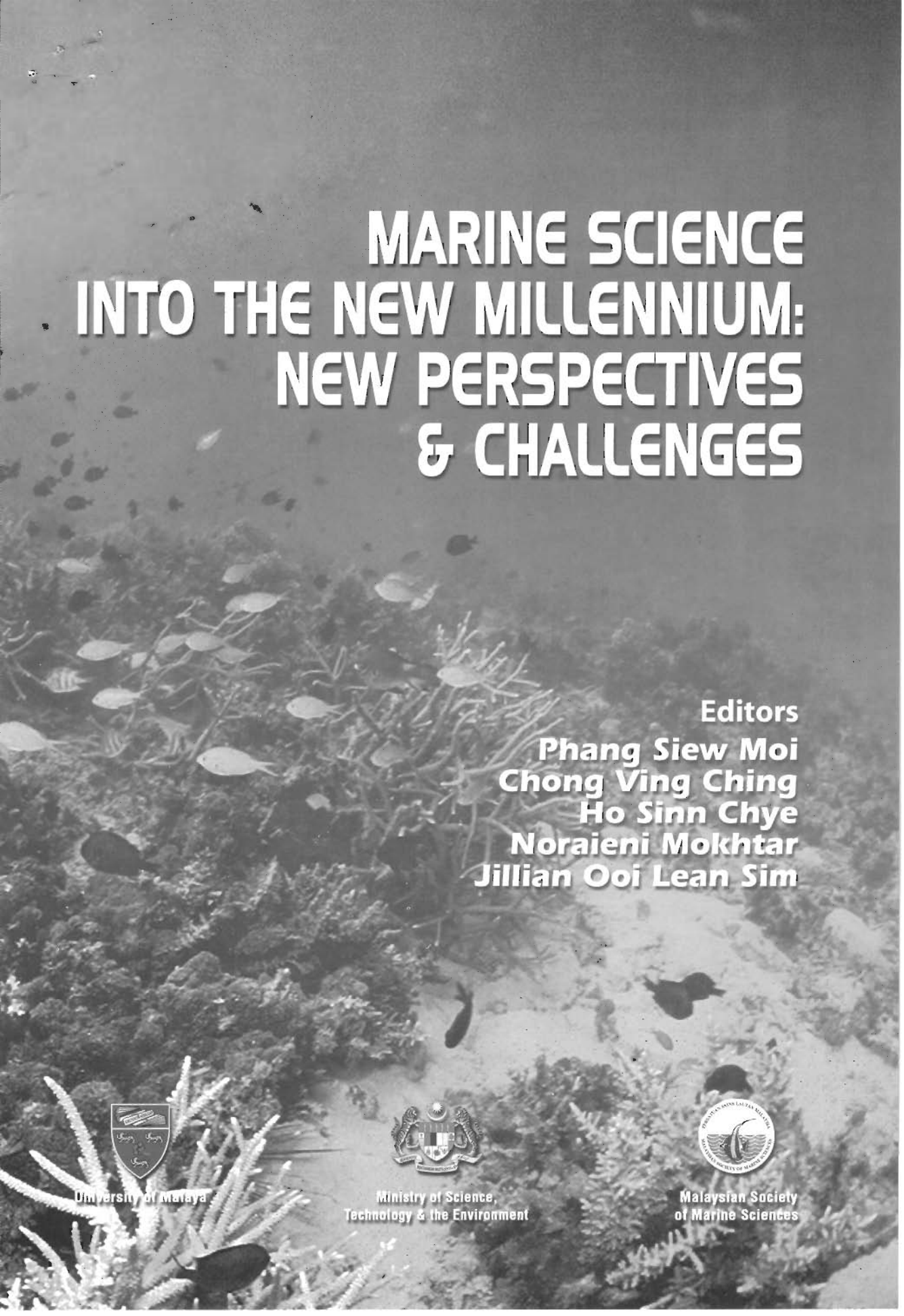

MARINE SCIENCE INTO THE NEW MILLENNIUM: NEW PERSPECTIVES & CHALLENGES

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First incidence of paralytic shellfish poisoning on the east coast of Peninsular Malaysia

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Abstract

The first incidence of paralytic shellfish poisoning (PSP) on the east coast of Peninsular Malaysia was reported in Tumpat, Kelantan. In September, 2001, six persons were hospitalized and one fatality was reported following consumption of contaminated toxic lokan, *Polymesoda* sp. A plankton survey was conducted at the affected area a week after the incident. The area is a semi-enclosed lagoon formed by sand bars at the mouth of Sungai Golok. The lagoon is fringed by *Nipah* palms, water depths ranged from 3 - 6 m and salinity ranged from 21‰ at the mouth to 12‰ in the inner part of the lagoon. Plankton samples were collected using a 20-μm mesh size plankton net from six locations. Lokan samples were also collected from the site where the contaminated shellfish were found. Morphological observation of plankton samples showed that the dinoflagellate, *Alexandrium minutum* was most probably the progenitors of this incident. The results also showed that most of the *Alexandrium* cells were found in the inner part of the lagoon, whereas no *Alexandrium* cell was found in the other five seaward locations. Laboratory cultures of the dinoflagellate have been successfully established. Toxicity testing by mouse bioassays and HPLC confirmed the presence of PSP toxins in extracts from the lokan and the cultured *Alexandrium* cells.

Introduction

Harmful algal blooms (HABs) and paralytic shellfish poisonings (PSPs) are not new phenomena in Malaysia. Blooms of the toxic dinoflagellate *Pyrodiium bahamense* var. *compessum* occur almost annually on the west coast of Sabah. Actual figures are difficult to ascertain, these event may have caused more than a hundred poisonings including some twenty fatal cases. Since the 1990s, however, poisonings due to *P. bahamense* have been very rare due to an efficient shellfish toxicity monitoring programme and increased public awareness of the problem.

Until 1990, PSPs in Malaysia were considered problems unique to the state of Sabah. In 1991, however, three people were taken ill after consuming green mussels from a recently established mussel farm in Sebatu in the Straits of Malacca on the west coast of Peninsular Malaysia. For several years the toxin producer was not known, but there is now strong evidence to suggest that it is the dinoflagellate *Alexandrium tamiavanichi.*

The latest in the development of PSP in Malaysia occurred in September, 2001. Six people were taken ill, including one fatality, after consuming the benthic clam (lokan) *Polymesoda* sp. collected from a coastal lagoon on the east coast of Peninsular Malaysia. The victims also displayed symptoms of PSP. Here we report on the species most likely responsible for the PSP events at Kelantan as well as some toxicity data obtained from analyses of toxic shellfish and culture dinoflagellate originating from the location.

Materials and Methods

Plankton samples were collected from Sungai Geting, Tumpat Kelantan at six sampling sites shown in **Fig. 1**. Sampling was carried out with a 20-µm mesh-size plankton net. One set of samples was immediately preserved in neutral Lugol's iodine solution and another set was brought back to the laboratory unpreserved.

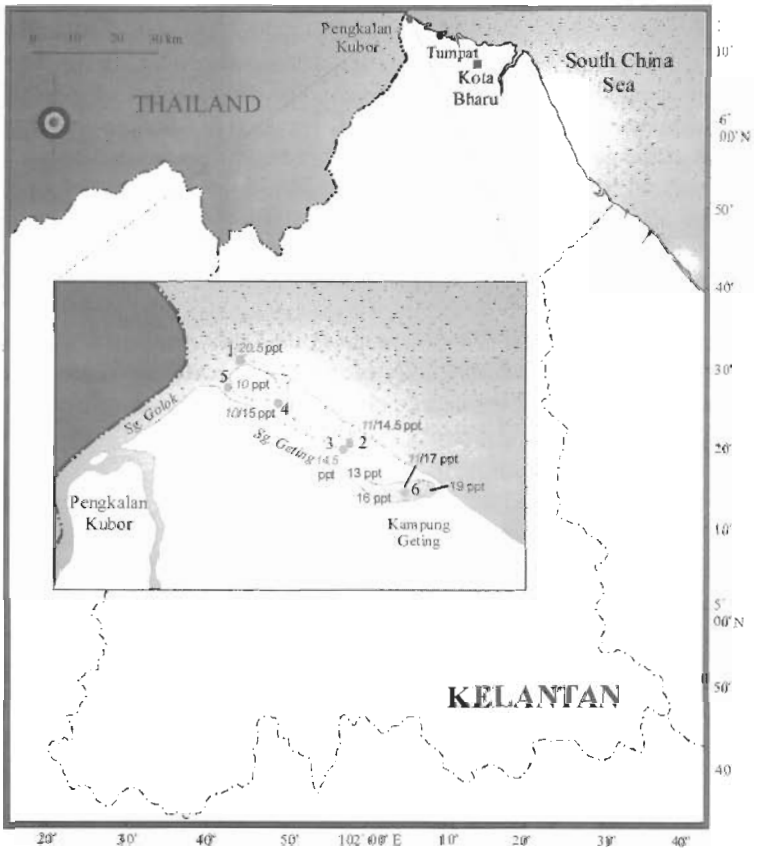

Fig. 1. Location of Sungai Geting Tumpat and the sampling sites. Salinity in the lagoon during low tide (italised) and high tide at each sampling site.

The plankton samples were examined on an Olympus BX51 microscope fitted with a Soft Imaging System cooled CCD camera and software set (SiS GmbH, Germany). For detailed examination of theca morphology, the cells were stained with 1% calcofluor white solution and viewed under epifluorescence.

Six clonal cultures of *A. minutum* were developed from water samples taken at the lagoon. The isolates were maintained in ES-DK medium (Kokinos and Anderson, 1995) at 26°C under a 14:10 hrs light:dark photoperiod.

Clam samples were collected from the area and extracted for PSP toxins according to the AOAC protocol. The shellfish extracts were further purified using ISOLUTE C18 SPE columns. Dinoflagellate cultures were harvested by centrifugation. The toxins were extracted by adding 0.05 M acetic acid to the cell pellet. Toxin extracts were analysed by HPLC using isocratic elution method (Oshima *et al.*, 1989).

Results

The coastal lagoon, locally known as Sungai Geting, is located in the state of Kelantan on the northeast coast of Peninsular Malaysia (see **Fig. 1**). It is situated at the mouth of the Golok River which forms the border between Malaysia and Thailand to the north. The lagoon runs parallel to the coast and was most probably formed by deposition of sandbanks near the mouth of the river. At present the only connection with the South China Sea is through the river mouth. The lagoon is shallow, ca. 6 m maximum depth. It is fringed by mangroves and *nipah* palms. Salinity in the lagoon at high tide is ca. 20‰. Close to its mouth there are several cages of reared sea perch *L. calcarifer*. The mudflats exposed during low tides are popular shellfish collecting areas for the local fisher folks.

Cell of *Alexandrium minutum* Halim (**Fig. 2**) are subspherical and oval in shape. The most distinct theca plates that differentiate *A. minutum* from other species of *Alexandrium* are the posterior sulcal plate (s.p.) and the sixth precingular plate (6"). The s.p. is wide and almost symmetrical whereas the 6" is narrow (**Fig. 2**).

Extracts of the culture cells killed mice with typical symptoms of PSP. For both shellfish and culture samples, four toxin components of gonyautoxins (GTX1, 4 and GTX 2, 3) were detected by HPLC, no GTX 5, 6 were detectable. Among the four GTXs detected, GTX 1, 4 are the most abundant GTXs in culture samples whereas in shellfish samples GTX 1, 4 was about as abundant as GTX 2, 3 (**Fig. 3**). Minor or no proportion of STX was detected in both shellfish and dinoflagellate culture samples but there was very high proportion of neoSTX in both samples.

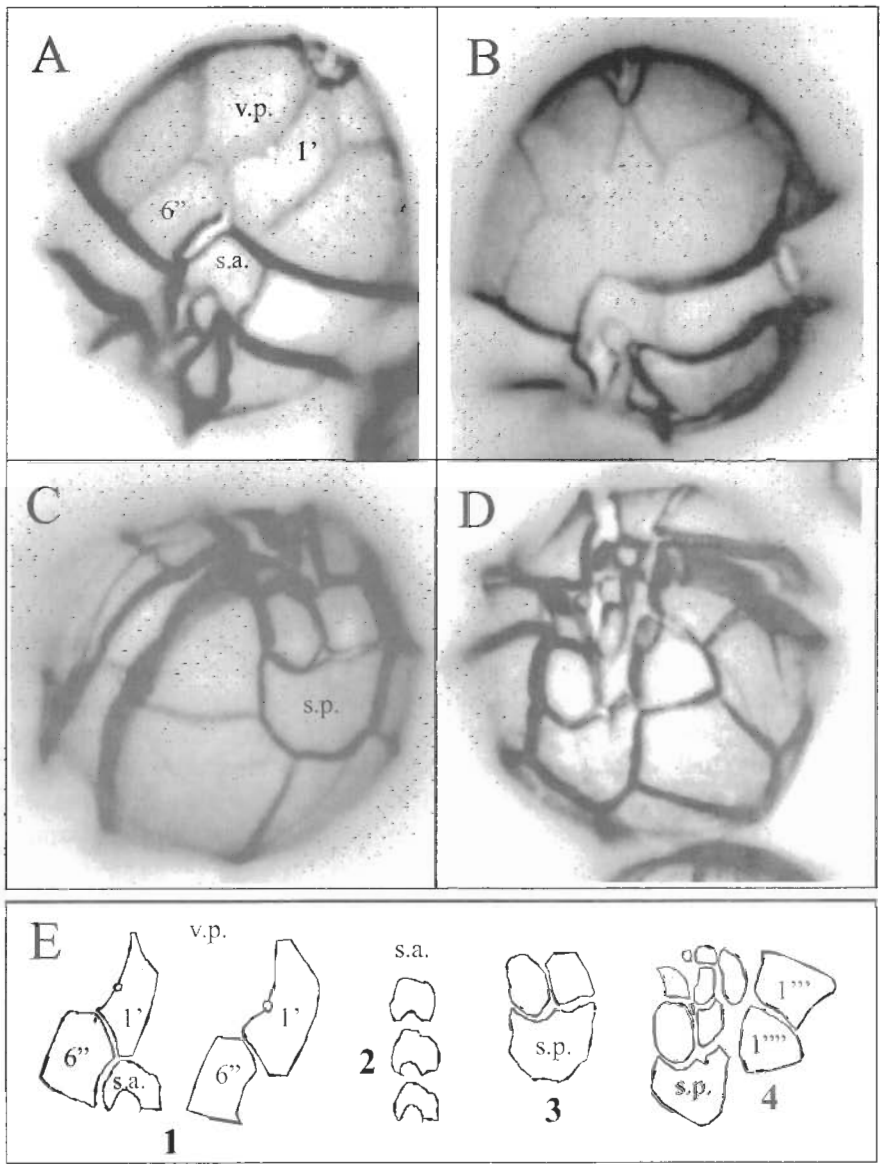

Fig. 2. Epifluorescence micrographs of *Alexandrium minutum* vegetative cells. (A, B) Ventral view shows first apical plate (1') with ventral pore (v.p.). The sixth precingular plate (6") is narrow. Anteriar sulcal plate (s.a.) with typical minutum shape. (C, D) Antapical view of cells. The posterior sulcal plate (s.p.) is wide and symetrical. (E) Diagram of plates tabulation of *A. minutum* from Tumpat with details of 1', vp., s.a. and s.p.

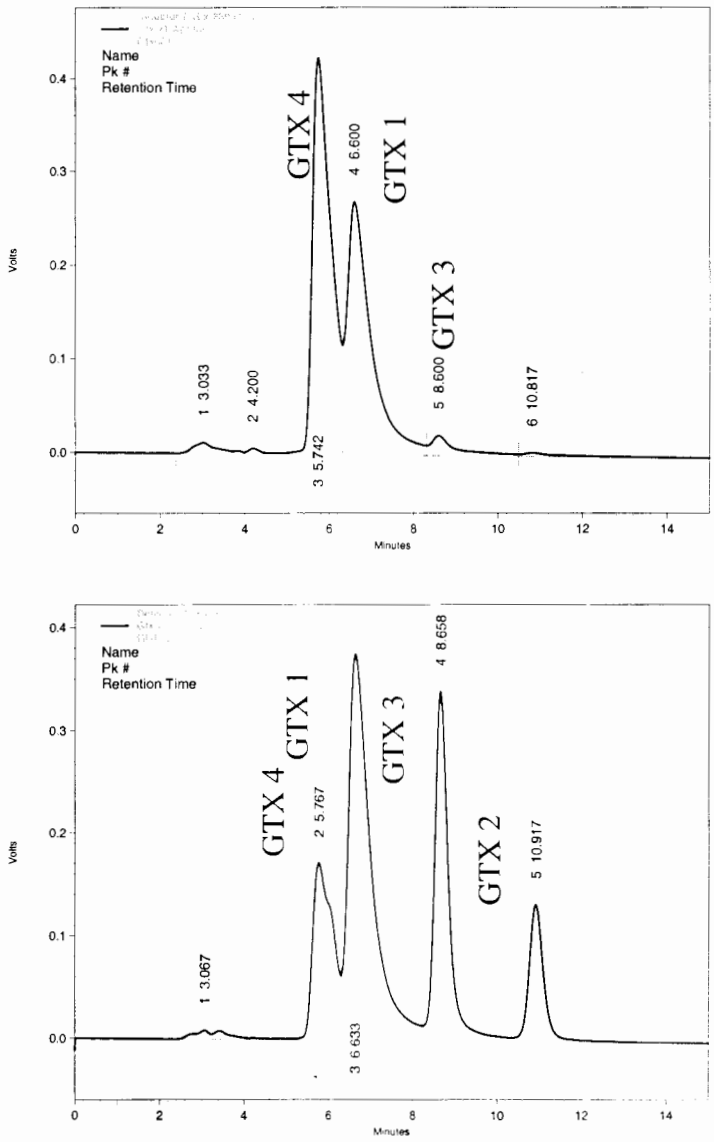

Fig. 3. HPLC chromatogram of GTXs from culture sample (A) and shellfish extract (B).

Discussion

During the September 2001 toxicity event in Sungai Geting, the most abundance dinoflagellate species found in this area is *Alexandrium* spp. They were found in the inner part of the lagoon (Sites 2 and 6, **Fig. 1**). Occurrence of various species was unexpectedly patchy for a relatively small body of water. *Dinophysis* spp. was also found at the same time at the mouth of the lagoon (Site 1) but no toxicity of DSP was tested.

Observation and enumeration of plankton and culture samples with calcoflour staining confirm the species as *Alexandrium minutum* Halim. The species was distinguished from other *Alexandrium* species by the wide posterior sulcal plate and narrow sixth precingular plate. The species was found at very low salinity (i.e. 12‰) and can tolerate very high salinity (up to 30‰, data not shown).

Toxins analysis with HPLC on culture samples and clam extracts confirmed the occurrence of PSP in this area. Four toxin components of gonyautoxins (GTX 1, 4 and GTX 2, 3) were detected in shellfish extracts whereas dinoflgellate culture samples contained only two components of GTXs (GTX 1, 4). No peaks were identical to GTX 5, 6 in both samples. Among the four GTXs detected, GTX 1, 4 were the most abundant in culture samples whereas in shellfish samples GTX 1, 4 were about as abundant as GTX 2, 3. Although the toxin profile of culture samples was different from the shellfish extracts, GTX 1, 4 could be transformed to GTX 2, 3. Kotaki *et al.* (1985) reported that a marine bacterium *Pseudomonas* sp. could significantly transform GTX 1 to GTX 2 and 3. Thus, toxins analysis reaffirmed the finding of the plankton analysis that *A. minutum* was the main and sole causative species responsible for the PSP event and casualty in this area.

Toxin composition of *A. minutum* isolates was similar to *A. minutum* isolated from Taiwan (Hwang and Lu, 2000). Surprisingly both isolates can tolerate very low salinity. Hwang and Lu (2000) reported that the best growth condition for Taiwan isolates was at 15‰.

Although the occurrence of various potentially toxic dinoflagellates species (i.e. *Dinophysis* spp.) were found at the same area, possibility of poisoning caused by these dinoflagellates species was dismissed. This is due to the fact that *Dinophysis* spp. which potentially caused dirrheatic shellfish poisoning (DSP) never caused fatality in the past. In addition, they are found relatively far from the area where the toxic lokan has been collected by the victims.

Conclusion

This is the first report of toxic dinoflagellate, *Alexandrium minutum* found in Malaysia. Toxicity of this species was analysed and confirmed responsible for the PSP event in Tumpat, Kelantan. This increased the number of PSP causative species in Malaysian waters to three species (*Pyrodinium bahamense* var. *compressum, Alexandrium tamiyavanichi* and *Alexandrium minutum*).

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