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PROBABLE TOXIN PRODUCER RESPONSIBLE FOR THE FIRST OCCURRENCE OF PARALYTIC SHELLFISH POISONING ON THE EAST COAST OF PENINSULA MALAYSIA

USUP, G.[1]*, PIN, L.C.[1], TEEN, L.P.[2] and AHMAD, A.[3]

[1]Program Sains Laut
[3]Program Biosains
Fakulti Sains dan Teknologi
Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

[2]Fakulti Sains dan Teknologi Sumber
Universiti Malaysia Sarawak
94300 Kota Samarahan, Sarawak, Malaysia

ABSTRACT

A study was carried out to determine the presence of potentially toxic dinoflagellate species in the Sungai Geting coastal lagoon in Tumpat, Kelantan. This followed the occurrence of paralytic shellfish poisoning (PSP) in the area in September 2001. Only 12 dinoflagellate species were found in net plankton samples collected from the lagoon during the week of the event. Of these, four were potentially toxic, namely *Dinophysis caudata, D. rotundata, D. miles*, and *Alexandrium minutum*. Among these species only *A. minutum* is known to produce PSP toxins, and the absence of other potential toxin producers strongly suggests that the species was responsible for the PSP event. The toxicity of the *A. minutum* clones established in laboratory cultures was subsequently proven. This is the first record of the presence of this toxic species in Malaysian waters. The presence of this species and the occurrence of PSP warrants the setting up of a shellfish toxicity monitoring program to cover the northeast coast of Peninsula Malaysia.

ABSTRAK

Satu kajian telah dijalankan untuk mengesan kehadiran spesies dinoflagelat yang mungkin beracun di lagun air payau Sungai Geting di Tumpat Kelantan. Ini berikutan kejadian keracunan kerangan yang melumpuhkan (PSP) di kawasan tersebut dalam bulan September 2001. Cuma 12 spesies dinoflagelat telah ditemui dalam sampel plankton yang diambil dari lagun berkenaan dalam minggu kejadian kes PSP. Empat dari spesies yang dikesan berpotensi menghasilkan toksin, iaitu *Dinophysis caudata, D. rotundata, D. miles*, dan *Alexandrium minutum*. Antara spesies tersebut hanya *A. minutum* diketahui boleh menghasilkan toksin PSP. Ketiadaan spesies penghasil toksin yang lain mencadangkan *A. minutum* bertanggung jawab ke atas kejadian PSP tersebut. Selanjutnya, ketoksikan klon *A. minutum* yang dikultur di makmal telah dapat dibuktikan. Ini adalah rekod pertama kehadiran spesies toksik ini di perairan Malaysia. Kehadiran spesies ini dan kejadian PSP menunjukkan perlunya diadakan satu program pemonitoran keracunan kerangan untuk kawasan perairan timur laut Semenanjung Malaysia.

Key words: *Alexandrium minutum*; Dinoflagellates; Harmful algal blooms; Malaysia; Paralytic shellfish poisoning

INTRODUCTION

Malaysia is one of many countries affected by harmful algal blooms (HABs) and associated seafood poisoning. Currently, paralytic shellfish poisoning (PSP) is the only HAB related shellfish poisoning that has been documented in the country. Until 1990, PSP was confined to the west coast of Sabah, where the dinoflagellate *Pyrodinium bahamense* Plate var. *compressum* Böhm form blooms almost annually. This species has long been considered the most important PSP toxin-producing

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species in southeast Asia (Malaysia and the Philippines) and along the Pacific coastline of central America (Usup & Azanza, 1998). In Malaysia alone *P. bahamense* has caused many poisoning events including several fatalities (Usup *et al.*, 1989).

In early 1991 PSP occurred for the first time outside Sabah. Three people were poisoned after consuming mussel collected from a mussel farm in Sebatu in the Straits of Malacca. Naturally *P. bahamense* was suspected to be the toxin producer, but to date the species has never been found in plankton samples collected from several locations in the Straits of Malacca. The toxic species was eventually shown to be *Alexandrium tamiyavanichii* (Usup *et al.*, 2002). In the most recent event in September 2001, six people were poisoned, including one fatally, after consuming the benthic clam *Polymesoda* sp. collected from a coastal lagoon in Tumpat on the northeast coast of Peninsula Malaysia. All victims displayed symptoms typical of PSP. Analysis of clam samples collected from the site during the event showed very high levels of PSP toxins (unpublished data). Bivalves are the main vectors for these toxins since their high rates of filtration feeding make them highly efficient accumulators for these toxins (Shumway, 1990). In this study the identity of the probable toxin producer for the PSP event in Sungai Geting was investigated.

MATERIAL AND METHODS

Study site

The toxic clams involved in the PSP event in Tumpat were collected from Sungai Geting in Kelantan. The site is a brackishwater lagoon probably formed by deposition of sandbanks parallel to the coast near the mouth of Sungai Golok that forms the boundary between Malaysia and Thailand. The lagoon is shallow and is fringed by mangroves and nipah palms. Salinity in the lagoon at high tide ranged from 22 $\degree/_{oo}$ at the mouth to 12 $\degree/_{oo}$ at the innermost part. Maximum water depth is ca. 5 meters. Several cage cultures of the sea bass (*Lates calcarifer*) are located near the mouth of the lagoon where the water is deepest. Local inhabitants collect shellfish from intertidal mudflats for personal consumption and also to be sold at markets.

Sampling

Plankton samples were collected from four stations located in the lagoon and two stations in the coastal water near the mouth of Sungai Golok (Fig. 1). Non-quantitative samples were collected during slack high tide by vertical trawling with a 20 μm mesh plankton net. Samples for microscopic examination were preserved in neutral Lugol's iodine solution. Live samples were also brought
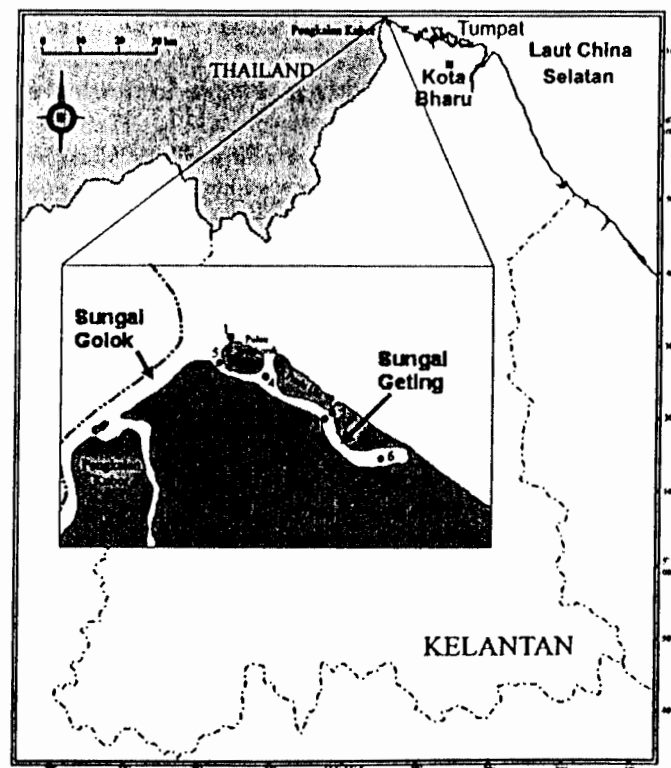

Fig. 1: Sungai Geting lagoon in Tumpat district, Kelantan.
Stations 1 – 6 indicate locations where phytoplankton samples were collected.

back to the laboratory for isolation of cells into cultures.

Species identification

Samples were examined on a microscope fitted with normal, phase contrast, DIC, and epifluorescence lighting. For more detailed examination of dinoflagellate cells, samples were stained with calcofluor white and viewed under epifluorescence lighting with a UV filter set. Images were captured with a cooled CCD camera (SiS Colorview, Germany). Identification of species was based primarily on Steidinger and Tangen (1997) and Balech (1995). Rough estimates of abundance of each species was made based on the number of cells found in each slide. Cells of interest were isolated to start cultures in ES-DK medium (Kokinos & Anderson, 1995).

Toxicity screening

Potential PSP toxin producers were screened for toxicity by the receptor binding assay. Protocols for the assay were as previously described (Usup *et al.*, 2000). Tritium-labeled saxitoxin standard was obtained from Amersham while unlabeled saxitoxin standard was obtained from the NRC, Canada.

RESULTS

Overall, the diversity of dinoflagellate species present was lower than expected (Fig. 2). A total of 12 species were found, of which four could potentially be toxic. Among those, only *Alexandrium minutum* is known to produce PSP toxins. This was confirmed by the receptor binding assay, although the amount of toxin per cell and the toxin profile of the *A. minutum* clones were not determined. *Dinophysis caudata, D. rotundata* and *D. miles* are potential producers of okadaic acid which can cause diarrhetic shellfish poisoning in humans. However, the toxicity of these three species were not determined in this study. There was a certain degree of patchiness in the distribution of certain species within the lagoon and in the coastal water outside the lagoon. *A. minutum* was only found inside the lagoon while the *Dinophysis* spp. were found predominantly at the two outer stations. Detailed morphological description is given for *A. minutum*.

Alexandrium minutum Halim (Fig. 3)

Material examined: Wild and cultured cells originating from Sungai Geting lagoon in Tumpat, northeast coast of Peninsula Malaysia.

Morphology: Cells are roughly oval in shape with a deep cingulum. The posterior sulcal plate is much wider than long, with curved margins. The anterior margin is slightly wavy in appearance. The first apical plate is of medium width with slightly curved left and right margins. The plate touches the apical pore complex (APC) directly. A ventral pore is located midway down the right margin. The sixth precingular plate is narrow and long. The APC is large, with straight right and dorsal margins. The left margin tapers to a point on the ventral end. The apical pore is centrally located. The third apical plate is assymmetrical, with the left anterior margin longer than the right. Other characteristic features are the rhomboidal shape of the left anterior lateral plate (S.s.a.) and the short left posterior lateral plate (S.s.p.) in the sulcus.

Dimensions: length 21 – 26 μm, transdiameter 15 – 20 μm.

Toxicity: Toxic, PSP toxin producer.

Distribution in Malaysia: The species has only been found in samples from the northeast coast of Peninsula Malaysia to date.

DISCUSSION

The presence of HABs and related seafood toxicity imposes a heavy burden on the affected country. An effective seafood toxicity monitoring program needs to be established to safeguard public health and fulfill seafood export requirements. A successful monitoring program depends in a large part on knowing HAB species that are present and locations where toxicity problems are most likely to arise. In Malaysia, monitoring of shellfish toxicity is carried out by the Department of Fisheries.

In this study the focus was only on the identification of dinoflagellate species, based on the fact that only dinoflagellates are known to produce PSP toxins (Levin, 1992). Only one potential PSP toxin producer was found in the samples, that is *A. minutum*. This is a toxic species that has a wide distribution and it has been reported in cold to tropical waters (Oshima *et al.*, 1989; Franco *et al.*, 1994; Hwang *et al.*, 1999). While the toxicity of the Malaysian isolate has been proven by receptor binding assay its detailed toxin profile has yet to be determined. This is an important aspect since it has been suggested that toxin profile could be used as a biogeography marker for this species (Franco *et al.*, 1994; Chang *et al.*, 1997).

The presence of toxic *A. minutum* on the east coast of Peninsula Malaysia raises at least two important points. First is regarding the origin of this population, and secondly is the apparent absence of PSP cases on the east coast of the peninsula until
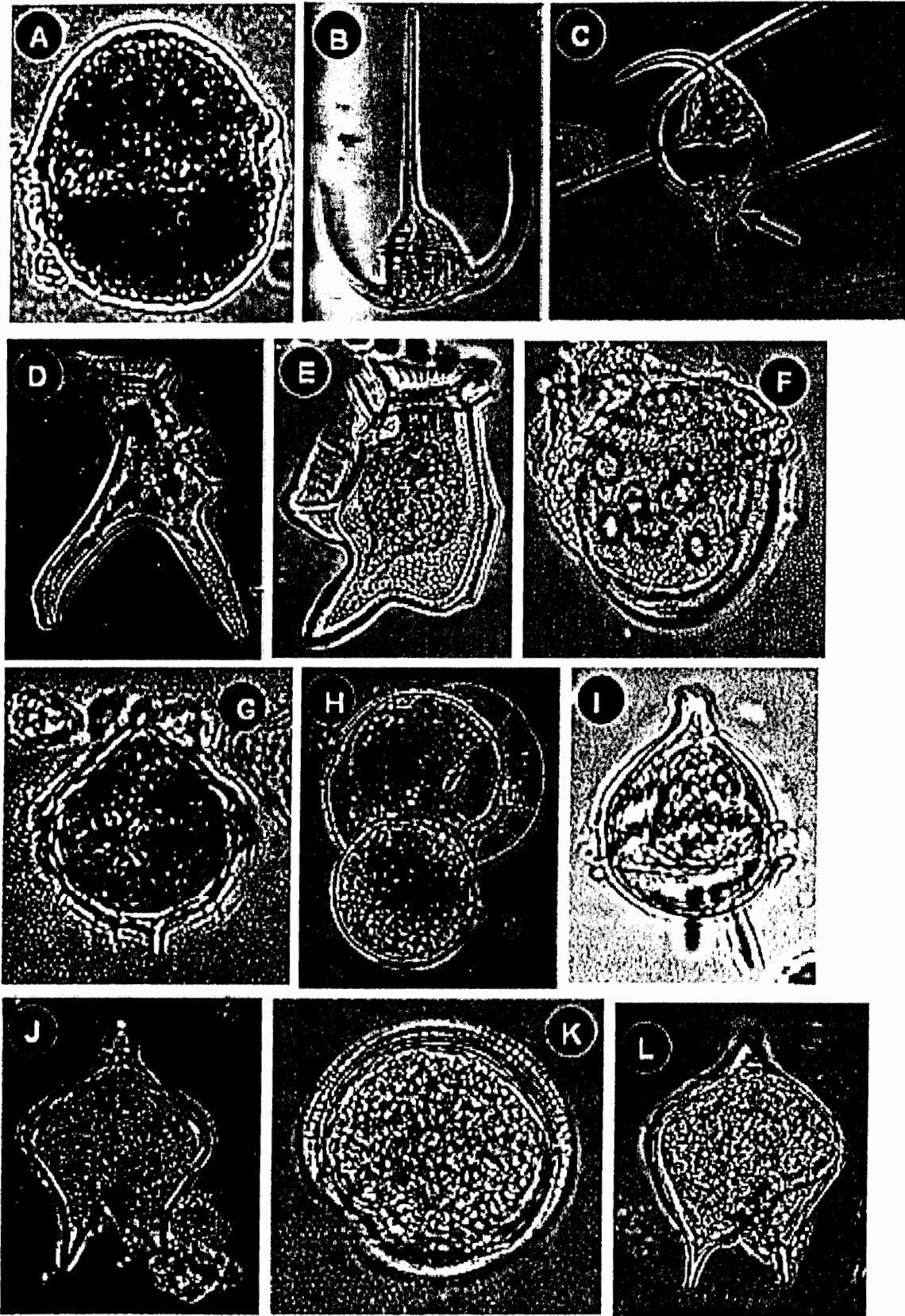
Fig. 2: Species of dinoflagellates present in the Sungai Geting lagoon during the period of shellfish toxicity in the lagoon. All images at 400X magnification. (A) *Alexandrium minutum*, (B) *Ceratium tripos*, (C) *Ceratium tripos* and *C. trichoceros* (arrow), (D) *Dinophysis miles*, (E) *Dinophysis caudata*, (F) *Dinophysis rotundata* (the cytoplasm has leaked out of the cell), (G) *Peridinium quinquecorne*, (H) *Pyrophacus steinii*, (I) *Protoperidinium latispinum*, (J) *Protoperidinium depressum*, (K) *Zygabikodinium* sp., and (L) *Protoperidinium* sp.
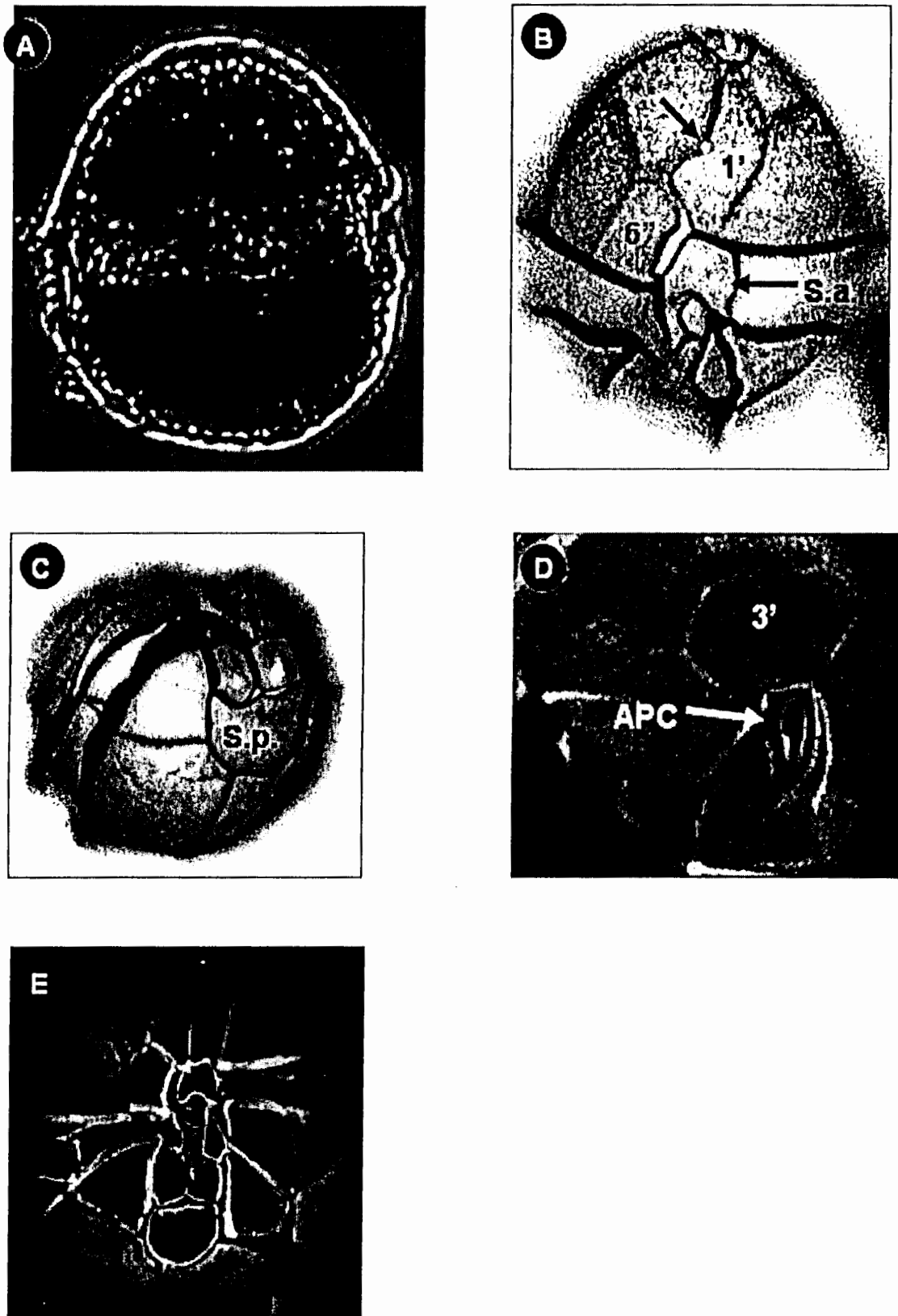
Fig. 3: Detailed morphological characteristics of *Alexandrium minutum* isolated from Sungai Geting. (A) shape of a typical vegetative cell, (B) ventral view of a cell showing the first apical plate (1'), the sixth precingular plate (6''), and the anterior sulcal plate (S.a.), (C) antapical view showing the wide posterior sulcal plate (S.p.) typical of the species, (D) apical view showing the apical pore complex (APC) and the third apical plate (3'), and (E) ventral view showing individual sulcal plates.

recently. With regards to origin, it would be tempting to speculate that *A. minutum* could have been introduced through discharge of ships' ballast water, considering that the South China Sea and the Straits of Malacca are among the busiest sea lanes in the world. Ballast water has been shown to be a significant means of dispersal of harmful algal species (Hallegraeff & Bolch, 1992; Carlton & Geller, 1993). However this speculation would be very difficult to prove. It is very likely that this species has been present in Malaysian waters for a long time, since it has been reported from the Gulf of Thailand much earlier (Fukuyo *et al.* 1988).

The apparent absence of PSPs on the east coast of Peninsula Malaysia until recently could be attributed to several factors. Firstly, there may well have been PSP incidences but were not reported or were misdiagnosed. Secondly, the toxic dinoflagellate species probably very rarely achieve densities sufficient to cause toxicity. Indeed there has never been any report of red tides along the east coast of the peninsula, although it should be noted that *Alexandrium* spp. very seldom, if ever, form visible blooms. The third and probably most likely reason has to do with the pattern of shellfish resource utilisation. In Peninsula Malaysia, the only bivalve mollusc that is consumed on a wide scale is the mud-dwelling blood cockle *Anadara granosa*. Mussels are still not so popular, and mussel farming is still on a very small scale compared to other countries. The only significant mussel farming areas are in Selat Tebrau, Sebatu and Port Dickson, and these cater mainly for export. On the east coast of the peninsula, mussel farming is non-existent. It is noteworthy that the first incidence of PSP in Sebatu in 1991 resulted from consumption of mussels from the newly established mussel farm there. Mud-dwelling bivalves such as cockles and clams are less prone to be toxic since they seldom come into contact with high densities of toxic phytoplankton which tend to form blooms in the upper water column. However, in a shallow area like the lagoon in Tumpat, high densities of toxic phytoplankton could get to the bottom, especially during low tide.

It was also found in this study that the bulk of the *A. minutum* population during the sampling period was located in the middle portion of the lagoon. This was the same location from where the toxic clams were collected. In a small water body like this lagoon, however, it is expected that a phytoplankton bloom patch would shift locations according to the tidal cycle. This is an important aspect since more shellfish populations could be exposed to the toxic species.

The other species of note found in the samples were *Dinophysis* spp. Several species of this genus are proven producers of okadaic acid that causes diarrhetic shellfish poisoning (DSP) in humans (Yasumoto, 1980; Cembella, 1989). Indeed, in major mussel growing countries such as Ireland, Spain and France, DSP is considered the most important shellfish toxicity (Maestrini, 1998). It is quite difficult to ascertain whether or not species found in Malaysia are toxic, since *Dinophysis* is very difficult to culture in the laboratory. However, their presence in large numbers in areas where shellfish are collected is cause for concern and it may be prudent to monitor the presence of okadaic acid in the shellfish.

In all three first time occurrences of PSP in Malaysia, the country was caught unaware. This should not be construed as a failure or weakness of the shellfish toxicity monitoring program since such a situation is also common for other countries. The reality of the matter is that a monitoring program is very costly to run and in most cases is not implemented in areas where there is no evidence that a problem exists. What is needed is a good baseline data on occurrence and distribution of potentially harmful species so that monitoring efforts could be directed to areas where significant risks of toxicity events exist. A good example of an effective monitoring program is in Sabah, where toxicity events have been very rare since the early 1990s even though blooms of toxic *P. bahamense* var. *compressum* occur annually. Results from this study suggests that a shellfish toxicity monitoring program is warranted for the northeast coast of Peninsula Malaysia, and the program should include both PSP and DSP toxins.

ACKNOWLEDGEMENTS

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