Finding of Toxic *Gymnodinium catenatum* Graham and *Alexandrium tamiyavanichii* Balech (Dinophyceae) from Coastal Waters of Selangor, Peninsular Malaysia

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Abstract: From plankton and sediment samples collected from the Selangor region of western Peninsular Malaysia, two dinoflagellate species that cause paralytic shellfish poisoning (PSP), *Gymnodinium catenatum* and *Alexandrium tamiyavanichii*, were identified. Cysts of *G. catenatum* were also detected from the sediments and were widely distributed in areas within the Selangor region where a blood cockle, *Anadara granosa*, is cultured. Since this region is important for bivalve culture, these findings warrant the inclusion of Kuala Selangor in the phytoplankton monitoring program.

Keywords: toxic, *Gymnodinium catenatum, Alexandrium tamiyavanichii,* Selangor, blood cockle

Abstrak: Dari sampel plankton dan sedimen yang dikutip dari negeri Selangor di Pantai Barat Semenanjung Malaysia, dua spesies dinoflagelat yang menyebabkan keracunan paralitik kerang-kerangan, *Gymnodinium catenatum* dan *Alexandrium tamiyavanichii* telah ditemui. Sista *G. catenatum* juga ditemui dalam sedimen dengan taburan yang meluas di kawasan ternakan kerang, *Anadara granosa* di Selangor. Memandangkan kawasan ini penting untuk ternakan kerang-kerangan, penemuan ini mewajarkan Kuala Selangor dimasukkan dalam program pemantauan fitoplankton kebangsaan.

Introduction

The control and management of food safety are essential issues in shellfish culture, especially culture of bivalves, which are well known to accumulate biotoxins. A major issue in this field is shellfish poisoning, which is mainly caused by blooms of micro-algae (mainly phytoplankton); therefore, monitoring of these algae occurrences is an important primary step for preventing such cases of poisoning. Among several different types of shellfish poisoning, in this region, paralytic shellfish poisoning (PSP) is regarded as the most serious type because it can be associated with human mortality caused by neurotoxic symptoms.

To date, several phytoplankton species in the coastal waters of Southeast Asia have been identified as causative agents of PSP. All of these are dinophycean species, including *Pyrodinium bahamense* Plate var. *compressum* (Böhm) Steidinger, Tester et Taylor, *Alexandrium minutum* Halim, *A. tamiyavanichii* Balech, *A. lucitanicum* Balech, *A. tropicale* Balech, and *Gymnodinium catenatum* Graham (Fukuyo *et al.*, 2011). In Malaysia, *A. minutum, A. tamiyavanichii*, and *G. catenatum* (Usup *et al.*, 2002; Mohamad-Noor *et al.*, 2002; Lim *et al.*, 2004) have occurred so far. More recently, Lim and Leaw (2012) reported additional occurrence locations of *P. bahamense* var. *compressum* at Port Dickson in the Strait of Malacca and of *A. tamiyavanichii* at Samariang in Sarawak. The current report is the first describing the presence of *P. bahamense* var. *compressum* in Malaysian waters on the Indian Ocean side of the country. Su-Myat *et al.* (2012) also reported the presence of *A. tamiyavanichii* and *G. catenatum* in the Myanmar coastal waters of the Malay Peninsula (Andaman Sea). These new findings of species that cause PSP, strongly suggest that such organisms are more widely distributed than previously thought and have also spread throughout the Malaysian coastal waters (Fig. 1).

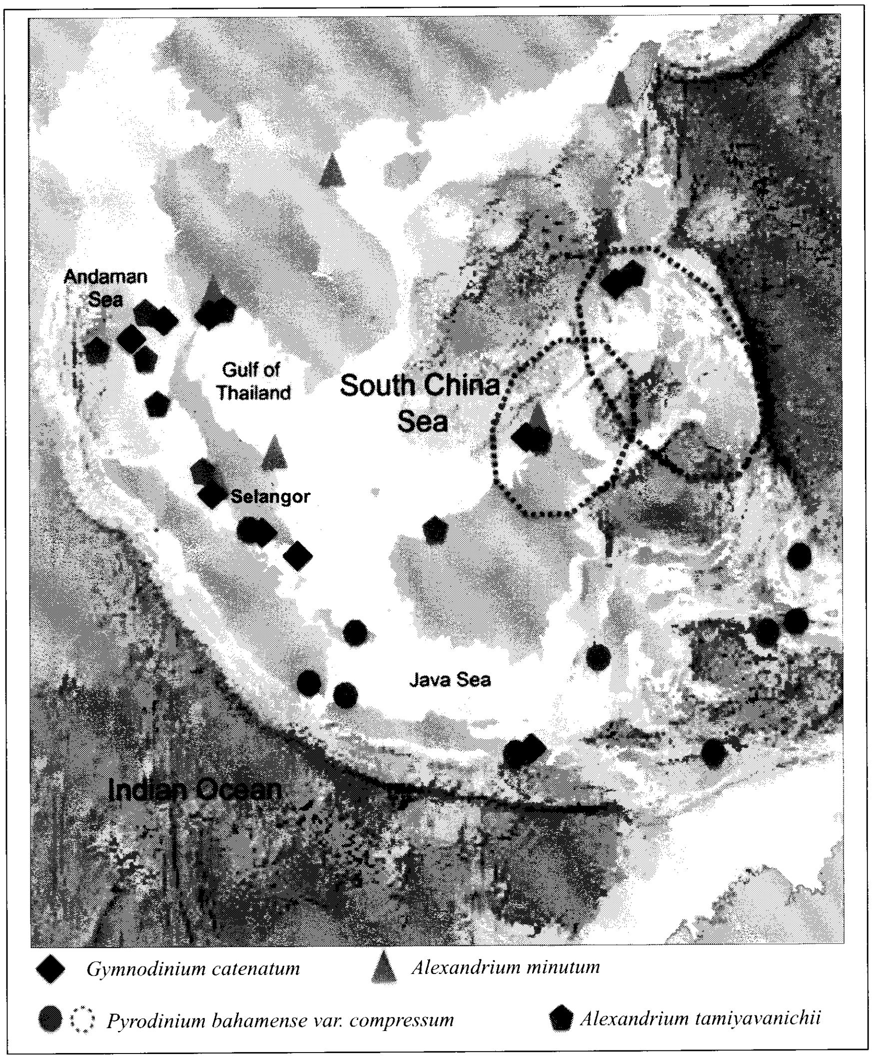

Figure 1: Geographical distribution of PSP-causative dinoflagellates in coastal waters of Southeast Asia. Compiled from Boonyapiwat *et al.* (2007), Fukuyo *et al.* (1993, 2011), Furio and Gonzales (2002), Lim and Leaw (2012), Lim *et al.* (2003, 2004), and Su-Myat *et al.* (2012)

The blood cockle *Anadara granosa* (Linnaeus, 1758) is one of the most important sources of food cultured in Malaysia (Broom 1985). The areas of culture are concentrated in Selangor region on the west coast of Peninsular Malaysia, and the cultured cockles are supplied to the domestic market and also exported to neighboring countries such as Thailand and Indonesia (personal communication with local fishermen). However, it should be noted that plankton-feeder bivalve cultures might always be along with risks of contamination of shellfish toxins. Therefore, sustaining or even promoting bivalve fisheries is heavily reliant on risk management of exposure to shellfish toxins and on a strict consensus for regulating contaminated shellfish. In this context, regular monitoring for toxic plankton is also required, as well as analyzing the toxicity of harvested products. As mentioned above, PSP is a potent neurotoxin for vertebrates, which is associated with paralysis and can be life-threatening for humans; outbreaks of PSP can also inflict serious economic damage on the bivalve culture industry. However, there is no information about causative dinoflagellates of PSP in the areas of bivalve culture within the Selangor region owing to the lack of a monitoring system. Therefore, in order to establish a plankton monitoring system in the Selangor region in the future, we first conducted a study on the detection of toxic dinoflagellate cysts and vegetative cells using field surveys. In this paper, we report the identification of two new PSP-causative phytoplankton species from the Selangor region of Peninsula Malaysia.

Materials and Methods

Sediment samples were collected from five sampling transects spaced at intervals of approximately 14-23 km named as follows: A) Bagan Nakhoda Omar, B) Sungai Basar, C) Sekinchan, D) Kuala Selangor, and E) Sungai Buloh (3° 45'-3° 13' N latitude, 100° 51'-101° 14' E longitude), located along the Selangor coast, Peninsular Malaysia, in September and December 2011 except for the station C5, which was collected on October because of bad weather on the sampling date of September. Five sampling points were set at each sampling transect (Fig. 2). Twenty-five samples were collected for each survey using a handy core sampler (TFO corer) equipped with an inner tube of 2.6 cm diameter. The upper 2 cm of core samples was cut and preserved with neutralized formalin at a final concentration of 2%. All preserved samples were transported to Hiroshima University, Japan, for analysis of dinoflagellate cysts.

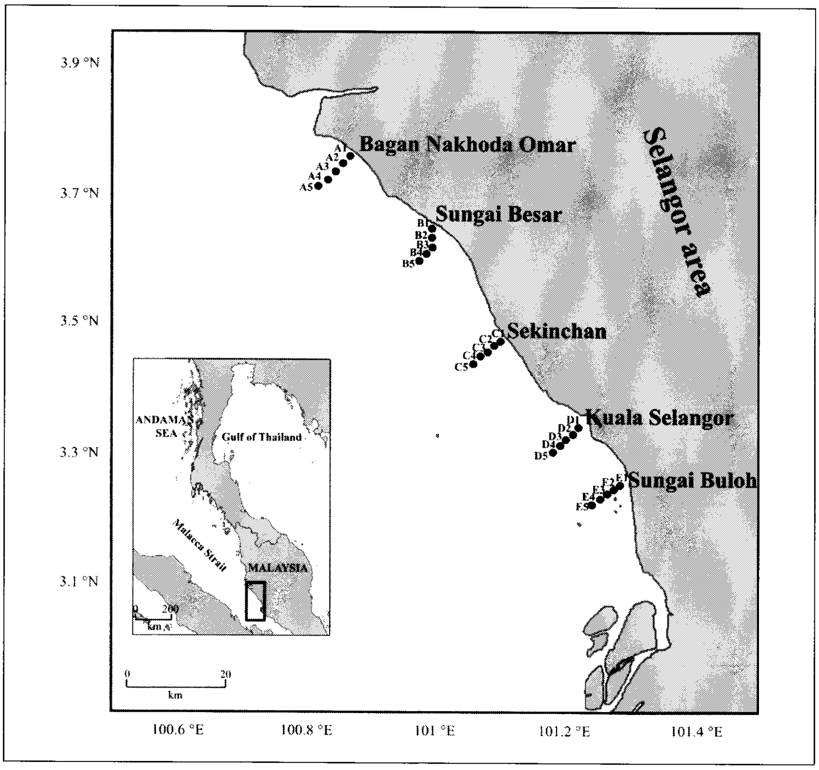

Figure 2: Map of the Selangor region along the west coast of Peninsular Malaysia, Malacca Strait, showing the sampling stations

Sediment samples were processed in accordance with Matsuoka and Fukuyo (2000); 6-29 g of sediment from each sample was chemically treated with 50 ml of 10% hydrochloric acid and 30% hydrofluoric acid (each for 24 hours) to remove carbonate and silicate particles. After each chemical treatment, samples were neutralized with distilled water overnight. The neutralized samples were sieved with meshes of 100 μm and 20 μm in size. The concentrated residue on the 20 μm mesh was suspended in 5 ml of distilled water and kept in a vial. Cysts were observed under an inverted light microscope (Olympus IX71) with DIC illumination and identified in accordance with previous reports (Bolch *et al.*, 1999; Matsuoka and Fukuyo, 2000; Matsuoka *et al.*, 2006). Dinoflagellate cysts were counted under an inverted light microscope at magnifications of ×200 to ×600. Cyst abundance is represented as number of cysts g^{-1} sediment dry weight.

Plankton sampling was carried out five times at stations A1, B1, C1, D1, and E1 from September 2011 to February 2012. Samples were collected by vertical hauling of a plankton net (20 μm mesh size, 20 cm in diameter, 80 cm in side length) from the bottom of the water column to the surface; the samples were then fixed with neutralized formalin solution at a final concentration of 2%. The fixed samples were observed under a light microscope and/or a fluorescence microscope after staining with Calcofluor White M2R (Fritz and Triemer, 1985) to visualize thecal plate tabulation. However, unarmored dinoflagellate *Gymnodinium catenatum* cells, for which adequate fixing using any fixative solution is difficult, were observed in the samples surveyed in January and February. For this reason, plankton samples without fixation were observed at a temporary laboratory after the sampling for the March and May surveys. Only in these monthly surveys and a preliminary survey of only site D on late February of 2012, plankton sampling at the offshore stations (A3, A5, B3, B5, and so on) was carried out in addition to that at the regular coastal stations (A1, B1, C1, and so on).

Results and Discussion

Gymnodinium catenatum Graham-Cysts

Cysts were identified as *G. catenatum* on the basis of the following features: reticulate ornaments (Fig. 3a, b), two rows of finer mesh developed along the paracingulum, chasmic archeopyle along the paracingulum (Fig. 3a), and larger cyst diameter (50–60 μm; Fig. 3a, b)

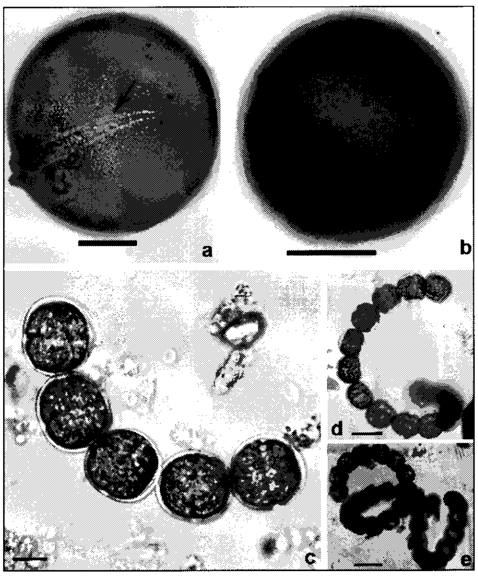

Figure 3: Light microphotographs of cysts and plankton of *Gymnodinium catenatum* found in the Selangor region. a, Cyst showing a large chasmic archeopyle (arrow, scale 20 μm); b, cyst showing surface reticulate ornamentation (scale 20 μm); c, plankton forming a chain composed of five cells (scale 20 μm); d, chain consisting of ca. 16 cells (scale 50 μm); e, chain consisting of ca. 32 cells (scale 50 μm). All plankton cells were deformed during transportation

The cysts of *G. catenatum* were detected at low numbers from the samples from 11 stations collected in September (Fig. 4a) and 8 stations in December (Fig. 4b). The highest numbers of *G. catenatum* cysts (1.28 cysts g^{-1} dry weight sediment in September and 0.78 cysts g^{-1} dry weight sediment in December) were found at stations A3 and A5, respectively. The average density of *G. catenatum* cysts was high (< 0.6 cysts g^{-1} dry weight sediment weight) at station A (Bagan Nakhoda Omar) compared with those at the other stations (Fig. 4a, b). Although the occurrence of *G. catenatum* was not clearly different between September and December, the density of cysts was lower at stations in the southerly part of the study area (Fig. 5).

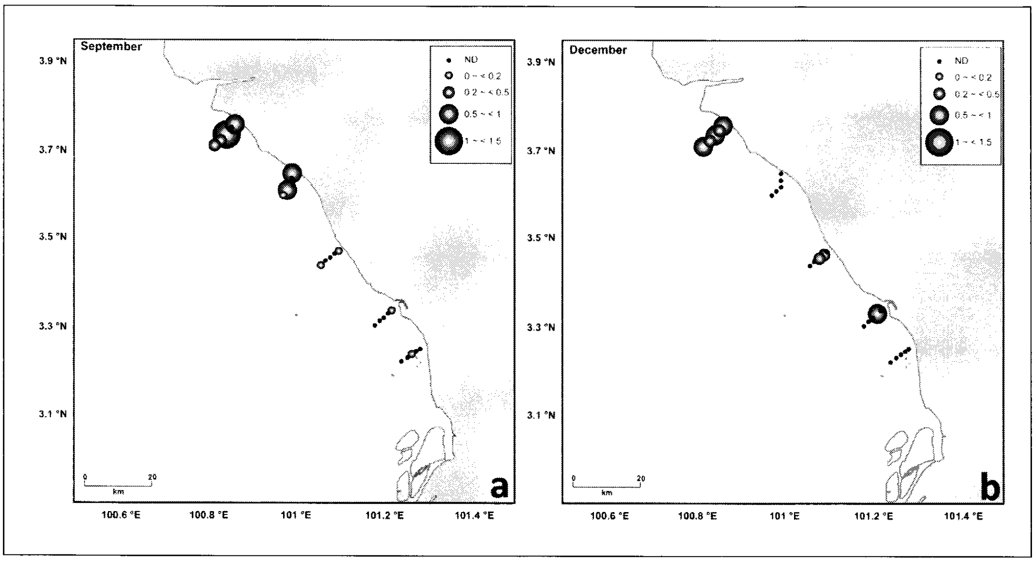

Figure 4: a: Map of the Selangor region showing the occurrence of cysts of *G. catenatum* in the September survey. b: Map of the Selangor region showing the occurrence of cysts of *G. catenatum* in the December survey (ND: not detected) (cysts g^{-1} dry weight sediment)

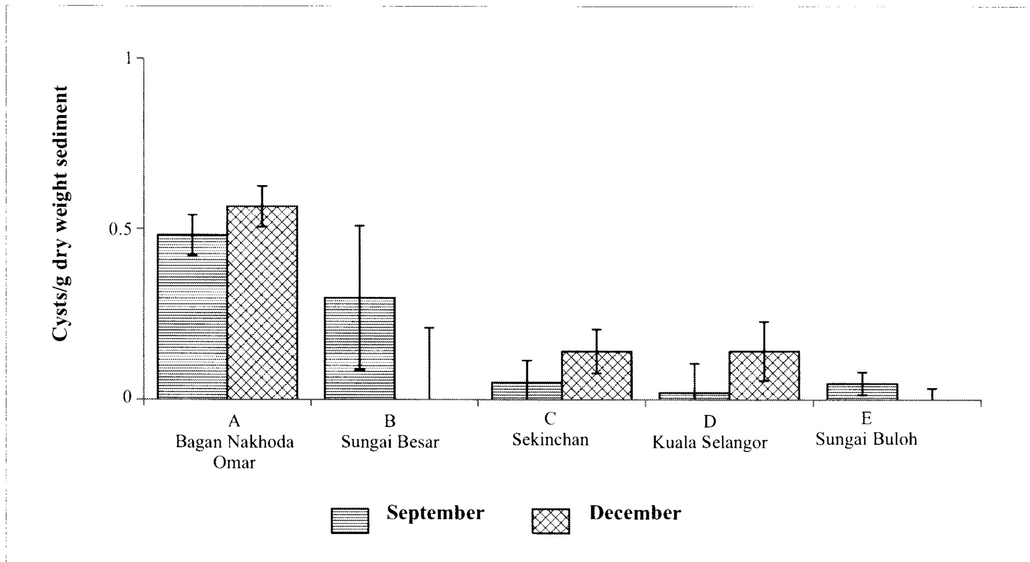

Figure 5: Average density of *G. catenatum* cysts at five sampling locations in the Selangor region

Within the total dinoflagellate cyst assemblages, *G. catenatum* cysts were a minor component and their density was low at 1.28–0.6 cysts g^{-1}. No quantitative data of plankton cells of *G. catenatum* in the Selangor coast at the moment. These cyst densities are low in comparison with those in other areas such as Western Japan and the southern coast of Korea, where the densities of these cysts were around 8% and 5.3% of the total cysts, respectively (Matsuoka *et al.*, 2006; Shin *et al.*, 2007). These low cyst densities of *G. catenatum* in the Selangor region probably reflect the low plankton cell densities.

Plankton of G.catenatum

At the moment to date, monitoring of plankton-monitoring with a focus oning harmful phytoplankton species has been not conducted regularly within the Selangor region; however, possible plankton cells bearing a strong resemblance to *G. catenatum* were found in a formalin-fixed sample collected at the station E1 on in January of 2012. Therefore, unfixed samples for the observation of potential *G. catenatum* cells were collected in a wide area of along the Selangor coast in March and May 2012, and the presence of *G. catenatum* the cells was confirmed at the stations A5, D1, and D5 in March and at the stations C3, C5, and E3 in May, respectively. However, unfortunately, these *G. catenatum* cells had already become rounded and were had thus not kept as theretained their original morphology when they were observed under a light microscope after transfer (Fig. 3c, d, e). However, the spherical nucleus positioned at the center of each cell and chains composed of over four cells suggested that this plankton was *G. catenatum* (Fig. 3c, d, e).

G. catenatum has been reported in Manila Bay (Fukuyo *et al.*, 1993), Singapore (Holmes *et al.*, 2002), the Gulf of Thailand (Lirdwitayaprasit *et al.*, 2008), and the Andaman Sea coast of Myanmar (Boonyapiwat *et al.*, 2007; Su-Myat *et al.*, 2012) (Fig. 1). This geographical distribution of *G. catenatum* suggests that the occurrence of this species in the Selangor region is reasonable.

A. tamiyavanichii Balech

Plankton samples collected at station D5 in Selangor in late February 2012 contained *Alexandrium* species. On the basis of the following morphological features, this plankton was identified as *A. tamiyavanichii* (Fig. 6): triangular apical pore complex with small connecting pore (Fig. 6e), the first apical plate directly contacting the apical pore complex, development of curtain (Fig. 6b, c), additional suture running along the anterior part of the anterior sulcal plate (Fig. 6a, d), well-developed sulcal sutures, and chains of more than eight cells (Fig. 6f). *A. tamiyavanichii* shares these features with *A. cohorticula*, but is different in terms of the lower epitheca, diagonal direction of the additional suture in the anterior sulcal plate, and a small connecting pore in the Po plate (Balech 1995). On the basis of these features, we identified the thecate dinoflagellates in the plankton samples as *A. tamiyavanichii*. This species has also been found in Manila Bay (Furio and Gonzales, 2002), the Gulf of Thailand as *Protogonyaulax cohorticula* (Fukuyo *et al.*, 1989), Sebatu in the Strait of Malacca, Malaysia (Lim *et al.*, 2004), and the Merugi Archipelago, Myanmar (Su-Myat *et al.*, 2012). *A. tamiyavanichii* is also known to produce resting cysts (Nagai *et al.*, 2003). However, it is actually very difficult to identify the cysts correctly because simple spherical cysts characterized by a transparent cyst wall without any surface ornamentation have also been found in *A. affine*, *A. fraterculus*, *A. pseudogonyaulax*, and *A. hiranoi* (Matsuoka and Fukuyo, 2000). We also observed such simple spherical cysts in surface sediments collected from the Selangor coast, but these have yet to be identified conclusively as cysts of *A. tamiyavanichii*.

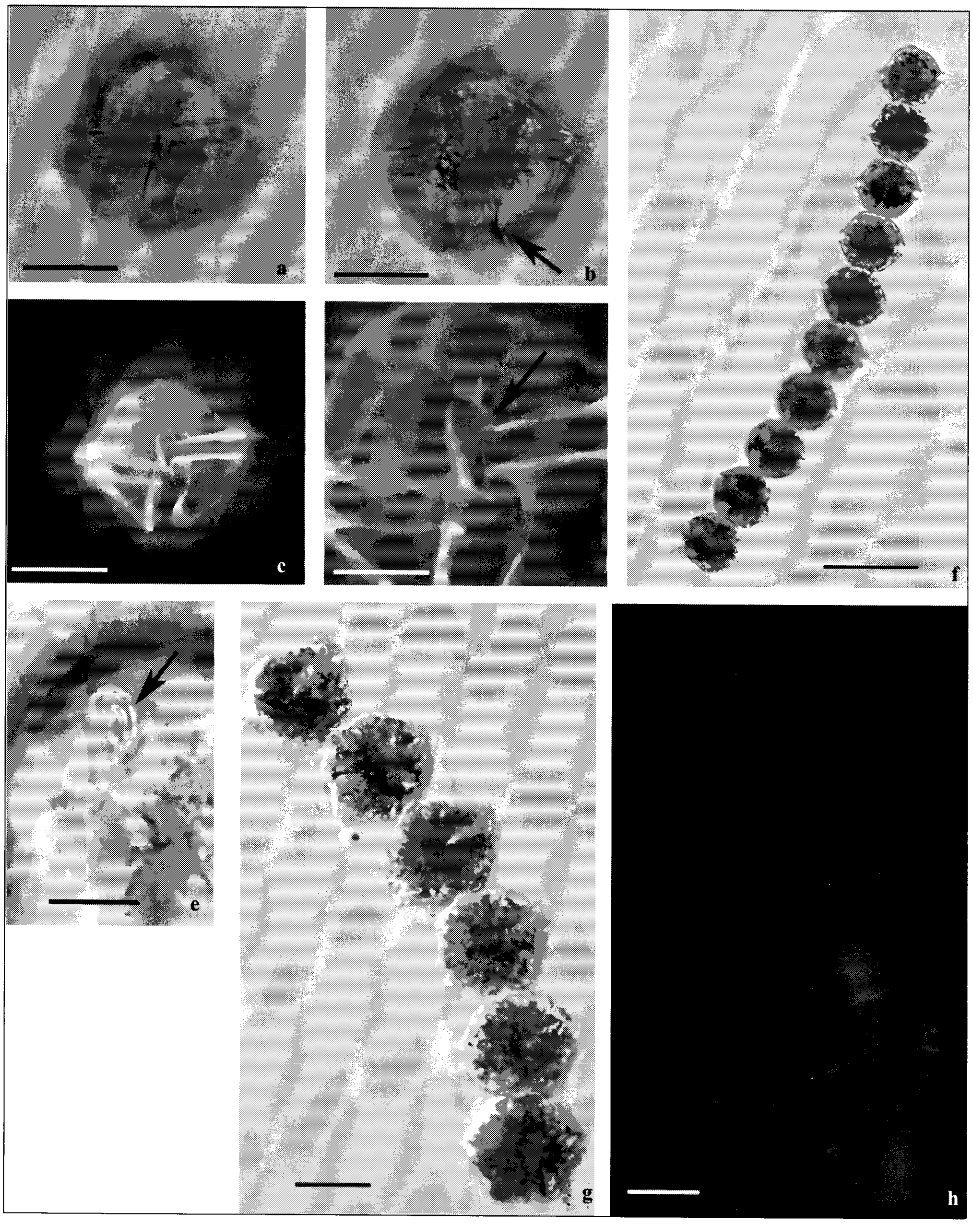

Figure 6: Light microphotographs of plankton of *Alexandrium tamiyavanichii* collected from the Selangor region. a, b, Outline of a single cell and well-developed extension of sulcal lists (arrow); c, fluorescence microphotograph of a single cell; d, fluorescence microphotograph showing anterior sulcal plate (arrow); e, light microphotograph showing apical pre-plate (arrow); f, chain consisting of ten cells; g, normal light microphotograph showing six cells; h, fluorescence microphotograph showing six cells. Scale bars in a, b, c, g, and h represent 20 µm, bar in f represents 60 µm and those in d and e represent 10 µm

Influence on blood cockle cultures

The Selangor region is one of the most important areas for the cockle culture industry in not only Malaysia but the whole of Southeast Asia. The natural spats of blood cockles in the region occur at high density several times a year. These spats are selected as seeds for culture and exported to neighboring countries as well as being used domestically because these seeds grow faster and contain higher levels of nutritious substances than others regions (personal communication with local fishermen). However, the occurrence of toxic dinoflagellates, *G. catenatum* and *A. tamiyavanichii*, in the cockle culture grounds suggests that there is a potential risk for PSP in the Selangor region (Fig. 7). In addition, the wide distribution of *G. catenatum* cysts may also suggest a risk of dispersion of *G. catenatum* cysts through transportation of the cockle spats from the area. Thus, precautions should be taken to prevent cyst contamination when transporting cockle spats. On the basis of the findings of this study, it is important to establish systems for monitoring toxic phytoplankton in cultured blood cockles in the Selangor region.

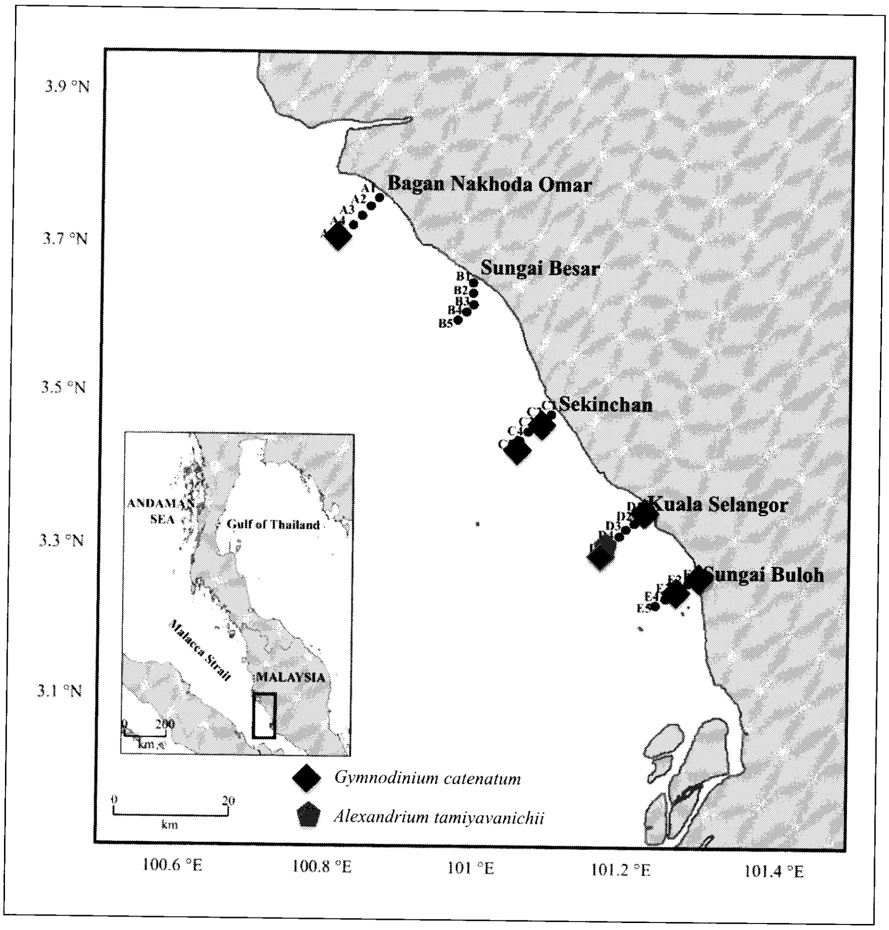

Figure 7: Map showing sites of *Gymnodinium catenatum* and *Alexandrium tamiyavanichii* along the Selangor coast

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