Potentially Harmful Microalgae From Cockle Culture Area in Sg. Jarum Mas, Perak

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Abstract: Phytoplankton study was carried out at cockle culture area in Sg. Jarum Mas, Perak, from February to October 2008. A total of 64 water samples were collected from eight sampling stations. Using an inverted microscope, a total of eight genera of potentially harmful microalgae were identified belonging to two different classes; dinoflagellates and diatoms. The potentially harmful dinoflagellates observed were *Alexandrium* sp., *Dinopyhsis* sp., *Dinophysis caudata, Prorocentrum* sp., *Ceratium furca, Noctiluca scintillans* and *Akashiwo sanguinea.* Meanwhile the potentially harmful diatom observed was *Pseudo-nitzchia* sp. The enumeration of the cell density for each of the potential harmful microalgae have also been carried out. The results show that the density of cells for each of the potential harmful microalgae is low, but, *Alexandrium* sp. found relatively high concentrations in April of ~ 25, 000 cells / L. However, no cases of food poisoning due to marine microalgae or red tide incidents reported during the period of this study.

Keywords: harmful dinoflagellates, diatoms, cells density, Sg. Jarum Mas

Abstrak: Kajian fitoplankton telah dijalankan di kawasan ternakan kerang di Sg. Jarum Mas, Perak bermula dari Februari hingga Oktober 2008. Sejumlah 64 sampel air telah diambil dari lapan lokasi sekitar Sg. Jarum Mas. Dengan menggunakan "inverted microscope", sebanyak 8 genera mikroalga yang berpotensi bahaya telah dikenalpasti iaitu dari kelas dinoflagelat dan diatom. Dinoflagelat yang berpotensi bahaya yang ditemui adalah *Alexandrium* sp., *Dinopyhsis* sp., *Dinophysis caudata, Prorocentrum* sp., *Ceratium furca, Noctiluca scintillans* and *Akashiwo sanguinea.* Manakala diatom yang berpotensi bahaya ditemui adalah *Pseudo-nitzchia* sp. Pengiraan kepadatan sel bagi setiap mikroalga yang berpotensi bahaya juga telah dilakukan. Keputusan mendapati bahawa kepadatan sel bagi setiap mikroalga yang berpotensi bahaya adalah rendah, namun begitu *Alexandrium* sp. didapati agak tinggi kepekatannya pada bulan April iaitu ~25, 000 sel/L. Walau bagaimanapun tiada kes keracunan makanan laut akibat mikroalga bahaya atau kejadian ledakan mikroalga dilaporkan sepanjang tempoh kajian dijalankan.

Introduction

Cockles are among popular seafood in Malaysia and its cultivation is a good revenue earner. In the year 2007, Malaysia produced about 49,620.16 tonnes of the cockles valued at RM 62.30 million (Annual Fisheries Statistic, 2007). State of Perak was the top producer in Malaysia contributing about 68% of the whole production, amounting to 33,711.51 tonnes valued of RM 41 million. Sg. Jarum Mas, Perak has been identified as one of the major cockle producing areas under the Department of Fisheries (DOF), Balance of Trade (BOT) program for mollusc. At present, the safety of the cockles harvested from Sg. Jarum Mas is monitored under the DOF Marine Sanitary and Phytosanitary (SPS) Program. The main objective of this program is to ensure that cockles are harvested from safe areas. Potential harmful microalgae are one of the parameters monitored in this program.

About 300 of the approximately 5,000 currently described microalgae species are considered to be harmful, while only 80 species have the capacity to produce potent toxins (Hallegraeff, 2003). Harmful microalgae are normally found in low numbers, but under certain condition may form extensive blooms that are capable of causing devastating effects on the environment (Moestrup and Larsen, 1992).

Harmful algal Blooms (HABs) are natural phenomena due to the increase of phytoplankton cell density in the water column (10^6-10^7 cells/L), there is a total dominance of a single species and the events are unpredictable in nature (Usup *et al.*, 1989). However, it is still difficult to define a cell concentration that constitutes HAB; some species such as *Dinophysis* can be dangerous, causing poisonings at cell densities of only several hundred cells per liter (Kevin *et al.*, 2003).

It is still a matter of debate as to the causes of HAB, the possibility of human activities such as nutrient enrichment (from industrial waste, human settlements and agricultural pratises, climate changes, or transport of algal species via ship ballast water (Anderson *et al.*, 2001; Mercedes and Esther, 2006). Some species may blooms causes by abundance nutrients sources from upwelling of water from below and river flow (Kevin *et al.*, 2003).

HAB association with human illness or damage to aquaculture operations is receiving growing attention in the newspapers, electronic media and scientific literature. As a result, more and more researchers are now surveying the local waters for the nuisance algae (Hallegraeff, 2003). The harmful species include some species of dinoflagellates, diatoms, cyanobacteria, and 'naked' flagellates. HABs are often accompanied by shellfish toxicity events because the algae are often consumed in large numbers by filter-feeding bivalves. Toxins eventually get transferred to humans through the food chain (Usup *et al.*, 2002a). Table 1 summarizes the three types of HABs problem, together with examples of causative algal species.

The first HABs and shellfish toxicity event in Malaysia was reported in Sabah in 1976 which was caused by *Pyrodinium bahamense* var. *compressum* (Taylor and Fukuyo, 1989; Choo, 1994; Anton *et al.*, 2000). A number of 202 people were reported to suffer from Paralytic Shellfish Poisoning (PSP) cases, with seven deaths recorded. Since then, this phenomenon has continued to occur almost annually in the state (Usup and Azanza, 1998). On the other hand, the first reported PSP case in the Peninsular took place in Sebatu, Malacca in 1991, triggered by *Alexandrium tamiyavanichii*. Three people were taken ill after consuming green mussel (Usup *et al.*, 2002b). The latest reported cases of PSP, occurred in 2001 in Tumpat, Kelantan where a youth died and six persons were hospitalized after eating benthic bivalve 'lokan' (*Polymesoda sp.*) contaminated with *Alexandrium minutum* (Lim *et al.*, 2004, Wan Norhana *et al.*, 2005).

To our knowledge, there has been no report on HABs occurrence or shellfish toxicity cases in Sg. Jarum Mas, Perak. Thus, the objective of this paper is to document the presence of potentially harmful microalgae in cockle culture areas in Sg. Jarum Mas, Perak.

Materials and methods

Sampling locations

Phytoplankton sampling was carried out monthly from eight sampling stations in Sg. Jarum Mas, Larut-Matang, Perak, (Fig. 1) from February to October 2008. Jetty and Station 1 are situated in area with a large population. Stations 2 to station 6 are located in the cockle culture area. While, Station 7 is situated away from the cockle culture areas and open sea area, it was chosen as the control.

Sample collection and analysis

Phytoplankton samples were collected using Van Dorn water sampler for phytoplankton identification and enumeration. For each sample, 2 L of sea water was filtered through 20 μm mesh size plankton net. Samples were taken at 2 m depths. The samples were fixed in Lugol's solution and observations of microalgae were carried out using an inverted microscope (Olympus Model IX71). Sedgewick Rafter Counting Cell Slide was used to quantify the cells by using a Lieca Compound Microscope (Lieca, Germany). Toxic phytoplanktons were identified based on several taxonomic manual (Taylor 1976; Hasle and Syverstsen, 1996; Steidenger and Tangent, 1996).

Results and Discussion

A total of 41 taxa of phytoplankton were documented in this study from Sg. Jarum Mas (Tables 2 and 3). A total of eight potentially harmful microalgae were observed, belonging to two different groups; dinoflagellates (Table 2) and diatoms (Table 3). Fig. 2-9 shows the cell density of each species at eight sampling station. *Alexandrium* sp. (Photo 1a), *Dinophysis* sp. (Photo 1c) and *Ceratium furca* (Photo 1f) were detected at most of the sampling stations throughout the sampling period.

Table 1: Different types of harmful algal bloom (from Hallegraeff, 2003)

1. Species that produce basically harmful water discolorations; however, under exceptional conditions in sheltered bays, blooms can grow so dense that they cause indiscriminate kills of fish and invertebrates through oxygen depletion. Examples: dinoflagellates *Akashiwo sanguinea, Gonyaulax, Noctiluca Scintillans, Scrippsiella trochoidea* ; cynobacterium *Trichodesmium erythraeum*

2. Species that produce potent toxins that can find their way through the food chain to human, causing a variety of gastrointestinal and neurological illness, such as;

- Paralytic shellfish poisoning (PSP)
  (Examples:Dinoflagellates *Alexandrium catenella, A. cohorticula, A. fundyense, A. fraterculus, A. leei, A.minutum, A. tamarese, Gymnodinium catenatum, Pyrodinium bahamense var compressum*)
- Diarrthetic shellfish poisoning (DSP)
  (Examples: dinoflagellates D*inophysis acuta, D. acuminate, D. caudata, D. fortii, D. norvegica, D. mitra, D. rotundata, D. sacculus, Prorocentrium lima*)
- Amnesic shellfish poisoning (ASP)
  (Examples: diatoms *Pseudo-nitzschia australis, P. delicatissima, p. multiseries, P. pseudodelicatissima, P. pungens* (some strain), *P. seriata*)
- Ciguatera fish poisoning (CFP)
  (Examples: donoflagellate *Gambierdiscus toxicus*,? *Coolia* spp., *Ostreopsis spp.*,? *Prorocentrim* spp.)
- Neurotoxic Shellfish poisoning (NSP)
  (Examples: dinoflagellate *Karenia brevis* (*Florida*), *K. papilionacea in editus, K. selliformis in editus, K. bidigitata in editus* (New Zealand)
- Cynobacterial toxin poisoning
  (Examples: cynobacteria *Anabaena circinalis* (Freshwater) *Microcystis aeruginosa* (Freshwater), *Nodularia spumigena*
- Estuarine associated syndrome (through aerosols from dinoflagellates *Pfiesteria piscicida, P. shumwayae*)

3. Species that are non-toxic to human but harmful to fish and invertebrates (especially in intensive aquaculture systems) by damaging or clogging their gills.
Examples: diatoms *Cheatoceros concavicorne, C. convolutes*; dinoflagellates *Karenia mikimotoi, K. brevisulcata, Karlodinium micrum*; prymnesiophytes *Chrysochromulina polyepis, Prymnesium parvum, P. patelliferum*; raphidophytes *Heterosigma akashiwo, Chattonella antique, C. marina, C. verruculosa.*

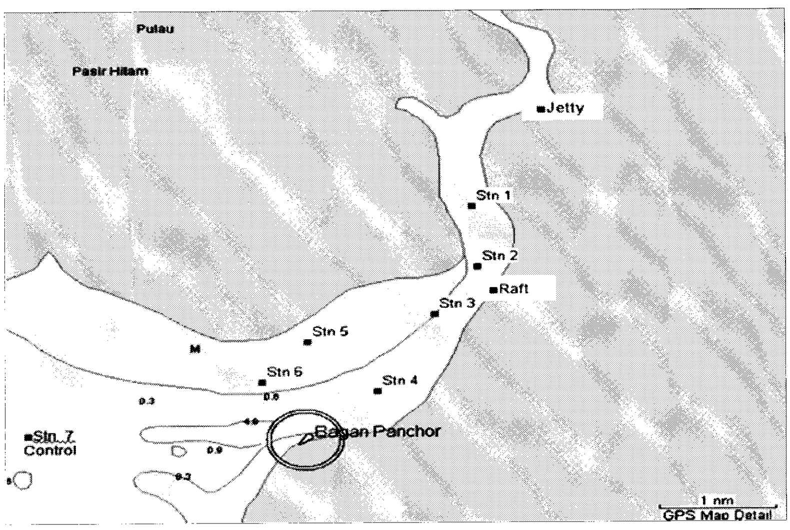

Figure 1: Sampling stations determined/selected/identified in this study

Table 2: List of dinoflagellates observed from February to October 2008

	Month								
Species identified	**F**	**M**	**A**	**M**	**J**	**A**	**S**	**O**	**Stations**
Alexandrium sp.	X	X	X	X	X	X	X	X	Jetty, 1, 2, 3, 4, 5, 6, 7
Ceratium furca	X	X	X	X	X	X	X	X	Jetty, 1, 2, 3, 4, 5, 6, 7
Dinophysis caudata	X	X	X	X	X		X		1, 2, 3 ,4, 5, 6, 7
Dinophysis sp.	X	X	X	X	X	X	X	X	Jetty, 1, 2, 3, 4, 5, 6
Akashiwo sanguinea		X				X			5, 7
Noctiluca scintillans				X					7
Prorocentrum sp.	X	X	X	X	X	X	X		Jetty, 1, 2, 3, 4, 5, 6, 7
Protoperidinium sp.	X	X	X	X	X	X	X	X	Jetty, 1, 2, 3, 4, 5, 6, 7

Table 3: List of diatoms observed from February to October 2008

	Month								
Species identified	**F**	**M**	**A**	**M**	**J**	**O**	**S**	**O**	**Stations**
Amphora sp.	X								
Asterionella sp.	X				X				1, 3, 5, 6 ,7
Bacillaria sp.	X				X			X	1, 5, 7
Bacteriastrum sp.	X				X	X	X		Jetty, 1, 2, 3, 4, 5, 6, 7
Bel lerochea sp.	X						X		5
Chaetoceros sp.	X	X	X	X	X	X	X	X	Jetty, 1, 2, 3, 5, 6, 7
Corethron sp.	X								1
Cosnodiscus sp.	X	X		X		X	X		Jetty, 1, 2, 3, 4, 5, 6, 7
Cylindrotheca sp.	X		X	X	X	X	X		Jetty, 1, 2, 3, 4, 5, 6, 7
Ditylum sp.	X	X	X	X	X	X	X	X	Jetty, 1, 2, 3, 4, 5, 6, 7
Eucampia sp.						X			5
Guinardia sp.		X	X		X	X			1, 2, 3, 4, 5, 6, 7
Gyrosigma sp.	X	X	X	X	X	X	X	X	Jetty, 1, 2, 3, 4, 5, 6, 7
Helicotheca tamensis				X					6, 7
Hemiaulus sp.					X	X	X		1, 3, 4, 5, 6, 7
Hylodiscus sp.			X						5
Lauderia sp.					X	X			Jetty, 1, 3, 5, 6, 7
Leptocylindrus sp.	X	X	X		X	X	X		1, 2, 3, 4, 5, 6, 7
Lithodemium sp.	X				X	X	X	X	1, 4, 5, 6
Navicula sp.	X		X	X	X	X	X		Jetty, 1, 2, 3, 4, 5, 6, 7
Nitzschia sp.		X	X	X		X			Jetty, 1, 2, 4, 5, 6, 7
N. longissima sp.	X	X							1, 5, 6, 7
N. membranacea sp.						X	X		4, 5, 6
Odontella sp.	X			X	X	X	X	X	Jetty, 1, 2, 3, 4, 5, 6, 7
Pleurosigma sp.	X	X		X	X	X	X		Jetty, 1, 2, 5, 6, 7
Pseudo nitzschia sp.*	X			X					2, 3, 5
Rhizosolenia sp.	X		X	X	X	X	X		Jetty, 1, 2, 3, 4, 5, 6, 7
Rhizosolenia setigera sp.		X					X		1, 2
Skeletonema sp.	X	X	X	X	X	X	X	X	Jetty, 1, 2, 3, 4, 5, 6, 7
Surirella sp.	X	X	X	X	X	X	X	X	Jetty, 1, 2, 3, 4, 5, 7
Synedra sp.					X	X	X		Jetty, 1, 2, 4, 5, 6
Thallassionema sp.	X	X	X	X	X	X	X	X	Jetty, 1, 2, 3, 4, 5, 6, 7
Thallassiosira sp.	X	X	X	X	X	X	X	X	Jetty, 1, 2, 3, 4, 5, 6, 7

*Potentially harmful microalgae

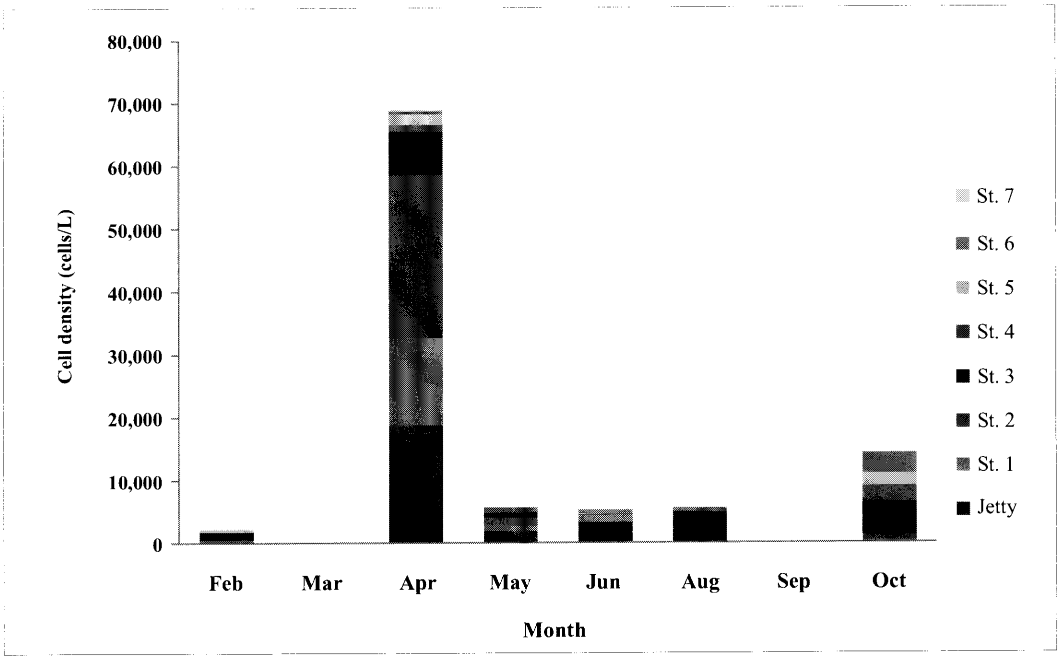

Figure 2: The cell density of *Alexandrium* sp. observed at eight sampling stations

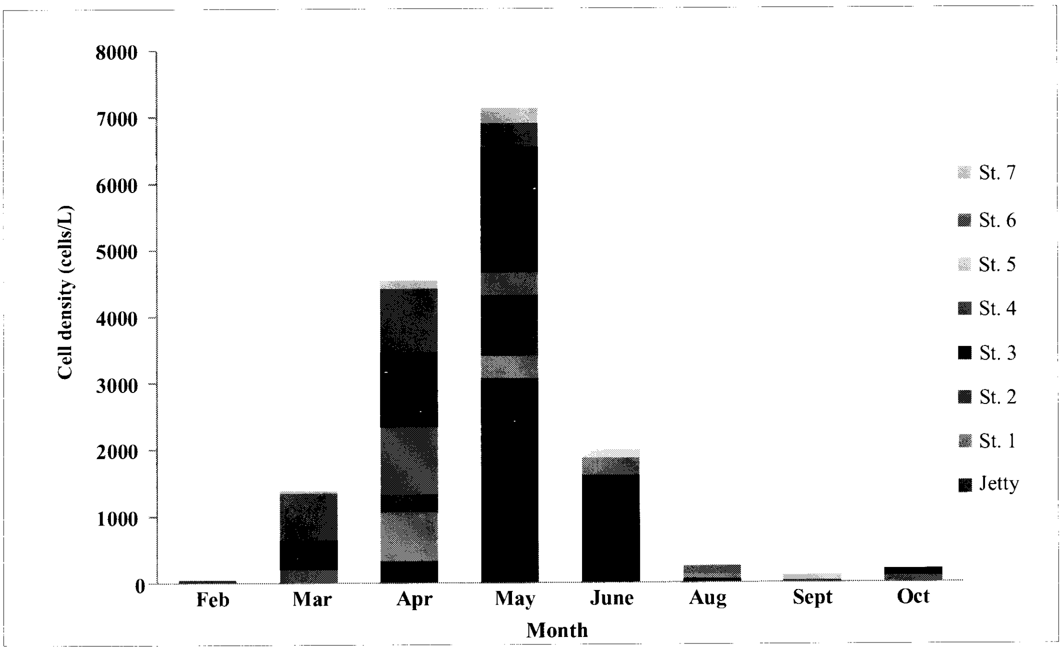

Figure 3: The cell density of *Dinophysis* sp. observed at eight sampling stations

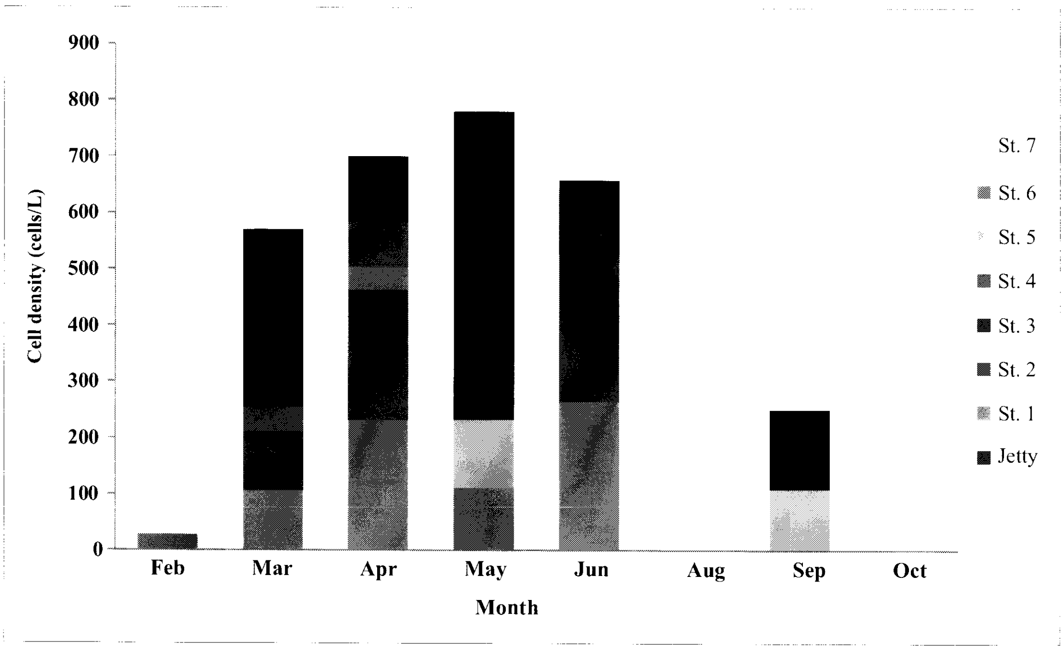

Figure 4: The cell density of *Dinophysis Caudata* observed at eight sampling stations

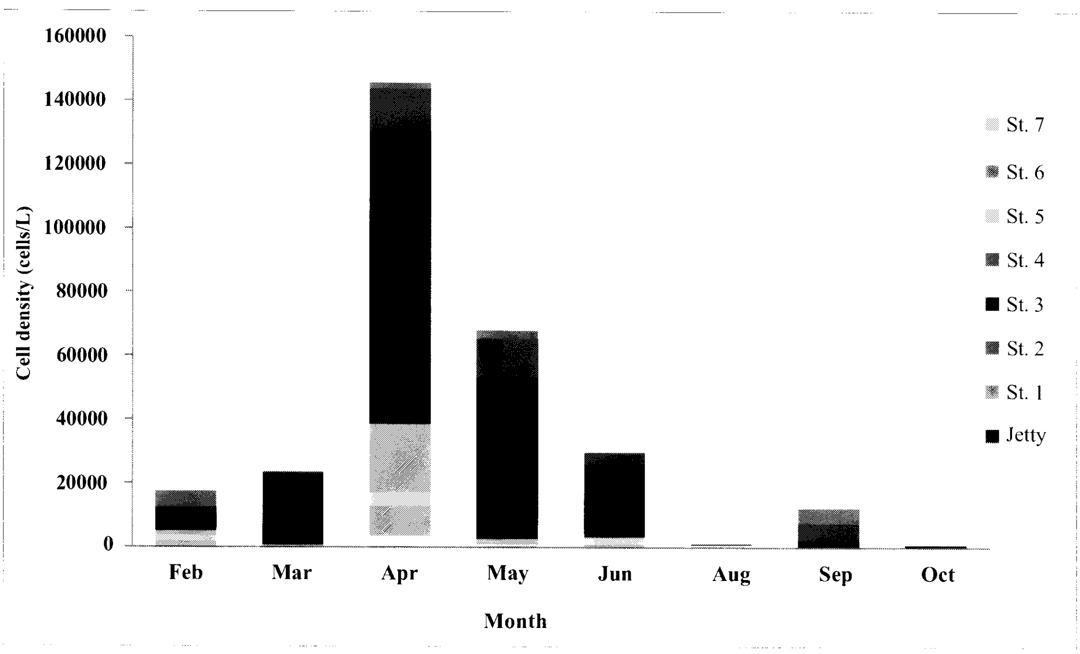

Figure 5: The cell density of *Ceratium furca* observed at eight sampling stations

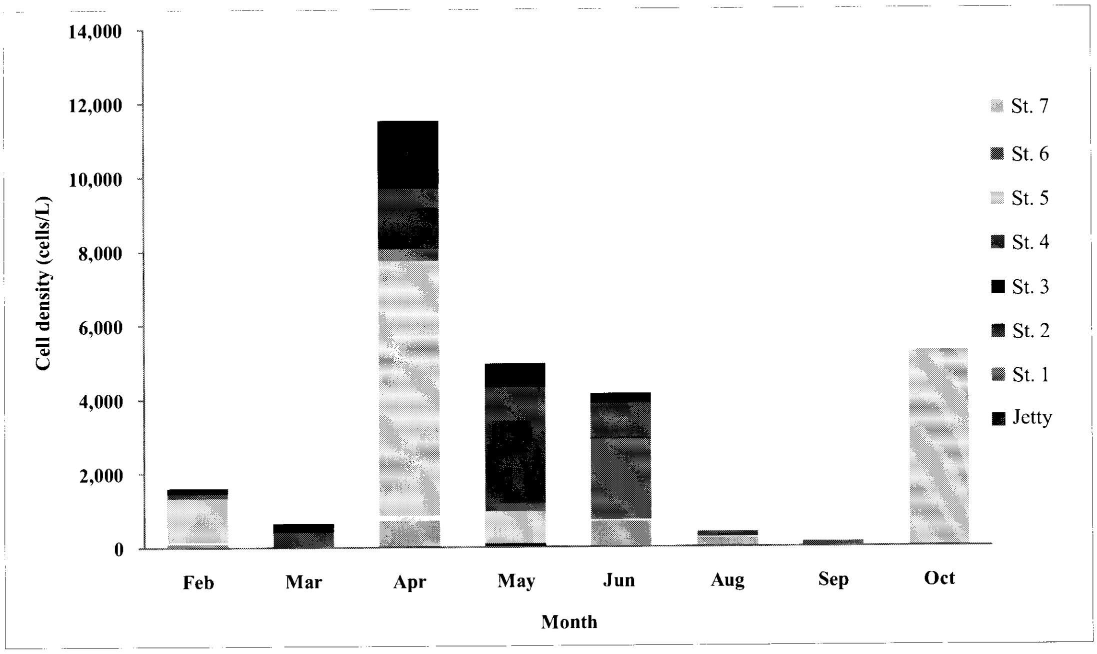

Figure 6: The cell density of *Prorocentrum* sp. observed at eight sampling stations

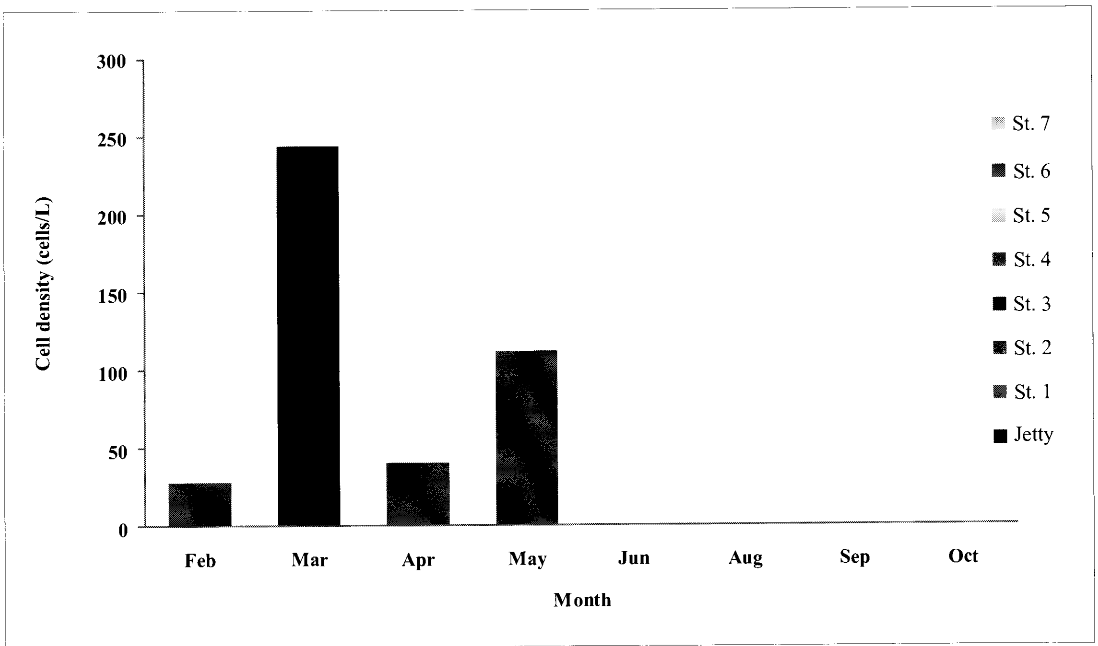

Figure 7: The cell density of *Akashiwo sanguinea* observed at eight sampling stations

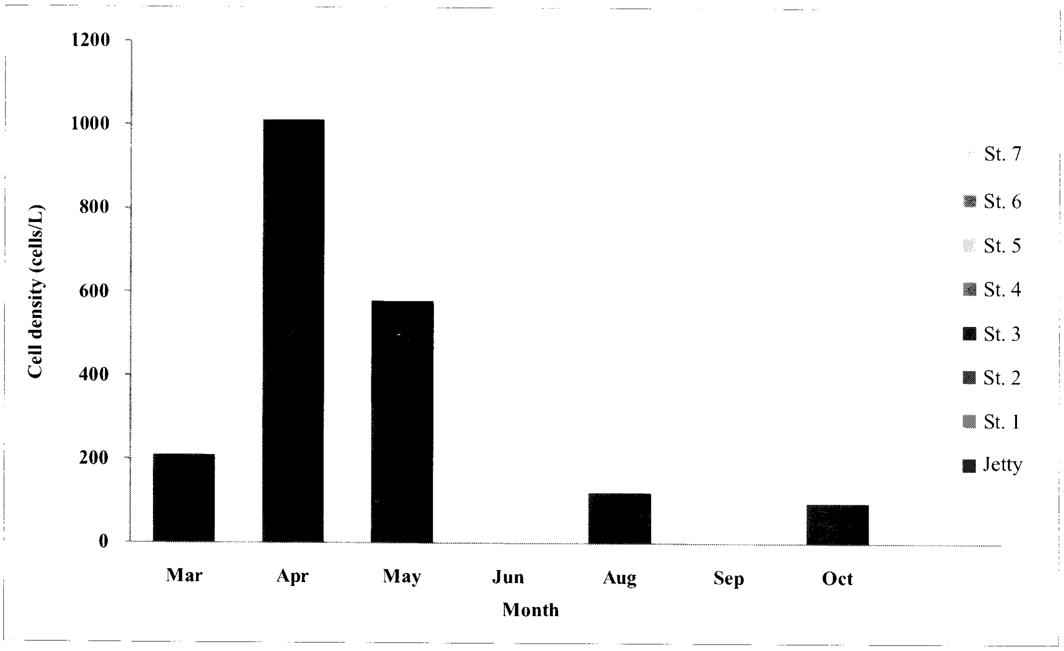

Figure 8: The cell density of *Noctiluca scintillans* observed at eight sampling stations

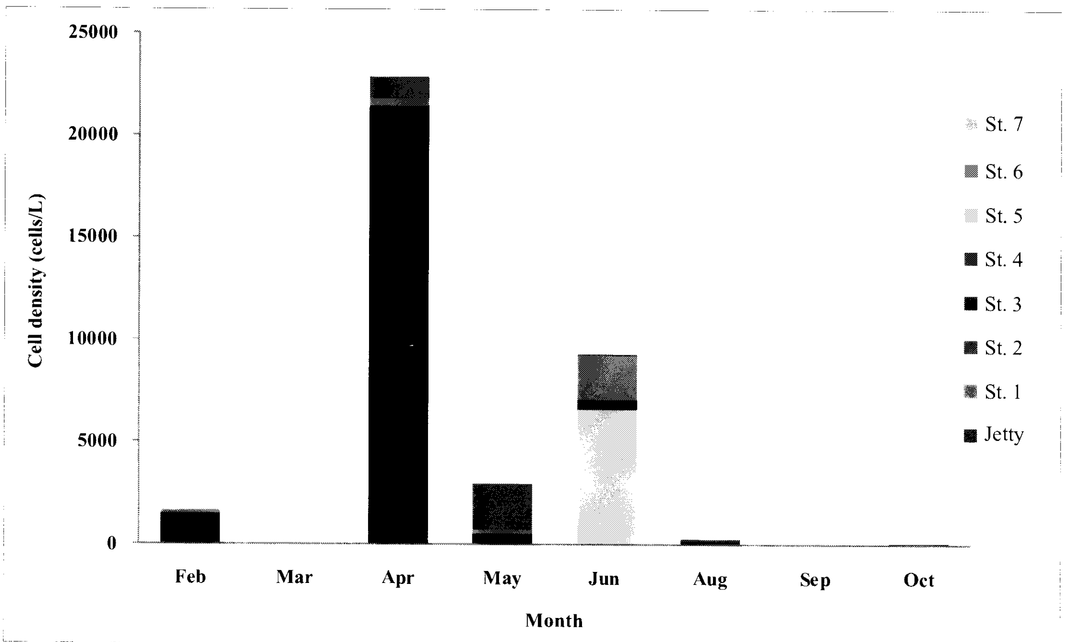

Figure 9: The cell density of *Pseudo-nitzchia* sp. observed at eight sampling stations

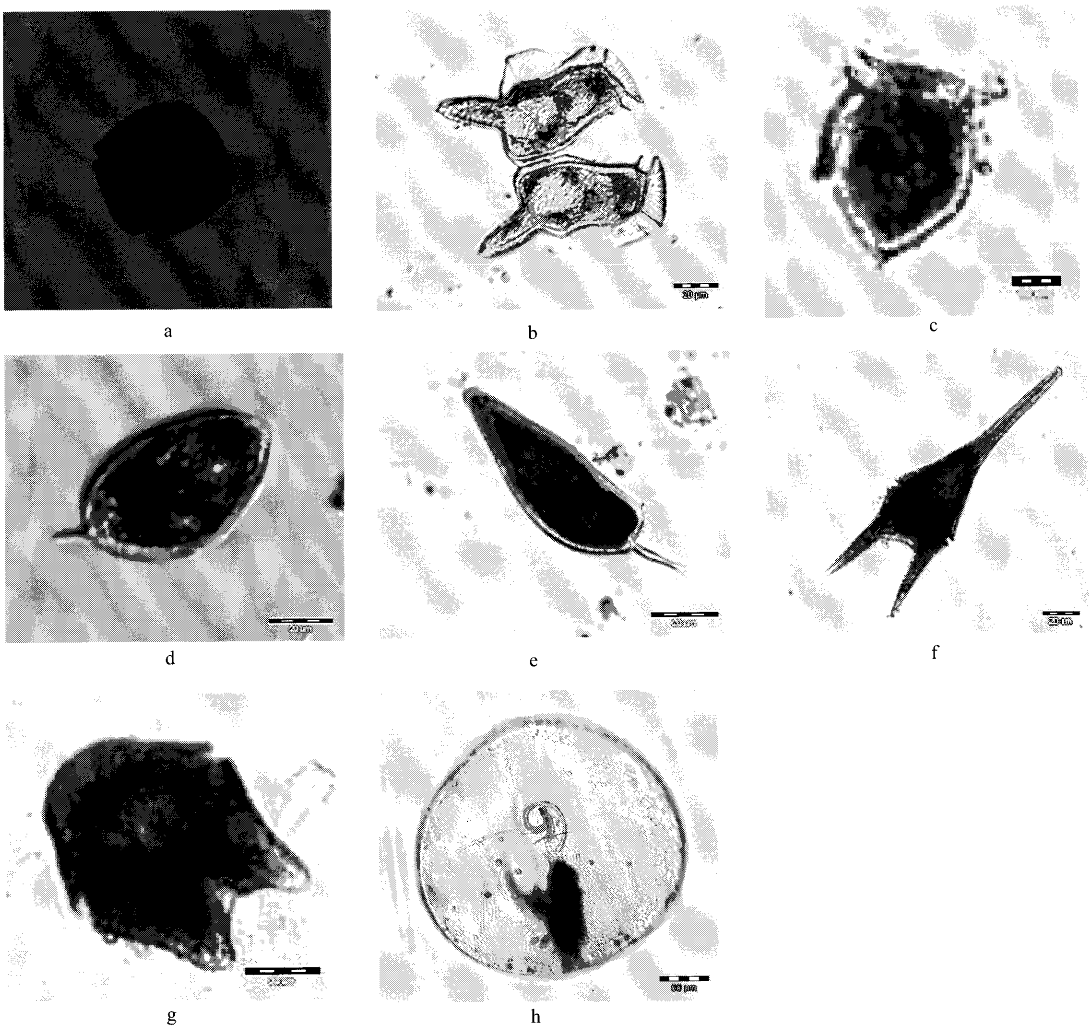

Photo 1: Potentially harmful finoflagellates. a: *Alexandrium* sp. b: *Dinophysis caudata*, c: *Dinophysis* sp. d: *Prorocentrum mican* e: *Prorocentrum gracile*, f: *Ceratium furca*, g: *Akashiwo sanguinea*, h: *Noctiluca scintillans*

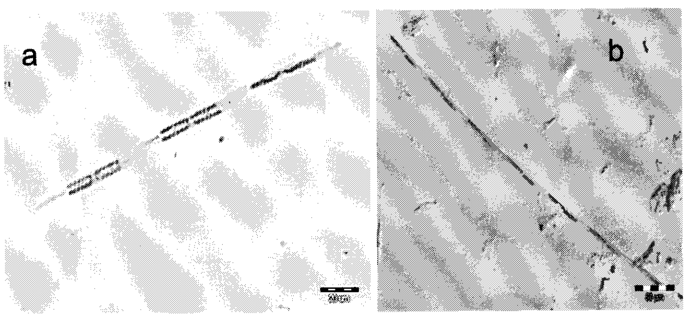

Photo 2: Potentially harmful diatoms a; b; *Pseudo-nitzchia* sp.

Alexandrium sp. was observed at each sampling station in April with density ranging from 200-25,000 cells/L and the highest density was recorded at station 2. *Alexandrium* sp. was not found in September in any station except at station 7 at a density of ~35 cells/L. Several species of this genus have been indicated to produce a number of neurotoxins, which lead to PSP cases in human (Backer *et al.*, 2003). PSP toxins are fast-acting neurotoxins that inhibit transmission of nerve impulse by blocking the sodium channels. These toxins may result in death caused by respiratory arrest within a few minutes to a few hours (Hallegraeff, 2003). At present, PSP is the only HAB-related shellfish poisoning that has been documented in Malaysia. Death due to eating bivalve 'lokan' (*Polymesoda sp.*) contaminated with toxic *Alexandrium* has been reported (Lim *et al.*, 2004) in Tumpat, Kelantan. At least two species of PSP toxin producers of *Alexandrium* sp. are known to be present in Malaysia waters. They are *A. minutum* in Tumpat and *A. tamiyavanichi*, in Sebatu Melaka (Usup *et al.*, 2002b; Usup *et al.*, 2006).

Two species of *Dinophysis* were observed in study i.e. *Dinophysis caudata* (Fig.3b) and *Dinophysis* sp. *D. caudata* were observed at most of the sampling stations with density ranging from 0-300 cells/L. *Dinophysis* sp. was observed at all sampling stations except station 7. The highest cell density of *Dinophysis* sp. was recorded at station Jetty (~3,000 cells/L). *D. caudata* is a toxic dinoflagellates, which produce okadaic acid that causes diarrhetic shellfish poisoning (DSP) (Backer *et al.*, 2003). Symptoms of DSP are very similar to diarrhea caused by bacterial poisoning. In Malaysia, no incidence of DSP has yet been reported. The under reporting of DSP could be due to the difficulty in differentiating whether the poisoning caused by DSP or bacteria. This toxic species may cause harmful effects without forming dense blooms. Concentrations as low as 200 cells/L of *D. fortii* can cause toxification of shellfish that is enough to affect humans (Yasumoto *et al.*, 1980). In Singapore waters, low concentration of *D. caudata* may cause persistent low concentrations of DSP toxin in mussels (Holmes *et al.*, 1999).

Several species of *Prorocentrum* are also known to be toxic. For example, *Prorocentrium lima* produces okadoic acid which causes DSP. In addition, *P. lima* has been associated with ciguatera syndrome. In this survey, two species of *Prorocentrium* were observed; *P. micans* (Photo 1d) and *P. gracile* (Photo 1e). Cells of *P. micans* are rounded anteriorly and taper towards the posterior. A distinct apical spine is also present. *Prorocentrum gracile* on the other hand, has a very robust apical spine and longer in length than *P. micans*. *Prorocentrum micans* is common in Malaysian waters and could present at high density (Usup *et al.*, 2002a). The highest cell density (~1,800 cell/L) of *P. micans* was recorded in April 2008 at station 7. Although these species are known to be non-toxic, they can result in fish mortality when present at high densities. In the year 2002, reddish-brown patches caused by *Prorocentrum minimum* were observed in the Straits of Johore. (Usup *et al.*, 2003). Fortunately, no harmful effect was reported.

Ceratium furca (Photo 1f) was detected at most of the sampling stations with density ranging from 0-49,000 cells/L. *C. furca* are relatively harmless microalgae. They are non-toxic and are required by the aquatic organisms in their food web. However, they may cause red tide if conditions allow for excessive blooming. A high density of *C. furca* could cause fish and invertebrates mortality due to insufficient oxygen by damaging or clogging their gills rather than causing intoxication or poisoning (Hallegraeff, 2003). In 2007, the Department of Fisheries reported a first red tide event caused by *C. furca* in Lumut, Perak. Their effect on fisheries however has not been studied. (Fisheries research Institute Anual Report, 2007).

Akashiwo sanguinea (Photo 1g) was present at stations 5 and 7 in March and at station 4 in August, however at a very low density (~30-200 cells/L). *A. sanguinea* is among the most common red-tide dinoflagellates in many coastal waters of the world. They form extensive blooms that color the water red and are associated with shellfish and fish kills (Mania and Rose, 2002).

Noctiluca scintillans (Photo 1h) was not found in any station except at station 7 in May at a density of ~200 cells/L. The cells are very large (app. 250-300 μm in diameter), balloon-like and subspherical. Although this species does not produce toxin, it accumulates ammonia in the vacuole, which may be toxic to fish (Taylor *et al.*, 2003). It has also been reported that in the food vacuoles of *Noctiluca scintillans* containing toxigenic microalgae may act as a vector of phycotoxins to higher trophic levels (Escalera *et al.*, 2007).

Most of the microalgae species identified during this monitoring were diatoms and most diatoms are not known to be harmful. Of the 33 species of diatoms observed, only one species of potentially harmful diatom, *Pseudo-nitzschia* sp., was identified (Photo 2a and 2b). The *Pseudo-nitzschia* at stations 2 (~50 cells/L) and 5 in February (~1,000 cells/L) and at stations 2 (~ 6,000 cells/L), 3 (~400 cells/L) and 5 (~2,600 cells/L) in June. Species of *Pseudo-nitszchia* form colonies that are characterized by overlapping cells. The genus *Pseudo-nitzschia* comprises more than 30 taxa of which 11 are associated with production of domoic acid, the agent responsible for amnesic shellfish poisoning (ASP) such as *P. multisiries*, *P. pseudodelicatissima* and *P. australis* (Miller and Scholin, 1998; Lundholm *et al.*, 2003). Domoic acid poisoning was first reported in 1987, where several intoxications including three persons died after eating cultured blue mussels from Prince Edward Island in Canada contaminated with *P. multiseries* (Lizzy, 2001). In Malaysia, however no incidence of ASP has yet been reported.

The results from this survey suggest that Sg. Jarum Mas could be considered as potential area for shellfish farming due to low density cell of potential harmful algae found and no HAB has been reported from this area. However, regular monitoring of phytoplankton should be continued in this area especially at stations Jetty, 1 and 2, which are situated at the narrowest part of Sg. Jarum Mas with the heaviest population on both sides of the riverbanks. Human settlements and the agriculture practices around these areas might be the sources of continuous influx of nutrient (from treated and untreated human wastes, livestock and pets) into the water environment and ultimately may stimulate harmful algae growth. Accordingly, *Dinophysis* sp. and *Alexandrium* sp. were present the highest in the stations Jetty, 1 and 2 compared to the other stations. While in the stations 6 and 7, potentially harmful microalgae were found in low cell density and may be due to the areas which are situated away from human settlements.

Conclusion

Monitoring carried out at eight locations show that potentially toxic *Alexandrium* sp., *Dinophysis caudata, Prorocentrum micans* and *Pseudo-nitzchia* sp. are quite common in Sg. Jarum Mas, Perak. Presence of non toxic species such as red tide forms sp. *Akashiwo sanguinea*, *Ceratium furca* and *Noctiluca scintillans* are also noted. Although non-toxic, they may cause red tide or excessive blooming if conditions allow. In the future, identification of species by scanning or transmission electron microscopy could be carried out to confirm the identity of the species. Establishment of laboratory cultures of these potentially toxic is important in order to verify the toxicity of this species. Cockle samples should also be sampled from time to time for marine biotoxins monitoring. Regular monitoring of phytoplankton should be continued in addition with the water quality parameters to better understanding seasonal distribution of these species.

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