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INCREASING IMPORTANCE OF HARMFUL ALGAL BLOOMS IN MALAYSIA

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ABSTRACT

Up until 1990, problems related to harmful algal blooms and paralytic shellfish poisoning (PSP) in Malaysia were relatively simple, being confined to the west coast of Sabah. PSPs in that area are primarily due to *Pyrodinium bahamense* var. *compressum*. A sufficiently effective shellfish toxicity monitoring program was established by the Malaysian Department of Fisheries, greatly reducing the occurrences of PSP despite recurring bloom events. In early 1991 PSP occurred for the first time in Peninsula Malaysia when three people were taken ill after eating farmed mussel from Sebatu in the Straits of Malacca. The dinoflagellate *Alexandrium tamiyanavichi* was eventually confirmed as the toxin producer. This event prompted the government to establish an additional shellfish toxicity monitoring facility based at the Fisheries Research Institute in Penang. The latest development of PSP events in Malaysia took place in September 2001 when six people were taken ill after eating clams harvested from a coastal lagoon in Kelantan on the east coast of Peninsula Malaysia. One of the victims eventually died. Mouse bioassays and the receptor binding assay confirmed high levels of toxin in the clams. Inspection of plankton samples collected from the affected area showed high density of the dinoflagellates *Alexandrium minutum*, *Alexandrium lusitanicum* and possibly *Alexandrium tropicale*. Laboratory cultures of these three species have been established. These events indicate the growing significance of PSP in Malaysia and it is a problem that could increase in importance as shellfish aquaculture intensifies. Logistical considerations warrant the establishment of a shellfish toxicity monitoring facility on the east coast of Peninsula Malaysia. In the long term, these monitoring facilities should also include monitoring of DSP and ASP in their programmes.

INTRODUCTION

Algal blooms refer to events during which the density of phytoplankton in the water column reaches a level far above normal. Sometimes blooms are referred to by colour. For example, 'red tides' refer to blooms of some dinoflagellate species that cause seawater to appear reddish due to high concentrations of the photopigment peridinin. Others are 'green tides' and 'brown tides' (Figure 1). The term harmful algal bloom (HAB) is used when the to other life, including humans, either through the production of toxins, physical damage, or degradation of water quality. HABs differ from normal blooms in that cell densities are unusually high (10^6 – 10^7 cells L^{-1}), there is total dominance of a single species, and the events are unpredictable in nature (Usup *et al.* 1989). HABs are often accompanied by shellfish toxicity events because the algae are often consumed in large numbers by filtration-feeding bivalves. Toxins eventually get transferred to humans through the food chain. In addition there are also HABs in which the toxin vectors are fishes. Over the last two decades there has been marked increase in HABs, both in geographical coverage and frequency.

Reasons for this apparent increase are not entirely known. Unfortunately, Malaysia is one of the countries affected by HABs. HABs pose serious threats to development of fisheries resources, particularly in aquaculture. In this review, proven HABs in Malaysia are discussed, together with other HABs which could become significant in future based on the presence of toxic species.

A

B

C

Figure 1. (A) A red tide of the dinoflagellate *Noctiluca scintillans*, (B) a cyanobacteria green tide, and (C) a brown tide of the dinoflagellate *Aureoumbra*

HISTORICAL DEVELOPMENT OF HABs IN MALAYSIA

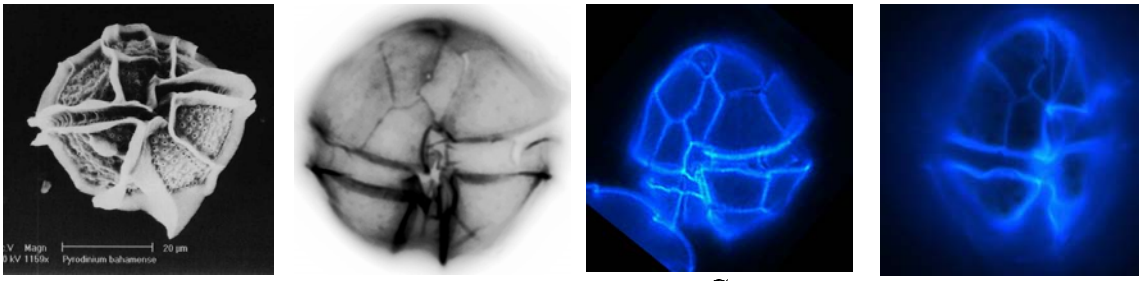
The first report of HABs and shellfish toxicity in Malaysia was in 1976 when the marine dinoflagellate *Pyrodinium bahamense* var. *compressum* bloomed in Brunei Bay on the west coast of Sabah (Roy 1977). Several people were poisoned during this event. The bloom eventually spread to other parts of the Sabah west coast. Blooms of this species have continued to occur almost annually in the state (Usup and Azanza 1998). Over the years many poisoning cases have been reported, including several fatalities. These events also resulted in significant economic losses to fishermen, because the public are afraid to consume all types of seafood during a bloom event, which normally lasts two to three weeks.

For many years HABs and shellfish toxicity in Malaysia were considered problems unique to the west coast of Sabah only. In 1991, however, three people were taken ill after consuming green mussel (*Perna viridis*) cultured at a newly established mussel farm in Sebatu Melaka. Symptoms suggested that intoxication was due to algal toxins. Subsequent testing of extracts from mussels collected during the event confirmed the presence of toxins. In 1997 the most likely toxin producer was established in laboratory cultures and identified as the dinoflagellate *Alexandrium tamiyavanichi*. Analysis of these cultures confirmed that the dinoflagellate produced toxins similar to those found in the toxic mussels (Usup *et al*. 2001). The latest development in HABs in Malaysia took place in September 2001. Six people were taken ill after consuming ‘lokan’ (*Polymesoda* sp.) collected from a coastal lagoon (Sungai Ubi) in Tumpat Kelantan. One of the victims died. Again the symptoms suggested intoxication due to algal toxins. Clam samples were collected during the week of the event and analysed by mouse bioassay and receptor binding assay (Usup et al. 2000). They were found to contain very high levels of toxins. At least two known toxic dinoflagellate species have been isolated from the lagoon and established in culture. Analysis of these cultures have confirmed their toxicity. These events show the probability of HABs and shellfish toxicity occurring in more locations in Malaysia, especially since known HAB species have been found in several locations even though no poisoning cases have been reported from those areas.

SIGNIFICANT HABs AND SEAFOOD TOXICITY IN MALAYSIA

Paralytic shellfish poisoning (PSP)

Globally, PSP is the most important form of seafood toxicity due to HABs (Hallegraeff 1993). The toxins are produced predominantly by marine dinoflagellates, although there have been reports of toxin production by the cyanobacteria *Aphazinomenon* sp. and *Anabaena* spp (Humpage *et al*. 1994; Jones and Negri 1997). The toxins, collectively referred to as saxitoxin, are blockers of voltage-gated sodium channels found on nerve cells and smooth muscle (Shimizu 1994). Action of these toxins inhibit depolarization of nerves and smooth muscles resulting in paralysis and death due to respiratory failure. In Malaysia at least four PSP toxin producers are currently known, viz. *P. bahamense* var. *compressum* in Sabah, *A. tamiyavanichi* in Sebatu Melaka, and *A. minutum* and *A. lusitanicum* in Tumpat Kelantan (Figure 2). There is also the possibility of a third toxic species in Tumpat, namely *A. tropicale*, but the taxonomy still needs to be confirmed. More extensive surveys may well reveal the presence of other PSP toxin-producing *Alexandrium* species. The primary vector for PSP toxins are bivalve mollusks, although planktivorous fish such as 'tamban' (*Sardinella* sp.) can also contain the toxin.



A **B** **C** **D**

Figure 2. PSP toxin-producing marine dinoflagellates in Malaysian waters. (**A**) *Pyrodinium bahamense* var. *compressum* (SEM), (**B**) *Alexandrium tamiyavanichi*, ordinary light microscopy, (**C**) *Alexandrium minutum*, stained with calcofluor white and viewed under epifluorescence microscopy, and (**D**) *Alexandrium lusitanicum*, stained with calcofluor white and viewed under epifluorescence microscopy.

Diarrheic shellfish poisoning (DSP)

DSP is the second most important seafood toxicity due to algal toxins. Experiences of several other countries (e.g. Spain, France, Portugal, Chile) showed that DSP emerged with the establishment of large scale mussel farms (Lembeye *et al*. 1993; Van Egmond et al. 1993; Gestal-Otero, 2000). This is because mussels are such efficient filter feeders that they can accumulate high amounts of toxins. The toxin that causes DSP is the polyether okadaic acid and its congeners, produced my several species of the cosmopolitan marine dinoflagellates *Dinophysis*, *Prorocentrum lima* and *P. micans*. As the name implies, DSP is a gastrointestinal malady, whose main symptom is diarrhea. Okadaic acid is a highly potent inhibitor of serine/threonine protein phosphatases (Gauss *et al*. 1997). These phosphatases are critical components of signaling cascades in eukaryotic cells that regulate other cellular processes (Cohen *et al*. 1990). Diarrhea associated with DSP is most probably due to the hyperphosphorylation of proteins, including ion channels, in the intestinal epithelia, resulting in impaired water balance and loss of fluids.

Surveys carried out at several locations show that potentially toxic *Dinophysis* spp., *P. lima* and *P. micans* are quite common in Malaysian waters and can occur in high densities (Figure 3). It is, however, difficult to prove the toxicity of these species since laboratory cultures have not been established. Shellfish in this country have also never been tested for okadaic acid. Symptoms of DSP are very similar to gastrointestinal ailments caused by bacteria poisoning and can be easily confused. It is also likely that DSP would not be reported since the diarrhea lasts for only 2-3 days and so far the poisoning has never been reported as fatal.

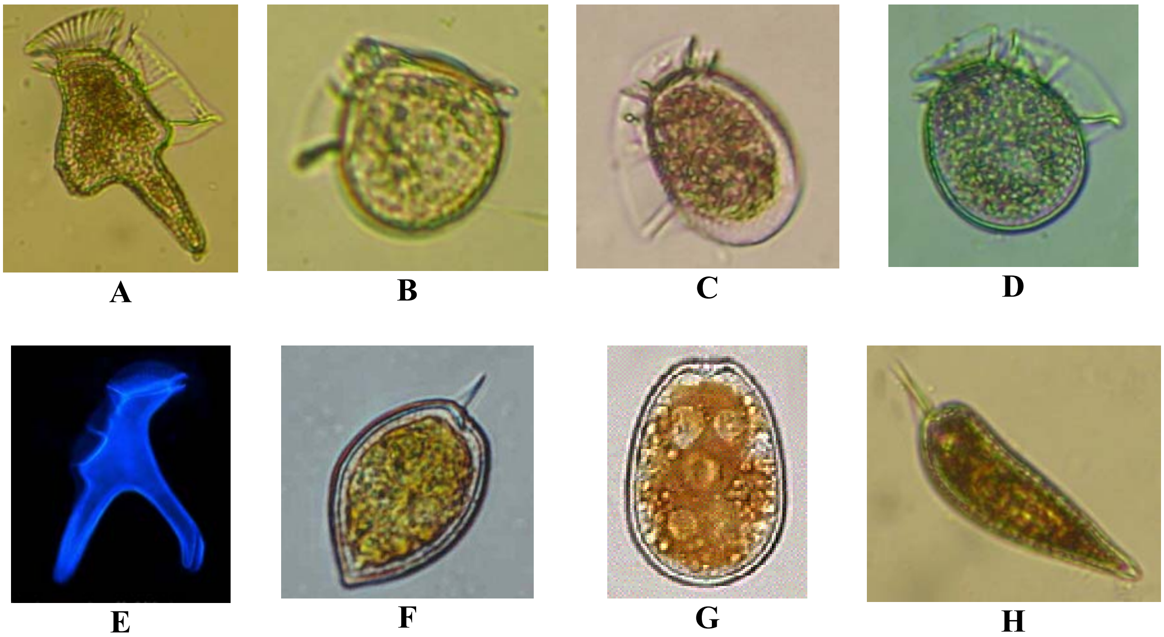


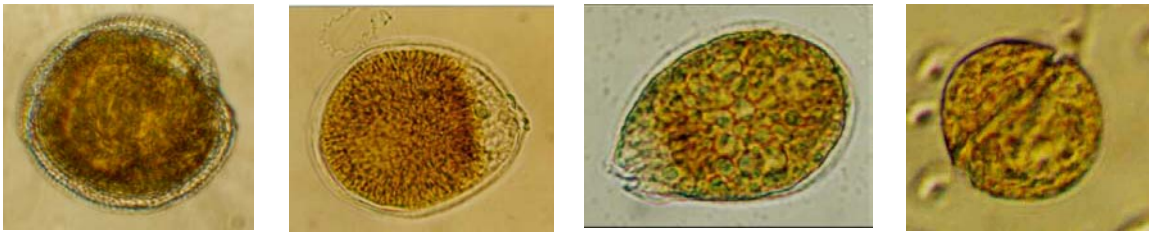
Figure 3: Potential DSP toxin-producers present in Malaysian waters. (**A**) *Dinophysis caudata*, (**B**) *Dinophysis rotundata*, (**C**) *Dinophysis acuminate*, (**D**) *Dinophysis* sp., (**E**) *Dinophysis fusus*, (**F**) *Prorocentrum micans*, (**G**) *Prorocentrum lima*, and (**H**) *Prorocentrum triestinum*.

Ciguatera fish poisoning (CFP)

This is one of the most interesting seafood poisoning, in that it is of solely tropical origin, toxins are produced by benthic dinoflagellates, and the vectors are various species of marine fish. Export of fishes to other countries can result in the occurrence of CFP in places far from where the ciguatoxic fishes originate (Geller et al. 1991). There are several species of benthic dinoflagellates which may be involved in CFP, the primary ones being *Gambierdiscus toxicus*, *Ostreopsis* spp. and *Coolia* spp (Lewis and Holmes, 1993). These species produce various toxins and bioactive compounds, and when the dinoflagellates are grazed upon by herbivorous fishes which are in turn preyed upon by larger carnivorous fishes such as barracuda, snappers and groupers, the toxins eventually get transferred to humans.

The three major CFP toxins are the polyethers ciguatoxin and palytoxin and the water soluble maitotoxin. Ciguatoxin is an extremely potent sodium channel toxin and its binding results in continuous depolarisation of the sodium channels leading to paralysis and in extreme cases death (Gordon 1997). Palytoxin meanwhile is even more lethal since it is a specific inhibitor of Na+, K+-ATPase (Ishida *et al*. 1983). Maitotoxin induces nonselective cation current leading to rise in intracellular calcium (Putney, Jr. 1990). Highly elevated intracellular calcium can lead to cell death. The multiplicity of toxins involved makes CFP symptoms very complex. Previous studies have shown that a victim never fully recovers from CFP since symptoms can reappear under certain conditions such as stress or alcohol intake (Anderson and Lobel 1987). Most probably this is because ciguatoxin is stored in body fat and could be released into the blood stream.

Samplings that have been carried out have resulted in the isolation and culturing of several clones of potentially ciguatoxic dinoflagellate species from many seaweed beds and coral reefs in Malaysian waters (Figure 4). However, *G. toxicus* has so far only been found in the waters of Kota Kinabalu in Sabah. Interestingly there has been at least one incident of ciguatera in Sabah involving consumption of ciguatoxic red snappers caught from a coral reef on the west coast. A new species, *Coolia malayense* has also been described. All cultures have been tested for biological activities. Extracts of *G. toxicus, O. ovata* and *O. lenticularis* were lethal to mice and all species also produce hemolysins. Identities of the toxic factors are currently being investigated.



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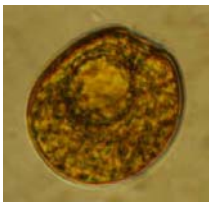

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Figure 4. Potentially ciguatoxic dinoflagellates found in Malaysian waters. (**A**) *Gambierdiscus toxicus*, (**B**) *Ostreopsis lenticularis*, (**C**) *Ostreopsis ovata*, (**D**) *Coolia malayense*, and (**E**) *Prorocentrum emarginatum*.

Amnesic shellfish poisoning (ASP)

ASP is the only seafood poisoning currently known to be caused by diatoms. It is also a relatively new poisoning, first described in 1987 from Prince Edward Island in Canada where it caused several intoxications including three fatalities (Perl *et al*. 1990). The toxin responsible for ASP is domoic acid, produced by several species of the cosmopolitan marine diatom *Pseudo-nitzschia* (Bates 1998).

Domoic acid is a water-soluble tricarboxylic amino acid that acts as an analog of the neurotransmitter glutamate and is a potent glutamate receptor agonist. Domoic acid binds with high affinity to both kainate and α-amino-3-hydroxy-5-methyl-4-

isoxazoleproprionic acid subtypes of the glutamate receptor, leading to greatly elevated levels of intracellular Ca^{2+} (Hampson and Manalo 1998). This causes neuronal cell death and lesions, particularly in the regions of the hippocampus responsible for learning and memory processing (Xi and Ramsdell 1996). This leads to short-term memory loss, thus the name ASP. Memory deficits can occur at doses below those causing structural damage of the brain. Domoic acid also causes intoxications in marine animals. In California, for example, every year mass mortalities of seabirds and marine mammals occur after the animals feed on anchovies containing high levels of domoic acid (Work *et al*. 1993; Scholin *et al*. 2000).

Domoic acid is a particularly worrisome toxin. Effects are cumulative and permanent. A person can suffer low level intoxication without displaying any symptoms. Furthermore studies on razor clams (*Siliqua patula*) have shown that domoic acid can persist in the shellfish for up to a year (Horner and Postel 1993). Samplings carried out in Selat Melaka have shown that several species of *Pseudo-nitzschia* are present (Figure 5). Efforts are currently underway to isolate, culture and identify these species and eventually test them for toxicity.

Figure 5. An unidentified *Pseudo-nitzschia* species found in Selat Melaka.

Cyanobacteria toxins

The final group of HAB species discussed in this review are the cyanobacteria or blue-green algae. These are ubiquitous prokaryots occurring in all sorts of aquatic and semi-aquatic environments. Many cyanobacteria species examined to date have been found to contain bioactive compounds including toxins. Most of the species studied originated from temperate waters. There have been very few studies on tropical cyanobacteria and there are probably many species yet to be discovered. From a public health perspective, the most important species are those occurring in freshwater, particularly in drinking water reservoirs, farmland and aquaculture ponds.

No one knows for certain the full diversity of cyanobacteria toxins, but three that are considered most important and have been widely studied are anatoxin-a, microcystin, and nodularin. Anatoxin-a is an extremely potent inhibitor of the nicotinic and muscarine acetylcholine receptor, causing postsynaptic cholinergic depolarization, that is it mimics acetycholine (Carmichael *et al.* 1979). Anatoxin-a is not broken down by acetylcholinesterase and thus it will cause prolonged depolarization leading to convulsions and possible death. Anatoxin-a is produced by *Anabaena* spp., *Aphazinomenon* sp. and *Cylindrospermum* sp. Microcystins constitute a family of monocyclic heptapeptides of great structural diversity. They are produced by several species of *Microcystis*, *Oscillatoria*, *Anabaena*, *Nostoc* and *Hapalosiphon*. The nodularins are a smaller family of cyclic pentapeptides structurally related to microcystins. They are produced by several species of *Nodularia*.

Both microcystin and nodularin are potent inhibitors of protein phosphatases PP1 and PP2. They are also hepatotoxins and tumor promoters (Fujiki and Suganuma 1993). Microcystin and nodularin that enter the body will eventually end up in the liver where they cause loss of normal cell-to-cell adhesion, disruption of intracellular architecture, intrahepatic hemorrhage and cell death. This liver disease is called Caruaru Syndrome, after a town in northern Brazil where in 1996 more than 50 diabetic patients died in a single event. The filtration system used to purify the water used in dialysis machines failed, resulting in very high levels of microcystins in the water (Jochimsen *et al*. 1998). Microcystins and nodularins not only affect humans but also livestock. In the United States, Finland, and Australia for example, livestock fatalities are common due to contamination of drinking water by the cyanobacterial toxins (Codd *et al*. 1994). More worrisome is the finding from some studies that suggest strong association of primary liver cancer with contamination of drinking water supplies by these cyanobacterial toxins (Ueno *et al*. 1996). Such is the importance of microcystin that the World Health Organisation has recommended a guidance value for microcystin of 1 $\mu g\ L^{-1}$ in drinking water (WHO 1997).

The extent of occurrences of toxic cyanobacteria and their toxins in Malaysia has never been studied to date. Samplings carried out in several freshwater bodies in Hulu Langat have shown several potentially toxic species are present, including *Anabaena, Nostoc, Microcystis, Oscillatoria* and *Cylindrospermum*. Some of these isolates have been established in laboratory cultures for identification and toxicity testing.

PUBLIC HEALTH ASPECTS OF HABs

Presence of HAB species imposes a severe burden on the affected country. This is compounded by wide adoption of the HACCP protocols which require that seafood is certified as safe and wholesome for consumption. Contamination by natural toxins is one of the parameters that need to be monitored. Ideally all high risk seafood such as bivalves and reef fishes should be tested before being marketed or exported, and in the case of cyanobacterial toxins, drinking water supplies should be routinely monitored for microcystins. However, the extent of the coastline, the unpredictable nature of these events, and shortage of manpower and facilities make regular, large-scale monitoring almost impossible. Thus monitoring activities are currently concentrated in areas where problems have been proven to exist. Nonetheless, the Department of Fisheries have made commendable efforts in this regard. In Sabah for example PSP cases have been very rare since the 1990s and in Semenanjung Malaysia no new PSP cases have been reported from Sebatu after the first event. Rarity of PSP events may also be due to increased public awareness of the HABs and shellfish toxicity. Problems will arise when HABs and toxicity emerge in a new location, like the recent event in Tumpat. In this situation, humans tend to be the detector organism.

It should also be realised that many HABs never truly disappear. Many of the species involved form resting cysts that can germinate and start new blooms when conditions are suitable. Areas where high densities of cysts occur would be highly unsuitable for shellfish aquaculture. It is strongly suggested that the current shellfish toxicity monitoring programs should be allocated more resources and expanded in geographical coverage. The programs should also not only concentrate on PSPs but also include monitoring of other toxins discussed here.

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