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Dinoflagellates: Ecological Approaches and Spatial Distributions in Malaysia Waters

Hamdan NA[1], Hassan MSA[1], Noor NM[2], Hamid SA[3] and Bunnori NM[1]*

[1]*Department of Biotechnology, Kulliyyah of Science, International Islamic University Malaysia, Pahang, Malaysia*
[2]*Department of Marine Science, Kulliyyah of Science, International Islamic University Malaysia, Pahang, Malaysia*
[3]*Department of Chemistry, Kulliyyah of Science, International Islamic University Malaysia, Pahang, Malaysia*

Abstract

Dinoflagellates are microscopic planktonic organisms that lead up the food chain in aquatic ecosystems. The naturally occurring phenomenon of *Dinoflagellates* blooming at the water surface brings detrimental effects to the economy as well as the environmental health of the affected ecosystem. Different types of *dinoflagellates* require different approaches in monitoring the excessive growth phenomenon. Identifying the vicinity of targeted species like environmental conditions is beneficial to overcome the blooming event. The tropical warm water of Malaysia eventually promotes the proliferation of harmful microalgae. Fish killings and human intoxication case reported in Malaysia to be precise are noteworthy effects brought by massive growth of *Dinoflagellates*. Shellfish poisonings are the silent killer to human as the outbreaks of harmful algae are obscured. Intoxication from *Dinoflagellates* may bring to death without prior notice. This paper provides information on *Dinoflagellates* growth mechanism and action potential induced by the toxins which are efficacious for mitigation and treatment purposes. Monitoring the pattern of blooming is a practical approach as no solid solution to the sudden outbreak has yet been clarified.

Keywords: *Dinoflagellates;* Harmful algal blooming; Shellfish poisonings; Fish killing; Malaysia

Introduction

Dinoflagellates: Multifaceted microorganisms

Marine ecosystem is rich in organisms with various sizes and shapes. The strata levels of sea depth assemble different types of marine creatures that adapt well at different pressure, temperature and light penetration. Phytoplankton contributes a lot in the food web of the marine ecosystem due to its role as primary producer in the food web. *Dinoflagellates* are a type of phytoplanktons, categorized under Kingdom Protista and further grouped into phylum *Pyrrophyta* as it is a single-celled organism with flagella [1].

The size of this tiny creature varies according to species, but most of them are larger than 20 µm which eventually classified them as microphytoplankton [2]. *Dinoflagellates* are differentiated from one another by the pattern of thecate or amphiesma which is made up of cellulose plates that protect the cells from the tugor pressure [1,3]. Previous study on the fossils of ancient *dinoflagellates* revealed six patterns of plate that classified the species into different major groups; gymnodinoid, suessioid, peridinioid, gonyaulacoid, dinophysoid, and prorocentroid [4].

Ecological Importance

Planktonic *Dinoflagellates* most likely reflect terrestrial plant as part of the carbon source to the aquatic ecosystem. The presence is crucial to create a balanced aquatic ecosystem via carbon fixation and also endosymbiosis with the coral reef population.

Dinoflagellates are important producer of the marine environment because of the large amount of carbon fixed from the photosynthesis [5,6]. These microorganisms are characterized by their reliance towards light intensity since they are exposed to UV light at the pelagic area and rich in chlorophyll contents, which explained the significant of photosynthesis [7]. Chlorophyll a and c, β-carotene and peridinin are major components within the plastids of *dinoflagellates* [8,9]. The light energy captured and later synthesized into chemical energy is important to higher trophic level organisms for continuous survival under water. The transfer of energy occurs via the food web where the secondary groups members are vary in sizes; from zooplankton to larger protozoan and small fish. Besides that, these *dinoflagellates* absorb nutrients and inorganic carbon from the moving water and fixed the carbon via Calvin cycle to produce carbon dioxide molecule as the end product [5,10].

On the other hand, heterotrophic type of *dinoflagellates* like *Noctiluca scintillans* and *Gyrodinium* spp. compensate the lack of photosynthesis system by preying on other smaller microorganisms like bacteria and other protists [11]. This prey-predator relationship is fundamental to control populations within the ecosystem. The feeding process in some cases results in existence of characteristics that are unusual to their origins. For instance, pigmentation analysis on some species from genera *Karenia* showed the presence of fucoxanthin unique to other organisms [12]. There is high possibility for the pigment molecule evolves from feeding on other organisms plastids that eventually resides and develops within the *dinoflagellates*. Both photosynthetic and heterotrophic *dinoflagellates* are crucial to keep the balance of the marine ecosystem either as the primary producer or the secondary user.

Symbiosis is an interaction between organisms which is beneficial for the endurance of smaller organisms like *dinoflagellates* in a vast marine ecosystem. Benthic *dinoflagellates* are well-known

***Corresponding author:** Bunnori NM, Ph.D., Assistant Professor, Department of Biotechnology, Kulliyyah of Science, International Islamic University Malaysia, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia, Tel: +60123873798, +6095705015 Ext. 5057; Fax: +6095716781; E-mail: noraslinda@iium.edu.my

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endosymbionts to coral reef ecosystem due to their developed mutual relationship [13]. Zooxanthelle, a type of dinoflagellate from *Symbodinium* spp. resides within corals and eventually gives coloration to the Ref. [14]. The symbiosis involves nutrient recycling of wastes from corals where zooxanthelle uses up the wastes to carry out photosynthesis [15]. Photosynthetic products are important for the continuum of coral metabolism, growth, reproduction and survival [10,16,17]. This is because oxygen-byproduct of *dinoflagellates* fuels up the rate of calcification by the reef-building corals [1,10]. In return, the reefs provide protection for zooxanthelle against grazers and free access of nutrients in the nutrient-poor environment [16]. This symbiosis is a good indicator for the early detection of reef deterioration as coral bleaching indicates the loss of zooxanthelle due to environmental stress like global warming and ocean acidification [13,18].

The cycle of life continues until the degree where dinoflagellate starts to outnumber as the environmental conditions change. Overloading of nutrients into coastal water from terrestrial runoff introduces a whole new phenomenon of algal blooming. Excess nutrients provide extra food thus speeding up the cell growth hence comes the blooming. The survival rate for *dinoflagellates* is species-specific hence emergence of new species in new coastal water is plausible. The blooming of these microorganisms is considered harmful under certain consequences which are further explained in the subsequent topic.

Worldwide Occurrences of Harmful Algae Bloom (HAB)

Harmful algal bloom phenomenon is referring to a condition where there is an accumulation of toxic as well as non-toxic phytoplankton in large number and capable of bringing harm effects to the ecosystem. It is a world phenomenon due its wide range of occurrence around the globe in regards of the environmental conditions. This phenomenon can be dangerous to aquatic wildlife as well as human being who consumed seafood such as clams, mussels, and oysters that have been affected from the toxicity of *dinoflagellates*' byproducts. There are many recurring factors that favor the excessive growth and also toxicity attributes of *Dinoflagellates* which include nutrients enrichment: 1) Physical oceanography changes; 2) Environmental parameters; and 3) Anthropogenic factors of the coastal area. However, these factors are interchangeable in accordance with the preferences of the *Dinoflagellates*.

Physical oceanographic changes

Geographical layouts along the coastline are the major factor that favors the excessive growth of both benthic and planktonic *dinoflagellates*. The most prevalent element is the water dynamic of the assemblage area as the colonization of the *dinoflagellates* will disperse upon strong wave action [19]. This is coincides with the fact that most of blooming cases were reported in sheltered area where there is less influence of vertical mixing. *Alexandrium catenella* bloom was recorded in Thau Lagoon of Mediterranean Sea where the wave action is restricted hence fostering the growth rate of the microalgae [20,21]. The lagoon was also furnished with harbors and jetties that provide full protection of the embayments from tidal actions [21]. Lack of water flushing is one of the conditions that encourage eutrophication to occur. A study by Hall et al. [22] claimed that poor mixing of the water column contributed to the stratification of Neuse River Estuary of North Carolina (USA) where dinoflagellate *Karlodinium veneficum* was found dominating the region. All these external circumstances accidentally boost up the cell division where eventually promotes the extensive blooming of *Dinoflagellates*.

Environmental parameters

Physical variables like water temperature and salinity of the blooming region also support the initiation of blooming and these parameters usually differ with corresponding species. The high temperature is favorable for benthic species and it is further verified when there is no blooming reported during winter [23]. An experimental study by Granéli et al. [24] concluded that elevated sea temperature was stimulating the bloom of *Ostreopsis ovata* in Brazil and Italy. The species grew well beyond 26°C with the influence of other promoting factors. A monitoring study in Golden Horn Estuary, Turkey revealed an attack of blooming species *Prorocentrum* minimum within two consecutive years [25]. Both incidents were recorded during late spring and summer seasons with tremendous increase of cell counts within weeks of occurrence.

Water salinity affects blooming activity differently and it usually varies in time due to rainfall and freshwater runoff. It is rational to the mixing fact mentioned earlier where blooming was intensified under calm water. A three months blooming of *Karenia mikimotoi* at the southwest coast of India had caused fish mortalities and the salinity of water recorded during the outbreak was more than 33 ppt [26]. A laboratory culture of *Cochlodinium polykrikoides* from Japan isolates grew best at salinity range between 30 to 35 ppt which corresponds to the in situ condition [27]. Meanwhile, *dinoflagellates* thrive at the estuary explains the ability of some species to grow in wide range of salinity. For instance, a reliable study on salinity effects of toxic *Alexandrium minutum* isolated from Bay of Morlaix in France showed optimum growth rate between 20-37 p.s.u. [28].

Altogether, water temperature and salinity are highly species-specific. Some *Dinoflagellates* adapt well to temperate as well as tropical water and they also can be grouped into euryhaline and stenohaline. All these data are crucial to predict blooming reoccurrence in the future.

Anthropogenic factors

Excessive nutrients loading from land is the ground to massive blooming of harmful microalgae along the coastline. Nitrogen and phosphorus are the two main elements that regulate the growth of *Dinoflagellates* apart from common requirement such as light. The ratio however differs according to species. Water pollution discharged from the industrial effluents, agricultural wastes and domestic sewages may provide excess nutrients that could cause *Dinoflagellates* to grow excessively in response to the high nutrients input [29]. Intensive fish farming technique also brought detrimental effect towards blooming of *Dinoflagellates* due to excessive usage of feed and chemicals [30].

Blooming initiation also becomes part of many studies in order to find the cause. It is also possible that one species is introduced to other part of coastal waters through ship's ballast water. The introduction of nonindigenous phytoplankton via ballast water has been discussed since 1800s when the harbor water was started to be used in place of solid ballast [31,32]. Dinoflagellate cysts might be transported from other countries and deported to different location during ballast water exchange activity. It thus explains on the similar fingerprints of Alexandrium sp. detected in Australian port (1986) with the one confined to Japanese coastal waters [33,34]. In Peninsular Malaysia, several species of Alexandrium have been reported to cause Paralytic Shellfish Poisoning (PSP) problems. The outbreaks of PSP were originally restricted to the temperate water of Europe, North America and Japan [35] but started to disseminate in 1970 to the southern part of the globe where the probability of being transshipped became the main logical argument. The idea was further justified with the discovery of Asian strain of A. catenella where the species was found blooming in Mediterranean Sea [36]. The different colonizing area indicates that some of these species adapt well to different environmental conditions. The bloom initiation somehow took from months to years for the cysts to germinate as it relies on the favorable environmental conditions as well as the nutrients availability.

Impacts of Excessive *Dinoflagellates* Blooming in Malaysian Waters

The natural occurrence of *dinoflagellates* is tolerable but becomes a nuisance once they start to outgrow the normal population. There are different consequences took place during blooming events. Some of them include disturbances to the aquatic wild life and eventually cause loss to the aquaculture industry [37]. The worst impact experienced from HAB outbreak was food poisoning as a result of consuming contaminated shellfish from affected area. The first case of seafood poisoning in Malaysia was recorded in 1976 with fatalities [37,38] and HAB events occurred afterwards throughout the coastal water of Malaysia. The summary on some of the HAB cases was presented in Figure 1.

Shellfish poisoning

Shellfish like bivalves and molluscs are the common vectors for most *dinoflagellates* poisoning cases apart from fish and other sea creatures. This is because shellfish feeds by straining particulate matters suspended in the water column including *dinoflagellates* [39]. Filter feeders like shellfish are naturally insensible to the toxic *dinoflagellates* as contaminated shellfish give no difference in terms of smell and taste. Toxin-related diseases caused by *dinoflagellates* have been extensively encountered yet no definite cure is manufactured worldwide. There are a few numbers of poisonings prevalence to seafood products with highlights in Malaysia waters and that includes Paralytic Shellfish Poisoning (PSP), Diarrheic Shellfish Poisoning (DSP), Neurotoxic Shellfish Poisoning (NSP) and Ciguatera Shellfish Poisoning (CFP) as shown in Table 1.

Dinoflagellates are obligated to stimulate most of the endemic seafood poisoning. The toxins synthesized by *dinoflagellates* are classified according to its symptoms expressed on patients inclusive of paralysis, diarrhea and neurological episodes pertaining to the central nervous system as well as gastrointestinal function. There is no specific threshold on the cell abundance of toxic *Dinoflagellates* for the outbreak to be considered harmful yet other methods have been used for the toxin monitoring such as mouse bioassay technique. This is because different species vary in terms of toxin synthesis regardless of its cell density. *Alexandrium tamiyavanichii* from Japan isolates produced high toxins in low cell count [35]. Malaysia strains of *A. tamiyavanichii* also showed high toxin level at low cell count of approximately between 20 to 40 cells per litre [44]. Malaysia has been confronted with the outbreak of PSP and has identified a few potential species of inducing NSP, DSP and CFP in Malaysia waters. This is probably due to the prevalence of some species to endure the tropical weather of Malaysia. These four types of poisonings were further discussed in terms of mode of action and symptoms developed.

Paralytic Shellfish Poisoning (PSP) Paralytic shellfish poisoning is a neurological malfunction of human immune system which developed right after consuming contaminated shellfish. Saxitoxins (STX) is the prototype of congeners mainly from *Dinoflagellates* and responsible of inducing PSP to human [45,46]. The congeners developed from the skeletal structure of 3,4,6-trialkyltetrahydropurine compound into carbamate, decarbamoyl, N-sulfocarbamoyl and hydroxybenzoate with each varied in their toxic potential [47]. These potent toxins act through blocking of sodium voltage-gated of sodium channel thus stimulating paralysis to the body parts and functions. An illustration of the binding site was presented in Figure 2. Depolarization of membrane potential is suppressed due to the binding of STX at the receptor of membrane protein [48]. The resting state of the membrane potential signals

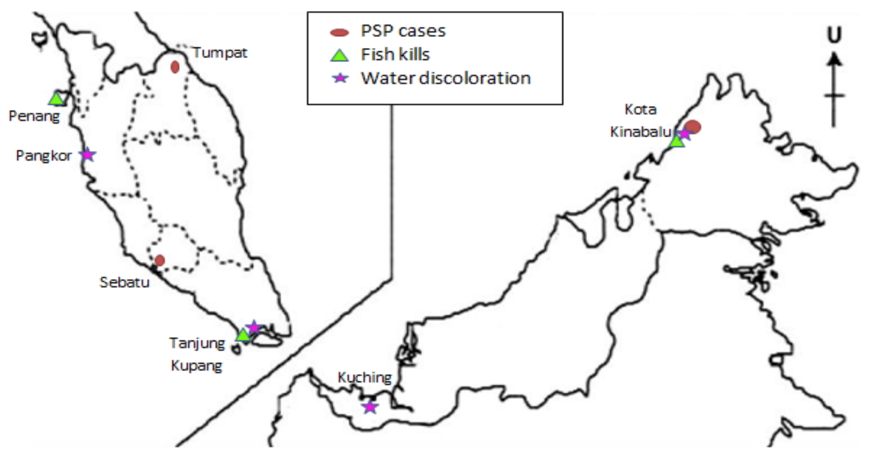

Figure 1: Summary on the blooming events of harmful microalgae throughout Malaysia coastal water.

Poisoning	Toxin	Organisms
Paralytic Shellfish Poisoning (PSP)	Saxitoxin	*Alexandrium* spp. [40]
Diarrheic Shellfish Poisoning (DSP)	Okadoic acids	*Dinophysis*, *Procentrum* [41]
Neurotoxic Shellfish Poisoning (NSP)	Brevetoxin	*Karenia brevis* [42]
Ciguatera Shellfish Poisoning (CFP)	Ciguatoxin	*Gambierdiscus toxicus* [43]

Table 1: Poisoning prevalence in Malaysia waters.

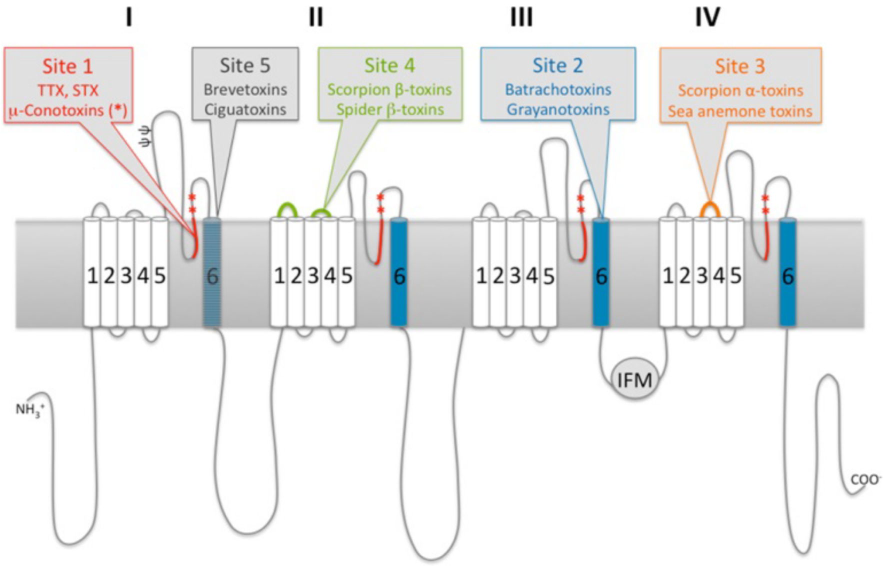

Figure 2: Two dimensional structure of voltage-gated sodium channel with binding sites for STX and BTX [55,56].

the onset of paralysis. Blocking of sodium conductance eventually generates symptoms of numbness, tingling sensation of the oral parts and nausea to the patient. The after affects took minutes to be expressed depending on the toxins concentrated within the shellfish [46].

Pyrodinium, Alexandrium and Gymnodinium are the three genus of *Dinoflagellates* affiliated to cause PSP worldwide [46]. In Sabah, PSP cases have been extensively reported since the first outbreak of Pyrodinium bahamense in 1976 [49]. Two-hundred and two cases of shellfish poisoning with seven deaths prompted the researchers to find the cause of the occurrence. Few more cases of algal blooms were reported several years later with a total of 31 PSP cases and 11 fatalities in 1988 after consuming shellfish from Sabah coastal water [50]. Meanwhile in Peninsular Malaysia, the most frequent cases of HABs reported were in Tebrau Strait where the rate of blooming is highly influenced by the water turbulence [51]. *Dinoflagellates* from genus Alexandrium predominate the West and East coasts of Malaysia with severe occurrences of PSP cases being reported [52]. *A. tamiyavanichii* was identified during an outbreak in Sebatu Malacca (1991) where green mussel breeding project was once established with three cases of poisoning [53]. However, the agent of toxicity of PSP cases in Tumpat was *A. minutum* where six persons were hospitalized with one casualty after consuming benthic bivalve (lokan).

Neurotoxic Shellfish Poisoning (NSP): Neurotoxic shellfish poisoning is known for causing failure of both gastrointestinal and neurological functions [42]. There is no lethal case reported to date during the outbreak of NSP. The major potent toxin released is brevetoxin with molecular weight of 900 Da. This heat stable toxin was isolated originally from dinoflagellate genus Karenia. The action mechanism of this toxin is totally opposite to the STX as brevetoxins stimulates the opening of sodium channel rather than blocking the inflow [54]. The potential mechanisms of action involve binding of brevetoxins at site 5 of voltage-gated sodium channel as shown in Figure 2 [55]. The binding leads to continuous influx of Na+ thus prolonged the depolarization of action potential [56].

The outbreak of NSP was found predominant in Florida since mid-1800s but not being specific to the species of Karenia brevis [57]. Fortunately, NSP was not documented in the Malaysia history of HAB events. The effects of NSP extended towards impairing of the immune system thus explained the various symptoms of poor coordination and organ numbness. In some cases, the poisoning may spread via exposure to the contaminated aerosols [58]. Onshore wind further aid the spreading of the toxin molecules 1 mile inland which was occupied with residents [59,60]. The congestion of airways with the minute toxin particles contributed to the dysfunctional of respiratory system of living things such as breathing difficulties, bronchitis and pneumonia [54,60].

Diarrhetic Shellfish Poisoning (DSP): Diarrhetic shellfish poisoning is the less severe type of intoxication for human. As the name implies, the toxins aimed for the dysfunctional of human digestive tracts. Species identification was carried out during the outbreak and *dinoflagellates* from genus Dinophysis and Prorocentrum were found to be responsible for the toxicity incursion. The first occurrence of DSP was recorded in the Netherlands during 1960s which then dispersed to Japan [61]. The frequent toxins causing DSP are found to be Okadaic Acid (OA) and dinophysiotoxins. Woo and Bahna [62] also stressed on the ability of OA to inactivate protein phosphatase function which can lead to tumor growth of the mammalian cells.

Hyperphosphorylation may result from the suppression of phosphatase and eventually caused fluid loss at human cytoskeletal junctions [54]. Diarrhea and vomiting are common indications developed by the patients prior to clinical diagnosis. In some cases the symptoms may be misconceived with symptoms of infections due to Vibrio chlorella. In Malaysia, no lethal case yet reported from DSP although an outbreak of its potential causative agent, Prorocentrum minimum had caused massive water discoloration in Tebrau Straits. However, the cell count was quite low to induce toxicity of shellfish [51].

Ciguatera Fish Poisoning (CFP): As for benthic species, the intoxication is detected from eating reef fish during the blooming event of HAB species mainly from genus *Gambierdiscus* [63]. Severe cases of Ciguatera Fish Poisoning (CFP) were reported among populations in small islands where deep water fish are known as a signature dish for such community [64]. The two common toxins inducing CFP are synthesized; maitoxin and ciguatoxin which attack calcium and sodium channels respectively. Specific binding site for these toxins has not been studied in details due to the insufficient amount of toxins extracted from the causative species [65]. However, it can be inferred that voltage-dependent sodium channel is the target for the activation of the toxicity.

There was no CFP cases reported in Malaysia to date but several identifications of the causative agents have been justified throughout monitoring process. *Gambierdiscus belizeanus* and *Gambierdiscus toxicus* were identified from the water bed of east coast of Sabah [66]. Meanwhile other potential species of CFP from genus Coolia and Ostreopsis [67] were detected in most Malaysia benthic ecosystem based on the samplings collected from coral fragments, seaweed and sediments yet their presence bring no harm to the ecosystem.

Water discoloration and fish killings

The blooming of non-toxic *Dinoflagellates* could also brought harm to the locality, Its dense accumulation capable of impairing aquaculture industry as well as tourism sector. Malaysia water experienced economical loss due to phenomenon of massive blooming apart from poisoning casualties. The aquaculture industry is largely affected due to massive fish killings resulted from oxygen depletion and physical infection [68]. The hypoxia condition induced by excessive growth of microalgae at the water surface leads to insufficient oxygen supply for fish respiration. Planktonic *dinoflagellates* move independently within the water column and commonly assemble at the water surface for maximum UV light exposure. The energy from the sunlight is essential for photosynthesis to occur. However excessive growing of these *dinoflagellates* create nuisance to other marine organisms as they start to cover the water surface thus reducing the oxygen availability for the ecosystem. In severe cases the bloom capable of developing 'dead zone' where the marine life prone to death due to low levels of dissolved oxygen.

Massive fish killing due to oxygen depletion and also clogging of the gills due to excessive slime production by the *Dinoflagellates* were reported in Sabah with the blooming of Cochlodinium polykrikoides [52,69,70]. The same species was also responsible for the huge loss of caged finfish in Penang with minimum loss of 6 million USD recorded [51]. During that incident, the fish were found dead due to suffocation from the excess mucus from *dinoflagellates* that smothers the gills. Apart from that, fish killing also resulted from continuous ingestion on the epidermis tissue of the fish by dinoflagellate species like *Pfiesteria piscicida* [68]. These *dinoflagellates* are armored with peduncle that helps their attachment to fish organs like gills and fins where ingestion happens gradually. Unfortunately, this can be lethal when toxic microalgae attack speeds up the toxin release upon contact with the ruptured gills [71]. Recent cases of fish killing were documented in Tanjung Kupang, Johor that involved economical loss to 250 fishermen and fish farmers due to *Karlodinium australe* [53,72]. Economical loss due to mass mortality of finfish is alarming as it concerns the aquaculture industry as well as food industry in Malaysia.

Conclusion

Many efforts were developed and keep piling up throughout monitoring and mitigating process in response to the negative impacts of *Dinoflagellates* blooming. Annual events of HABs in Malaysia have led to different ways of reducing the impacts of HABs which include early warning on possibility of blooming reoccurrence based on monthly water quality analysis. Apart from that, shellfish banning procedures were also announced during sudden outbreak of *dinoflagellates* to avoid further casualties due to consumption of contaminated shellfish [39]. Preventing reoccurrence is quite a challenge as there is no way to control the laws of nature. However, controlling is currently the only possible solution for the damaging effects of HABs with few undergoing researches on mitigation of the problems. There are a number of studies recently focus on the characteristics and behavioral of the causative agents so that a great solution on preventing reoccurrence can be developed. Fundamental studies of *dinoflagellates* from its DNA to protein mapping as well as byproduct analysis are useful to understand the biochemistry and hence cease the blooming. Awareness among the publics on the cause and consequences of HABs should be organized frequently so that mutual understanding on securing the balance of the ecosystem can be achieved.

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