SUBSTRATE PREFERENCES AND SEASONAL VARIATION OF CIGUATERA FISH POISONING DINOFLAGELLATES IN KOTA KINABALU COASTAL WATERS

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ABSTRACT

Ciguatera fish poisoning (CFP) is a food poisoning endemic to tropical and subtropical regions caused by eating fish that have been contaminated with toxins produced by dinoflagellates mainly of the genus *Gambierdiscus.* The toxin may be found in large reef fish, generally barracuda, red snapper, eel, grouper, sea bass, amberjack and Spanish mackerel. It is the most common seafood poisoning affecting the human population worldwide with 50,000 to 500,000 global incidences annually. Since 1984, there were a few suspected cases of CFP in Sabah, but these were never confirmed. This study aims to identify the CFP species with emphasis to the distribution and substrate preferences as well as the seasonal effects on the cell abundance of CFP dinoflagellates in the reefs of Kota Kinabalu, Sabah, Malaysia. Sepanggar Island, Manukan Island and Gaya Island were chosen as the sampling sites. Sampling duration was done in 12 months which involved 24 diving trips in total. A total of 149 fiberglass screens were used as an artificial substrate to collect the benthic epiphytic-CFP associated microalgae for 24 hours. Samples were then brought back to the laboratory for sample processing and cell enumeration. Photoquadrat survey method was adapted for habitat mapping by taking photographs underwater using a 0.25 m^2 quadrate at the sites of where devices were deployed. Bray-Curtis similarity was used to construct cluster analysis based on relative abundance data and relationships between substrate types were visualized using cluster analysis. One-way ANOVA (Kruskal-Wallis test) and Spearman correlation tests were done using GraphPad Prism 5 (GraphPad Inc.) for data analysis. The results recorded all five genera of CFP causing dinoflagellates *Amphidinium, Coolia, Gambierdiscus, Ostreopsis* and *Prorocentrum* were found in coral reef marine environments in low abundances at Kota Kinabalu coastal waters. *Prorocentrum* was found dominant in Sepanggar Island (62.2%), while *Ostreopsis* dominates in Manukan Island (73.6%) and Gaya Island (75.7%). Four major distinct geomorphic zones (rubbles, sand, dead corals and macroalgae) were indicated from cluster analysis results. The results showed dinoflagellates in this study were epiphytic on dead corals, and low densities were detected on sands. Two identified CFP dinoflagellates species showed statistically significant substrate preferences. For instance, *Gambierdiscus* was mainly founded on sand, *Prorocentrum* mainly encountered at macroalgae, while *Ostreopsis, Coolia* and *Amphidinium* showed no preferences on the four main substrate types. Mean total abundance of CFP dinoflagellates varied significantly between seasons (Southwest Monsoon>Northeast Monsoon>Inter-Monsoon). Throughout the study, gradual increases of *Gambierdiscus* cell numbers were observed during Southwest Monsoon (SWM). A significant positive correlation was observed between *Gambierdiscus* cell and sea surface temperature. *Prorocentrum* cell numbers were stable between seasons but showed an increasing pattern during Northeast Monsoon (NEM). *Coolia* and *Ostreopsis* did not show any pattern related to seasonal changes but average cell abundances of both dinoflagellates were significantly different between SWM and Inter-Monsoon (IM), while *Amphidinium* cell numbers showed no significant difference between seasons. The results observed highest average abundances of total CFP dinoflagellates during SWM (65.22 ± 149.5 cells/ 100 cm^2), while the lowest abundances were during IM. In conclusion, this study can be used as a reference in studying and managing benthic harmful algal bloom dinoflagellates by safeguarding public health in order to reduce seafood poisoning cases.

ABSTRAK

KEUTAMAAN SUBSTRAT DAN VARIASI MUSIM BAGI DINOFLAGELLAT KERACUNAN IKAN CIGUATERA DI PERAIRAN PANTAI KOTA KINABALU

Keracunan Ikan Ciguatera (CFP) adalah keracunan makanan endemik di kawasan tropika dan subtropika yang disebabkan oleh ikan yang tercemar dengan toksin yang dihasilkan oleh dinoflagellat genus Gambierdiscus*. Toksin tersebut boleh dijumpai pada ikan karang bersaiz besar, seperti barakuda, kerapu, ikan merah, belut, "amberjack", ikan siakap dan ikan tenggiri Sepanyol. Ia merupakan keracunan makanan laut yang menjejaskan populasi manusia di seluruh dunia dengan jumlah insiden global sebanyak 50,000 hingga 500,000 setiap tahun. Sejak tahun 1984, terdapat beberapa kes yang disyaki CFP di Sabah, tetapi kes tersebut tidak pernah disahkan. Kajian ini bertujuan untuk mengenalpasti spesies CFP dengan menjurus pada taburan dan keutamaan substrat serta kesan musim ke atas kelimpahan sel dinoflagellat CFP di terumbu karang Kota Kinabalu, Sabah, Malaysia. Pulau Sepanggar, Pulau Manukan dan Pulau Gaya telah dipilih sebagai tapak kajian. Pensampelan telah dijalankan selama 12 bulan yang melibatkan 24 selam skuba. Sebanyak 149 skrin gentian kaca digunakan untuk mendapatkan dinoflagellat CFP selama 24 jam. Sampel kemudian dibawa balik ke makmal untuk pemprosesan sampel dan penghitungan sel. Kaedah 'photoquadrat' telah diaplikasi untuk pemetaan habitat dengan mengambil gambar menggunakan kuadrat bersaiz 0.25 m^2 di dalam air. 'Bray-Curtis similarity' digunakan untuk membina analisis kluster berdasarkan data kelimpahan relatif dan hubungan di antara jenis substrat didapatikan melalui analisis kluster. Semua data analisis dijalankan dengan kaedah ANOVA Sehala (Kruskal-Wallis test) dan 'Spearman correlation test' menggunakan GraphPad Prism 5 (GraphPad Inc.). Hasil kajian merekodkan semua lima genera dinoflagellat CFP* Amphidinium*,* Coolia*,* Gambierdiscus*,* Ostreopsis *and* Prorocentrum *ditemui di persekitaran terumbu karang marin dalam jumlah yang rendah di perairan pantai Kota Kinabalu.* Prorocentrum *didapati dominan di Pulau Sepanggar (62.2%), manakala* Ostreopsis *mendominasi di Pulau Manukan (73.6%) dan Pulau Gaya (75.7%). Hasil analisis kluster menunjukkan terdapat empat zon utama geomorfik yang berbeza (pecahan karang, pasir, karang mati dan makroalga). Hasil kajian mendapati dinoflagellat dalam kajian ini adalah epifit pada karang mati dan kepadatan rendah dikesan pada pasir. Dua spesies dinoflagellat CFP yang dikenal pasti menunjukkan keutamaan substrat yang signifikan. Sebagai contoh,* Gambierdiscus *ditemui pada pasir,* Prorocentrum *didapati di makroalga, manakala* Ostreopsis*,* Coolia *dan* Amphidinium *tidak menunjukkan keutamaan terhadap empat jenis substrat utama. Purata jumlah kelimpahan dinoflagellat CFP berubah secara signifikan antara musim (SWM> NEM> IM). Sepanjang kajian, bilangan* Gambierdiscus *didapati meningkat semasa SWM. Korelasi positif yang kuat diperhatikan antara sel* Gambierdiscus *dengan suhu permukaan laut. Bilangan sel* Prorocentrum *adalah stabil sepanjang musim tetapi menunjukkan corak peningkatan semasa NEM.* Coolia *dan* Ostreopsis *tidak menunjukkan corak yang berkaitan dengan perubahan musim tetapi purata kelimpahan sel bagi kedua-dua dinoflagellat antara SWM dan IM menunjukkan perbezaan signifikan. Bilangan sel* Amphidinium *tidak menunjukkan perbezaan yang signifikan di kalangan musim. Hasil kajian menunjukkan purata kelimpahan tertinggi jumlah dinoflagellat CFP yang direkodkan adalah semasa SWM (65.22 ± 149.5 sel / 100 cm^2), manakala yang paling rendah adalah semasa IM. Sebagai kesimpulan, kajian ini boleh digunakan sebagai rujukan*

dalam kajian dan pengurusan dinoflagellat ledakan alga bentik berbahaya dengan menjaga kesihatan masyarakat untuk mengurangkan kes-kes keracunan makanan laut.

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BHAB	:	Benthic harmful algal bloom
CFP	:	Ciguatera fish poisoning
CTX	:	Ciguatoxin
DO	:	Dissolved oxygen
DSP	:	Diarrheic Shellfish Poisoning
DTX	:	Dinophysistoxin
HAB	:	Harmful algal bloom
IM	:	Inter-Monsoon
MOH	:	Ministry of Health
MTX	:	Maitotoxin
NEM	:	Northeast Monsoon
OA	:	Okadaic acid
PAST	:	Paleontological statistics software package
PSP	:	Paralytic Shellfish Poisoning
SWM	:	Southwest Monsoon

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CHAPTER 1

INTRODUCTION

1.1 Overview of ciguatera fish poisoning

During the Spanish Conquest, there was an occurrence of a food poisoning caused by consumption of a marine snail*, Turbo pi*ca (termed cigua by the natives in Cuba) in the Caribbean and to illustrate this intoxication, a term ciguatera fish poisoning (CFP) was used (de Fouw *et al.,* 2001). Guzman-Perez *et al.,* (2000) stated that the word CFP is used today to name the intoxication instigated by consuming reef fish which have gained specific toxins from their diet. The fish usually originates from the tropical and subtropical areas of the Caribbean Sea and the Pacific Ocean.

CFP is a seafood illness endemic to tropical and subtropical coral reef areas of the world. Dinoflagellates of the genus *Gambierdiscus* produces lipid-soluble toxins, accumulates in finfish and consumption of certain reef fish causes intoxication (Dickey & Plakas, 2010). According to Mattei *et al.,* (2014), the most common seafood illness worldwide is ciguatera with 50,000 to 500,000 global occurrences per annum and it is mostly restricted to endemic areas which are located between 35° northern and 35° southern latitude.

de Fouw *et al.,* (2001) stated that a lot of countries such as Australia, Bahamas, Canada, China, Haiti, Hawaii, Madagascar, Mexico, Tonga, US, and even

in Germany and the Netherlands have reports of ciguatera intoxication. A theory made by Lehane & Lewis (2000) mentioned that ciguatera may develop at areas which are far from tropical oceans, where alive, fresh or preserved reef fish are exported to other areas and this might lead to a world health problem. Throughout the year, due to the expansion of international seafood trade from tropical fisheries worldwide, ciguatera has become a threat to consumers in non-endemic countries (Kibler *et al.,* 2015). Mattei *et al.,* (2014) suggested that the globalization of fishing industry and tourism in Europe and the lack of awareness among medical personnel which causes under-reporting are the other factors which contributed to the huge increase in the amount of ciguatera cases.

The causative agents of CFP in human are marine biotoxins produced by dinoflagellates (Mattei *et al.,* 2014). Ciguatera causes gastrointestinal and neurological disorders and may cause human death in rare cases (Faust, 2009). According to Xu *et al.*, (2014), *Gambierdiscus* produces gambiertoxins which are the precursors for the ciguatoxins (CTX) and are responsible for ciguatera. The toxins enter the coral reef food web through grazing by herbivores and detritivores, and when these animals are eaten by predators, accumulation and biomodification of these toxins take place. Coral-reefs are nurseries for harmful dinoflagellates and possible sites for ciguatera outbreak world-wide (Faust, 2009). According to Bienfang *et al.,* (2008), the dinoflagellates *Gambierdiscus* spp. produces CTX and these dinoflagellates are commonly found in association with numerous macroalgae in the coral reef ecosystems. Herbivorous fish eat up these dinoflagellates, initiates the bioaccumulation, biomagnification, and biomodification processes in the food web, as the herbivores are eaten by carnivores and, ends up as human consumption.

The symptoms of ciguatera seem to differ geographically, between individuals and incidents and differ temporally within an area. It mostly consists of early-onset (two to six hours) gastrointestinal disturbance, such as nausea, vomiting, and diarrhea, and may be followed by other neurological illnesses (18 hours), including numbness, reversal of temperature sensation, muscle and joint aches, itching,

tachycardia, blurred vision and paralysis. On some rare occasions, ciguatera may cause death (Botana, 2000).

Unfortunately, due to under-reporting and misdiagnosis, the real extent of illness is not well documented (Radke *et al.,* 2015). Berdalet *et al.,* (2015) stated that the cases of CFP often have been traditionally 'managed' by native fishermen via their local and traditional knowledge. In tropical areas, CFP is a threat to public health, and it the most widespread, and hence best described, in the island population of the Pacific and the Caribbean. There has been a consistent increase in the number of known harmful species, algal toxins, toxic outbreaks, and fisheries and ecosystem impacts, all at a time when human dependence on the coastal area for food, recreation, and commerce is rapidly growing (Anderson*,* 1998).

1.2 Problem statement

Department of Fisheries Sabah has been doing monitoring on CFP and based on their unpublished reports from 1984 to 1992, there have been 38 complaints regarding fish poisoning mainly due to red snappers, particularly *Lutjanus bohar*. Most of the samples were collected from the Kota Kinabalu Fish Market, SAFMA Fish Landing Jetty and several places on the West Coast of Sabah. In April 1989, red snapper *Lutjanus bohar* was totally banned either for local or overseas market to safeguard public health and fisheries industry in Sabah. In year 2017, an unpublished report by Ministry of Health (MOH) Sabah stated that there were three suspected cases of CFP. In December of the same year, one person was confirmed by laboratory test and medical practitioner as a case of CFP. All these reports raised a lot of questions such as the origin of the sample and the toxin itself. How were the CFP dinoflagellates distributed in Kota Kinabalu throughout the years? Up until now, there were lack of information on where and what is the CFP pattern in Sabah. Thus, the purpose of this study was to (1) check the presence of other CFP dinoflagellates in coastal waters of Kota Kinabalu, (2) find out if the CFP dinoflagellates show any substrate

preferences and (3) observe if seasonal factors have any effects on the cell abundance of CFP dinoflagellates.

1.3 Significance of study

Seafood poisoning cases in the west coast of Sabah has started since 1970s. To date, monitoring of paralytic shellfish poisoning by Department of Fisheries Sabah (DOFS) was successful in lowering the risk of seafood poisoning. By knowing the pattern and distribution of CFP dinoflagellates, monitoring plan can be carried out effectively in HAB management programs. Therefore, this study can be used as a reference in studying and managing BHAB dinoflagellates by safeguarding public health.

1.4 Objectives of study

The objectives of this study are:

i. To identify the causative dinoflagellates species responsible to Ciguatera Fish Poisoning (CFP).
ii. To determine the substrate preferences of CFP Dinoflagellates in Gaya, Manukan and Sepanggar Islands.
iii. To determine the effects of seasonal factors on cell abundance of CFP Dinoflagellates.

CHAPTER 2

LITERATURE REVIEW

2.1 Harmful algal blooms in Malaysia

Harmful algal blooms (HABs) or commonly known as "red tides" are a worldwide problem which affects not only to human but also to our natural ecosystem. HABs can be categorized to three different types which are (1) dinoflagellate species that produce harmless water discolorations but cause mass mortality of fish due to oxygen depletion, (2) toxic to humans which causes gastrointestinal and neurological illnesses and (3) non-toxic to humans but harmful to fish by clogging their gills (especially fish in cage aquaculture).

The first report of HABs and shellfish toxicity in Malaysia was in 1976 when the *Pyrodinium bahamense* var. *compressum* bloomed in Brunei Bay on the west coast of Sabah and this event poisoned several people (Roy, 1977). The bloom eventually spread to other parts of Sabah but focuses mainly at the west coast of Sabah. Over the following few decades, many poisoning cases have been reported including fatality but with the establishment of monitoring programme, these cases were under control.

In Sabah, HABs caused by *P. bahamense* and *Cochlodinium polykrikoides* took place almost every year on the west coast of Sabah (Wang *et al.,* 2008), especially in coastal areas of Kota Kinabalu. Many poisoning cases have been reported over the years including casualty. Other unidentified toxic dinoflagellates may exist in waters of Sabah and current environmental conditions are not favorable for bloom to happen. The number of HAB species in Sabah are on the increase with toxic *Gymnodinium catenatum* identified off the west coast of Sabah (Mohammad-Noor *et al.,* 2008), *Gonyaulax polygramma* in Kota Kinabalu (Lim *et al*., 2014) and *Noctiluca scintillans* in Kota Marudu and Kudat (Department of Fisheries Sabah).

Previous research concluded that the waters in Malaysia have a high diversity of marine benthic dinoflagellates flora and the highest species diversity among the sites examined was Sipadan Island (Mohammad-Noor *et al.,* 2007). A survey conducted in Singaporean waters showed that species diversity was low, with *O. ovata* was the dominant species (Holmes *et al.,* 1998). *Gambierdiscus yasumotoi*, a new toxin-producing species was identified in the reef around the Singapore island of Pulau Hantu (Holmes, 1998). In Malaysia waters, several species of benthic dinoflagellates which may be involved in CFP have been identified, they were *G. toxicus*, *O. ovata*, *O. lenticularis*, and *Coolia* sp. Toxicity screening based on hemolytic assay, antimicrobial and mouse bioassay has proven the existence of biological activities in aqueous and organic phase extract of these cultures. However, the toxins compound is yet need to be determined (Lim *et al.,* 2003). Department of Fisheries Sabah took the initiative to conduct a preliminary study on distribution of CFP dinoflagellates in Sabah between 1994 – 1997 with the help of a consultant from the Asean-Canada Cooperative Programme and the results showed that Sepanggar Island, Kota Kinabalu recorded the highest number of *G. toxicus* cells (383 cells/ g FW) (Department of Fisheries Sabah). To date, there were no reported blooms caused by benthic dinoflagellates in coastal waters of Sabah.