Marine Biotoxins in Malaysia: Occurrence, Toxicity Cases, Analytical Capabilities and Regulatory Limits

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Abstract: Marine biotoxins are naturally occurring chemicals produced by certain types of toxic algae. Exposure to marine biotoxins can occur through consumption of toxin-contaminated seafood, direct contact by swimming or breathing in aerosolised toxins in water droplets. Symptoms of food poisoning can vary depending on the types of toxins ingested. Symptoms may vary from severe gastrointestinal intoxication symptoms such as diarrhoea, nausea, vomiting, and abdominal cramps to neurological disorders such as ataxia, dizziness, partial paralysis, and respiratory distress. In Malaysia, paralytic shellfish poisoning and tetradotoxin poisoning are the two most frequently reported cases of marine biotoxins poisoning. Poisoning cases are more often reported in Sabah and Sarawak than in the Peninsular Malaysia. This paper presents information on marine biotoxins in general as well as cases related to biotoxins poisoning that have been reported particularly in Malaysia. This paper also highlights biotoxins monitoring programs, analytical capabilities in the country as well as reference safety standards for biotoxins in shellfish.

Keywords: Marine toxins, shellfish, Malaysia, cases, food poisoning, toxic algae, monitoring

Abstrak: Biotoksin marin adalah bahan kimia alami yang dihasilkan oleh jenis alga toksik tertentu. Pendedahan kepada biotoksin marin boleh berlaku melalui memakan makanan laut yang tercemar dengan biotoksin marin, bersentuh atau berenang di dalam air laut yang tercemar atau bernafas dengan titisan air yang mengandungi aerosol toksin. Gejala keracunan makanan berbeza-beza bergantung kepada jenis toksin. Gejala mungkin berkaitan dengan keracunan saluran usus yang teruk dengan cirit-birit, mual, muntah, dan kekejangan perut hingga gangguan saraf seperti ataksia, pening, lumpuh separa, dan gangguan pernafasan. Di Malaysia, keracunan kerangan paralitik dan keracunan tetradotoksin adalah dua kes keracunan biotoksin marin yang paling kerap dilaporkan. Kes keracunan lebih banyak dilaporkan di Sabah dan Sarawak berbanding Semenanjung Malaysia. Kertas ini membentangkan maklumat-maklumat mengenai biotoksin marin secara umum serta kes-kes berkaitan keracunan biotoksin yang pernah dilaporkan, khususnya di Malaysia. Kertas ini turut menyentuh tentang program pemantauan biotoksin, kemampuan analitikal dalam negara serta rujukan piawaian keselamatan untuk biotoksin dalam kerang-kerangan.

Introduction

Marine biotoxins may cause serious pathologies in humans especially during the harmful algae bloom events as demonstrated in many reports. Their frequency and severity have both increased, indicating a serious global threat to public health. Marine biotoxins are naturally produced chemicals by microalgae in the sea. Based on the chemical structure, marine biotoxins are divided into two types (hydrophilic and lipophilic toxins) and eight groups (saxitoxin (STX), domoic acid (DA), azaspiracid (AZA), brevetoxin (BTX), okadaic acid (OA), pectenotoxin (PTX), yessotoxin (YTX), and cyclic imine (CI). The European Food Safety Authority (EFSA) (2009) added two more groups' i.e Palytoxin (PlTX) and ciguatoxin (CTX) to the existing groups. Table 1 shows types of marine biotoxins, its producer, clinical symptoms, and possible treatment. Marine biotoxins-related pathologies are usually named after the effects they cause or the species that produces the toxin. For instance, Paralytic Shellfish Poisoning (PSP) causes paralysis and brevetoxins is named after the producing microalgae, *Karenia brevis*.

Toxic microalgae are consumed by marine organisms, particularly filter-feeding species such as shellfish and mollusc which accumulate biotoxin and transmit it to humans and animals through the food chain (Farrell et al., 2013). Humans are primarily exposed to marine biotoxins through the consumption of contaminated seafood. In addition, exposure can occur through the ingestion of contaminated water (Batoréu et al., 2005), physical contact with water during an active bloom (Weirich and Miller, 2014), and inhalation of aerosolized toxins when a wave breaks up the cells of certain harmful microalgae (Batoréu et al., 2005; Fleming et al., 2006).

Larger animals like dolphins (Fire et al., 2011), seals (Jensen et al., 2015), sea lions (Brodie et al., 2006), and marine birds (Shumway et al., 2003) were also affected by the toxins. In short, besides being a public health concern, marine biotoxins also impose an economic consequence due to the closure of contaminated fisheries grounds and costs of running monitoring programmes.

Marine biotoxin is a worldwide issue, and Malaysia is not exempted. There is still limited published information about this subject, specifically on the local analytical capabilities and capacities. Hence, this paper focuses to report on the toxicity cases, analytical capabilities and regulatory limits of marine biotoxins in Malaysia. In this paper, we also cover tetrodotoxin (TTX) poisoning from puffer fish consumption as one of the marine biotoxins in Malaysia as intoxication due to TTX is the second most popular poisoning besides PSP.

Table 1: Types of clinical related poisoning due to marine biotoxins, toxins involved, producing microalgae, associated symptoms and treatment

Clinical related poisoning	Toxins	Producer	Symptoms	Other information
Paralytic Shellfish Poisoning	Saxitoxins and analogs	*Alexandrium tamarense*, *A. catenella,* *A. minutum, A. fundyense* *Gymnodinium catenatum*, *Pyrodinium bahamense* var. *compressum*, Cyanobacteria species (Lyngbya, Anabaena, Cylindrospermopsis, Aphanizomenon, Phlanktothrix)	Mild symptoms include tingling sensation or numbness around the lips, gradually spreading to the face and neck, pins and needles in fingertips and toes, headache, dizziness and nausea. Moderate symptoms include incoherent speech, progression of pins and needles to arms and legs, stiffness and non-coordination of limbs, general weakness and feeling of lightness, then slight respiratory difficulty and rapid pulse plus backache as late symptoms. Severe symptoms include muscular paralysis, pronounced respiratory difficulty and a choking sensation may occur. In fatal cases, death is caused by respiratory paralysis occurring within 2–12 h after the consumption of contaminated shellfish, in absence of artificial respiration (FAO/IOC/WHO, 2004).	No antidote Artificial respiration
Amnesic Shellfish Poisoning	Domoic Acid and analogs	*Pseudo-nitzschia* spp., Red algae such as *Chondria armata*, *Digenea simplex*, and *Alsidium corallinum*	In humans, symptoms range from gastrointestinal effects (nausea, vomiting, diarrhea, or abdominal cramps) and/or neurological signs (confusion, lethargy, disorientation, paresthesia, and short-term memory loss) and in extreme cases coma or death. (Visciano et al., 2016)	No antidote
Diarrhetic Shellfish Poisoning	Okadaic Acid, Dinophysis toxins	*Dinophysis* spp., *Prorocentrum* spp.	Nausea, vomiting, diarrhoea, abdominal pain	No antidote
Azaspiracids Poisoning	Azaspiracids and analogs	*Azadinium* spp., *Protoperidinium* spp.	Nausea, vomiting, diarrhoea, abdominal pain	No antidote

Neurotoxic Shellfish Poisoning	Brevetoxin	*Karenia brevis*	Nausea, vomiting, diarrhoea, chill, sweating, dysesthesia, hypotension, paraesthesia of lips, face and extremities, cramps, paralysis, seizures and coma after ingestion. Rhinorrhoea, cough, bronchoconstriction after inhalation	No antidote
Ciguatera Fish Poisoning	Ciguatoxin	*Gambierdiscus* spp., *Ostropesis* spp., *Prorocentrum* spp., *Coolia* spp.	Gastrointestinal symptoms, cardiovascular or neurological problems	No antidote. Activated charcoal
Puffer fish Poisoning	Tetrodotoxin	Puffer fish, newt, gobies, ribbon worm, lined moon shell, starfish, xanthid crabs, horseshoe crab, frogs, blue-ringed octopus, flatworms, trumpet shell, grey side-gilled sea lug, nematodes, gastropods, ocean sunfish, porcupine fish, sediment, bacteria	Perioral numbness and paraesthesia, nausea, lingual numbness, numbness of face, early motor paralysis and incoordination, respiratory failure, aphonia, hypoxia, cardiovascular symptoms	No antidote. Activated charcoal, anti-cholinesterase. Maintaining adequate respiration, endotracheal intubation and mechanical ventilation.

Marine biotoxins occurrence and intoxication cases in Malaysia

Paralytic Shellfish Poisoning

PSP is among the main intoxication cases reported due to marine biotoxin in Malaysia (Table 2) and also globally. The highest number of PSP cases (2124 with 120 deaths) was reported in the Philippines from 1983 to 2002 (Ching et al., 2015). The main producers of PSP toxins are dinoflagellates of the genera *Alexandrium* and *Gymnodinium*. More than 30 STX analogs have been identified and grouped into four subgroups: carbamate, N-sulfo-carbamoyl, decarbamoyl, and hydroxylated saxitoxins (EFSA, 2009).

The first case of PSP in Malaysia where 22 people were poisoned and seven died was reported on the west coast of Sabah in 1976 and was linked to *Pyrodinium bahamense var. compressum* (Roy, 1977). Since then, PSP have been regularly reported in Sabah and the Department of Fisheries (DOF) Sabah regularly conducts routine HAB monitoring to ensure the safety of seafood (Jipanin et al., 2019). On the other hand, the first documented PSP occurrence in Peninsular Malaysia was reported in 1991 in Sebatu Melaka, on the west coast of Peninsular Malaysia. The PSP was caused by *Alexandrium tamiyavanichii* bloom where three people were hospitalized after consuming contaminated green mussels (Usup et al., 2002). A PSP case due to *Alexandrium minutum* bloom which led to one death and 6 others hospitalised was later reported in September 2001 at Sg Getting, Tumpat, Kelantan (Lim et al., 2004). Besides Melaka and Kelantan, ten cases with typical symptoms of PSP intoxication from consumption of oysters contaminated with *A. tamiyavanichii* at Kuantan, Pahang were reported in November 2013 and recurred in August 2014 (Mohammad-Noor et al., 2017).

A. minutum bloom is common in Tumpat, Kelantan and is almost a yearly event. It was reported to recur in September 2015 (Lau et al., 2017). The selling and collection of shellfish from the area was banned because of the high level of saxitoxin detected in the shellfish tissue (Borneo Post Online, 2015). The most recent case of *A. minutum* in Tumpat, Kelantan was in August 2020 with cell densities ranging from 9, 250 cell/L to 867,500 cell/L. The Fisheries Research Institute, Batu Maung, Penang monitoring of the shellfish culture area around Sg. Geting, Tumpat, Kelantan recorded shellfish tissue with PSP toxin content ranging from 13.4 to 21.9 µg eq STX/100g, however no poisoning was observed (unpublished data).

Table 2: Reported human intoxications cases due to Paralytic Shellfish Poisoning in Malaysia (1976-2014)

Year	Location	Species	Number of patients	Reference
1976	West Coast of Sabah	*Pyrodinium bahamense* var *compressum* (Böhm) Steidinger, Tester et Taylor)	7 deaths	Roy (1977)
1976-1988	Sabah	*P. bahamense* var *compressum*	31 deaths	Ting and Wong (1989)
1991	Sebatu, Melaka	*Alexandrium tamiyavanichii*	3 persons hospitalized	Usup et al. (2002)
2001	Tumpat, Kelantan	*Alexandrium minutum*	1 death, 6 hospitalized	Lim et al. (2004)
2009	Kota Kinabalu, Sabah	*P. bahamense*	No data	DOF, Sabah (2009)
2013	Sepangar Bay or Kuala Penyu, Sabah	*P. bahamense* var *compressum*	58 cases 4 deaths	Suleiman et al. (2017)
2013 2014	Kuantan, Pahang	*A. tamiyavanichii*	10 hospitalized	Mohammad-Noor et al. (2017)

2015	Labuan	Not mentioned	2 cases	The Star, 13 April, 2015

Puffer fish poisoning

Puffer fish, mainly from the Tetraodontidae family is known to possess a neurotoxin or tetrodotoxin (TTX) which can cause poisoning and adverse effect to human health. Almost all puffer fish are poisonous and contain the poison (TTX) in their body parts. Puffer fishes are very common in Malaysian waters, often caught in large numbers by trawlers. There are at least 185 species of puffer fishes which are distributed in 28 genera in the family Tetraodontidae (Oliveira et al. 2006). They are 32 species of puffer fish that can be found in Malaysia, mainly in marine, brackish and freshwater in tropical and subtropical seas (Ambak et al., 2010). The most common species in Malaysia are *Lagocephalus lunaris*, *L. sceleratus* and *L. spadiceus*, consumed by some locals (Kan et al. 1987). In Sarawak, *Xenopterus naritus* or locally known as 'ikan buntal kuning' is considered a delicacy by the local community. Besides PSP, puffer fish poisoning is another main cause of intoxication reported due to marine biotoxin in Malaysia (Table 3). Food poisoning incidents associated with puffer fish consumption occur when consumers are unaware of the toxicity of the species. This could also be due to the consumption of toxic puffer fish that have not been handled properly. The latest case of puffer fish poisoning in Nov 2021 in Selangor where elderly couples were admitted to ICU after consuming puffer fish obtained through online shopping.

Table 3: Reported cases of puffer fish poisoning in Malaysia

Year	Location (cases)	Reference
1985	Sabah (4)	Lyn (1985)
1987	Sabah (9)	Kan et al. (1987)
1997	Terengganu (1)	Loke & Tan (1997)
2008	Johor (34), Sabah (1), Sarawak (1)	Chua and Chew (2009)
2009	Sabah 6, Terengganu (5), Sarawak (3)	Murali (2009), Razak et al. (2009)
2013	Sarawak	Pers. Comm.
2021	Selangor (2)	Pers. Comm.

Amnesic Shellfish Poisoning (ASP)

Domoic acid (DA) and other toxic DA isomers which are responsible for ASP are produced mostly by marine diatoms of the genus *Pseudo-nitzschia*. The data relating to cases of human poisoning caused by DA is limited, except for a unique ASP outbreak in Canada in 1987. The event involved 150 people with 19 hospitalizations and 4 deaths after consumption of contaminated mussels (Jeffery et al., 2004; Álvarez et al., 2015). Other than this, there are no reported cases of human illness associated with DA in any European countries or regions. Although there is scarce formal reporting, it cannot be assumed that mild cases have not occurred.

In Malaysia, so far there are no confirmed or reported cases of poisoning due to ASP. Hence, there is no active surveillance program for ASP. However, under the Japanese Trust Fund VI (JTF VI) Project "Chemical and Drug Residues in Fish and Fish Products in Southeast Asia – Biotoxins Monitoring and Harmful Algae Blooms in the ASEAN region" from 2009 to 2019, a survey has been conducted in 2015 and 2016 to determine the prevalence of DA in shellfish samples from Peninsular Malaysia.

Table 4 presents the level of DA in the shellfish samples analysed. In general, the levels of ASP in clams (*Polymesoda expansa*), oysters (*Crossostrea* sp.) and cockle (*Tegillarca granosa*) samples are well under the permissible level of 20 mg/kg. Shellfish samples from the east coast of Peninsular Malaysia (Johor, Kelantan and Terengganu) showed low levels of DA. The highest amount of DA (8.38 mg/kg) was detected in clam samples from Johor. Most of the shellfish samples from Kelantan also contained detectable levels of DA.

Table 4: Domoic acid levels in shellfish samples

Location	Date of sampling	Sample type (N)	Range (mg/kg of meat)
Johor	2015	Clams (2)	1.22-8.38
Kelantan	2015	Clams (15)	0.08-1.67
		Oysters (1)	0.52
	2016	Clams (12)	*Not detected- 0.95
Kedah	2016	Cockles (2)	Not detected
Terengganu	2015	Oysters (7)	Not detected-0.35
Perak	2016	Cockles (10)	Not detected

*Not detected – the instrument has the lowest detection limit which is not 0

Diarrheic Shellfish Poisoning (DSP)

Diarrheic shellfish poisoning toxins are polyether compounds with distinctive chemical structures grouped into four structural classes (Berti and Milandri, 2014): okadaic acid (OA) and its derivatives (dinophysistoxin or DTX); pectenotoxin (PTX); yessotoxin and its derivatives (YTX) and azaspiracid (AZA). Among the other mentioned classes, PTX and YTX were first believed to be relevant in DSP syndrome due to their co-occurrence in shellfish with OA group, but they have not been implicated in human illness (Li et al., 2012), whereas AZA causes a form of poisoning. Of the 21 different analogs identified, AZA1, AZA2, and AZA3 are the most important ones depending on occurrence and toxicity (EFSA, 2018).

DSP toxins were detected most frequently and abundantly in shellfish harvested in China (Li et al., 2012; Chen et al., 2013) and Europe (Braga et al., 2016). In Malaysia, so far there are no confirmed or reported cases of poisoning due to DSP. Hence, there is no active surveillance program for this marine biotoxin. Under the JTF VI, a survey had been conducted to determine the prevalence of AZA in shellfish samples from Peninsular Malaysia in 2015 and 2016. Random sampling method was adopted by the State Fisheries Assistant under the National Shellfish Sanitation Program (NSSP). Locations of the sampling include major natural and cultured shellfish areas. Shellfish samples collected for PSP testing under the NSSP Program were used in this project. Figure 1. illustrates the sampling locations for this program. Samples examined for ASP, AZA and BTX determination in this project consisted of clams (*Polymesoda expansa*), cockles (*Tegillarca granosa*), oysters (*Crossostrea* sp.) and green mussels (*Perna viridis*). About 1-2 kg of commercial size shellfish were collected from each sampling locations and were immediately transported to the laboratory in ice cooled insulated boxes.

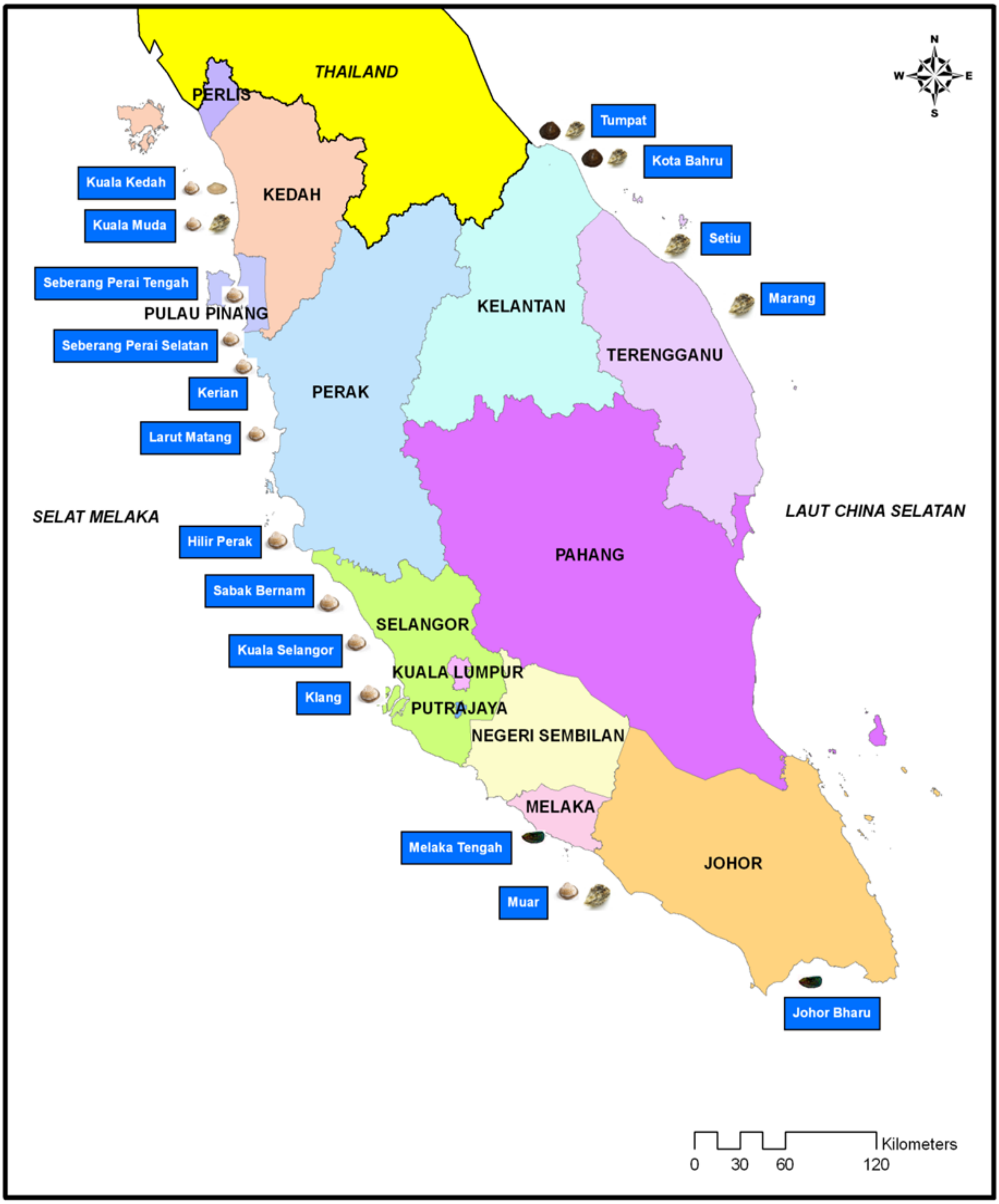

Figure 1: Sampling locations determined under the National Shellfish Sanitation Program (NSSP)

Table 5 indicates the level of AZA in the shellfish samples analysed. Similarly, like ASP, the level detected for AZA is also well under the permissible level of 160 µg/kg. Less than 5% of the samples showed some level of AZA. The highest amount of AZA detected is only 0.02 mg/kg in clams from Kelantan. However, the source of AZA was not determined in this study.

Table 5: Azaspiracids acid (AZA) levels in shellfish samples

Location	Year of sampling	Sample type (N)	Range (mg/kg of meat)
Terengganu	2015	Oysters (3)	Not detected
Johor	2015	Mussels (5)	Not detected
		Oysters (1)	Not detected
		Cockles (5)	Not detected
		Clams (4)	Not detected
Pahang	2015	Oysters (3)	Not detected-0.001 (AZA 1,3)
		Mussels (2)	Not detected
		Clams (3)	Not detected
Kelantan	2015	Oysters (3)	Not detected-0.003 (AZA 1,3)
		Clams (10)	Not detected
	2016	Clams (15)	Not detected-0.02 (AZA 1,3)
Perak	2016	Cockles (10)	Not detected

Neurotoxic Shellfish Poisoning (NSP)

Brevetoxins (BTX) are the cause of NSP. These are produced by an algae species of the *Karenia* genus (Watkins et al., 2008). Until now NSP intoxications have been restricted to the United States of America (Gulf of Mexico and Florida) and New Zealand (Heil, 2009; Ishida et al., 2004). As these toxins have not been discovered in Malaysia, no regulations or monitoring programs have been established for them.

The level of BTX in the shellfish samples is shown in Table 6. The level observed for BTX in all samples is below the safety limit of 0.8 mg/kg (Food and Drug Administration, 2001). The highest amount of BTX detected is only 2.0 μg/kg in cockles. However, the samples number were very limited in this survey.

Table 6: Brevetoxin levels in shellfish samples

Location	Date of sampling	Samples		Level
		Type	Number	μg/kg
Perak	5/5/2016	Cockles	9	2.00
	3/8/2016	Cockles	2	<1.00
Bako Sarawak	15/7/2016	Cockles	1	0.121
Sematan, Sarawak	16/5/2016	Cockles	1	2.00
Sematan, Sarawak	16/5/2016	Clam	1	2.00
Samarahan, Sarawak	3/5/2016	Cockles	1	2.00
Bintulu, Sarawak	15/5/2016	Clam	1	2.00
Semera, Sarawak	10/8/2016	Cockles	2	<1.00
Penang	3/7/2016	Cockles	5	<1.00
Kedah	24/7/2016	Cockles	10	<1.00
Kota Kinabalu, Sabah	10/8/2016	Cockles	2	<1.00

Ciguatera Fish Poisoning (CFP)

Ciguatera Fish Poisoning is a term used to describe the intoxication caused by consumption of fish, primarily reef fish from tropical and subtropical areas, which have accumulated ciguatoxins (CTXs). CTXs is a natural toxin produced by benthic dinoflagellates such as *Coolia* sp., *Gambierdiscus* sp., *Ostropesis* sp. and *Prorocentrum* sp. (Caillaud et al., 2010). There are eight potentially toxic species in Malaysian waters, including *Prorocentrum arenarium*, *Prorocentrum lima*, *Prorocentrum concavum*, *Prorocentrum cf. faustiae*, *Gambierdiscus pacificus*, *Ostreopsis labens*, *O. ovata* and *Coolia* sp., indicating that the country may face ciguatera and/or DSP problems (Mohammad-Noor et al., 2007). These dinoflagellates toxins are mostly found on the seabed and attached to corals, seaweeds, sediments or other macro algae that feed on small fish (herbivorous fish). These small fish will be consumed by large predatory fish (carnivorous fish) such as sea bass, groupers, red fish, sturgeon, barracudas, amberjack, parrotfish or other coral reef fish and will thus remain in the food chain system (Heimann et al., 2011, Chan, 2015).

Most cases of CFP occur in countries with tropical and subtropical zone coral reefs such as the Indian Ocean, the Caribbean Ocean and the Southern Pacific (Thomas, 2015). It is established that differences exist between the toxin profiles from fish collected at different sites throughout the world. Consequently, CTXs are divided into toxins from the Pacific (P-CTXs), the Caribbean (C-CTXs) and the Indian Ocean (I-CTXs) (Lewis et al., 1998). A total of 39,677 cases of CFP were reported in the Pacific Islands from 1998 to 2008 (Mark et al., 2011). In 2010, one case of CFP was reported in Malaysia in which 11 persons were hospitalized after consumption of the head and viscera from contaminated red fish imported from China. Ciguatera toxin levels in the fish were determined to be low and moderate (Chan, 2015).

Currently there is no specific monitoring or research on ciguatera conducted by the DOF, as the incidence of CFP in Malaysia is almost non-existent or unreported. However, researchers from local universities such as Universiti Kebangsaan Malaysia (UKM), Universiti Malaya (UM) and International Islamic University Malaysia (IIUM) have done investigations on the presence of dinoflagellates species capable of causing CFP. Moreover, the facility for analysing ciguatera toxins is not yet fully developed in the department.

Capability and capacity for marine biotoxin analysis

Since most intoxication cases in Malaysia due to marine biotoxin involve STX and TTX, therefore the capability and capacity for these marine biotoxin analysis are quite established. Table 7 depicts the capabilities and capacity for marine biotoxin analysis in Malaysia. In 1990's, biological method i.e mouse bioassay (MBA) was the main method used in Malaysia for the detection of PSP, particularly in Likas Fisheries Research Centre, DOF Sabah and FRI, Batu Maung, Penang. The MBA used for detecting PSP toxins is an official AOAC method. However, the limitations of MBA are that they are not very sensible, subjected to interferences, do not give information about the concentration of the different toxins and is difficult to identify which toxin causes the death of the mice. They are also time consuming, expensive and ethical restriction. Besides, the large amount of the toxin standards needed for MBA also makes this method a very unpopular choice. The chemical analytical methods are the current most powerful analytical tools able to identify multiple toxins. They are based on liquid chromatography (LC) to separate marine biotoxins by an extraction step, followed by the toxin-specific detection by UV (LC–UV), fluorescence (LC–FLD), or mass spectrometry (LC–MS/MS). Numerous LC-MS/MS methods are now available for determining most or all toxin classes associated with marine biotoxins.

Table 7: Capabilities and facilities for biotoxin analysis in Malaysia

Research Institute/Biosecurity Centre/University	Toxin	Method of Analysis
Likas Fisheries Research Centre, DOF Sabah	STX	Mouse Bioassay, ELISA
Fisheries Research Institute (FRI), Batu Maung, Penang (DOF)	STX, TTX	HPLC, LC-MS/MS
Fisheries Biosecurity Laboratory, Kuala Lumpur (DOF)	STX	ELISA (Takata Kit)
Fisheries Biosecurity Laboratory, Kuantan, Pahang (DOF)	STX, ASP, AZA	HPLC, LC-MS/MS
Fisheries Research Institute (FRI), Bintawa, Sarawak	BTX	ELISA Kit
Public Health Laboratory, Johor (Ministry of Health)	TTX	LCMS
National Poison Centre, Universiti Sains Malaysia (USM),	TTX	GCMS
Dept. of Aquatic Science, Universiti Malaysia Sarawak (UNIMAS)	TTX, STX	HPLC

Although not all marine biotoxin analysis capabilities are in place, Malaysia do carry out harmful algae monitoring programme and R&D on harmful algae. Table 8 lists the agencies that are involved in this scope of work. The harmful algae monitoring programme in Sabah started much earlier compared to Peninsular Malaysia. In Peninsular Malaysia, the monitoring program has been executed by the DOF since the year 1999 under the Sanitary and Phyto Sanitary (SPS) Program (2000-2012) and continued under the NSSP from 2013 until the present. Under the NSSP, marine biotoxin (particularly PSP toxin) analysis would only be carried out if the cell counts of toxin producer microalgae was high in water samples. This is in accordance with the Regulation of the European Union, where the shellfish production areas were periodically monitored to check the presence of toxins-producing planktons (EC Regulation No 854/2004). The European Union's also use MBA or rat assay (RBA) to determine the presence of OA, YTXs, PTXs, and AZAs in their shellfish monitoring activities.

If there is a case of PSP intoxication in Malaysia, the frequency of microalgae sampling and examination will be increased and biotoxin analysis in shellfish from affected areas will be initiated. If the results of monitoring exceed the regulatory limits, the production area shall be closed by the DOF and may be re-opened when at least two consecutive results of biotoxin levels in shellfish are within the safety limit (EC Regulation No 854/2004). The closure of the production area is necessary in order to ensure that molluscs harmful to human health are not placed on the market. In fact, the prevention of contaminated seafood reaching the markets is currently an effective way to protect human health. DA, AZA and BTX are not prioritised under the NSSP because there are no reported cases of ASP, DSP or NSP in Malaysia in addition to limited resources. However, studies pertaining to the presence of the responsible groups or toxin producers of ASP, DSP and NSP, especially *Pseudo-nitzschia* sp. have been carried out (Table 9).

Table 8: Capabilities and facilities for HAB work

	Agency	Activities
1.	FRI, Batu Maung, Penang	Monitoring, R&D
2.	Fisheries Biosecurity Laboratory, Dept. of Fisheries Malaysia - Kuantan, Pahang - Kuala Lumpur - Bintawa, Sarawak	Monitoring
3.	Likas Fisheries Research Centre, Dept. of Fisheries Sabah	Monitoring

4.	Public Universities - Universiti Malaya (UM) - Universiti Malaysia Sarawak (UNIMAS) - Universiti Kebangsaan Malaysia (UKM) - Universiti Antarabangsa Islam (IIUM) - Universiti Malaysia Sabah (UMS)	R&D

Table 9: Reported Domoic acid, Azaspiracid acid and Brevetoxin producer algae in Malaysia

	Toxin producer species	Location	Reference
	Domoic acid, Azaspiracid acid producer		
1	*Pseudo-nitzschia batesiana*	Miri, Telok Batik	Lim et al. (2013), Teng et al. (2016)
2	*P. abrensis*	Miri	Teng et al. (2016)
3	*P. kodamae*	Telok Batik, Port Dickson, Johor strait, Bintulu, Miri, Pulau Banggi, Semporna	Teng et al. (2013), (2014), (2016)
4	*P. lundhomiae*	Telok Batik, Miri	Lim et al. (2013), Teng et al. (2016)
5	*P. subfraudulenta*	Telok Batik, Port Dickson, Grigat, Miri, Pulau Banggi	Teng et al. (2013), (2016)
	Neurotoxic Shellfish Poisoning/ Brevetoxin producer		
1	*Karenia brevis*	Johor strait	Tan et al. (2016),

Regulatory Limits

The monitoring of marine biotoxins in molluscs is essential to manage the risk because it may cause serious diseases in humans. Various factors need to be considered when establishing regulatory limits for marine biotoxins including the readiness of survey and toxicological data, the distribution of the toxins throughout sampled lots, stability in the samples and the availability of analytical methods. Since there is limited data on marine biotoxins occurrences and toxicity in Malaysia, there are currently no established national regulatory limits for marine biotoxins. International limits or guidelines used as reference as indicated in the Table 10.

Table 10: Safety limits or guidelines for marine biotoxins by other countries and regulation bodies in the world

Marine biotoxin	Type of poisoning	Safety limit	Reference
Saxitoxin (STX)	Shellfish poisoning	800 μg/kg	EC Regulation No 854/2004
Azaspiracid (AZA)	Shellfish poisoning	160 μg/kg	EC Regulation No 854/2004
Domoic acid (DA)	Shellfish poisoning	20 mg/kg	EC Regulation No 854/2004
Breveatoxin (BTX)	Shellfish poisoning	0.8 mg/kg	Food and Drug Administration, 2001

Tetrodotoxin (TTX)	Fish poisoning	2.2 µg TTX eq/g	Japan Food Hygiene Association, 2005
Ciguatoxin (CTX)	Fish poisoning	0.01 ppb P-CTX-1 (Pacific ciguatoxin) 0.1 ppb C-CTX-1 (Caribbean ciguatoxin)	Food and Drug Administration, 2001

Conclusion

Marine biotoxins are a worldwide problem, including in Malaysia. Intoxication by saxitoxin and tetrodotoxin are the most common cause of death and hospitalization. Other intoxications have not been reported despite the presence of toxic algae in our waters. Monitoring seafood for toxicity is essential to manage the risks. However, due to several limitations in the capability and capacity of monitoring and determining marine biotoxins in Malaysia, the shellfish production areas are periodically monitored to check the presence of toxins-producing microalgae. However, microalgae populations are patchy and ephemeral, it is difficult to make a quantitative correlation between numbers of toxic microalgae and levels of toxins in seafood and the amount of toxin per cell can vary widely. Data on the occurrence of toxic microalgae species may point to which toxins may be expected during periods of algal blooms and which seafood products should be considered for analytical monitoring. A problem is that certain algal species, which have never occurred in a certain area, may suddenly appear and then rapidly cause problems. The plankton observations are used to focus toxicity testing, but are not in themselves used for regulatory decisions. Moreover, most monitoring and regulatory programmes often are not adequate to meet the expanding threat of new harmful algal blooms. As a result, when new outbreaks occur, the response is often uncoordinated and slow.

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