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Short Communication

The Embryonic Development of the Giant Freshwater Mountain Crab, *Isolapotamon bauense* (Ng, 1987)

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Abstract: The embryonic development of the giant freshwater mountain crab, *Isolapotamon bauense* (Ng ,1987) endemic to Sarawak, Malaysia which is an endemic species listed as Vulnerable under the IUCN redlist, is described for the first time in this paper. Mature female *I. bauense* were observed to spawn small clutches of large macrolecithal eggs typical of other Potamid primary freshwater crabs. Embryonic development occurred within the eggs corresponding to other primary freshwater crabs. The developing embryo goes through 3 major development stages within the egg before hatching into a fully formed crab hatchling at the end of the gestation period. Eggs had a gestation period of between 36-45 days from spawning until hatching. The findings of the present study provide useful insight into the life cycle and breeding biology of *I. bauense* which will be applicable for both conservation and aquaculture studies in the future.

Keywords: Giant freshwater crab, Borneo and embryonic development.

Abstrak: Perkembangan embrionik ketam gunung air tawar gergasi, *Isolapotamon bauense* (Ng ,1987) yang merupakan spesis yang endemik di Sarawak, Malaysia, yang tersenarai dalam senarai merah IUCN diterangkan buat julung kalinya di dalam kertas ini. *I. bauense* betina yang matang akan mengeluarkan sejumlah kecil telur "macrolecithal" seperti ketam-ketam Potamid air tawar yang lain. Perkembangan embrionik berlaku di dalam telur sepertimana ketam-ketam air tawar primer yang lain. Embrio yang sedang berkembang melalui 3 peringkat fasa perkembangan utama dalam telur sebelum menetas menjadi anak ketam kecil yang terbentuk sepenuhnya pada akhir tempoh pengeraman. Telur mempunyai tempoh pengeraman antara 36-45 hari dari pengeluaran sehingga penetasan.

Introduction

Primary or true freshwater crabs are an important ecological component of ecosystems of which they are found in (Dobson et al., 2007). The main feature distinguishing primary freshwater crabs from terrestrial and marine crabs is the direct development of offspring, whereby larval development happens within the eggs which hatch directly into young crabs (Sternberg and Cumberlidge, 2001; Ng, 2017). There are currently three families, 14 genera and 48 species of primary freshwater crabs recognized in Sarawak (Ng et al., 2008; Grinang et al., 2016). Under the IUCN Red List of Threatened Species there are four species of Sarawakian primary freshwater crabs listed as endangered and two as vulnerable (IUCN, 2021). The largest of the primary freshwater crab's endemic to Sarawak is *Isolapotamon bauense* (Grinang et al., 2016, Lirong et al., 2020). Taxonomically, the genus *Isolapotamon, Bott* (1968) is relatively well understood with seven species native to Sarawak. (Ng and Yang, 1986; Ng, 1987; Ng and Tan, 1998). *I. bauense* is currently classified as "Vulnerable" under the IUCN Red list (IUCN, 2021). *I. bauense* is an aquatic potamid crab narrowly endemic to the Bau District in Kuching, Sarawak with populations recorded in restricted habitats of mountainous regions in the areas of Singai, Serumbu, Krokong and Serapi (Ng, 1987; Grinang et al., 2016; Grinang et al., 2017). There is a current lack of research into ecological and conservation aspects of primary freshwater crabs in Borneo (Mcfarlane et al., 2011; Zhang et al., 2020). All species of *Isolapotamon* are large in size (Grinang et al., 2016) but *I. bauense* is the largest Potamid crab in South East Asia, capable of reaching a maximum carapace width of 9.5cm (Lirong et al., 2021). It's large size, abbreviated life cycle and good meat quality makes it an interesting prospective species for commercial aquaculture. Current research on *I. bauense* has mainly focused on the taxonomic and morphological characteristics of the species. There have been few studies concerning the basic biology of this species and currently no prior reports on the embryonic development of *I. bauense.* Thus, this paper presents a first report of the embryonic development of *I. bauense*.

Materials and methods

Mature female crabs were collected from 3 principal sampling sites known to be the habitat of *Isolaptamon bauense* in the Bau region namely: Gunung Serumbu (01° 25' 55" N, 110°13' 27" E), Gunung Singai (01° 50' 39" N, 110°17' 25" E) and Gunung Podad (01° 35' 58" N, 110°12' 75" E) as shown in Figure 1.

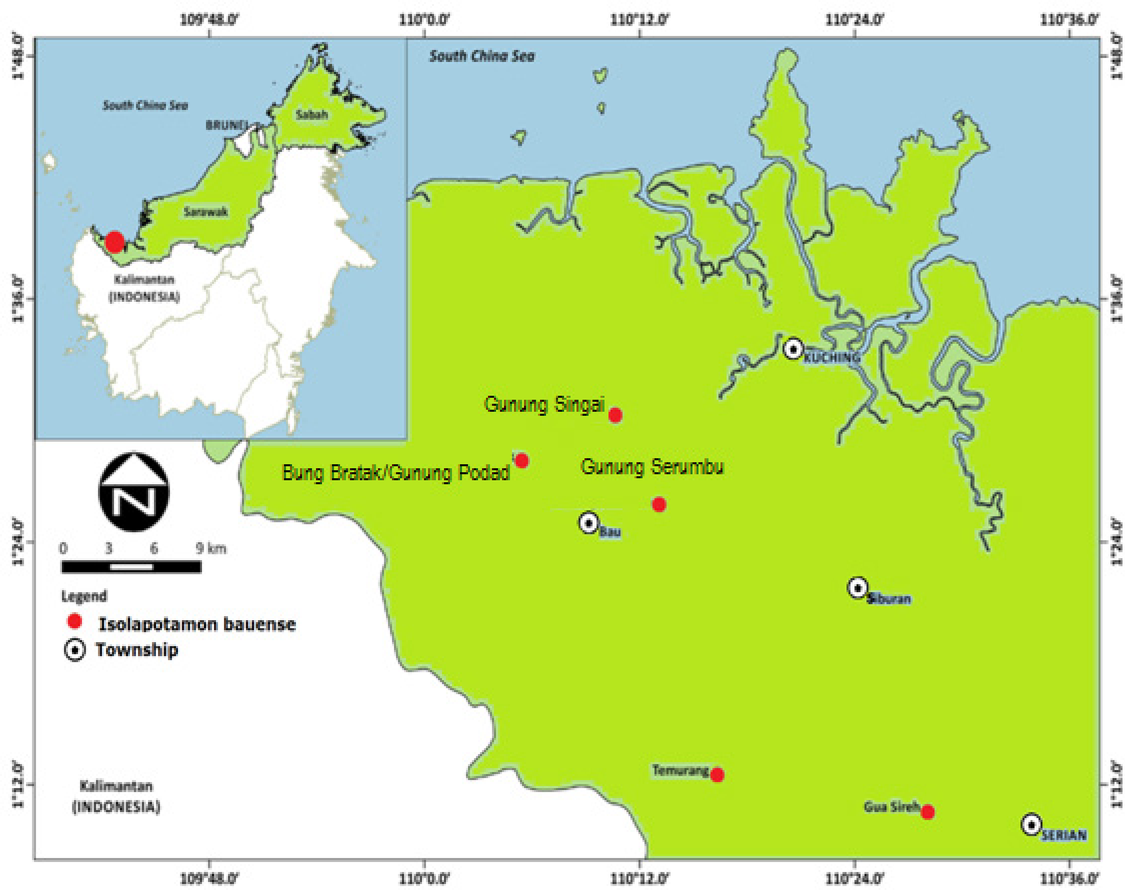

Figure 1: Map displaying the three main sampling sites of the study

Subsequently live experimental animals were transported to the Aquatic Nutrition Laboratory, Department of Animal Science and Fisheries, Faculty of Agriculture and Forestry, Universiti Putra Malaysia Bintulu Campus (UPMKB) where the breeding trials were conducted. After a one-week acclimatization period, Methyl Farnesoate (Mf) (Echelon Biosciences, Salt Lake City, Utah, USA) was injected at a dose of 10 µl/g bodyweight into experimental animals through the arthrodial membrane of the coxa of the left cheliped to encourage spawning. The experimental animals (n=10) were kept together at ambient room temperature 24°C - 27°C in two High Density Polyethylene (HDPE) tanks (179 cm length, 119 cm width, and 60 cm height) with a water depth of 10cm. Tanks were equipped with adequate aeration (using a Hailea HAP 60 air blower system) and overhead filtration system (a Boyu UF-130 8watt submersible pump and overhead filter system) to provide circulation as well as filtration. Substrate and shelter were provided in the form of river gravel, cut lengths of PVC pipe and river rocks protruding above the water line. Crabs were fed once daily with freshly chopped fish meat and any excess food and crab exuvia was removed 2 hours after feeding through siphoning of 20% of rearing volume with new fresh water topped up after each cleaning cycle. Crabs were observed over a 60 day study period and any spawning females were moved to individual containers for egg monitoring and hatching. Hatching containers were lined with sterile moistened peat moss and humidity was kept high mimicking natural burrow conditions using two water saturated sponges in each unit. The hatching containers were exposed to the natural ambient lighting conditions of the laboratory fluctuating accordingly to the natural daylight cycle. Sponges were drained and re-saturated with freshwater every day. Embryonic development was observed by gently removing an egg from the pleopods of each ovigerous female using dissecting tweezers for viewing under a digital microscope (MUSTOOL Digital USB Microscope, 2-megapixel, 1000 X max magnification) at 7-day intervals until hatching occurred. Embryonic development was categorized into 4 general categories as adapted from Ramach et al. (2009): I- Initial (Yellow), II-(Intermediate), III –pre-hatching (dark brown with eye spot) and IV-(Crab 1).

Results

The embryonic development of *I. baunse* eggs from spawning until hatching is photographically chronicled (Figure 2). The first subsection (A) (Category I- Initial) shows the large bright yellow yolk of the newly spawned macrolecithal egg, the second subsection (B) (Category II- Intermediate) shows an egg 36 hours after spawning whereby the yolk has

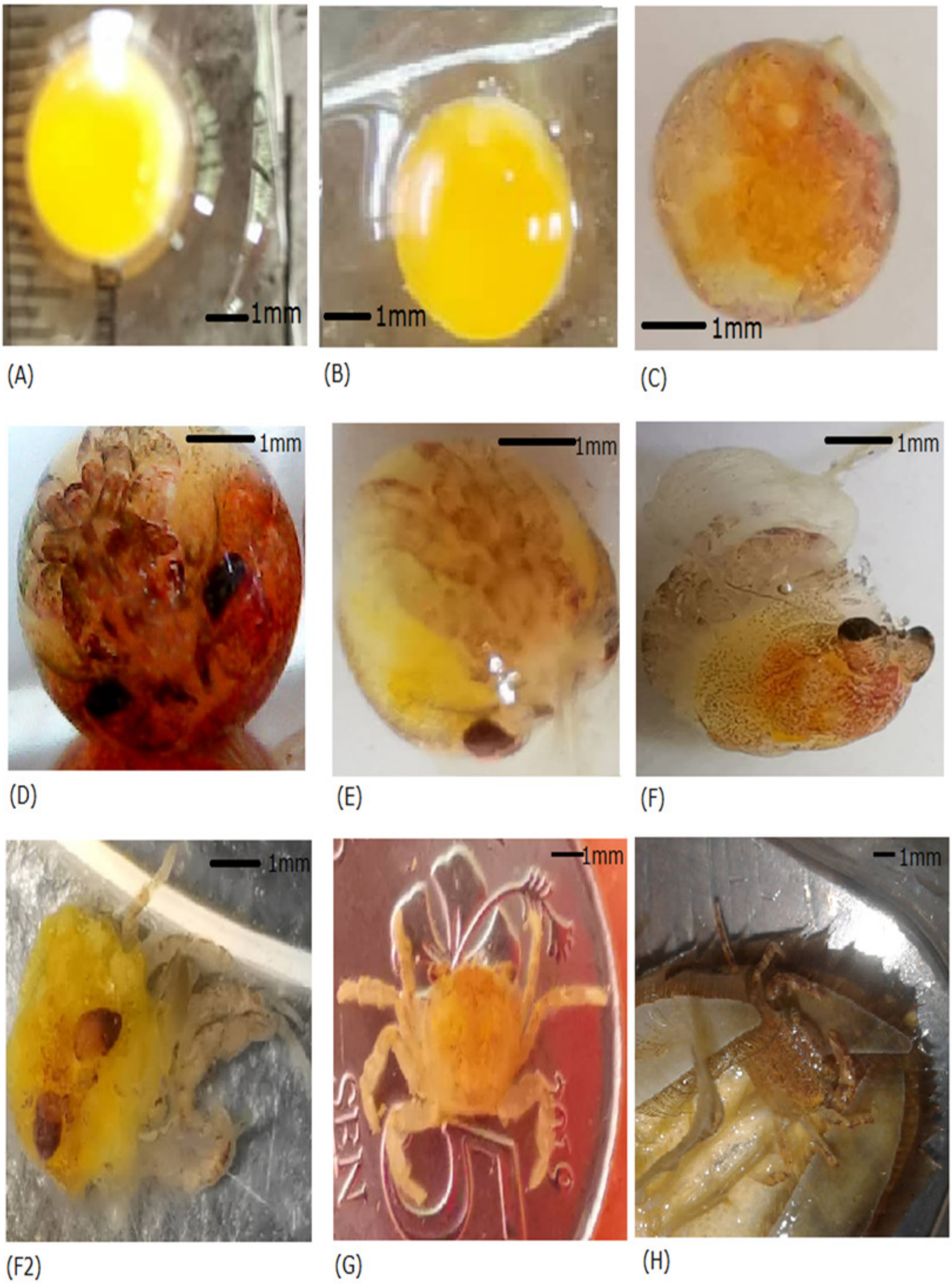

Figure 2: The embryonic development of *I. bauense* (I- Initial, II- Intermediate, III-Pre-Hatching, IV- Crab 1)

become a deeper orange hue and the blastula is visible on one side of the egg indicating embryonic development. The second stage of development continues on into the first and second week, as presented in the third sub-section (Category II- Intermediate) whereby the developing embryo can now be distinguished. By the third week eyespots and appendages are now visible within the egg as shown in fouth sub-section (D) (Category III-Pre-Hatching) which corresponds to the zoeal stage of development and by the 4th week of embryonic development the imprisoned megalopa stage can be ascertained by the appearance of chelipeds. By the 5th week of embryonic development (Category III-Pre-Hatching) the egg juvenile crab can be discerned by the appearance of stalked eyes, chelipeds and folded telson within the egg membrane. By the 36th day of gestation some of the crablings begin to break out of the egg membrane as shown in the sixth sub-section (F). The hatching process takes a little over 30 minutes with the emerging hatchling having a soft gelatinous exoskeleton (the seventh sub-section (F2) with curly pereiopods which hardens and straightens within an hour after hatching. The first crab stage (Category IV- Crab 1) as shown in eighth sub-section (G) has an average carapace width (CW) of 3.5 mm. Under captive conditions it was observed that the hatchlings remained in the abdominal apron of their mother for 14 days, during which they underwent several molting cycles (2-4 molts) before being released with a size of between 4-5 mm as shown in Figure 2 (H). Once the young crabs had been released from the abdomen of the mother, maternal care ceased and one female was observed cannibalizing her newly released offspring.

Discussion

The earliest scientific record of the embryonic development in a Potamid crab species was by Pace et al. (1976) for *Potamon fluviatile*. The present study records the first observation of the embryonic development of *I. bauense* bred under laboratory conditions. The embryonic development of this species corresponds closely to the embryonic development of other Potamid primary freshwater crab species such as *Sinopotamon yangtsekiense* as observed by Wu et al. (2010), with an extended gestation period (36-45 days) and zoeal development within the egg. Rachata et al. (2020) recorded a relatively short 12-day gestation period for a smaller species of true freshwater crab *Esanthelphusa nani,* although the embryonic development stages corresponded closely to *I. bauense*. Further studies are necessary to collect more information on the breeding biology of this species for use in both conservation and aquaculture purposes.

Acknowledgement

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Baseline Study on the Quantity, Composition and Density of Plastic from the Sea-bed Associated with Trawling Grounds in Kuala Pahang, Malaysia

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Abstract: There is very limited information regarding plastic pollution on the sea-bed especially in Malaysia since substantial cost and resources are required to undertake such studies. Thus, in the recent trawl survey to determine the nursery grounds and species composition of sharks and rays at Kuala Pahang, Pahang, East Coast of Peninsular Malaysia, an added activity on the quantification of plastic materials trapped in the trawl net was implemented. This paper presents the quantity, composition and density of macro-plastic from the sea-bed of Kuala Pahang, conducted during the monsoon period using Monsoon Season Trawl Net at nine pre-determined stations. At each station, trawl was conducted approximately 0.75-1.48 km from the coastline for about an hour with a trawling distance of around 3.15 km. Plastic materials collected at each station were categorized into five groups; bags, bottles, fishing gears, household items and others. All plastics were weighed in wet and dry conditions. 'Plastic bags' was the most abundant material collected in this study with the highest weight of 2.844 kg followed by 'bottles' (0.602 kg), 'household items' (0.37 kg), 'fishing gears' (0.25 kg) and 'others' (0.183 kg). The stations with the highest 'plastic bags' contents were Station 3 (0.943 kg) followed by Station 1 (0.668 kg) and Station 2 (0.546 kg). The overall density of plastic observed in the present study was 146.52 kg/km^2 comprising of plastic bags (98.07 kg/km^2), bottles (20.76 kg/km^2), household items (12.76 kg/km^2), fishing gears (8.62 kg/km^2) and others (6.31 kg/km^2).

Keywords: Plastics, Pollution, Solid waste, Trawl net

Abstrak: Maklumat mengenai pencemaran plastik di dasar laut sangat terhad terutamanya di Malaysia memandangkan kos dan sumber yang diperlukan untuk menjalankan kajian berkaitan adalah tinggi. Terdahulu, kajian untuk menentukan kawasan nurseri dan komposisi spesies ikan yu dan pari menggunakan pukat tunda telah dijalankan di Kuala Pahang, Pantai Timur Semenanjung Malaysia. Aktiviti tambahan mengenai kuantiti bahan plastik yang terperangkap di dalam pukat tunda ketika aktiviti penangkapan ikan telah dijalankan. Kertas ini membentangkan kuantiti, komposisi dan ketumpatan bahan plastik yang diperolehi di dasar laut perairan Kuala Pahang. Kajian dijalankan ketika musim tengkujuh menggunakan Pukat Tunda Musim Tengkujuh di sembilan stesen tundaan yang telah ditetapkan. Tundaan di setiap stesen dilaksanakan pada jarak antara 0.75-1.48 km daripada persisiran pantai dengan kelajuan lebih kurang satu jam serta jarak tundaan lebih kurang 3.15 km. Bahan plastik yang diperolehi di setiap stesen dikategorikan kepada lima kumpulan iaitu 'beg', 'botol', 'peralatan menangkap ikan', 'sisa dari dapur' dan 'lain-lain'. Berat kesemua plastik telah ditimbang ketika dalam keadaan basah dan kering. 'Beg plastik' merupakan bahan yang paling banyak dikumpul dengan berat sebanyak 2.844 kg diikuti 'boto' (0.602 kg), 'sisa dari dapur' (0.37 kg), 'peralatan menangkap ikan' (0.25 kg) dan 'lain-lain' dengan berat sebanyak 0.183 kg. Stesen yang merekodkan 'beg plastik' tertinggi ialah Stesen 3 (0.943 kg) diikuti Stesen 1 (0.668 kg) dan Stesen 2 (0.546 kg). Secara keseluruhannya, ketumpatan plastik yang diperolehi di kawasan kajian ialah 146.52 kg/km^2

yang terdiri daripada 'beg plastik' (98.07 kg/km^2), 'botol' (20.76 kg/km^2), 'sisa dari dapur' (12.76 kg/km^2), 'peralatan menangkap ikan' (8.62 kg/km^2) dan 'lain-lain' (6.31 kg/km^2).

Introduction

Since its first introduction in 1960s, plastic production has amplified exponentially due to worldwide demand because of its versatility, sturdiness and it is relatively cheap to produce (Geyer et al., 2017). Plastic production is over 300 million tons every year for use in a wide variety of applications (IUCN, 2021). Generally, about 14 million tons of plastic end up in the ocean every year. The most abundant type of litter in the ocean is plastic debris that making up 80% from the total marine debris found from surface waters to deep-sea sediments (IUCN, 2021). The source of plastic pollution ranges from land-based waste disposal, waste from vessels, abandoned fishing gears and natural and anthropogenic disasters, among others. With the ability to persist in the environment, plastic is harming freshwater and marine ecosystems, marine fauna including zooplankton, cetaceans, seabirds and marine reptiles (Eriksen et al., 2014). The effects of plastic pollution are not only aesthetic but could have serious consequences to marine life either through ingestion or entanglement with these harmful objects. Furthermore, it also affects human health and well-being through ingestion of micro plastics.

Malaysia has the highest annual plastic use per capita, at 16.78 kg per person compared to China, Indonesia, Philippines, Thailand and Vietnam (Kneefel, 2020). In terms of plastic waste, Malaysia ranks second highest in overall generated waste (National Solid Waste Management Department, 2011). A first step to tackle the problem of plastic pollution is to quantify the existing volumes. However, data on the quantification of plastic pollution is generally limited in Southeast Asia including Malaysia (Lyons et al., 2019).

The composition, abundance and quantification of plastic from beaches (Khairunnisa et al., 2012; Fauziah et al., 2015; Mobilik et al., 2015; James Noik and Mohd Tuah, 2015; Chong and Narayanan, 2016; Mobilik et al., 2017; Yin et al., 2019, Azman et al., 2021; Fauziah et al., 2021;) and mangrove forests (Barasarathi et al., 2014) in Malaysia have been reported since 2010. The reports on plastic washed up on shorelines is justifiable because of the presence of sources, ease of access and aesthetic reasons (McGranahan et al., 2007). According to Engler, (2012) deep-sea surveys are equally important because approximately 50% of plastic litter sink to the seafloor and even low-density polymers such as polyethylene and propylene will eventually sink due to the weight of fouling. Information regarding plastic litter on the sea-bed is very limited because of the sampling difficulties, inaccessibility and substantial cost and resources required to undertake such study. That is why there is almost no report on quantification of plastic obtained through trawl surveys in Malaysia although other countries have reported macro-debris (debris with size larger than 20 mm) load including plastic from the sea-bed as early as the 90s (Bingel et al., 1987; Galil et al., 1995; Galgani et al., 2000). On the other hand, literatures on the detection of micro plastic in seawater and seafood from Malaysia were published recently (Sarijan et al., 2018; Khalik et al., 2018; Mat Issa and Ab Rahim, 2018; Hashim et al., 2019; Najihah et al., 2020).

The increase use of plastics warrants greater concern and Malaysia need baseline information in quantification of plastics pollution obtained from the sea-bed although it is noted that the cost to conduct specific trawl survey for this purpose is not inexpensive. Thus, we took the opportunity during the recent survey carried out by the Institute Sains Marine Asia Tenggara (ISMAT), Chendering, Kuala Terengganu to determine the composition of sharks (Hemiscyllidae) and rays (Dasyatidae and

Gymnuridae) species and their nursery grounds in Kuala Pahang, Pahang, the East Coast of Peninsular Malaysia using Monsoon Season Trawl Net or locally known as 'Pukat Tunda Musim Tengkujuh' to record the quantity, composition and density of plastic trapped in the net throughout trawling. To our knowledge, this is the first work that provides the first record of benthic marine plastic from the coastal areas of Kuala Pahang, Pahang, Malaysia.

Materials and Methods

Study areas

Kuala Pahang is the river mouth of Sungai Pahang, which is the longest river in the Peninsular Malaysia (459 km in length). Sungai Pahang starts from the upper slopes of Banjaran Titiwangsa and enters the Jelai river flowing in a south-easterly direction, passing through Kuala Lipis before merging with Sungai Tembeling near Pahang and Terengganu state border. It then flows in a south-westerly direction passing through Kuala Tahan. Jerantut, Kuala Krau, Kerdau and Temerloh. At Mengkarak, the river turns northeast, passing through Chenor and then turning east at Lubuk Paku and Lepar into the floodplain of Paloh Hinai, Pekan and Kuala Pahang before draining into the South China Sea (Figure 1). Throughout its course, Sungai Pahang flows through agriculture farms and human settlements.

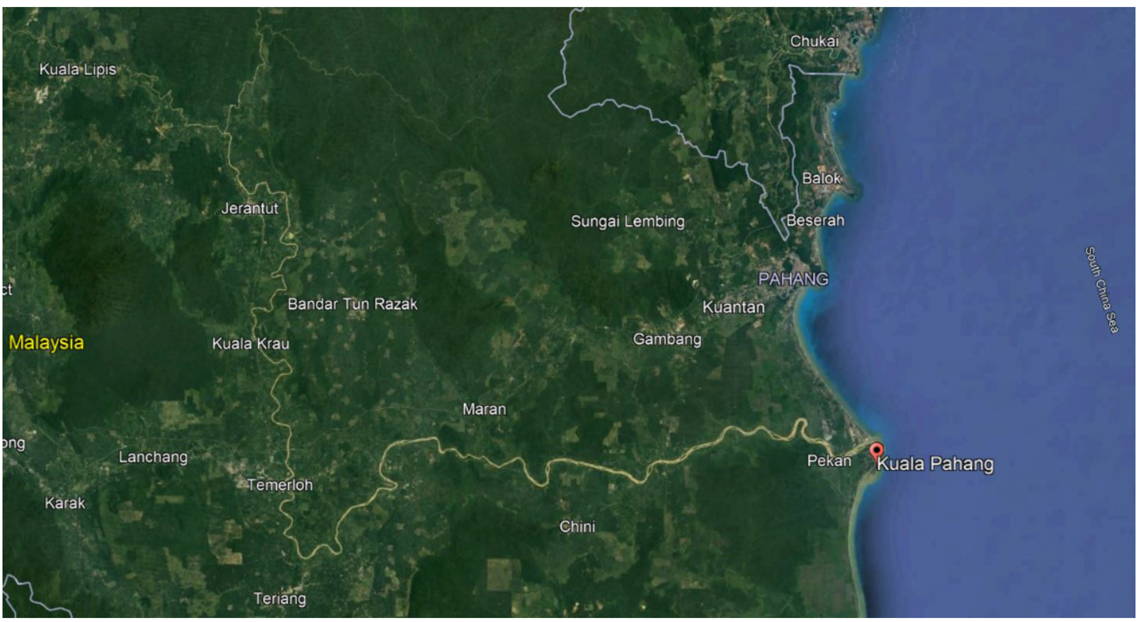

Figure 1: The area of Sungai Pahang

Trawling activities

The data on the quantity, composition and density of plastic was acquired during three trawling activities conducted on the 30th December 2019, 29th January and 30th January 2020 in Kuala Pahang Zone A waters using a private fishing vessel (GRT of 22.03 tones, 13.9 m length, 4 m width with 190 HP engine and equipped with Garmin GPSMAP 585 Plus GPS chart plotter with chirp fish finder and Sonar with GT20 transducer). The gear used was the monsoon season trawl net with a net length of 25.6 m, net opening width of 9.14 m, 36.6 m warp wire, 4.6 m sweep line, 10 m head line and 25 mm cod-end mesh size.

Table 1: Details information trawl activities carried out in the present study

Date	Trawl station	Depth at start (m)	Depth at end (m)	Latitude at start	Longitude at start	Latitude at end	Longitude at end
30/12/2019	1	7.9	7.6	3°32.620′	103°28.311′	3°33.594′	103°26.937′
30/12/2019	2	8.1	7.9	3°33.494′	103°26.965′	3°34.714′	103°25.579′
30/12/2019	3	8.2	7.9	3°34.400′	103°26.115′	3°32.577′	103°27.313′
29/01/2020	4	6.7	4.8	3°32.440′	103°28.293′	3°33.506′	103°26.617′
29/01/2020	5	4.8	7.3	3°33.466′	103°26.606′	3°34.940′	103°25.606′
29/01/2020	6	7.3	8.0	3°34.786′	103°26.703′	3°33.368′	103°27.150′
30/01/2020	7	4.8	6.4	3°31.532′	103°29.398′	3°29.718′	103°29.679′
30/01/2020	8	6.4	3.3	3°29.279′	103°29.685′	3°30.149′	103°29.105′
30/01/2020	9	3.3	9.1	3°29.986′	103°29.225′	3°31.234′	103°29.877′

Trawling time was about 60 min at around 2.5 knots trawling speed. The study was conducted in waters between 3.3 – 9.1 m depth and about 0.75-1.48 km (0.4 – 0.8 nautical miles) from coastline. Trawling distance at each station was about 3.15 km (1.7 nautical miles) with swept area of approximately 0.029 km^2 (0.0091 km x 3.15 km). Information on the trawling stations, exact locations and illustration are provided in Table 1 and Figure 2.

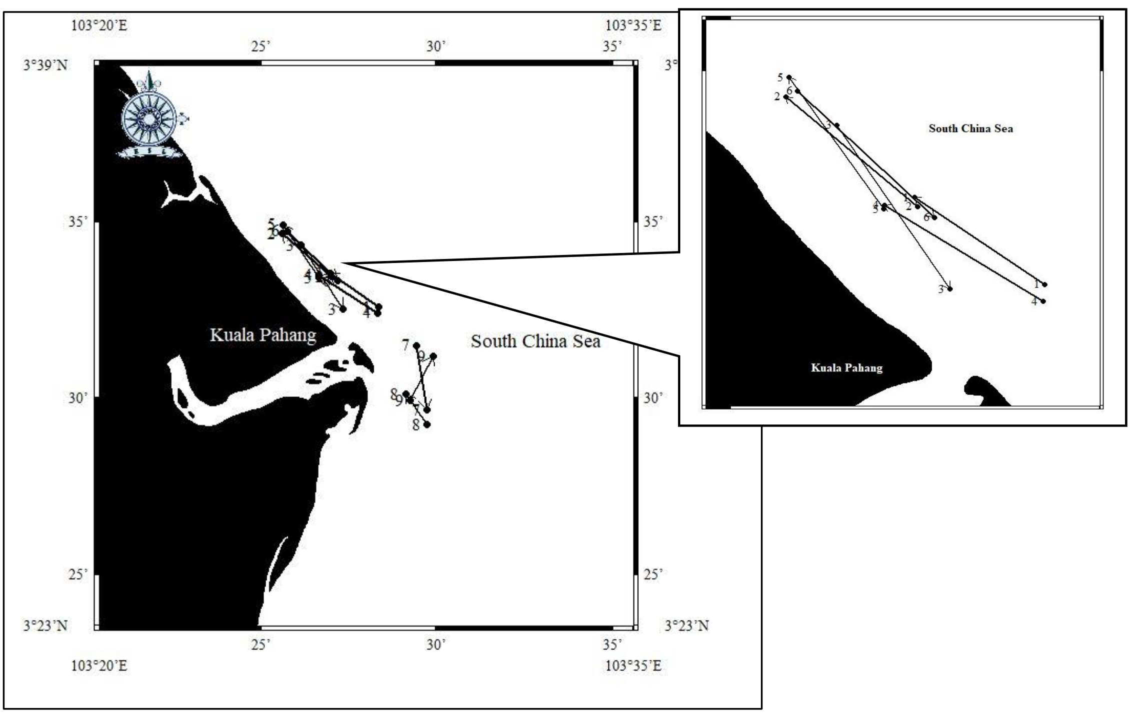

Figure 2: Study area showing trawl stations and trawling direction

Data collection

After trawling, the plastic debris were classified into five categories, namely plastic bags, plastic bottles, fishing gears (nets and ropes), household items and others. Some of the pictures of the plastic debris gathered are shown in Figure 3. Plastic bags group included snack wrap, food packaging, bags and garbage bags. Any types of kitchen stuff made of plastic were categorized under household items. On the other hand, pieces of plastic toys, sharpeners, measuring tapes, agriculture polybags, nursery pots, cassettes, and compact disc (CD) holders were placed under 'others' category. All plastic materials were weighed by categories in wet and dry conditions. This is done because plastic materials in wet condition are mostly covered with mud, sand and sometimes with biofouler (algae and barnacles) and plastic bags may contain water. All these elements would increase the actual weight of the plastics. After removing the water, mud and biofouler, all plastic materials were sun-dried and weighed again.

Results

The quantity of plastic materials collected at each station in wet and dry weight is presented in Table 2. Station 3 recorded the most weight of plastic (1.444 kg) collected, followed by Station 1 (1.158 kg) and Station 2 (0.704 kg). Besides Station 9 (0.290 kg), Station 5 (0.279 kg) and Station 4 (0.174 kg), all others stations counted less weight of plastic debris (< 0.1 kg).

Representative images of the plastic debris gathered are shown in Figure 3. In term of composition, 'plastic bags' recorded the highest weight at 2.844 kg followed by 'bottles' (0.602 kg), 'household items' (0.37 kg), 'fishing gears' (0.25 kg) and 'others' (0.183 kg). The stations with the highest 'plastic bags' contents were Station 3 (0.943 kg) followed by Station 1 (0.668 kg) and Station 2 (0.546 kg). Other stations recorded between 0.039 – 0.285 kg. Understandably for dry weight measurement the pattern was similar to wet weight. Station 3 recorded the highest dry weight of plastic debris of 0.61 kg followed by Station 1 (0.536 kg) and 2 (0.232 kg). Other stations recorded between 0.035 – 0.162 kg.

The details on plastic density from the study is shown in Table 3. The total plastic density (in wet weight) recorded was 146.52 kg/km^2 comprising of plastic bags (98.07 kg/km^2), bottles (20.76 kg/km^2), household items (12.76 kg/km^2), fishing gears (8.62 kg/km^2) and others (6.31 kg/km^2). The greatest density of plastics (in wet weight) was collected at Station 3 with 49.79 kg/km^2, followed by Station 1 (39.93 kg/km^2), Station 2 (24.28 kg/km^2) and Station 9 with 10 kg/km^2. Other stations recorded densities between 1.69 – 9.62 kg/km^2.

The proportion of plastics (wet weight) to total catches by stations is shown in Table 4. In general, plastic constituted only about 1.06% percent of the total catches in this study. The percentage of plastic by stations was in the range of 0.1-7.65% of the total catch. Station 1, 3 and 2 recorded the highest percentage of plastic in the catches of 7.65%, 6.74% and 4.60% respectively.

Table 2: Wet and dry weight of plastic materials collected at each station (weight in kilogram)

	Station																			
	Station 1		**Station 2**		**Station 3**		**Station 4**		**Station 5**		**Station 6**		**Station 7**		**Station 8**		**Station 9**		**TOTAL**	
Category	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	**wet**	**dry**
Plastic bags	0.668	0.172	0.546	0.098	0.943	0.222	0.13	0.088	0.092	0.035	0.039	0.028	0.049	0.035	0.092	0.058	0.285	0.147	**2.844**	**0.883**
Bottles	0.188	0.17	0.058	0.05	0.347	0.245	0	0	0	0	0.009	0.008	0	0	0	0	0	0	**0.602**	**0.473**
Fishing gears	0.172	0.071	0.029	0.018	0.018	0.015	0.031	0.025	0	0	0	0	0	0	0	0	0	0	**0.25**	**0.129**
Household items	0.03	0.029	0.037	0.033	0.103	0.1	0.013	0.01	0.187	0.127	0	0	0	0	0	0	0	0	**0.37**	**0.299**
Others	0.1	0.094	0.034	0.033	0.033	0.028	0	0	0	0	0.006	0.004	0	0	0.005	0.002	0.005	0.003	**0.183**	**0.164**
Total	**1.158**	**0.536**	**0.704**	**0.232**	**1.444**	**0.61**	**0.174**	**0.123**	**0.279**	**0.162**	**0.054**	**0.04**	**0.049**	**0.035**	**0.097**	**0.06**	**0.29**	**0.15**	**4.249**	**1.948**

Figure 3: Representative images of the plastic debris, a. Plastic materials obtained from trawling before sorting, b. Sorting of 'bottles', c. Pieces of toys under 'others', and d. 'Bottles', 'fishing gear' and 'plastic bags' after drying

Table 3: Density of plastic materials in wet and dry condition collected at each station (kg/ km^2). Swept area was 0.029km^2

Category	Station																		TOTAL	
	Station 1		Station 2		Station 3		Station 4		Station 5		Station 6		Station 7		Station 8		Station 9			
	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry
Plastic bags	23.03	5.93	18.83	3.38	32.52	7.66	4.48	3.03	3.17	1.21	1.34	0.97	1.69	1.21	3.17	2.00	9.83	5.07	98.07	30.45
Bottles	6.48	5.86	2.00	1.72	11.97	8.45	0	0	0	0	0.31	0.28	0	0	0	0	0	0	20.76	16.31
Fishing gears	5.93	2.45	1.00	0.62	0.62	0.52	1.07	0.86	0	0	0	0	0	0	0	0	0	0	8.62	4.45
Household items	1.03	1.00	1.28	1.14	3.55	3.45	0.45	0.34	6.45	4.38	0	0	0	0	0	0	0	0	12.76	10.31
Others	3.45	3.24	1.17	1.14	1.14	0.97	0	0	0	0	0.21	0.14	0	0	0.17	0.07	0.17	0.10	6.31	5.66
Total	39.93	18.48	24.28	8.00	49.79	21.03	6.00	4.24	9.62	5.59	1.86	1.38	1.69	1.21	3.34	2.07	10.00	5.17	146.52	67.17

Table 4: Proportion of plastics in wet condition to total catches by stations. Weight in kilogram

Items	Station									Total
	1	2	3	4	5	6	7	8	9	
Other catches	13.97	14.61	19.99	52.70	37.80	34.74	87.01	96.14	39.71	396.66
Plastics	1.16	0.70	1.44	0.17	0.28	0.05	0.05	0.10	0.29	4.25
Total other catches and plastics	15.13	15.31	21.43	52.87	38.08	34.79	87.05	96.23	40.00	400.91
Plastic percentage (%)	7.65	4.60	6.74	0.33	0.73	0.16	0.06	0.10	0.72	1.06

Discussion

This study reports data on the quantity, composition and density of plastic from the sea-bed of Kuala Pahang. This study was essentially selective and, in many cases, do not provide definitive conclusions concerning geographical variations and the amounts of plastic actually present. As mentioned in the introduction, most of the previous studies on macro-debris load from the sea-bed, including plastic utilized bottom trawl fish stock assessments (Galgani et al., 2015) to optimise the resources. Similarly, in the present study we utilized the trawl survey to determine sharks and ray's species composition and their nursery grounds in Kuala Pahang to record the quantity, composition and density of plastic of the trawling grounds. According to Galgani et al., (2011), trawling was considered the one of most adequate method to quantify plastic debris when considering net mesh sizes and opening width. However, this method has some limitations especially for rocky habitats or hard substrates. In the present study, trawling was carried out in muddy sediment sea-bed which covers most of the area close to the coast line of Pahang as described in the National Offshore Sand Resources Survey Phase III (Amin Noorasid et al., 2020). This is clearly evidenced from our observation of all the plastic items collected which were covered with fine mud.

Stations 1, 2, 3, 4, 5 and 6 were located in the northern part of Kuala Pahang. Stations 3, 1 and 2 recorded the topmost weight of plastic debris of 1.444 kg, 1.158 kg and 0.704 kg respectively. Besides Station 9 (0.290 kg), Station 5 (0.279 kg) and Station 4 (0.174 kg) all others stations recorded weight of less than 0.1 kg. The main explanation of this observation is trawling at Station 3, 1 and 2 was conducted on 30th December 2019 which was at the peak of Northeast Monsoon season on the east coast of Peninsular Malaysia while samplings at Stations 4, 5, 6, 7, 8 and 9 were carried out after a month i.e. on 29th and 30th of January 2020. Similar observation was described by Lattin et al. (2004) who found that the concentration of plastic waste collected after the storm was the highest particularly at the sampling area closest to shore. Other reasons could be due to current patterns. According to Liew et al. (1987) and Yaacob et al. (1995), current patterns in the South China Sea are monsoon-controlled. The Northeast Monsoon in the east coast of Peninsular Malaysia started from November until March. During the Northeast Monsoon, currents flow southward along the east coast of Peninsular Malaysia with speed varying between 0.1 – 0.2 m/s. In this study, the result shows that plastic debris collected at Station 3 was higher than Station 1 and Station 2. The direction of trawling at Station 3 was towards the south as the current flow. Whilst, the trawling direction at Station 1 and Station 2 was towards north and opposite to the current flow. Additionally, plastic debris from mainland could also be transported to Stations 3, 1 and 2 not only through Sungai Pahang but also several rivers slightly north of Kuala Pahang i.e Sungai Kuantan, Sungai Terus and Sungai Penur. This is also suggested by Lechner et al. (2014) and Rech et al. (2014) who stated that although large rivers are responsible for substantial input of debris to the sea-bed in estuaries, small rivers also transport waste far offshore because of their high flow rate and strong currents.

In terms of composition, 'plastic bags' was the most abundant material collected in this study. Since there is no comparison to any published data of plastic pollution of sea-beds in Malaysia, we tried to compare our findings with studies that focus on the quantifications of plastic from the beaches in Malaysia. Fauziah et al. (2015) also found plastic bags or film as the main plastic debris on the beach of Telok Kemang Beach and Pasir Panjang Beach, Port Dickson, Tanjung Aru Sabah and Teluk Likas, Sabah. Similarly, Yin et al. (2019) reported plastic bags as the largest fraction (2,046 items; 30.3%), followed by plastic sheets (1,343 items; 19.9%) in their study on the anthropogenic marine debris at two urban and peri-urban mangroves in Penang, Malaysia.

Our findings were also in accordance with (Galil et al., 1995; Galgani et al., 1995, 2000; Ramirez-Llodra et al., 2013; Ioakeimidis et al., 2014) who also observed 90% of litter caught in benthic trawls on the seafloor of Eastern Mediterranean, north-western Mediterranean Sea, and Black Seas was plastic. This fact is further reinstated by Pham et al. (2014) whose assessments on the composition of litter in different marine regions of European Seas show that plastics, which include all petroleum-based synthetic materials, make up the largest proportion of overall litter pollution. Similar discoveries were also described in other parts of the world where plastic bags, fishing equipment, food and beverage containers were the most common items and constitute more than 80% of litter stranded on beaches (Topçu et al., 2013). Comparable findings were likewise noted by Law et al. (2010) in North Atlantic Ocean, Thiel et al. (2013) in northern-central Chile and Galgani et al. (2000) along European Coasts.

For 'fishing gears', Station 1 recorded the highest weight of 0.172 kg followed by Station 4 (0.031 kg), Station 2 (0.029 kg) and Station 3 (0.018 kg). 'Fishing gears' were not observed at other stations. Surprisingly 'fishing gears' were 4th in ranking of the type of plastic observed. The results are quite surprising as this stretch of coastal waters is considered as an active fishing ground. Fauziah et al. (2015) observed that fishing gears were the main type of plastic pollution found at a fishing beach compared to a recreational beach. Although the impacts caused by derelict fishing gear was not investigated in this study, numerous studies have shown diverse impacts including ghost fishing and entanglement by sessile invertebrates such as corals. Abandoned gears may also trap more litter resulting in a litter 'depot' that has a bigger impact. Furthermore, most fishing gear is mostly made of highly resistant plastics and will likely persist in the ocean.

The 'household items' and 'others' category were the least plastic materials observed in this survey. For 'household items', Station 5 recorded the highest weight of 0.187 kg followed by Station 3 (0.103 kg), 2 (0.037 kg), 1 (0.03 kg) and 4 (0.013 kg). The household items could be from the settlements along Sungai Pahang including Kampung Beruas, Kampung Permatang Arang, Kampung Pasir Panjang, Kampung Ketapang, Kampung Pulau Maulana and Kampung Pulau Tambun.

Density of plastic bags recorded the highest compared to other categories and the densities varied between stations. The greatest density of plastics (wet weight) was found at Station 3 (49.79 kg/km^2), followed by Station 1 (39.93 kg/km^2), Station 2 (24.28 kg/km^2). It is very difficult to compare plastic density of various coastal areas (with different population densities, hydrographic and geological conditions) obtained from various studies with different methodologies. Nevertheless, common patterns indicate the prevalence of plastics, greater loads close to urban areas and tourism regions (Barnes et al., 2009). As suggested from previous work, the geographical distribution of debris including plastic on the ocean bed is subjected to many factors such as hydrodynamics, geomorphology and human factors (Galgani et al., 1996; Pham et al., 2014). Moreover, there are notable temporal variations, particularly seasonal, with tendencies for accumulation and concentration of marine litter in particular geographical areas (Galgani et al., 1995).

This is our first attempt to quantify plastics debris from the sea-bed. As ISMAT regularly carried out fish resource survey, more attempts could be made in getting more information on plastic debris. Several shortcomings have been identified in the present study will be rectified in future endeavour. Among them is the method in quantifying the plastic. Quantification of plastics items is more appropriate than weighing the plastic. Data expressed as items m^{-2} or km^{-2} are more useful for comparisons with previous work. The interpretation of the available data is also challenging since the plastic distribution is influenced by a variety of factors i.e. human activities, seasonal changes in rivers flow rate, hydrodynamic conditions and sea-bed morphology.

Conclusion

'Plastic bags' was the most abundant material collected in this study followed by 'bottles', 'household items', 'fishing gears' and others. The stations with the highest 'plastic bags' contents were Station 3 followed by Station 1 and Station 2. Our results are very basic to explain the complexity of the problem and highlight the needs for concentrated efforts in order to collect sufficient data to fill the existing gaps in knowledge. The further study is going to be conducted focusing on composition of marine debris using various type of fishing gears.

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Short note

The Carbohydrate Profile of Riverine Fruits in the Natural Diet of Malaysian Mahseer, *Tor tambroides*

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The Malaysian mahseer, *Tor tambroides* (Bleeker, 1854), is a commercially important aquaculture species that is widely popular as ornamental and game fish, and its flesh touted as a tasty delicacy (Lau et al., 2021). A high-quality individual is priced at MYR600 kg^{-1} (approximately USD175 kg^{-1}) making it the highest valued freshwater fish cultured in Malaysia (DOF, 2021). Generally, fish from the genus *Tor* are omnivores with several species recognized as frugivores such as Semah mahseer (*Tor duoronensis*), Chinese mahseer (*Tor sinensis*), and Javan mahseer (*Tor tambra*) (Kamarudin et al., 2014). The roles of carbohydrates in the diet of the omnivorous Malaysian mahseer have not been fully understood, and this species is reported to have an optimal carbohydrate requirement of 23.4% (Ishak et al., 2016). The data collection in this article characterizing the carbohydrate profile of seasonal riverine fruits found in the diet of Malaysian mahseer in its natural habitat is shown in Figure 1. The profile comprised of: (i) total carbohydrate (made up of crude fibre and nitrogen-free extract (NFE)); and (ii) simple carbohydrates or sugars (monosaccharide (glucose and fructose) and disaccharide (sucrose and maltose). Fruits from six seasonal riverine tree species; Football fruit or "Kepayang" (*Pangium edule*), Pacific almond or "Dabai" (*Canarium odontophyllum*), "Neram" (*Dipterocarpus oblongifolius*), Guayabilla or "Jambu Ara" (*Bellucia pentamera*), Cottonfruit or "Sentul" (*Sandoricum koetjape*), and Paddy oats or "Melinjau" (*Gnetum gnemon*), were reported to be part of the natural diet of Malaysian mahseer (Tan, 1980; Misieng et al., 2015). These wild fruits are edible and both human and animal consume the fruits for its high carbohydrate and lipid content (Ambak and Jalal, 2006). Bami et al. (2017) studied the effects of *C. odontophyllum* oil incorporated in the Malaysian mahseer diet and found that although the fruit is consumed in the wild, nonetheless the extracted concentrated crude oil is not a suitable lipid source indicating a possible purpose of consuming for dietary carbohydrate. Samples for this study were obtained from the collection of the Department of Aquaculture, Universiti Putra Malaysia, Serdang, and subjected to high-performance liquid chromatography (HPLC) analysis following AOAC official method 982.14. Briefly, samples in powder form were subjected to direct extraction in acetonitrile:water (1:1, v:v) with no heat, subsequently filtered through a 0.45µm nylon syringe filter prior to chromatographic separation. Filtered samples were then injected into Prevail Carbohydrate ES column (250mm × 4.6mm i.d., 5µm) on a Varian 385-LC Evaporative Light-Scattering Detector ELSD (Agilent Technologies, USA); evaporator, 40°C; nebuliser, 40°C; equipped with a JASCO PU980 pump. The mobile phase was 3 parts acetonitrile to 1 part water (75:25, v/v) with a nitrogen flow rate of 1.0 mL min^{-1}. The sugar composition was determined as the percentage of area under chromatographic peaks over the total area of peaks. Data in Table 1 showed that *B. pentamera*, *D. oblongifolius* and *S. koetjape* have more than 70% of total carbohydrate compared to *P. edule, C. odontophyllum* and *G. gnemon*, with high crude fibre content in *C. odontophyllum* (33.10%) and *D. oblongifolius* (25.30%). Comparatively, *B. pentamera* was shown to have the highest content of glucose (2.391±0.142 g $100g^{-1}$) and fructose (8.168±0.286 g $100g^{-1}$), whereas the highest content of sucrose was *C. odontophyllum* (0.846±0.071 g $100g^{-1}$). Meanwhile, the highest NFE contents were the fruits *B. pentamera* (69.22%) and *S. koetjape*

(70.79%). No glucose was detected in the fruits of the *P. edule* and *C. odontophyllum*; whereas maltose was not detected in any samples. The non-digestible carbohydrate constituents may contribute to the fish dietary fibre intake, whereas the sugars are highly digestible energy source for vertebrates and vital in maintaining bodily functions. These analyses were carried out to add baseline data on the nutritional content of these fruits which subsequently will provide a basis in understanding the Malaysian mahseer's natural diet and its feeding behavior. Knowing the quality of natural diet can assist in better feed formulation for the aquaculture feed industry. Inadvertently, this data can also be used for the conservation of threatened riverine trees due to river inundation and other anthropogenic activities.

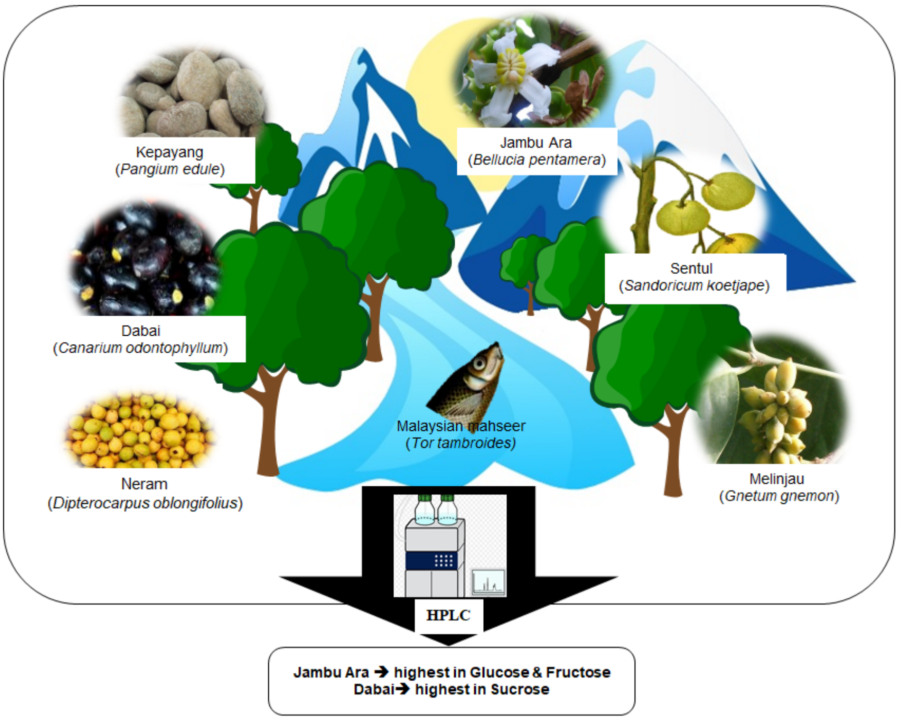

Figure 1. Graphic representation of the data characterizing the carbohydrate profile of seasonal riverine fruits found in the diet of Malaysian mahseer in its natural habitat.

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

Table 1. Carbohydrate composition (% as fed basis) of insoluble (crude fibre) and soluble carbohydrate (nitrogen free extract/NFE), and sugar composition (g $100g^{-1}$ dry matter of dried sample) of riverine fruits in *T. tambroides* diet

Riverine fruit	Crude fibre[1]	Nitrogen Free Extract[1]	Total carbohydrate[2]	Monosaccharide		Disaccharide	
				Glucose	Fructose	Sucrose	Maltose
Football fruit/ "Kepayang" *Pangium edule*	12.29	12.96	25.25	*	0.100 ± 0.001^{c}	0.017 ± 0.001^{d}	*
Pacific almond/ "Dabai" *Canarium odontophyllum*	33.10	17.39	50.49	*	0.100 ± 0.001^{c}	0.846 ± 0.071^{a}	*
"Neram", *Dipterocarpus oblongifolius*	25.30	45.57	70.87	0.100 ± 0.001^{c}	0.199 ± 0.141^{c}	0.498 ± 0.000^{b}	*
Guayabilla/ "Jambu Ara" *Bellucia pentamera*	0.95	69.22	70.17	2.391 ± 0.142^{a}	8.168 ± 0.286^{a}	0.099 ± 0.000^{c}	*
Cottonfruit/ "Sentul" *Sandoricum koetjape*	0.42	70.79	71.21	0.399 ± 0.141^{b}	0.349 ± 0.070^{b}	0.598 ± 0.141^{ab}	*
Paddy oats/ "Melinjau" *Gnetum gnemon*	13.73	52.23	65.96	0.100 ± 0.000^{c}	0.150 ± 0.070^{c}	0.150 ± 0.070^{c}	*

Each reported sample values were mean ± standard deviation, subjected to one-way analysis of variance (one-way ANOVA) and differences between means were tested using Duncan's new Multiple Range Test at $P<0.05$.
[1]For comparison purposes, data from Misieng *et al*. (2015) of the same fruit samples were incorporated.
[2]Total carbohydrate = crude fibre + NFE
*Not detected

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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.

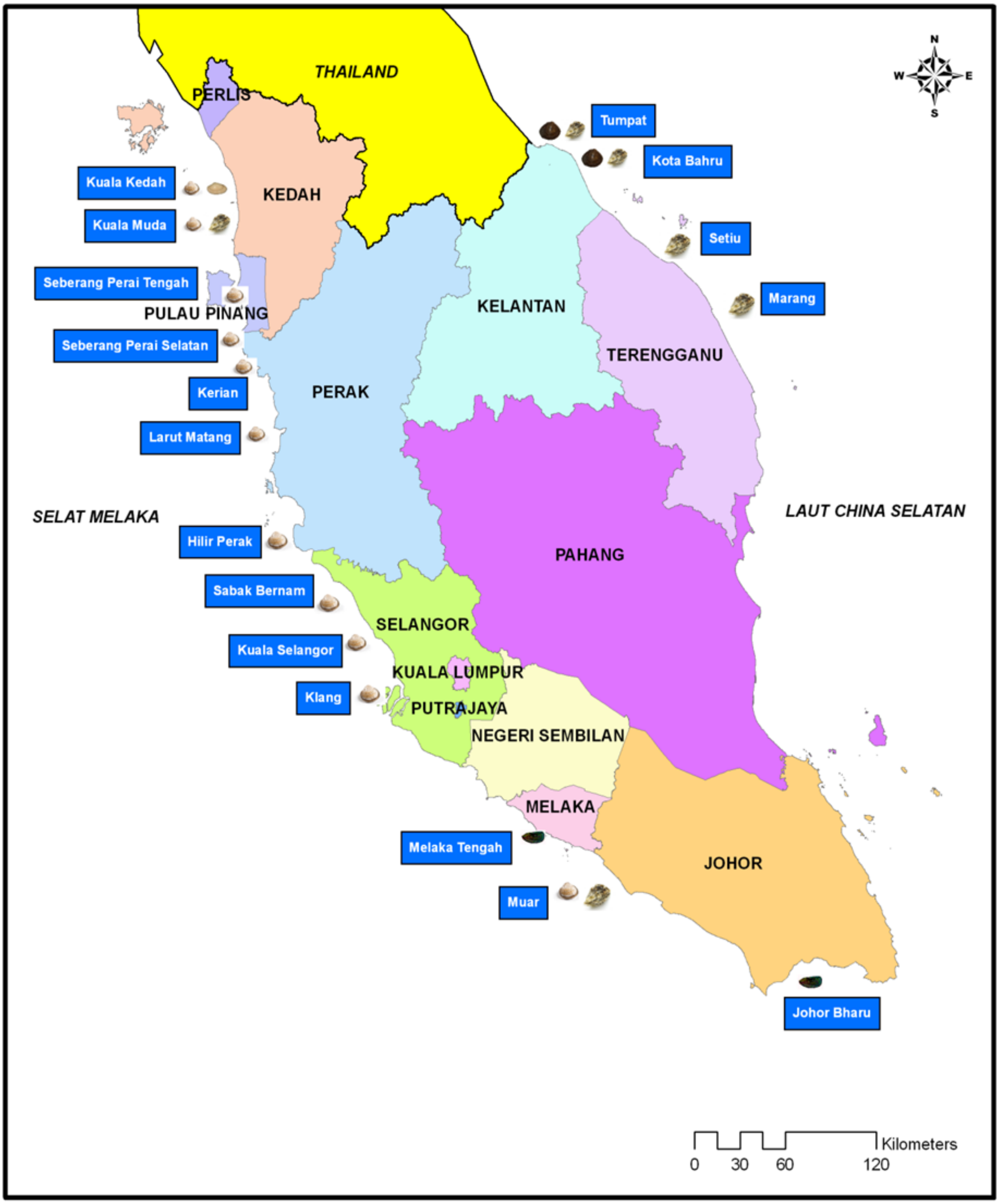

Figure 1: Sampling locations determined under the National Shellfish Sanitation Program (NSSP)

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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Effect of Rotifer (*Brachionus plicatilis*) Bioencapsulation with SirehMAX™ on Growth and Survival of Asian Sea Bass (*Lates calcarifer*) Larvae

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Abstract: SirehMAX™ is a product that has shown positive impact on prevention against bacterial infection in fish, in addition to the enhancement of growth performance. In this study, the effect of SirehMAX™, a plant-based product from *Piper betle* leaves on growth performance and survival of sea bass (*Lates calcarifer*) larvae against vibriosis were investigated. Cytotoxic bioassay of SirehMAX™ was assessed using rotifer. The LC_{50} value of rotifer was observed as 10 mg/L. After optimization, rotifer used in the study were enriched in 10 mg/L of herbal product for 3 h in a well aerated container and given to the fish twice daily. Survival and growth rate of the seabass larvae were monitored and compared to control. Results indicated that the addition of SirehMAX™ increased growth of seabass larvae at 47%. The survival result indicated a slightly different of 15% but there are no significant different between the group. The result presented suggested that SirehMAX™ could be used as enrichment feed for live feed to attain higher and faster growth during larval development, thus providing a new technology for mass production of fry and fingerlings.

Keywords: SirehMAX™, Rotifer, cytotoxicity, growth performance, survival, bioencapsulation

Abstrak: Kesan SirehMAX™, produk berasaskan daun sirih terhadap kadar tumbesaran dan hidup larva ikan siakap (*Lates calcarifer)* melawan jangkitan vibriosis dikaji. Kajian sitotoksik SirehMAX™ dijalankan menggunakan rotifer. Nilai LC_{50} terhadap rotifer didapati pada kepekatan 10 mg/L. selepas pengoptimuman, rotifer yang digunakan dalam kajian dikayakan dalam 10 mg/L produk herba selama 3 jam berserta pengudaraan dan diberi makan kepada ikan 2 kali sehari. Larva ikan dipantau dari segi kadar hidup dan tumbesaran berbanding dengan kawalan. Keputusan menunjukkan penggunaan SirehMAX™ meningkatkan kadar tumbesaran larva ikan, tetapi tiada perbezaan ketara dalam kadar hidup antara kumpulan rawatan. Keputusan yang dipamerkan mencadangkan SirehMAX™ boleh digunakan sebagai bahan pengkayaan untuk makanan hidup bagi mencapai kadar pertumbuhan larva yang lebih cepat, sekaligus menghasilkan teknologi baru dalam penghasilan larva dan anak ikan yang tahan penyakit.

Introduction

Live feed, rotifer is primary food for fish larvae. They are used extensively worldwide as live food for the larval stages of commercially marine fish species. The non-selective feeding behaviour makes these organisms a good biological carrier for transferring essential nutrients to predator larvae using bioencapsulation technique (Immanuel et al., 2003). Aneuvo et al. (2014) pointed out that during bioencapsulation, desired essential nutrients dissolved in water are ingested by the rotifer with minimal amount of leakage when given to fish larvae. This technique is not only being used for improving quality of live feed but also as means to deliver antibacterial agents such as oxytetracycline (Langdon et al*.,* 2008), oxolinic acid (Touraki and Niopas, 2012), florfenicol (Roiha et al*.,* 2010) and metronidazole (Rodrigue et al*.,* 2011) for treatment against bacterial diseases in larval hatcheries.

Vibriosis is a major infectious disease, affecting fry and fingerling production of marine fish and caused heavy losses to farm. These pathogens invade fish larvae and juvenile via rotifer feeding such as in Asian seabass and grouper culture (Iwata et al*.,* 1987, Masumara et al*.,* 1989, Abdullah et al., 2018). Elimination of this pathogenic bacteria in rotifer cultures using antibiotics proved effective and yielded better survival and growth of fish larvae but the use is undesirable as it could promote antibiotic resistance and threaten human safety (Battaglene et al*.,* 2006). For that reason, sustainable culture methods without applications of antibiotics are needed for mass production of fry.

Several studies have reported that herbal medicines can be used to enhance resistance of marine fish against pathogen including in the control of bacteria, fungi and viral diseases (Dhayanithi et al*.,* 2013). In addition, herbal enrichment diet was also reported to promote weight gain, immune response and recovery from stress for fish (Takaoka et al*.,* 2011). Regardless, there is little information on the effects of rotifer enrichment with medicinal herbs extract during larval rearing. Thus, in the present study, focused on the effects of SirehMAX™, an herbal product on; a) selected pathogenic bacteria (*Vibrios*); b) growth of larvae fed herb-enriched rotifer and subsequently c) survival of fingerlings after 25 days of culture. SirehMAX™, were chosen because earlier studies have shown that the product has positive impact on prevention against bacterial infection in fish (Abdullah et al., 2018). Mortality is low in fish given SirehMAX™ diet compared to control. In addition, SirehMAX™ diet was also reported to enhance growth performance (Abdullah et al., 2018).

Materials and Method

SirehMAX™

SirehMAX™, is an herbal product made from *Piper betle* leaf extract. The process for production of SirehMAX™, is patented (Patent No:MY-176273-A). The recommended dosage of this product is 1 mL for 1 kg of formulated feed.

Rotifer Toxicity Bioassay

Cytotoxicity bioassay was carried out as described by Meyer et al*.,* (1982) and McLaughlin et al*.,* (1991) on freshly harvested rotifer. Rotifer used were cultured indoor at Fisheries Research Institute Tanjung Demong (FRITD). The rotifer was disinfected following protocol of Bioencap ABI (unpublished report, 2020). Triplicate samples of SirehMAX™, were tested initially at concentrations of 1, 10, 100 and 1000 ppm (µg/mL) in vials containing 5 mL of brine solution and 10 individuals (ind.) of rotifers. Control contained only 5 mL brine solution. The rotifers were provided with sufficient light and aeration for 24 h. Survivors were counted after 24 h and the median lethal concentration (LC_{50}) with 95% confidence intervals calculated using Probit Analysis (Finney and Tattersfield, 1952)

Bacterial Counts in Rotifer by Herbal Enrichment

Rotifers were harvested from FRITD Rotifer culture system and washed with sterilized seawater. The rotifers were introduced into a 10 L container at 200 rotifer/mL containing 1 µL/mL and 10 µL/mL of SirehMAX®, 0.01 mg/mL l Elbaju (commercial name for nifurstyrenate-sodium) as positive control and brine solution as negative control. *Vibrio* sp. count in rotifer using thiosulfate citrate bile-salts sucrose (TCBS) media were compared at 1, 2, 3 and 4 h after SirehMAX®, and control enrichments. Treatments were conducted in triplicate. At each sampling, 50 mL was removed from the containers and rotifers were filtered off using 60-micron plankton net, washed thoroughly with sterilized seawater. Then rotifers were homogenized in 1 mL of sterile seawater at 4°C. Serial dilution was performed and 1 mL of diluted homogenate were inoculated in duplicate on TCBS medium and incubated at 25°C for 24 h. Bacterial colonies obtained were counted and total bacterial number was calculated as colony forming units (CFU).

Enrichment of Rotifer with SirehMAX™,

Rotifer enrichment was carried out by following the standard procedure of Sorgeloos and Kulasekarapandian (1984). Enrichment period was conducted for 3 h as in the previous study. The enrichment was performed in 10 L containers, with continuous aeration, and a density of rotifer of approximately 600 ind/mL. The seawater had a salinity of 30% and a temperature of 23°C. Presence of the leaf extract molecules could readily be assessed by the yellowish gut.

Bioencapsulation studies on larvae growth performance

Five thousand (5,000) sea bass fish larvae with initial weight of 0.5 mg were placed in a 300-L aerated tank. Six (6) 300-L tank were prepared for the experiment. Two feeding regimes were tested; i) Larvae were fed with enriched rotifer (10 ppm SirehMAX™) once a week and unenriched rotifer twice a day at (7 and 16 h); ii) larvae fed with unenriched rotifer twice a day at (7 and 16 h) which act as a control. Three replicates were maintained for each group. The culture period was for 25 days since after this period larvae were fed with pelleted feed. At the end of the experiment, both control and experimental fish fry were randomly selected and the total body weight was measured. The remaining fish available at the end of experiment were counted and growth measurements such as weight were also recorded individually.

Statistical Analysis

Data obtained were subjected to one-way ANOVA followed by Duncan's multiple range tests to calculate differences in growth rates among the different experimental treatments and survival rate.

Results and Discussion

Determination of the Median Lethal Concentration (LC_{50})

The lethal concentration (LC_{50}) of the standard SirehMAX™, was found to be at 10 ppm. At this concentration, rotifer could survive more than 24 h. At higher dose rotifer could survived only for 0.167 to 0.5 h in SirehMAX™. Rotifer could survive for more than 160 hours at low dose (1 ppm) (Figure 1). This correspond the findings by Krishnaraju et al*.,* (2005) that the degree of lethality was found to be directly proportional to the concentration of the extract. They observed that the maximum and least mortalities of brine shrimp were at 1000 µg/L and 10 µg/L respectively when placed in herbal solution. Final optimization of 10 µL/g SirehMAX™ solution was selected for enrichment of rotifer. The maximum uptake of SirehMAX™ by rotifer was observed at 180 and 240 minutes after immersion in the herbal solution (Figure 2). The photomicrograph shows that the increment of intensity of the color in the gut of rotifer which indicate the present of SirehMAX™ in there. As stated by Rico-Martínez et al., (2016), rotifer is sensitive to organic matter and pesticide which can lead to their mortality when expose to high concentration for long period of time.

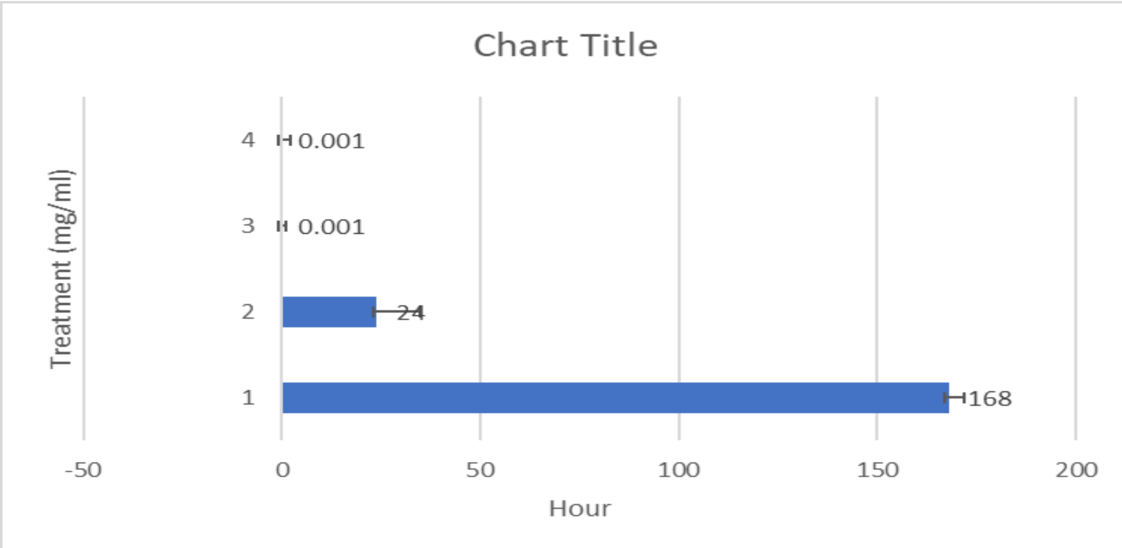

Figure 1. Mortality of rotifer (*Brachionus plicatilis*) against time and treatment concentration

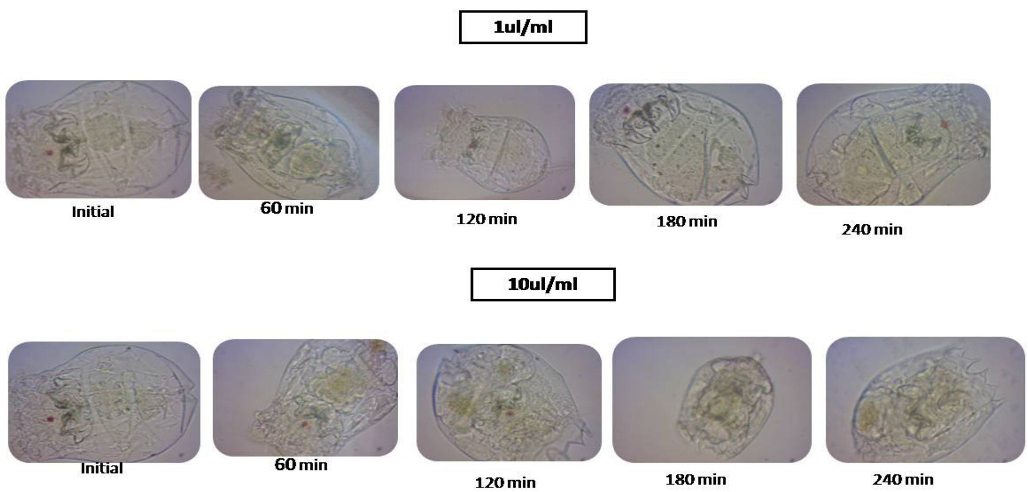

Figure 2. Photomicrograph showing the gut region of rotifer *(Brachionus plicatilis*) enriched with *SirehMAX*™

Bacterial Counts in Rotifers' body after SirehMAX™*, enrichment*

The bacterial load of rotifer in control and 1 µL/mL treatment with SirehMAX™ appeared relatively constant for 1 to 4 h ranging from 2.2 x 10^3 to 7.2 x 10^6 CFU/mL. However, in 10 µL/mL treatment with SirehMAX™, bacterial load was completely eliminated after 2 h. (Table 1). Treatment with Elbaju can completely remove the bacterial load in rotifer after 1 h treatment. This result indicated that SirehMAX™ can eliminate bacteria in rotifer by using doses of 10 µL/mL for 2 h treatment time.

Table 1. Number of bacterial loads in rotifer *(Brachionus plicatilis)* after received SirehMAX™

Treatment	1 h	2 h	3 h	4 h
Control	$2.4x10^6$	$1.8x10^6$	$5x10^6$	$7.2x10^6$
SirehMAX™ 1 µL/mL	$5.2x10^5$	$6x10^3$	$2.1x10^4$	$2.2x10^3$
SirehMAX™ 10 µL/mL	$5.5x10^2$	$3.2x10^2$	-	-
Elbaju 0.01 mg/mL	-	-	-	-

Growth Performance and Survival of Fish Larvae

The effect of herbal treatment on growth of seabass larvae are shown in Figure 3. Significant difference ($p<0.05$) was observed in seabass received with SirehMAX™, compared to control. The highest total length observed was 15 cm compared to 8 cm in control. This is consistent with findings by Daga et al. (2013) in turbot (*Scophthalmus maximus*) enriched with probiotics and Arulvasu et al., (2012) in *Poecilia sphenops* enriched with *Vitex negundo* leaf extract.

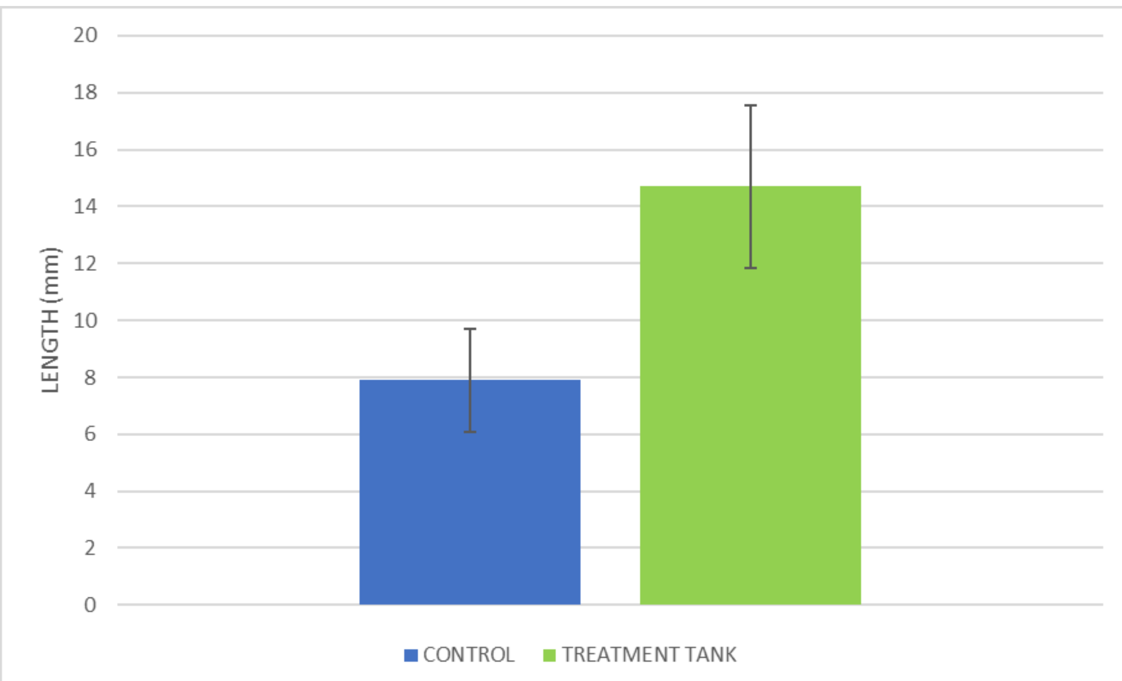

Figure 3. Graph showing growth of seabass (*Lates Calcarifer*) fry cultured for 25-days

At the end of the experiment, there were no significant differences in survival of larvae between both group (Figure 4). This corroborates findings by Takaoka et al., (2011) with no significant difference in survival of red sea bream larvae fed with rotifer enriched with *Crataegu fructus* and mixture of *Crataegu fructus*. Contrarily, Arulvasu et al., (2012) observed maximum survival in *Poecilia sphenops* treated with *Artemia* nauplii enriched with *Vitex negundo* leaf extract.

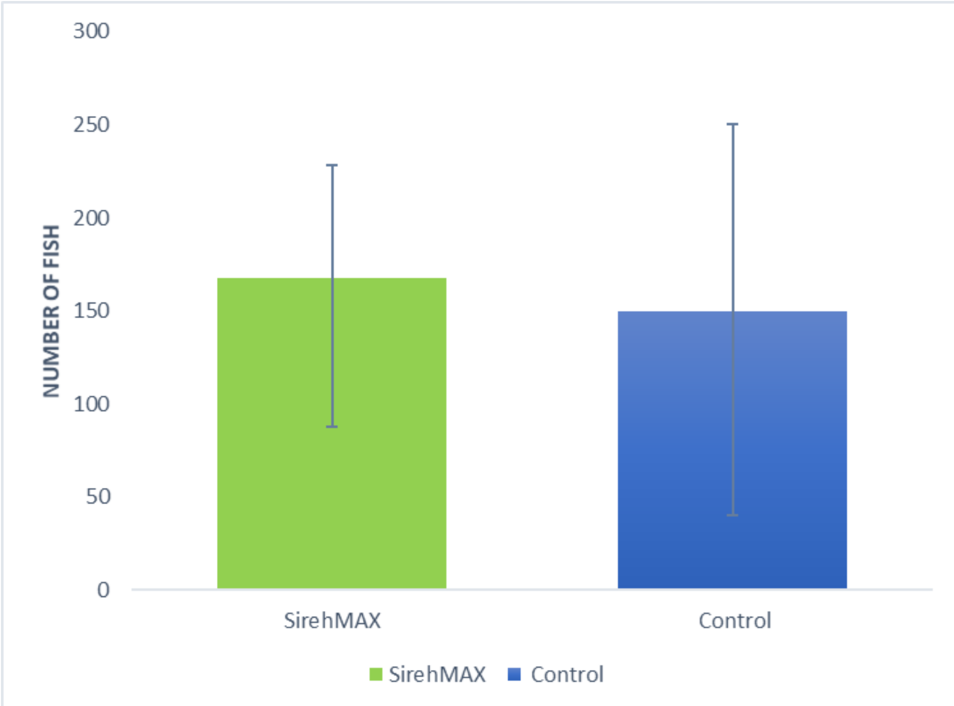

Figure 4. Number of fish fry fed with unenriched and enriched rotifer after 25 days of culture period.

Conclusion

The recommended concentration of SirehMAX™ use for bioencapsulation is at 10 ppm which seabass larvae fed with enriched rotifer with optimized dose herbal extract showed overall increase in growth and survival when compared to that of unenriched control. Further studies should be conducted to infer which ingredients in medicinal herbs promoted the growth and immune response of fish larvae.

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b) Gudding, R. (2014). Vaccination as a preventive measure. In R. Gudding, , A. Lillehaug, and O. Evensen, (Eds.), *Fish Vaccination* (pp. 12-21). John Wiley & Sons Ltd, West Sussex, UK.

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Rosenberg, G. (1997, March 31). Electronic discovery proves an effective legal weapon. *The New York Times.* http://www.nytimes.com

b) Composite works of serials:
Sequence of citation: author's name, initials, year of publication, publisher, place of publication, first and last page no.

Example:
Guilbot, A. and Marcier, C. 1985. Starch. *In:* Aspinall, G.O. (ed). The polysaccharides, Academic Press, New York, pp. 209-283.

c) Full publication details must be given for any citation that does not fit into any of the above categories such as unpublished in-house reports, contract reports, etc.

Acknowledgement
Brief of appreciation to whom it is due.

Table
Plain Tables should be used for data which cannot be described in the text. Type each table double spaced and position in the manuscript. Table number and caption should be positioned at the top. Explanatory footnotes in lower case letters should be concise to enable them to stand independent of the main text. Tables are numbered with Arabic numerals.

Figures
Figures should be selected to illustrate points which cannot be easily made in the text. They are numbered with Arabic numerals. Graphs, photos and diagrams with caption should be positioned in the manuscript. Diagrams must be drawn and lettered in black ink on good quality white paper for camera-ready use. Lettering should be parallel to the axes. Photocopies, hand-drawn diagrams and typewritten labels are not acceptable. Scale marks on graphs should be within the axes. Graphs should avoid as far as possible large areas of unused space.
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the purpose of printing.

Units, Abbreviations and Nomenclature

Use only recommended SI Units. Use superscripts presentation (mg mL^{-1}). Below are few examples of abbreviations of the most commonly used SI units:

Base quantity	**Name**	**Abbreviation**
Length	Meter	m
Mass	Kilogram	kg
Time	Second	s
Time	Minute	min
Electric current	Ampere	A
Area	square meter	m^2
Volume	cubic meter	m^3
Frequency	Hertz	Hz

The correct Latin names of organisms must be used on first mention in the text. A widely recognized and designated common name should be used for subsequent mention.

References

American Psychological Association. (2020). *Publication manual of the American Psychological Association* (7th ed.). https://doi.org/101037/0000165-000

Paper Title: A concise and informative title unobscured by taxonomic detail

FULL NAME ALL AUTHORS: AUTHOR[1,2], AUTHOR[3], AUTHOR[3], AUTHOR[1], AUTHOR[4,*]

[1]Institution with complete current address, including post code

[2] Institution with complete current address, including post code

[3] Institution with complete current address, including post code

[4] Institution with complete current address, including post code

**Corresponding author: author@institution.domain*

***Abstract**:* A concise and factual abstract is required and must be written in English (maximum length of 250 words). The abstract should state briefly the purpose of the research, the methods used, the principal results and major conclusions. Please try to keep each sentence as specific as possible and avoid such general statements as "The fisheries status are discussed'. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, they must be cited in full, without reference to the reference list. Any abbreviations should be avoided, but if needed they must be defined at their mentioned in the abstract.

Keywords: Provide a maximum of 6 keywords, using British spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abstrak: Abstrak di dalam Bahasa Malaysia mestilah mempunyai makna dan terjemahan maksud yang sama seperti dalam abstrak Bahasa Inggeris. Penggunaan 'google translate' dibenarkan dengan syarat penulis memeriksa kembali setiap patah perkataan dan membuat pembetulan mengikut tatabahasa yang betul.

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Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described. The correct Latin names of organisms must be used on first mention in the text. A widely recognized and designated common name should be used for subsequent mention.

Methods

Sub headings are used to itemize the main parts. Materials and methods should be written in the past tense either in active of passive voice. In this section, study dates, number of subjects, groups, evaluation criteria, exclusion criteria and statistical methods should be described sequentially. The origin of materials and/or suppliers of equipment should be named if necessary.

Sub-section

Third level section should be italic. We do not encourage additional sub-levels after the third level. Please try to make your paper concise and clear.

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Use only recommended SI Units. Use superscripts presentation (e.g: mg mL^{-1}) and common abbreviations such as ‘m’ for meter, ‘kg’ for kilogram, ‘min’ for minute and so on.

Results and Discussion

Results should be clear and concise. The discussion should explore the significance of the results of the work. Avoid extensive citations and discussion of published literature. If appropriate, Results can be written in a separate section from Discussion. This especially if the Discussion is extensive and includes all the Results of the study.

Table

Please submit tables as **editable text** and not as images. Tables use double space and 12pt Times New Roman fonts.

Table 1. Use Times New Roman 12 font

Component	Content (%, w/w)
Protein	44.9 ± 0.37
Carbohydrate	22.3 ± 0.94
Water content	13.7 ± 0.02
Ash	6.1 ± 0.19

Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules.

Figures

Please embed the figures in the text with minimum resolution of 300 dpi. Separate **figure files in JPEG or PNG formats can be supplied if it feels necessary**. Ensure that each figure has a caption. A caption should comprise a brief title (not on the figure itself) and a description of the figure. Keep text in the figure themselves to a minimum but explain all symbols and abbreviations used.

Figure 1. Left: Trap one funnel (1F)

Graphs

Graphs must be supplied in figure formats. The fonts of the graph must be clear and readable. Black and white graphs are preferred.

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All citations in the text should refer to:

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References should be arranged first alphabetically and then further sorted chronologically. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.

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The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of Results and Discussion section.

Author contribution (Optional)

Please list the contribution of each author here, e.g.: M.I. designed the research and supervised all the process, L.A. collected and analyzed the data and wrote the manuscript.

Acknowledgments

List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proofreading the article, etc.).

Conflict of Interest (Optional)

Please state any conflict of interest regarding the research or the research funding.

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