

FOOD SAFETY ASPECTS IN BLOOD COCKLES (*Tegillarca granosa*) CULTURED OFF SELANGOR, PENINSULAR MALAYSIA

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Received: 10 June 2016

Revised: 19 Oct 2016

Accepted: 2 Nov 2016

ABSTRACT This study evaluates some food safety aspects of the blood cockle (*Tegillarca granosa*) and its culture waters at Bagan Nakhoda Omar (BNO), Sungai Besar (SB) and Bagan Sungai Buloh (BSB) in Selangor. Samplings of cockles and seawater from the culture beds were carried out from Oct 2014 to Mar 2015 to analyse for fecal indicator bacteria, hepatitis A virus and saxitoxin in cockles and harmful phytoplankton and heavy metals in seawater. The results indicated that cockles from BNO, SB and BSB were of Class C category suggesting that the cockles could only be sold for human consumption after re-laying, followed by purification or heat treatment processes. Meanwhile, the cockle culture areas of BNO, SB and BSB were classified as 'conditionally approved'. The saxitoxin levels in cockle samples from BNO, SB and BSB were 0.12-0.80, 0.05-0.25 and 0.05-0.18 nmol/g, respectively and hepatitis A virus was not detected in the cockles samples. Phytoplankton composition was dominated by the diatom group (>96%). *Pseudo-nitzhia* spp. and *Dinophysis caudata* were commonly found but at low cell counts. The levels of Cr, Zn, Cu, As and Hg were higher than the Malaysian standards while Se, Cd and Pb were within the safe limits. Further investigation should be carried out to identify the pattern and source of pollution throughout a year so that appropriate and cost-effective corrective actions could be taken to prevent future pollution and adverse effect to the public.

Keywords: food safety, phytoplankton, cockles, culture waters, Selangor

ABSTRAK Kajian ini menilai beberapa aspek keselamatan makanan bagi kerang (*Tegillarca granosa*) dari Bagan Nakhoda Omar (BNO), Sungai Besar (SB) dan Bagan Sungai Buloh (BSB), Selangor. Persampelan kerang dan air laut dari kawasan ternakan kerang dibuat daripada Okt 2014 hingga Mac 2015 untuk diuji kandungan bakteria penunjuk pencemaran najis, virus hepatitis A dan saxitoxin dalam kerang serta komposisi fitoplankton dan logam berat dalam air laut. Keputusan yang didapati menunjukkan kerang dari BNO, SB dan BSB adalah dari Kelas C yang bermaksud kerang dari sini hanya boleh dipasarkan selepas dipindah dan dibiarkan dalam perairan yang bersih diikuti dengan samada depurasi atau proses pemanasan. Perairan ternakan kerang di BNO, SB dan BSB dikategorikan sebagai lulus bersyarat. Paras saxitoxin dalam sampel kerang dari BNO, SB dan BSB adalah 0.12-0.80, 0.05-0.25 dan 0.05-0.18 nmol/g, masing-masing sementara virus hepatitis A tidak dikesan dalam sampel kerang. Komposisi fitoplankton bagi semua kawasan persampelan didominasi oleh kumpulan diatom (> 96%) daripada jumlah kepadatan fitoplankton. Mikroalga penghasil toksin, *Pseudo-nitzhia* spp. dan *Dinophysis caudata* ditemui di setiap kawasan persampelan tetapi pada kepadatan yang rendah. Paras logam Cr, Zn, Cu, As dan Hg dalam air laut adalah lebih tinggi daripada paras yang dibenarkan dalam Piawaian Malaysia manakala Se, Cd dan Pb dikesan, tetapi tidak melebihi piawaian. Kajian lanjut sepanjang tahun perlu dijalankan untuk mengenalpasti corak dan punca pencemaran najis supaya tindakan pembedahan yang sesuai dan kos efektif dapat diambil agar pencemaran yang lebih teruk serta kesan negatif terhadap pengguna dapat dihalang.

INTRODUCTION

Selangor is one of the major blood cockle (*Tegillarca granosa*) producing states in Malaysia. According to the Annual Fisheries Statistics, the harvest of cockles in Selangor

remained at less than 10,000 tons in early 2000 but gradually increased from 2008 attaining a record harvest of 42,000 tons in 2010, produced from about 43 active production plots (Annual Fisheries Statistic, 2008; 2010). However, the production of

cockles had declined to about 25,000 tons in 2011 and further plunged to 5,407 metric tonnes in 2013. Meanwhile, the productivity of the culture plots along the coastal areas of Selangor had also deteriorated from 14.3 tons/hectare in 2010 to 2.9 tons/hectare in 2013 (Alias, 2015). The decline in cockle production in Selangor had been closely associated with pollution of the coastal waters from the intensive agricultural activities, the reduction of mudflats due to erosion of fine mud, deterioration of sea bed surfaces due to decline in the flow rate of rivers as a result of dam and reservoir developments and overstocking of cockle seed (Yurimoto et al., 2014). Other studies had associated high ammonia concentrations in the culture waters with the decline in cockle production (Mohd Fadzil et al., 2010; Shimoda, 2015).

The Department of Fisheries Malaysia and the Japanese International Research Cooperation (JIRCAS) initiated a research collaboration in 2011 to investigate the problems. A research project related to the declining cockle industry entitled “the development of a sustainable management plan for blood cockle culture deploying the carrying capacity analysis” was planned and executed. One of the components of this project was to assess the food safety aspects of the cockles for human consumption and the phytoplankton abundance especially the toxin producing algae and heavy metals contents in the cockles culture waters. This is important as cockles are prone to accumulate contaminants such as viruses, bacterial pathogens and toxic phytoplankton present in water due to their filter feeding behaviour. Thus, harvesting cockles from areas exposed to fecal pollution or areas with high numbers of toxic phytoplankton may pose health hazards to the public when they consume cockles (Ahmed, 1991).

To our knowledge, there is very little published information on the safety aspects of cockles or the sanitary quality of cockle culture waters from Selangor although some of the areas are being monitored under the National Shellfish Sanitation Program by the Department of Fisheries, Malaysia. The physicochemical water quality of cockle culture areas from Kuala Selangor has been considerably described in detail (Yurimoto et al., 2014; Mohd Fadzil et al., 2010; Shimoda, 2015). To acquire a complete baseline data for the cockle industry in Selangor, it will be valuable to assess the biological quality of the culture areas and the safety aspects of the cockles harvested for consumption. Hence, this study is carried out to i) determine the fecal concentration in cockle tissues and seawater from the cockle culture beds off Selangor ; ii) detect the presence of Hepatitis A virus in cockles tissues iii) detect the presence of Paralytic Shellfish Toxin (PSP) in cockles tissues; iv) determine the heavy metal levels in cockles tissues and v) determine the presence of potential harmful microalgae in seawater.

MATERIALS AND METHODS

Sampling

Seawater and shellfish (blood cockles, *T. granosa*) samples were collected from 3 stations; i) Bagan Nakhoda Omar (N 3° 44' 21.66", E 100° 52' 30.72"), ii) Sungai Besar (N 3° 38' 53.88", E 101° 58' 46.38") in Sabak Bernam district and iii) Bagan Sungai Buloh (N 3° 15' 31.14", E 101° 17' 9.18") in Kuala Selangor district, Selangor, Malaysia (Fig. 1) on a monthly basis from October 2014 to March 2015. Seawater was collected using sterile bottle (500 ml) in triplicate from each station for bacterial analysis. Cockles from each station were sampled using a core with a long handle (basket size: 60 cm wide, 15 cm high, 30 cm

deep; 1.5 cm mesh). The core was dragged for one to two minutes on the cockle bed until half full and brought up to the surface, rinsed vigorously in seawater to remove mud before placing the cockles on the boat deck. A total of 20-30 individuals were randomly selected and placed in a sterile bag for bacterial, hepatitis A virus and biotoxin analysis. This process was repeated three times. Sub surface water samples for heavy metal from each station were collected using polypropylene Niskin bottles (100 ml). Surface seawater samples from the 3 selected locations were collected using a water sampler and transferred to 1 L sampling bottles. A few drops of Lugol's solution were added to the seawater samples for fixing and staining the phytoplankton present. The samples were packed in ice-cooled insulated box and transported back to the laboratory to be analysed within 24 h.

Fecal coliform (FC) and E. coli (EC) analysis in seawater and shellfish samples

Cockle samples were rinsed clean with tap water and shucked using a sterilized knife. A total of 25 g of the cockle flesh was transferred to a sterile blender (Waring, USA) and a 1:10 dilution was prepared with a sterile phosphate buffer solution, followed by blending for 1 min. Serial dilution was performed and 1 ml of the homogenate was inoculated on a Petrifilm (3M) to enumerate FC and EC. The analysis of FC in seawater was carried out using the Most Probable Number (MPN) method as described in the APHA (1998). FC and EC in shellfish were determined according to MPN methods as detailed out in the APHA (2001). The shellfish culture waters in Selangor were classified according to a classification criteria as described by the US National Shellfish Sanitation Program (2013) (Table 1). Meanwhile the European Committee Directives (1991) (Table 2) was used to classify the cockles quality. The one-way analysis of variance (ANOVA) was performed on all data sets using the SPSS Version 16.0. (Chicago, SPSS Inc.) at 95% confidence level.

Table 1: Shellfish water classification criteria according to the US NSSP.

Classification	Criteria
Approved areas	When under the most unfavourable meteorological, hydrographic, seasonal or point-source conditions, the FC counts do not exceed 14/100 ml and ≤ 10% of the samples exceed a FC MPN of 43/100 ml, for a five-tube decimal dilution test. At least 15 samples must be analysed.
Conditionally approved areas	When there are specific, predictable events (such as rainfall) that can cause an area to exceed the water quality standards. The area is approved for shellfish harvest unless such an event occurs, at which time it is closed for harvest for a period of time pre-determined by the State. Areas with conditionally approved status must meet the standards set forth by the NSSP (see under approved status) outside of the specific, predictable events. Shellfish harvest is allowable in these areas when a closure is not in place.
Restricted areas	When the waters are subjected to limited amounts of pollution such that shellfish must be depurated or relayed prior to sale. Under the most unfavourable meteorological, hydrographic, seasonal or point-source conditions, water samples should not have total coliforms levels in excess of 700 per 100 ml with less than 10% of samples exceeding 2,300/100 ml for a 5 tube MPN. In addition, FCs must not exceed 88/100 ml with ≤ 10% of samples exceeding 260 per 100 ml for a 5 tube MPN.
Conditionally restricted areas	When the waters are subject to intermittent pollution which make them temporarily unsuitable as a source of shellfish for depuration or relaying. The waters are closed for harvesting until they can meet the sanitary criteria for restricted areas.

Prohibited areas	When the level of pollution is such that shellfish are likely to be unfit for human consumption even after depuration or relaying. The harvesting of shellfish is banned from such waters.
Unclassified areas	When no sanitary survey has been conducted. Harvesting of shellfish from such areas is banned.

Table 2: EC shellfish directives 91/492/EEC

Classification	Permitted levels	Outcome
A <230	Less than 230 EC/100g flesh or Less than 300 FC/100g flesh	May go direct for human consumption
B <4,600	Less than 4,600 EC/100g flesh (in 90% of the samples) or Less than 6,000 FC/100g flesh (in 90% of the samples)	Must be depurated, heat treated or relayed to meet category A requirement
C <46,000	Less than 60,000 FC/100g flesh (in 90% of the samples) Less than 46,000 EC/100g flesh	Must be relayed for a period of at least 2 months, followed where necessary by treatment in a purification centre to meet category A requirements
Above 60,000 FC		Unsuitable for production

Hepatitis A virus (HAV) analysis

The RNA extraction of the Hepatitis A virus from the cockle meat was carried out using the RNeasy® kit (Qiagen, Hilden, Germany) while the Qiagen® One-Step RT-PCR kit (Qiagen, Hilden, Germany) was used for the detection of HAV as described in Goswami et al. (1993). Hepatitis A Virus (Enterovirus 72), cytopathic HM 175 (Clone 2) in infected cell lysates obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA) was used as a positive control. Reverse Transcriptase PCR conditions: reverse transcription at 50°C/30 min; initial PCR activation at 95°C/15 min; 3-step cycling-denaturation at 94°C/40 s, annealing at 49°C/40 s and extension at 72°C/60 s for 25-40 cycles. Primers used were;
5' ATGCATCAACATGGATTCATCTCCT GG3' and

5'CACTCATGATTCTACCTGCTTCTCTA ATC3'

Paralytic Shellfish Toxin (PSP) analysis

A total of 25 g of cockle tissues topped up with 0.1 M HCL (made up to 50 ml) in a falcon tube was homogenized. The homogenate was boiled for 5 min and left to cool at room temperature. The homogenate was centrifuged at 3000 rpm for 5 min at 25°C. The PSP toxin was extracted from the supernatant using the S Kit ELISA for Paralytic Shellfish toxin. The concentrations of PSP toxin were quantified according to the method provided by the kit manufacturer (S Kit ELISA).

Harmful phytoplankton identification

In the laboratory, the seawater samples were filtered through a 20 µm pore size plankton

net and each of the concentrated samples was diluted to 100 ml. A volume of 1 ml concentrated sample was transferred to a Sedgewick Rafter Cell Counter using a 1 ml micropipette (Eppendorf Research). The samples were viewed under an Olympus IX51 inverted microscope (Olympus, Tokyo, Japan) at 20x magnification and cells counted. Harmful phytoplanktons were identified to the lowest taxa based on the morphological descriptions provided by Balech (1995) and Omura et al. (2012).

Heavy metal analysis

In the laboratory, the seawater samples were immediately filtered through a nylon filter paper (0.45 µm, 4 mm diameter, Acrodisc (USA)) to remove the suspended particles. A volume of 500 µl Nitric Acid (1%) was added to the filtered seawater sample to reduce the pH to less than 2. Sample preparation for heavy metal analysis was carried out as described in the EPA Method 200.8 (1994) and 6020 (CLP-M) (1998). The concentration of various heavy metals was determined using an Inductive Coupled Plasma Mass Spectrometer (ICP - MS) CX7500. A total of 8 metals: chromium (Cr), zinc (Zn), copper (Cu), arsenic (As), selenium (Se), cadmium (Cd), mercury (Hg) and lead (Pb) were examined.

RESULTS AND DISCUSSION

The geometric mean of FC counts in the seawater samples from the three stations along the coastal waters off Selangor is shown in Figure 2. There is no significant difference ($p < 0.05$) between FC counts in seawater samples from BNO, SB and BSB although the FC counts in SB was

significantly lower ($p < 0.05$) than the FC counts in BNO and BSB. All of the seawater samples from BNO and BSB had FC readings that exceeded 43 MPN/100 ml which is the criteria used for any shellfish culture area under the US NSPP (2013), and in this case $> 90\%$ of seawater samples from SB exceeded the stipulated value. There was no significant difference ($p > 0.05$) for FC counts in seawater samples at different sampling months. The FC counts was higher in Jan, Feb, Mar compared to Oct, Nov and Dec. Although from mid Oct to Mar was the northeast monsoon which usually brings heavy rainfall in the east coast, Jan to Mar on the west coast is the beginning of hot and dry season. Elevation of FC counts especially during summer has been reported by others (Latha & Ramachandra, 2013; Sasikumar & Krishnamoorthy, 2010). This is not surprising as FC has adapted from living inside the intestinal tract of the warm blooded animals thus making them able to survive better in warmer waters. Based on the NSSP, cockle culture areas at BNO, SB and BSB are categorized as “conditionally approved” based on their median FC counts which exceeded 14 MPN/100 ml and more than 10% of the samples exceeded of 43 MPN/100 ml. Our observation is supported by Othman et al. (2014) who reported a high total coliform and *E. coli* counts in Selangor River and its tributary. They observed the highest *E. coli* concentration in the urban area (Rawang sub basin) followed by industrial, residential and agricultural areas. Their results revealed that, the Selangor river water at Rawang sub basin was greatly affected by microorganism attributed to point and non-point sources of pollution.

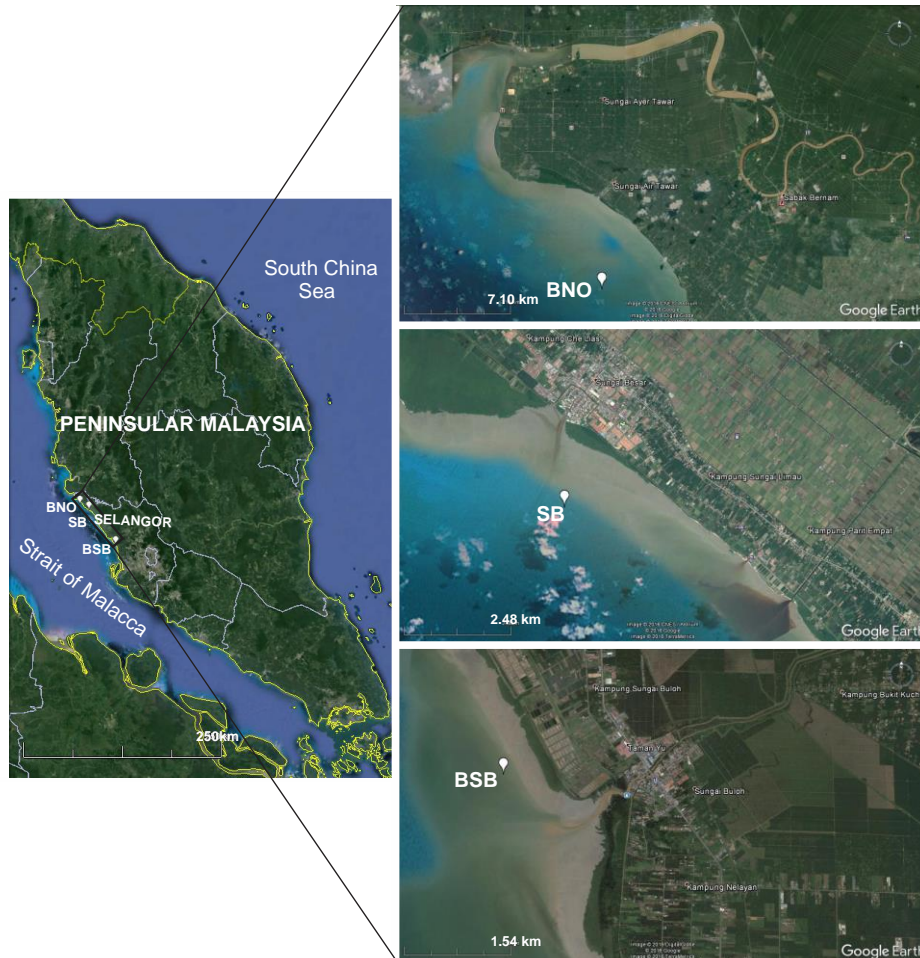


Figure 1: Map of study areas at Bagan Nakhoda Omar, Sungai Besar and Bagan Sungai Buloh.

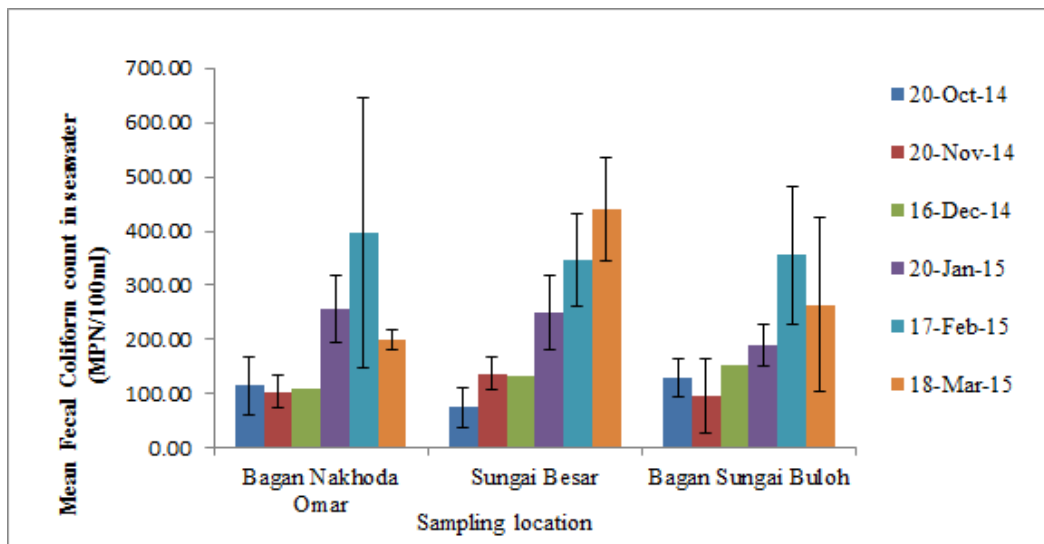


Figure 2: Fecal coliform (FC) counts in seawater from Bagan Nakhoda Omar, Sungai Besar and Bagan Sungai Buloh from October 2014 to March 2015

The mean FC counts in cockles from BNO, SB and BSB are presented in Figure 3. There was no significant differences ($p > 0.05$) in FC counts in cockles from SB, BNO and BSB or for different sampling dates although the cockles from SB generally had lower FC counts as compared to BNO and BSB. BNO and BSB are located very near to the river mouth. This might be the source of constant influx of FC into the waters. Meanwhile SB is located adjacent to a very densely populated area. Based on the Directives 91/492/EC, cockles harvested from BNO, SB and BSB fall under Class C as the samples harboured FC of more than 6,000/100g in 90% of the samples tested (Table 2). As such, shellfish harvested from these areas should not be consumed raw.

SB is one of the stations monitored under the National Shellfish Sanitation program by the DOF and had been classified as Class B during the years 1999 to 2012 (unpublished data). However in 2013 and 2015 (this study) it is categorised as Class C.

The finding indicates that the quality of cockles from SB had been deteriorating. This could be due to the anthropogenic activities (agricultural activities, human settlement) that have been affecting the physicochemical aspects of the water quality as reported by Yurimoto et al. (2014), Othman et al. (2014), and Shimoda (2015). Unfortunately there was no data for the classification of BNO and BSB for comparison under the same program.

Hepatitis A virus was not detected in any of the shellfish samples examined. The very low detection of HAV in shellfish from this study agrees with other reports (Tek, 2009; Vilarino et al., 2009). The saxitoxin content in cockle samples from BNO, SB and BSB ranged from 0.12-0.80, 0.05-0.25 and 0.05-0.18 nmol/g, respectively which were within the safety limit of 1.6 nmol/g (Table 3). However, the saxitoxin producing microalgae was not detected in this study.

Table 3: Saxitoxin concentrations in cockle tissues from Bagan Nakhoda Omar, Sungai Besar and Bagan Sungai Buloh, Selangor

Sampling Location	Date of sampling	PSP concentration (nmol/g)
Bagan Nakhoda Omar	20/10/2014	0.12 ± 0.02
	20/11/2014	0.12 ± 0.01
	16/12/2014	0.16 ± 0.02
	20/1/2015	0.14 ± 0.02
	17/2/2015	0.80 ± 0.06
	18/3/2015	0.20 ± 0.00
Sungai Besar	20/10/2014	0.11 ± 0.03
	20/11/2014	0.08 ± 0.02
	16/12/2014	0.25 ± 0.08
	20/1/2015	0.05 ± 0.01
	17/2/2015	0.08 ± 0.02
	18/3/2015	0.06 ± 0.01
Bagan Sungai Buloh	20/10/2014	0.16 ± 0.05
	20/11/2014	0.08 ± 0.04
	16/12/2014	0.18 ± 0.09

20/1/2015	0.15 ± 0.04
17/2/2015	0.05 ± 0.00
18/3/2015	0.05 ± 0.01

Cell densities of the potentially harmful microalgae at BNO, SB and BSB are shown in Figure 3. The phytoplankton community was dominated by the diatom group which is common in the coastal waters which comprised of more than 96 % of the total cell count at BNO, SB and BSB. Similar observation was recorded in previous studies where they noted diatoms to be the most abundant group of marine phytoplankton (Majbrit, et al., 2004; Booyapiwat, 1997; Carter et al., 2005). Diatoms are known for their rapid growth responses to nutrient enrichments as compared to others phytoplankton groups (Polat, 2007). Diatom proliferation also depends on the nutrient ratio and not just the nutrient concentration. However, most of the diatoms are non-toxic

and serve as a primary food source for the filter-feeding cockles. *Pseudo-nitzschia* spp. was observed in low densities at all the sampling sites throughout the sampling period, with a maximum of 0.25 % of the total count. Although *Pseudo-nitzschia* spp. has been reported to be widely distributed in Malaysian waters (Lim et al, 2012; Teng et al., 2013), their presence in cockle culture waters need to be heeded as almost one third of this species produce domoic acid, a type of neurotoxin that causes amnesic shellfish poisoning (ASP). However, to date, there is no incidence of ASP reported in Malaysia, although a toxic species, *P. kodamae* had been reported in the Straits of Malacca (Tong, 2014).

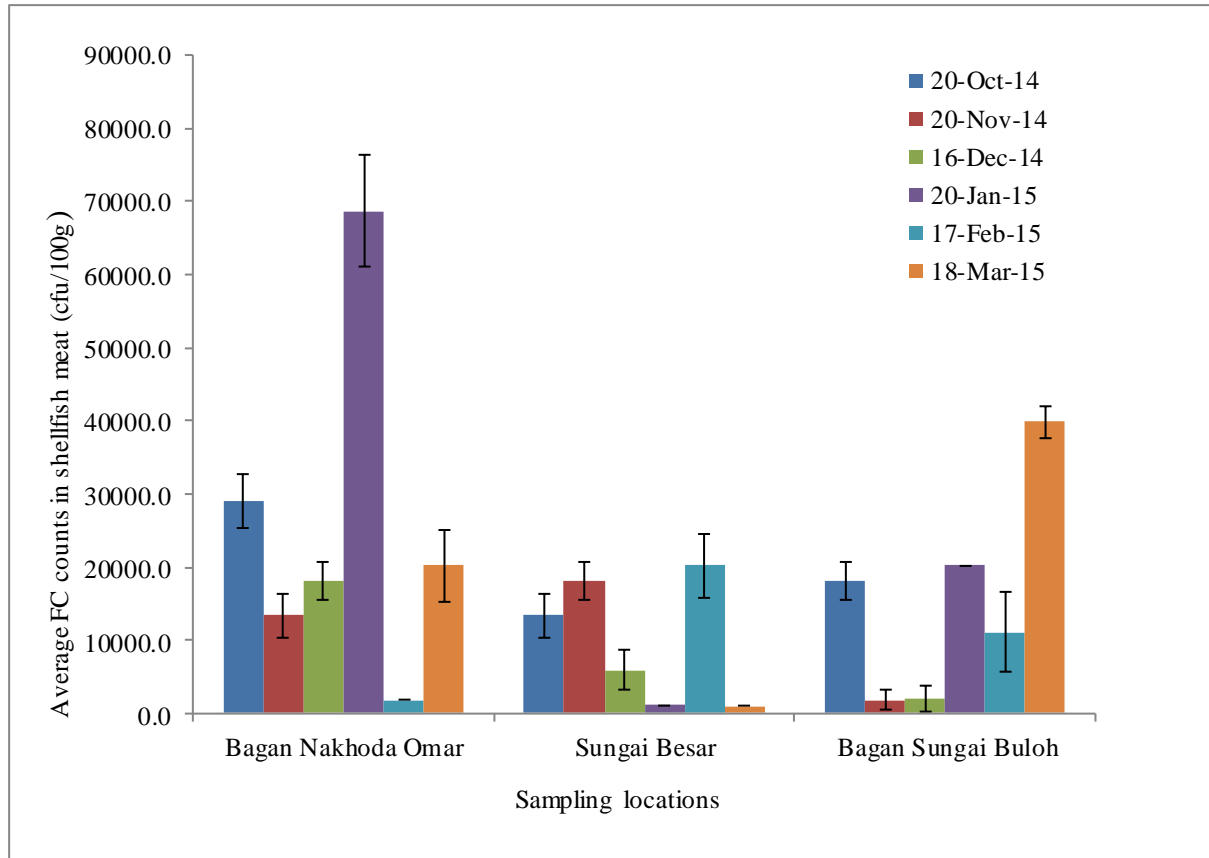


Figure 3: Fecal coliform (FC) counts in cockles from Bagan Nakhoda Omar, Sungai Besar and Bagan Sungai Buloh from October 2014 to March 2015

Besides *Pseudo-nitzhia* spp., other potentially shellfish poisoning toxin producers found in BNO, SB and BSB were *Dinophysis* spp. and *Prorocentrum micans*. *D. caudata* was present in BNO and SB with the highest cell density of 500 cells L⁻¹ and 100 cells L⁻¹ respectively, while the other *Dinophysis* spp. was only present at SB. The presence of *D. caudata* had been reported in the coastal waters off Malacca (Mohammad-Noor, et al., 2007), Sg. Jarum Mas, Perak (Roziawati and Faazaz, 2011) and Selat Tebrau, Johor (Toh et al., 2013). Several species of *Dinophysis* produce okadaic acid, a toxin that causes gastrointestinal illness, even at low cell densities (< 10³ cells L⁻¹). Even at low densities, *D. caudata* had been

known to produce persistently low concentrations of Diarrhetic Shellfish Poisoning (DSP) toxins in the green mussels (*Perna viridis*) off Singapore coast (Holmes and Teo, 2002). In Malaysia, no incidence of DSP had been reported so far. This could be due to the undetected or under-reporting of DSP cases as symptoms of DSP are almost similar to diarrhoea caused by bacterial poisoning. Although not detected in this study, other Paralytic Shellfish Poisoning (PSP) toxin producer such as *Gynmodinium catenatum* and *Alexandrium tamiyavanichi* had been reported by Su-Myat et al., 2012 in seawater and sediment samples from Selangor waters. Regular monitoring programs in these areas of Selangor should be implemented to ensure that cockles

harvested from these areas are safe for consumption.

The heavy metal concentrations in seawater samples from BNO, SB and BSB are shown in Table 4. The metal abundance in BNO and SB were Zn>Cu>As>Cr>Hg>Se>Cd>Pb. Meanwhile the metal abundance in cockle culture waters from BSB were Zn>As>Cr>Cu>Hg>Cd>Se>Pb. The level of Cr, Zn, Cu, As and Hg in seawater samples from BNO, SB and BSB collected in Nov, 2014 and Jan, 2015, were higher than the levels stated in the Malaysia Interim Water Quality Standards (MWQCS). These suggest that there could be a fresh disposal of heavy metal waste around the areas at that time. On the other hand, the level of Se, Cd

and Pb were low in concentrations and did not exceed the recommended levels. To date, data on heavy metals in shellfish growing waters in Malaysia remain scarce as compared to the availability of data on heavy metal contents in shellfish tissues. Our observation on the high Zn content in seawater samples from BSB, Kuala Selangor was similar to Koh et al. (2010) who reported concentrations (110.95-139.65 ppm) that exceeded the Malaysian Food Act (1985) permissible levels of Zn (100 ppm) in cockle's tissues from Kuala Selangor. According to Koh et al. (2010), cockle tends to accumulate light metals such as Zn and Cu in higher concentration in their tissue than Pb, Cd and Hg due to their significant role as precursors in various enzymatic reactions

Table 4: Heavy metal concentrations in seawater samples from cockle culture areas at Bagan Nakhoda Omar, Sungai Besar and Bagan Sungai Buloh, Selangor

Location	Sampling date	Concentrations (ppb)							
		Cr	Cu	Zn	As	Se	Cd	Hg	Pb
Bagan Nakhoda Omar	20/10/14	BD	BD	BD	0.01±0.0	0.08±0.0	BD	0.03±0.0	BD
	20/11/14	23.12±0.55	55.75±0.60	101.7±0.40	28.1±0.5	0	0.03±0.02	2.23±0.20	0.01±0.00
	16/12/14	BD	BD	0.002±0.00	BD	0.06±0.01	BD	1.20±0.06	BD
	20/1/15	20.91±0.882	13.22±1.09	58.80±3.06	24.6±0.82	ND	0.08±0.04	1.35±0.12	0.35±0.40
	17/2/15	BD	0	0.01±0.00	BD	0.10±0.01	BD	0.70±0.15	BD
	18/3/15	0.05±0.00	0.04±0.00	0.10±0.00	0.05±0.00	0.07±0.00	BD	0.04±0.00	BD
	20/10/14	BD	BD	0.19±0.00	0.01±0.00	0.08±0.00	BD	0.30±0.00	BD
	20/11/14	24.22±0.05	30.19±0.05	70.40±0.10	29.6±0.63	ND	0.01±0.02	2.22±0.20	BD
	16/12/14	22.70±0.06	14.90±0.90	54.82±1.00	22.87±0.32	ND	0.03±0.02	1.20±0.06	BD
	20/1/15	21.10±0.49	14.32±0.30	57.91±0.30	24.5±0.41	ND	0.05±0.01	1.32±0.10	BD
Sungai Besar	17/2/15	BD	0	0.01±0.00	BD	0.11±0.00	BD	0.77±0.10	BD
	18/3/15	BD	0	0.1±0.00	0.1±0.00	0.07±0.01	BD	0.01±0.00	BD
	20/10/14	BD	BD	BD	0.004±0.00	0.04±0.01	BD	0.29±0.21	BD
	20/11/14	24.70±0.20	19.30±1.20	71.50±1.70	28.4±0.44	ND	BD	0.5±0.10	BD
	16/12/14	BD	BD	BD	BD	0.07±0.04	BD	0.02±0.00	BD
	20/1/15	20.81±0.07	13.15±0.50	57.3±1.10	24.50±0.60	ND	0.06±0.10	0.70±0.30	BD
Bagan Sungai Buloh	17/2/15	BD	0	0.01±0.00	BD	0.11±0.00	BD	0.61±0.08	BD
	18/3/15	0.01±0.00	0.04±0.00	0.10±0.00	0.08±0.00	0.07±0.00	BD	BD	BD
	20/10/14	BD	BD	BD	0.004±0.00	0.04±0.01	BD	0.29±0.21	BD
	20/11/14	24.70±0.20	19.30±1.20	71.50±1.70	28.4±0.44	ND	BD	0.5±0.10	BD
	16/12/14	BD	BD	BD	BD	0.07±0.04	BD	0.02±0.00	BD
Malaysia Interim Water Quality Standards (MWQCS)		10.00	2.90	2.00	50.00	20.00	2.00	0.16	8.50

BD-below detection limit
ND-not detected

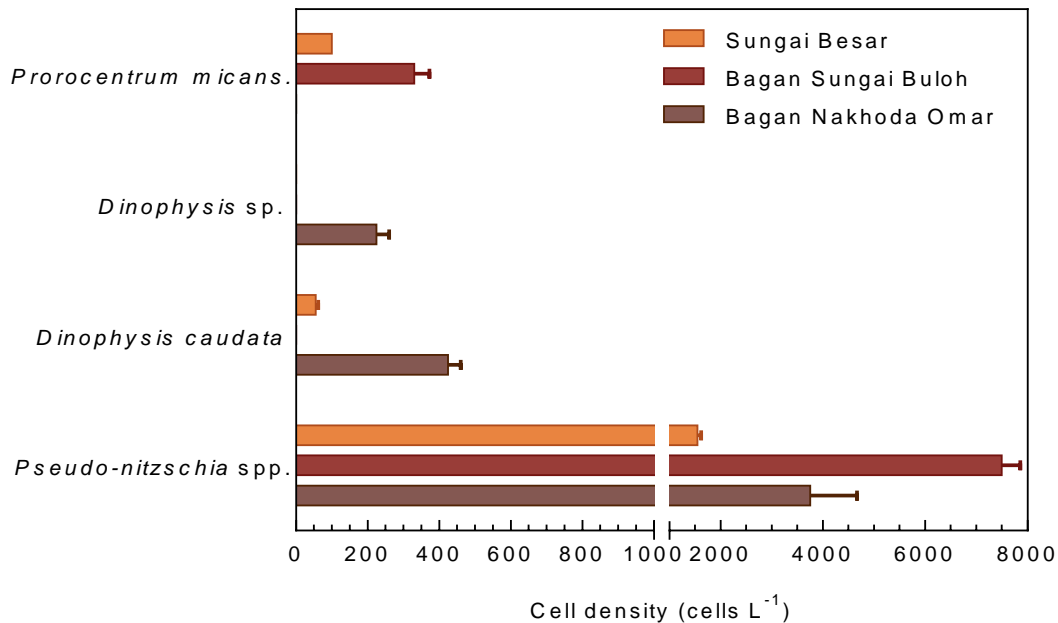


Figure 4: Mean cell densities of potentially toxic phytoplankton by sampling site

CONCLUSION

In conclusion, this study provides the baseline information on the food safety aspects of the blood cockle (*Tegillarca granosa*) and its culture waters at BNO, SB and BSB. The results from this study indicate that the cockles from BNO, SB and BSB could only be sold for human consumption after re-laying them in cleaner water and to be followed by purification or heat treatment processes. The shellfish areas at BNO, SB and BSB can be classified as conditionally approved. There is a trend indicating an increase of fecal pollution in SB. Further investigation should be carried out to identify the primary source of pollution and their occurrences throughout the year so that appropriate and cost-effective corrective actions could be taken to prevent future pollution and adverse effect to the public.

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