

Effect of Physicochemical Parameters and Phytoplankton Composition on Growth Performance of Green Mussel (*Perna viridis*) in Ambong Bay and Marudu Bay, Sabah, Malaysia

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ABSTRACT

This study was conducted to understand the effect of physicochemical parameters and phytoplankton density on the growth performance of green mussel, *Perna viridis* in Marudu Bay and Ambong Bay, Sabah, Malaysia. Physicochemical parameters recorded among stations were not significantly different ($p>0.05$), except that the river mouth station had slightly lower salinity, pH, temperature and dissolved oxygen. The phytoplankton community at the river mouth station was dominated by *Pleurosigma* sp., *Thalassionema* sp. and *Coscinodiscus* sp., while coastal and open sea stations were dominated by *Thalassionema* sp., *Prorocentrum* sp. and *Chaetoceros* sp. Mussels cultured in the river mouth station attained the highest mean meat yield (28.67%) but also showed the highest cumulative mortality (6.48%). The favorable meat yield and condition index of the mussels cultured at the river mouth station could be due to availability of favourable food items such as *Coscinodiscus* sp. and *Thalassionema* sp. However, mussels cultured in the river mouth recorded highest cumulative mortality. In conclusion, places with higher concentration of phytoplankton especially digestible diatoms can be good sites for green mussel farming. In contrast, places which are known to have high turbidity, low salinity and low dissolved oxygen, and recurrent of harmful algal blooms should be avoided. Hence, a thorough study is necessary to identify specific areas within the bays that conform to the site selection criteria suggested in this study before a large scale green mussel farming operation can be established.

Keywords: Bivalve, Phytoplankton, Sabah

INTRODUCTION

Green mussel (*Perna viridis*) provides an affordable source of protein for human consumption and has been cultured with great success using various methods at different places in both hemispheres (Srinibhadh, 1976). According to Choo and Ng (1990), there was about 60% protein in mussel soft tissue (dry meat weight). The harvesting of *P. viridis* can have economic, ecological, and human health impacts (Azman *et al.*, 2012). Besides food, mussels are also used as biomonitoring agents for heavy metals because of their worldwide

consumption (Farrington *et al.*, 1987). They are also sedentary organisms, long-lived, easily identified and sampled, reasonably abundant and available throughout the year, tolerant to natural environmental fluctuations and pollution, and have capacity to accumulate pollutants (Yap *et al.*, 2004).

Growth of *P. viridis* can be separated into shell and body growth. In mussels, growth is most commonly measured as the increase in length, which is the maximum distance between the anterior and posterior axis of the shell (Vakily, 1989; Gosling, 2003). The shell length does not necessarily reflect

the meat content. During spawning or food shortage, internal energy reserves are consumed while the shell may continue to grow (Power *et al.*, 2004; Tan and Ransangan, 2014).

The growth rate of *P. viridis* is high (about 6-10 mm per month) compared to other species of mussels (Shafee, 1979; Sallih, 2005) due to their high adaptability and tolerance to a wide range of environmental conditions (Power *et al.*, 2004). However, their growth is also highly influenced by fluctuations in environmental conditions (Gosling, 2003). This suggests that growth of *P. viridis* is the result of combined effects of a number of environmental factors such as temperature, salinity, food availability, current speed, particulate matter, and nutrient supply (Wilbur and Owen, 1964; Vakily, 1989). Among these factors, food availability is considered to be the most important, since it promotes sustainable growth (Seed and Suchanek, 1992).

According to Cheong (1982), suitable phytoplankton biomass for *P. viridis* culture is 17 to 40 $\mu\text{g}\cdot\text{L}^{-1}$. Rajagopal *et al.* (1998) suggested that a chlorophyll-*a* concentration ranging from 0.7 to 17 $\mu\text{g}\cdot\text{L}^{-1}$ is favourable for *P. viridis* culture. On the other hand, a study by Sivalingam (1977) reported that even 3.5 to 5.2 $\mu\text{g}\cdot\text{L}^{-1}$ of chlorophyll-*a* was sufficient for *P. viridis* culture. Recent study by Tan and Ransangan (2016) on *P. viridis* in Marudu Bay, Malaysia demonstrated poor growth and high mortality even though the phytoplankton density and the chlorophyll-*a* concentration were within the recommended values. This suggests that the feeding behaviour of mussels is not only affected by the quantity of food, but that food quality also seems to play an important role. According to Arifin and Bendell-Young (2001), mussels appear to be highly adapted to a dynamic food environment with negligible costs associated with the feeding process, even when significant pre-selection of organic-rich particles occurs.

P. viridis occurs widely in coastal waters along the west coast of Peninsular Malaysia (Al-Barwani *et al.*, 2007). Aquaculture of *P. viridis* in Malaysia started in Johor Strait and Melaka, where

natural spats are available. Later, the culture was extended to Perak, Kedah and Sabah through seed transplantation (Al-Barwani *et al.*, 2007; Sallih, 2005). In Sabah, *P. viridis* was first cultured in Tawau using broodstock originated from Johor Strait and was later extended to Marudu Bay (Tan and Ransangan, 2016). Although production of *P. viridis* in Sabah was significantly lower compared to Peninsular Malaysia, and only contributed two metric tonnes to the total national green mussel production in 2015 (DOF, 2015), it helps improve the livelihood of the coastal community. Unfortunately, a mass mortality of cultured *P. viridis* in Marudu Bay occurred in late 2009, and the fishery was still struggling to recover even while this study was conducted. Such incidents have affected the livelihood of local farmers (Taib *et al.*, 2016). Hence, this study was carried out to understand the effects of physicochemical parameters and phytoplankton composition on the growth performance of *P. viridis* in Marudu Bay and Ambong Bay in which small scale mussel farming are already in operation.

MATERIALS AND METHODS

Sampling sites

This study was conducted in Ambong Bay and Marudu Bay, Sabah over a 12-month period (October 2015 - September 2016). The sampling station in Marudu Bay was situated at Kampung Taritipan (river mouth station: N06° 34'43.1" E116° 51'17.1") (Figure 1), whereas sampling in Ambong Bay was carried out at two sites: near Kampung Baru-Baru, a coastal station (N06° 17'59.2" E116° 17'36.22") and an open sea station (N06° 18'16.9" E116° 18'16.6") (Figure 2). All sampling stations are sites selected for the 'Green Mussel Rearing Project' subsidised by the Department of Fisheries, Sabah.

Kampung Taritipan is located along the mouth of Taritipan River, which flows into Marudu Bay within Kota Marudu District. It is an aquaculture farm surrounded by mangrove forest, and thus may be subjected to anthropogenic wastes from the farm itself as well as domestic activities and organic

detritus runoff from the nearby areas. Kampung Baru-Baru is located at the southeast side of Ambong Bay, which falls under the administration of Tuaran District Office. As mentioned above, there were two sampling stations in this bay: coastal and open sea stations. There are also aquaculture

farms in these sites, mainly for culturing green mussels and oysters (*Crassostrea* sp.). The coastal station was located along the inner part of the bay and was surrounded by mangrove forest, whereas the open sea station was located approximately 2 km from the coastal station.

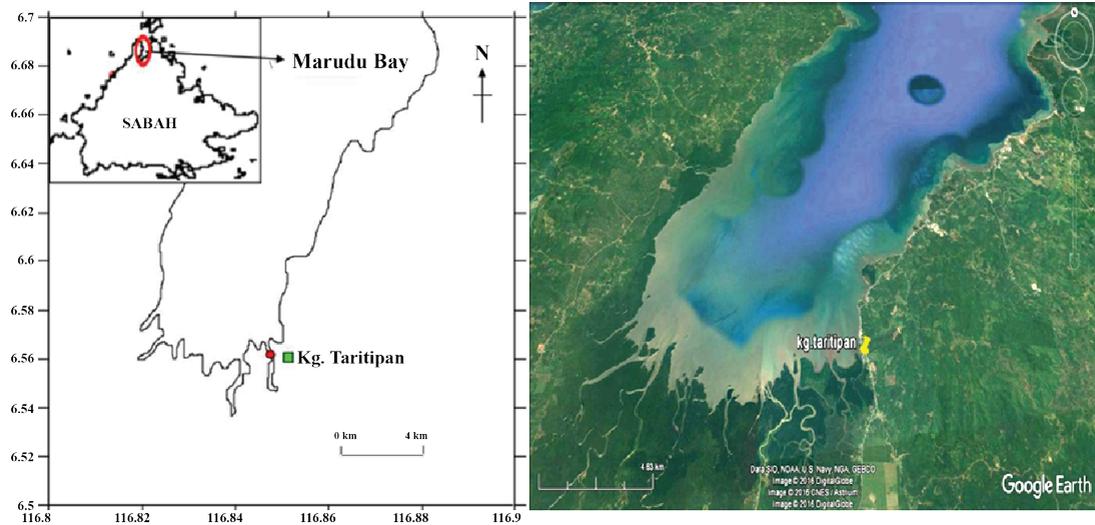


Figure 1. Location of sampling station (river mouth) in Marudu Bay, Sabah, Malaysia.

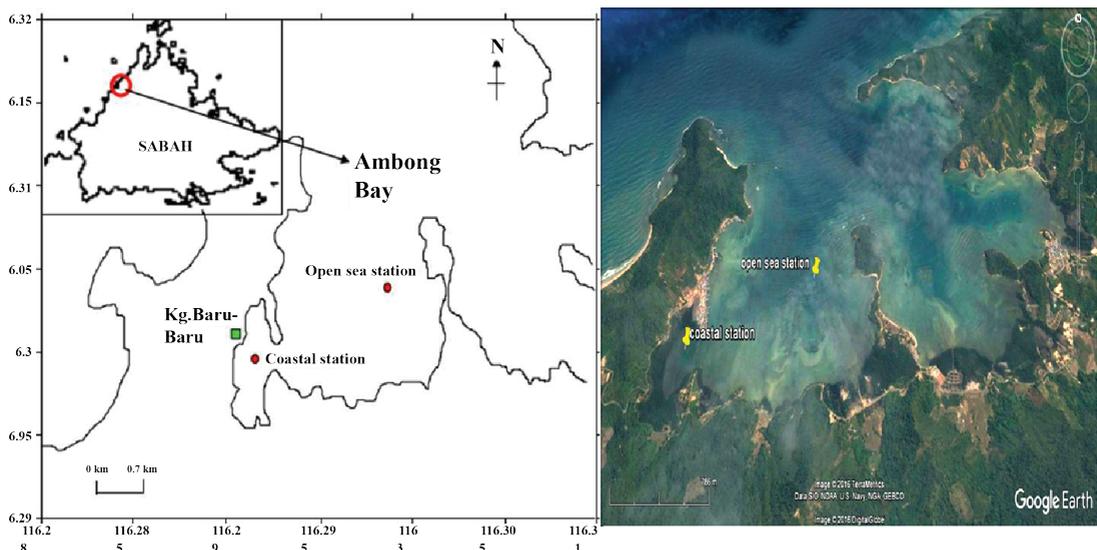


Figure 2. Location of sampling stations (coastal and open sea) in Ambong Bay, Sabah, Malaysia.

Phytoplankton analysis

Sampling of phytoplankton was carried out for a 12-month period, while green mussel collection for growth parameters was done monthly for six months, from April to September 2016. Phytoplankton sampling for identification purposes was done using a plankton net (20 µm) towed vertically from the depth of 1 m to the water surface. For phytoplankton cell counts, water samples were collected at 0.5 m below the water surface using a Van Dorn water sampler and preserved in Lugol's solution in the field.

In the laboratory, phytoplankton was observed under a Carl Zeiss light microscope at 400x and 1000x magnification and identified following Hasle and Syvertsen (1997), and Steidinger and Tangen (1997). For cell counts, 1000 mL of sampled water was poured into a measuring cylinder and left to settle for 24 hours using the Utermöhl sedimentation method (Utermöhl, 1958). The supernatant was then discarded while the bottom 50 mL was retained for phytoplankton quantification using a Sedgwick Rafter chamber under a Carl Zeiss microscope at 400x magnification (Andersen and Thronsen, 2003).

Water physicochemical analysis

Temperature (°C), salinity (psu), pH, and dissolved oxygen (mg·L⁻¹) of the seawater were measured in situ using a multi-function environmental sensor (YSI, Loveland Co, USA). Water samples were also collected for chlorophyll-*a* and dissolved nutrient (phosphate-phosphorus, ammonia-nitrogen, nitrate, and nitrite) analyses following the method by Parsons *et al.* (1984), as well as total particulate matter (including particulate organic matter (POM) and particulate inorganic matter (PIM)) following the method of Wong and Cheung (1999).

Growth performance analysis

Mussel spat settlement was first established in Kampung Baru-Baru at the open sea station using a raft culture system. The raft was constructed from wood beams and polypropylene pipe (Figure 3).

The spats were allowed to settle and grow on the rope for six months. Juvenile mussels of uniform size (mean shell length of 3.25±0.13 cm) were then selected and stocked into lantern nets. Lantern nets were constructed using 0.5-inch mesh nylon fishing net, stainless steel rings (30 cm in diameter) and nylon rope (Figure 4). Three lantern nets were hung randomly on the raft system at each sampling station. Thirty juvenile mussels were stocked into each lantern net and five mussels were randomly collected monthly for a period of six months from each lantern net for growth performance analysis.

Growth was estimated from monthly changes in shell length (SL), live weight (LW), wet meat weight (WMW), dry meat weight (DMW) and ash-free dry meat weight (AFDMW). Shell length was determined by measuring the maximum anterior-posterior axis to the nearest 0.1 mm with a Vernier calliper (Seed, 1968; Celik *et al.*, 2009). Live weight was determined by weighing live samples (total weight of mussels), and wet meat weight was obtained by weighing the meat after dissecting the mussels and blotting extra water with tissue paper. The dry meat weight was determined by drying the tissue for 24 hours at 105 °C in a drying oven. The ash content was determined by combusting the dry meat for 20 hours at 500 °C in a muffle furnace (Carbolite, UK). Ash-free dry meat weight was then calculated by subtracting the ash content from the dry weight (Gimin *et al.*, 2004).

Monthly specific growth rate (SGR) and cumulative mortality of mussels was calculated according to Celik *et al.* (2009) as follows:

$$\text{SGR (\%)} = [(\ln L_2 - \ln L_1) / (T_2 - T_1)] \times 100,$$

where L_1 and L_2 are the mean shell lengths at times T_1 and T_2 ($T_2 - T_1$ was 30 days).

$$\text{Cumulative mortality (\%)} = (N_t / N_0) \times 100,$$

where N_t is the number of empty mussel shells removed from the net after time t and N_0 is the number of mussels at the beginning of rearing.

Meat yield and condition index were calculated using formulae below:



Figure 3. Raft culture system used for green mussel cultivation.



Figure 4. Lantern net used for green mussel growth study.

$$\text{Meat yield (\%)} = (\text{wet meat weight/live weight}) \times 100$$

$$\text{Condition Index (CI)} = (\text{dry meat weight}/(\text{live weight} - \text{wet meat weight})) \times 100$$

The diversity and equitability of phytoplankton were expressed by the Shannon-Wiener index (H') (Shannon and Weaver, 1963) and Pielou's evenness index (J') (Pielou, 1966), respectively, using formulae as shown below:

Shannon-Wiener index (H')

$$H' = - \sum_{i=1}^R p_i \ln p_i$$

where p_i is the proportion of individuals found of each species (i).

Pielou's evenness index (J)

$$J = \frac{H'}{\ln(S)}$$

where H' = Shannon-Wiener index, S = Total number of phytoplankton taxa.

Statistical Analyses

Statistical analyses were performed using the SPSS software (version 21). Tests were judged to be significant at $p < 0.05$. Correlation coefficient tests were performed to evaluate the relationships between growth parameters with phytoplankton and other environmental parameters. One-way ANOVA test was applied followed by Tukey multiple comparison tests (Tukey HSD) to determine the significant differences and to compare the growth parameters and phytoplankton composition among sampling stations.

RESULTS

Phytoplankton abundance and cell density

A total of 36 phytoplankton genera, representatives of 30 families, were identified from the three sampling sites: 30 genera and 25

families at the river mouth station; 33 genera and 25 families at the coastal station; and 34 genera and 28 families at the open sea station. There were five phytoplankton genera which were only found at one station throughout the sampling period, namely *Asterionellopsis* spp. and *Scrippsiella* spp. at the river mouth station (Marudu Bay), and *Dictyocha* spp., *Pronocticula* spp., and *Pyrocystis* spp. at the open sea station (Ambong Bay).

The phytoplankton community at the river mouth station was dominated by *Thalassionema* spp. throughout the 12-month sampling period, with mean abundance of 23.40%. Both coastal and open sea stations were dominated by *Prorocentrum* spp., with mean abundance of 16.93% and 24.44%, respectively (Figure 5). The coastal station had higher phytoplankton cell density, with 5.66 ± 8.52 (mean \pm SD) cells \cdot mL⁻¹ as well as higher Shannon-Wiener diversity Index (H') and Pielou's evenness (J') of $H' = 2.08 \pm 0.70$ (mean \pm SD) and $J' = 0.72 \pm 0.24$ (mean \pm SD), respectively. However, spatial comparison of phytoplankton community structure showed no significant difference ($p > 0.05$) in total phytoplankton cell density, diatom cell density, Shannon-Wiener diversity index (H') or Pielou's evenness (J') among the three stations. Dinoflagellate cell density at the open sea station was found to be significantly different ($p < 0.05$) from the river mouth and coastal stations. However, there was no significant difference ($p > 0.05$) in dinoflagellate cell density between river mouth and coastal stations.

Physicochemical parameters

A summary of physicochemical parameters of waters at the sampling sites throughout the study period (October 2015 to September 2016) is shown in Table 1.

Growth parameters

A summary of growth parameters of green mussels at the river mouth station in Marudu Bay, and coastal and open sea stations in Ambong Bay from April 2016 to September 2016 is shown in Table 2.

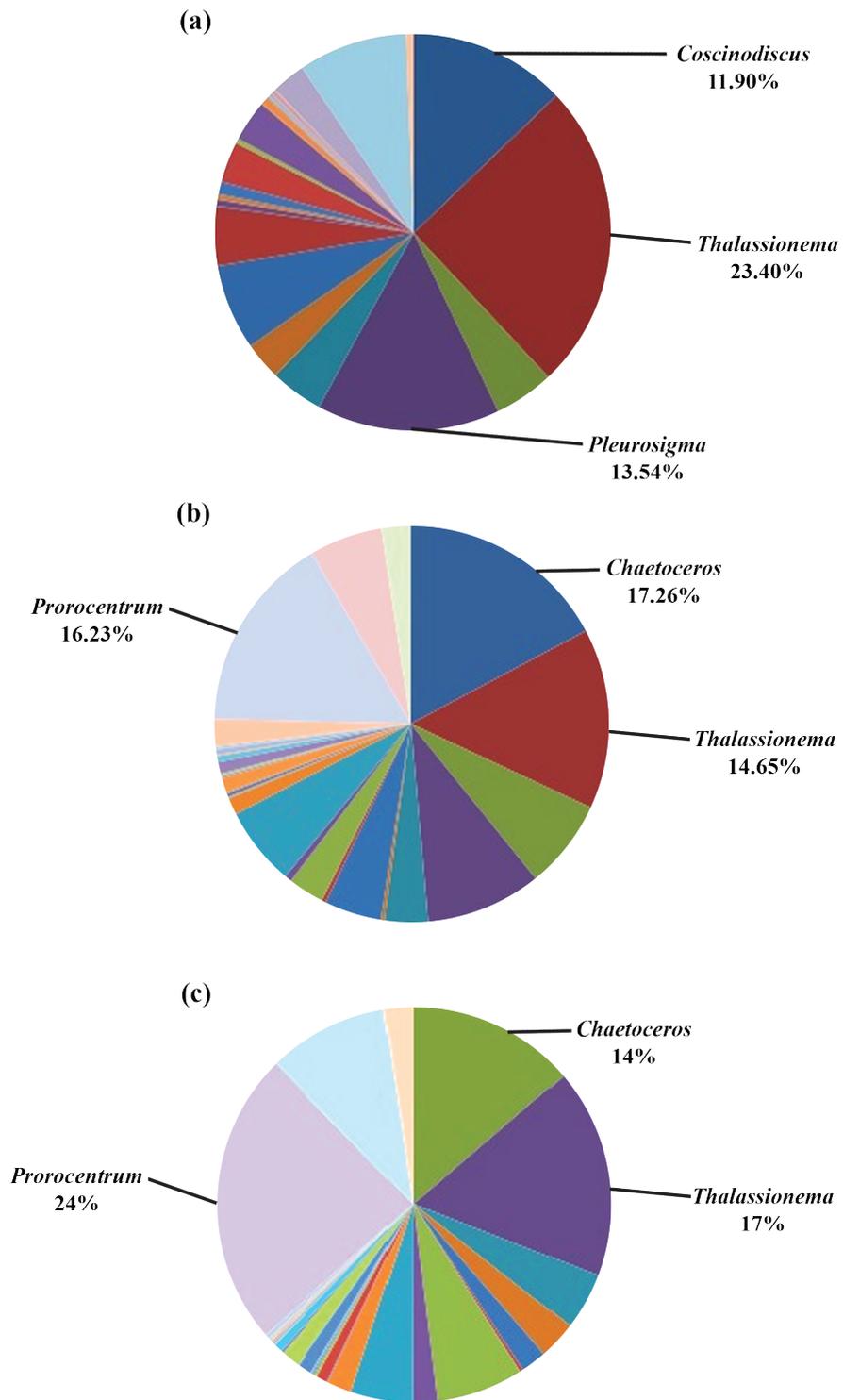


Figure 5. Dominant phytoplankton taxa (%) recorded in each sampling station: (a) river mouth station; (b) coastal station; (c) open sea station, in Sabah, Malaysia, from October 2015 to September 2016.

Table 1. Environmental parameters from three sampling stations in Sabah, Malaysia, recorded from October 2015 to September 2016.

Parameter	River Mouth Station		Coastal Station		Open Sea Station	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Temperature (°C)	29.45±1.41	27.3-31.8	31.48±1.07	30.0-33.2	30.76±1.23	28.3-33.1
pH	7.03±0.27	6.67-7.51	7.70±0.27	7.12-8.07	7.88±0.21	7.56-8.30
Salinity (psu)	31.14±2.01	27.31-33.96	33.45±1.22	31.75-34.94	33.28±1.13	31.24-34.61
Dissolved oxygen (mg·L ⁻¹)	3.66±1.04	2.21-5.41	5.21±0.66	4.58-6.60	5.77±0.78	4.70-7.00
Nitrate (mg·L ⁻¹)	0.5762± 0.4486	0.1005- 2.1090	0.3791± 0.3163	0.1105- 1.2496	0.1881± 0.1570	0.0205- 0.5801
Nitrite (mg·L ⁻¹)	0.0022± 0.0011	0.0004- 0.041	0.0010± 0.0006	0.0002- 0.0029	0.0005± 0.0003	0.0001- 0.0009
Total dissolved phosphorus (mg·L ⁻¹)	0.0116± 0.0530	0.0034- 0.0232	0.0066± 0.0036	0.0010- 0.0178	0.0083± 0.0049	0.0007- 0.0200
Total ammonia nitrogen (mg·L ⁻¹)	0.0704± 0.0292	0.0274- 0.1491	0.0488± 0.0196	0.0270- 0.1057	0.0407± 0.0101	0.0250- 0.0575
Chlorophyll- <i>a</i> (µg·L ⁻¹)	3.57±5.57	0.30-21.40	2.89±3.87	0.23-16.85	1.99±3.82	0.01-16.42
Total particulate matter (mg·L ⁻¹)	0.0572± 0.0703	0.0210- 0.2892	0.0537± 0.0728	0.0156- 0.2903	0.0459± 0.0712	0.0134- 0.2788
Particulate inorganic matter (mg·L ⁻¹)	0.0285± 0.0088	0.0142- 0.0440	0.0237± 0.0092	0.0066- 0.0445	0.0189± 0.0065	0.0080- 0.0348
Particulate organic matter (mg·L ⁻¹)	0.02876± 0.0702	0.0035- 0.2617	0.0299± 0.0705	0.0014- 0.2612	0.02701± 0.0713	0.0018- 0.2612

Table 2. Initial, final and increment in growth measurements (Mean±SD) of *Perna viridis* from sampling stations in Sabah, Malaysia, from April 2016 to September 2016.

Parameter		River Mouth	Coastal	Open Sea
Shell Length (cm)	Initial	2.98±0.25	3.31±0.17	2.90±0.19
	Final	5.28±0.42	6.46±0.26	5.18±0.24
	Increment	2.30	3.15	2.27
Live Weight (g)	Initial	2.48±0.41	3.34±0.39	2.41±0.34
	Final	9.53±0.76	14.56±0.24	7.38±0.27
	Increment	7.05	11.23	4.97
Wet Meat Weight (g)	Initial	0.62±0.03	1.01±0.08	0.66±0.06
	Final	2.70±0.28	3.38±0.31	2.02±0.23
	Increment	2.08	2.37	1.36
Dry Meat Weight (g)	Initial	0.08±0.01	0.12±0.01	0.08±0.01
	Final	0.30±0.02	0.35±0.02	0.23±0.02
	Increment	0.22	0.23	0.15
Ash-Free Dry Meat Weight (g)	Initial	0.06±0.00	0.16±0.00	0.07±0.01
	Final	0.22±0.02	0.27±0.02	0.22±0.01
	Increment	0.16	0.16	0.14

Monthly distribution of growth parameters of the mussels is depicted in Figure 6. There were decreases from May to June 2016 in both DMW (-7.39% at river mouth, -49.93% at coastal, and -10.92% at open sea station) and AFDMW (-19.19% at river mouth, -97.19% at coastal, and -42.68% at open sea station) at the three sampling stations, followed by small increments of SL (+4.35% at river mouth, +5.07% at coastal, and +4.76% at open sea station) and LW (+8.27% at river mouth, +6.40% at coastal, and +10.93% at open sea station) in June to July 2016 compared to the previous months. WMW also increased slowly from May to June 2016, with increment of only 6.90% at the river mouth station, 3.33% at the coastal station, and 4.88% at the open sea station.

The increment of all growth parameters at the coastal station was significantly higher ($p < 0.05$) than the river mouth and open sea stations. However, there was no significant difference ($p > 0.05$) in growth parameters between river mouth

and open sea stations.

Specific growth rate (SGR) and cumulative mortality

The monthly distribution of specific growth rate (SGR) of the mussels from the river mouth, coastal and open sea stations ranged from 0.15%-0.60% ($0.35 \pm 0.18\%$), 0.39%-0.63% ($0.51 \pm 0.16\%$), and 0.22%-0.70% ($0.38 \pm 0.19\%$) (mean \pm SD), respectively. Figure 7a shows that mussels at all three stations experienced a reduction of SGR from June to July 2016, coinciding with the small increment in SL and LW. From May to June 2016, the SGR for river mouth and open sea stations showed decrements of 0.18% to 0.35%, respectively, whereas at the coastal station, an increment of 0.10% was recorded. However, SGR of the mussels at the three stations exhibited similar trends from June to August 2016. From August to September 2016, SGR of mussels at the coastal and open sea stations decreased by 0.06% while SGR for mussels at the river mouth station increased by 0.33%.

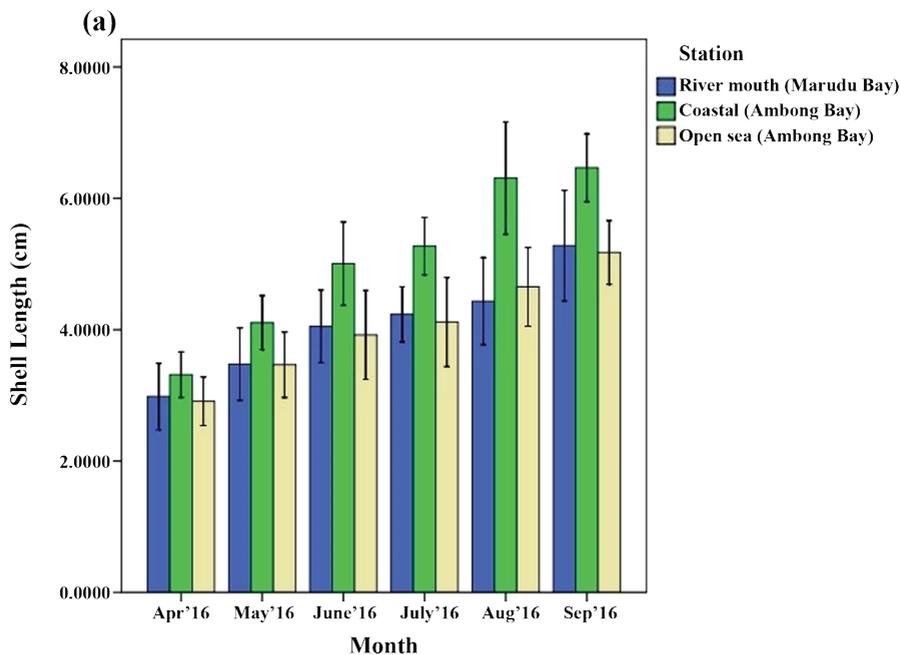


Figure 6. Monthly distribution of growth parameters of *Perna viridis*: (a) shell length; (b) live weight; (c) wet meat weight; (d) dry meat weight; and (e) ash-free dry meat weight at three sampling stations in Sabah, Malaysia, from April 2016 to September 2016.

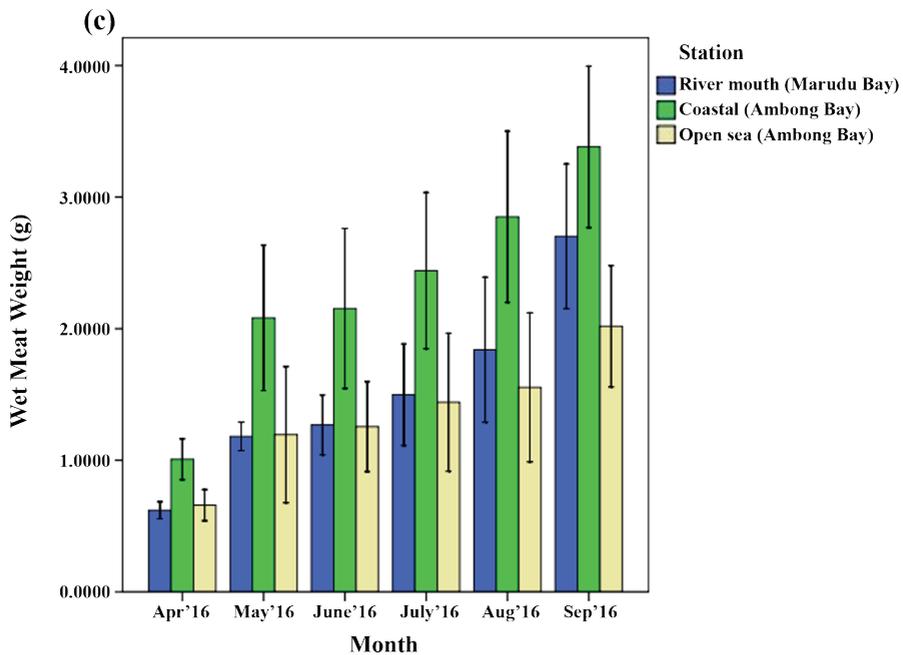
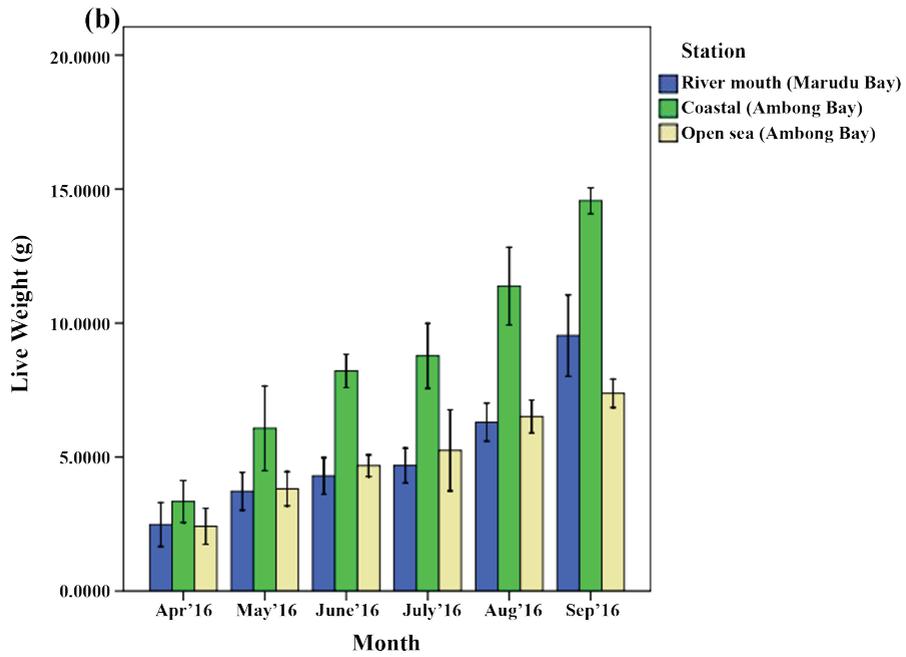


Figure 6. (cont.) Monthly distribution of growth parameters of *Perna viridis*: (a) shell length; (b) live weight; (c) wet meat weight; (d) dry meat weight; and (e) ash-free dry meat weight at three sampling stations in Sabah, Malaysia, from April 2016 to September 2016.

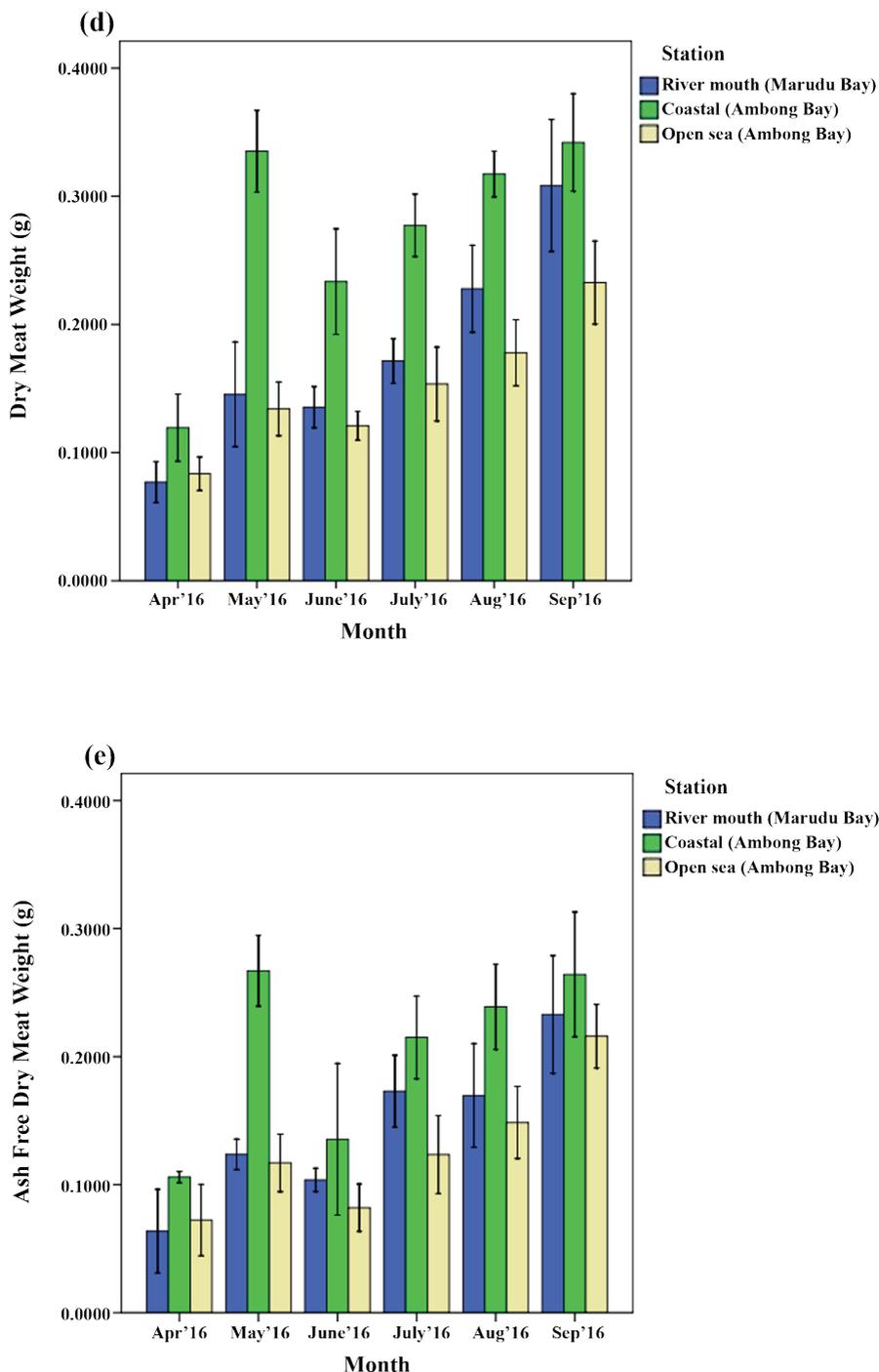


Figure 6. (cont.) Monthly distribution of growth parameters of *Perna viridis*: (a) shell length; (b) live weight; (c) wet meat weight; (d) dry meat weight; and (e) ash-free dry meat weight at three sampling stations in Sabah, Malaysia, from April 2016 to September 2016.

Cumulative mortality of mussels was highest at the river mouth station and lowest at the coastal station (Figure 7b). From April to July 2016, mortality of mussels at the three sampling stations exhibited similar trends. However, mussel mortality at the river mouth station increased suddenly from 5%

to 15% in August 2016. The mortality at coastal and open sea stations also increased, but only by 1% to 2% from the previous month. Statistical analysis showed that cumulative mortality experienced by the mussels at the river mouth station was significantly higher ($p < 0.05$) than at the coastal and open sea stations.

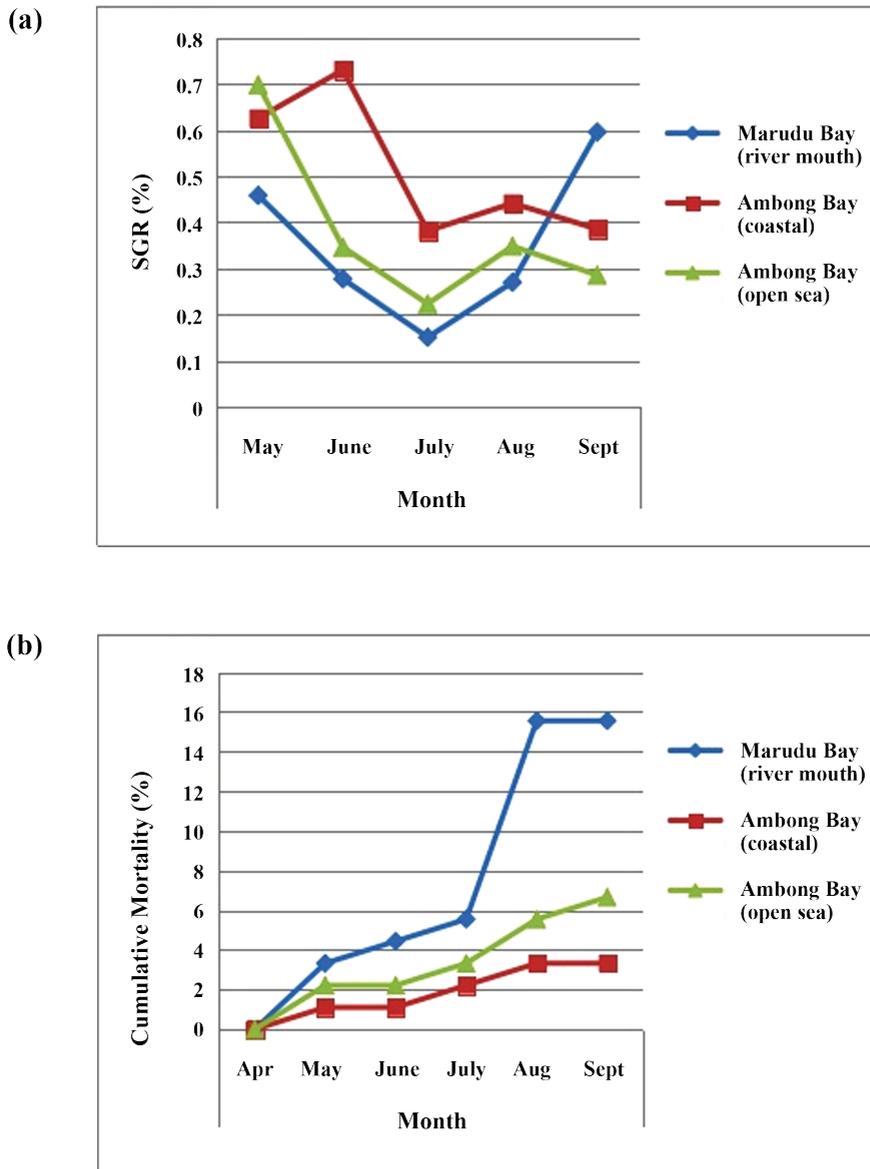


Figure 7. Monthly values for (a) specific growth rate (SGR) and (b) cumulative mortality of *Perna viridis* recorded at three sampling stations in Sabah, Malaysia, from April to September 2016.

Meat yield and condition index

In general, meat yield of mussels at the river mouth station was highest, with mean \pm SD of 28.67 \pm 3.80%, whereas yields in coastal and open sea stations were 27.86 \pm 4.84% and 27.23 \pm 2.21%, respectively. Meat yield of mussels was highest at the coastal station at the beginning of the sampling period (April and May 2016), but later was overtaken by the mussels at the river mouth and open sea stations (Figure 8a). Despite a high growth rate, the mussels at the coastal station had comparatively low meat content. Nevertheless, the meat yield of mussels among the three stations was not significantly different ($p>0.05$).

The condition index of mussels (Figure 8b) at the three stations showed a similar trend as the meat yield, where the coastal station had higher condition index at the beginning (April and May 2016) but was later overtaken by the river mouth station. Mussels from the river mouth station had the highest condition index, of 4.84 \pm 1.46 (mean \pm SD), followed by the coastal and open sea stations, with 4.78 \pm 2.16 and 4.66 \pm 1.65, respectively. Statistical analysis, however, showed that the condition index of the mussels from the three stations was not significantly different ($p>0.05$).

Relationship between growth, phytoplankton and physicochemical parameters

The results from Pearson's correlation analyses on physicochemical parameters of water and growth parameters of green mussels at the sampling sites demonstrated that SL, LW, WMW and DMW of mussels from all stations exhibited strong negative correlation with salinity. Furthermore, SL and LW were also found to be moderately negatively correlated with temperature, DO and chlorophyll-*a* at all stations. On the other hand, meat yield and condition index were strongly and positively correlated with chlorophyll-*a*, total particulate matter (TPM) and PIM in all stations.

The growth parameters (SL, LW, WMW, DMW, and AFDMW) of mussels at coastal and open sea stations were positively correlated with water current speed, except for the mussels at the river mouth station. All growth parameters of mussels at coastal and open sea stations also showed positive correlation with pH, while the mussels at the river mouth station did not.

In terms of growth, the mussels from the open sea station showed significant positive correlation with phytoplankton cell density (both diatoms and dinoflagellates), whereas the mussels from the coastal station showed significant correlation only between SL and LW with diatom, and DMW and AFDMW with dinoflagellates. On the other hand, the meat yield and condition index of mussels from the river mouth station showed positive correlation with H' and J' , but were negatively correlated with phytoplankton cell density (both diatoms and dinoflagellates). The meat yield and condition index of mussels from the open sea station were positively correlated with TPM and PIM. The SL, LW, and WMW of mussels from the coastal station, however, were noted to negatively correlate with TPM, PIM and POM. The SL, LW, DMW and AFDMW of the mussels at the river mouth station showed positive correlation with POM. The meat yield and condition index of the mussels at the coastal station were positively correlated with TPM, POM and PIM.

All growth parameters, except for meat yield and condition index of the mussels from the coastal station, showed positive correlation with NO_2^- and $\text{NH}_3^- \text{N}$. Growth parameters of mussels at the open sea station also showed positive correlation with NO_2^- , while it was noted that growth parameters of mussels at the river mouth station were negatively correlated with $\text{NH}_3^- \text{N}$. Although mussel growth at the river mouth station responded negatively to $\text{PO}_4^- \text{P}$, the mussels at the open sea and coastal stations did not show any correlation.

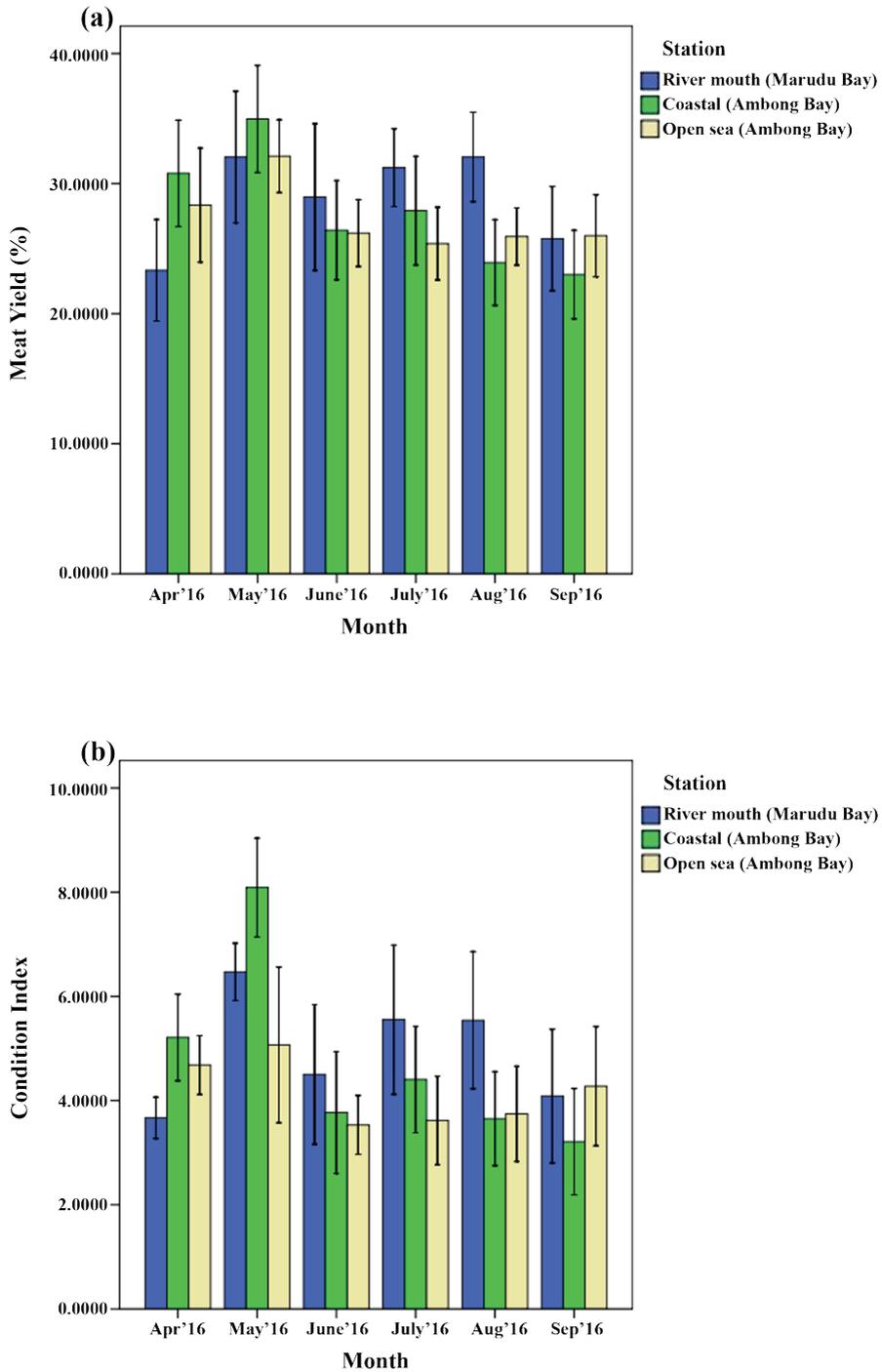


Figure 8. Monthly distribution of meat yield (a) and condition index (b) of *Perna viridis* recorded three sampling stations in Sabah, Malaysia, from April 2016 to September 2016.

DISCUSSION

This study revealed that phytoplankton density in the two bays where green mussels (*P. viridis*) are cultured did not show strong correlation with nutrients in the water, suggesting that nutrients were not the main factor influencing the spatial distribution of phytoplankton abundance in the bays. This is not a surprising finding, as many other factors have been suggested to influence the spatial distribution of phytoplankton, including magnitude and position of the turbidity maximum (Fisher *et al.*, 1988), tidal amplitude and freshwater discharge volume (Gao and Song, 2005), water column stratification and grazing rates of zooplankton (Cloern *et al.*, 1985), light (Parkhill and Cembella 1999; Mei *et al.*, 2009), temperature (Mohammad-Noor *et al.*, 2012) and salinity (Marcarelli *et al.*, 2006).

The high turbidity at the river mouth station could have prevented efficient light penetration into the water column and thus affected the phytoplankton cell density. However, the chlorophyll-*a* concentration at that station was higher, which disagreed with the low phytoplankton cell density in the area. This shows that the high chlorophyll-*a* at the river mouth station is not solely contributed by photosynthetic algae (Sinden and Sinang, 2015) but could also be due to the presence of photosynthetic bacteria, particularly cyanobacteria (blue-green algae). Cyanobacteria often form extensive and persistent blooms in aquaculture ponds during favourable conditions such as high nutrient loading, low rates of vertical mixing, and warm water temperatures (Paerl and Tucker, 1995).

The increment in growth parameters (SL, LW, WMW, DMW and AFDMW) of green mussels cultured at the coastal station of Ambong Bay was found to be significantly higher than the other two stations. This indicates that the physicochemical conditions in that area could be more favourable and thus promote good growth of mussels. The reduction in DMW and AFDMW of mussels recorded from May to June at all three stations could be explained by the consumption of reserved energy during spawning. According to Karayücel and Karayücel (1999), meat weights of mussels are usually affected

by gonadal development and gamete release, as well as by the growth changes in tissue and shell weight. The reproductive tissue extends nearly through the whole body of the mussel except the foot, gills and muscles, thus the gonad constitutes a considerable portion of the body weight prior to spawning (Sprung, 1983).

In most tropical countries, green mussels are noted to spawn all year-round; with peak spawning periods occurring during monsoon seasons in March to April and in October to November (Sivalingam 1977; Tan and Ransangan 2014). Therefore, the reduction in DMW and AFDMW in May to June could be due to the peak spawning in April, which caused the reduction of WMW. The spawning event also slows down the growth rate (SL and LW) of the mussels in the following months.

The high initial specific growth rate (SGR) recorded for the mussels at all stations was expected, as mussels tend to grow more rapidly at an early age than in later stages (Kamal and Khan, 1998). From June to July, SGR decreased, which coincided with the reduction in the increment of SL in the same months. The slow increment of SGR and SL during these months could be explained by energy exhaustion due to spawning of the mussels in the bays (Tan and Ransangan, 2016). According to Sreenivasan *et al.* (1989), green mussels start to mature as early as when they reach 25 mm in shell length. The range of initial shell lengths of the experimental mussels used in this study was 29 to 33 mm, which certainly exceeds the shell length of first maturity of green mussels. The highest SGR of mussels recorded from the river mouth, coastal and open sea stations was in September, August and June, respectively. This reflects the synergistic effects between availability of food and environmental conditions (Karayücel and Karayücel, 1999; Celik *et al.*, 2009) that might favor growth of mussels at the different culture sites. It was noted that a diatom, *Coscinodiscus* (Coscinodiscaceae) was abundant during these months, in which the SGR was higher. Other phytoplankton species which were also quite abundant during those months include *Thalassionema*, *Nitzschia*, *Lauderia*, *Rizosolenia* and *Pleurosigma*. These phytoplankton taxa have

been found to be selectively ingested by green mussels (Tan and Ransangan, 2016). Furthermore, study has shown that some *Coscinodiscus* species (eg. *C. socialis*) contain high levels of lipids (Artamonova *et al.*, 2017). Such a phytoplankton can serve as an important diet to green mussels, especially during gonad maturation, when energy requirements are highest (Gopalakrishnan and Vijayavel, 2009).

Cumulative mortality was found to be significantly higher at the river mouth station due to the sudden increase of mortality in August 2016. Natural mortality in mussel populations is the result of interaction between many biological and physical factors (Celik *et al.*, 2009). Metabolic stress related to spawning has also been found to contribute to mortality in mussels (Yancik *et al.*, 2003). High turbidity at the river mouth station in July and August 2016 could also contribute to the high mortality. Hypoxia condition coupled with low salinity has been shown to affect the byssus production in green mussels (Wang *et al.*, 2011). Thus, the low levels of dissolved oxygen and salinity at the river mouth station compared to the other two stations could have caused the mussels to fail to attach to substrates and hence be more susceptible to predation (Wang *et al.*, 2011).

Despite their slower growth rate and high cumulative mortality, mussels at the river mouth station still recorded the highest meat yield and condition index. Generally, food availability is the main factor which contributes to higher meat yield and condition index in mussels (Celik *et al.*, 2012). Filter feeding bivalves, such as *P. viridis* can alter their filtration, ingestion and absorption efficiency based on the quantities of food in the water (Bayne *et al.*, 1987). Higher concentrations of phytoplankton and organic matter in the water will require lower filtration rates to intake the maximum volume of food, and thus generates faster growth rates (Iglesias *et al.*, 1992; Hawkins *et al.*, 1998). However, in this study, we noticed that mussels at the coastal station exhibited lower meat yield despite having high phytoplankton density and organic content available. This condition may be explained by the food quality. According to

Manfrin *et al.* (2012), despite high concentration of phytoplankton in water during algal blooms, the closure of the shells has been observed and reduction of the filtration rate has been reported in various bivalve species in response to harmful and toxic algae. In addition to shell closure, alteration of feeding behaviour represents another means by which a bivalve may minimize contact with harmful algae by not digesting the cells ingested (Hégaret, 2007). Continuous exposure of mussels to potentially harmful algae at the coastal station may have resulted in the alteration of feeding behaviour and thus affected the meat production. In addition, some dinoflagellates such as *Ceratium* spp., *Dinophysis* spp., although abundant, may have low nutrition value and are poorly digested by mussels, and thus may not provide good growth.

CONCLUSION

Places with higher concentration of phytoplankton especially digestible diatoms can be good potential farming sites as they can enhance meat quality and condition index of the cultured mussels. In contrast, places which are known to have high turbidity, low salinity and low dissolved oxygen, and recurrent of harmful algal blooms should be avoided as these conditions not only affect survival and meat production but also create safety consumption issue of the cultured mussels.

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