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Original Article

Density, recruitment and growth performance of Asian green mussel (*Perna viridis*) in Marudu Bay, Northeast Malaysian Borneo, three years after a massive mortality event

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Abstract

Density, recruitment and growth performance of Asian green mussel (*Perna viridis*) in a particular coastal marine environment can be affected by many factors, including environmental change, pollution, disease outbreak and massive mortality event. The present study was conducted to determine the density, recruitment and growth performance of farmed Asian green mussel in Marudu Bay, three years after a mass mortality event. The study was carried out for 12 months between April 2013 and March 2014. The length frequency data of 1,308 individuals of green mussel were analyzed using the latest version of the FAO-ICLARM Fish Stock Assessment Tools (FiSAT II). The result showed that the green mussel recruitment in Marudu Bay occurs throughout the year with two major peaks i.e. February and July which coincided with the monsoon seasons. The asymptotic length ($L\infty$), growth coefficient (K) and growth performance index (φ') of the farmed Asian green mussel in Marudu Bay are relatively high at 113.4 mm, 1.7 year^{-1} and 4.34, respectively. However, despite good culture location, the settlement density of green mussel in the bay was low. We suspected that the low settlement density could be influenced by the ecological effects due to the long term suspension of the culture substrates and the physiochemical properties of the water in Marudu Bay. Nevertheless, chlorophyll-á measurement alone was not able to justify if food scarcity has resulted in high mortality of the farmed Asian green mussel in Marudu Bay.

Keywords: mussels, *Perna viridis*, density, recruitment, growth performance

1. Introduction

Asian green mussel (*Perna viridis*) is an important seafood resource and it is widely cultivated for commercial purposes especially in the Southeast Asian region. This species is extensively cultured due to its high productivity, high tolerance to a wide range of environmental conditions, and it requires less farm management (Rajagopal *et al.*, 2006; Al-Barwani *et al.*, 2007; McFarland *et al.*, 2013). *Perna viridis* is currently being recognized as a cheap protein sources, containing high nutritional values and it is popular for its delicious taste (Rajagopal *et al.*, 1998; Yap, 2012).

The production of green mussel in Malaysia reached the highest peak in 2010 with total production of 10,529.06 MT, but declined continuously until 2013 (1,070.88 MT) by 89.9% reduction compared to that in 2010 (DOF, 2010; DOF, 2013). One of the major causes of the decline was the occurrence of massive mortality event in many green mussel farms across the country. One of these farms was located in Marudu Bay, northeast Malaysian Borneo. The green mussel aquaculture was introduced in the bay in the late 1990s and became a commercially important activity in early 2000. Unfortunately, in late 2009 to 2012 the green mussel farm in Marudu Bay was seriously affected by massive mortality. The mortality event wiped out almost all the juveniles and adults mussel population, leaving only small quantity of survived

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mussels on culture ropes. Since then, the production of green mussel in the bay had drastically gone down, deserted and caused huge economic loss to farmers.

Previous studies described several factors which can cause mussel mortality including physiochemical, hydrodynamic, food, predation, and diseases outbreaks (Gulshad, 2003; Schiel, 2004; Peperzak and Poelman, 2008; Yap, 2012; Heinonen, 2014). Among these causes of mortality events, the environmental parameters and food availability are the most reported causes of massive mortality of farmed bivalve. For example, sudden increase in water temperature is often lead to mortality of green mussel and other bivalve species under experimental conditions (Hiebenthal *et al.*, 2012; Sreedevi *et al.*, 2014; Solomieu *et al.*, 2015). Furthermore, Alforo (2006) found the mortality of *Perna canaliculus* in northern New Zealand was due to limited food supply.

The of physiochemical parameters of water, water nutrients and chlorophyll are essential to establish the relationship between growth and abundance of green mussel in Marudu Bay and the environmental factors. Besides, information pertaining to recovery rates such as recruitment, growth and mortality after a mass mortality event is also essential for farm management to understand the extent of the potential risk and thus possible mitigation for future restocking. In this study, we investigated the density, recruitment pattern and growth performance of green mussel cultured using the hanging rope culture method in Marudu Bay, three years after the mass mortality event.

2. Materials and Methods

2.1 Study area

This study was conducted in a green mussel farm in Marudu Bay, (6°38’22" N, 116°53’17" E), Sabah, Malaysia; Figure 1). Marudu Bay is influenced by two monsoons, the Northeast Monsoon that occurs between November and April and the Southwest Monsoon that takes place between May and September. Heavy rainfall generally occurs during the Northeast Monsoon.

2.2 Field experiment and sampling

The experiment was carried out for 12 months from April 2013 to March 2014. The Asian green mussel was cultured using the hanging rope culture method. Nylon ropes (length x diameter: 1 m x 2 cm) wrapped with a fine filament of fish netting were used as substrate to facilitate the settlement of the mussel spats. A total of 250 ropes were hung on the raft and suspended at 0.5 m depth below the water surface with 30 cm rope spacing. Samplings were conducted once a month. Ropes with nine replicates were collected randomly on a monthly interval to estimate the density of the mussel. All analyses on the mussel samples were conducted within 48 hours of collection.

2.3 Length and weight measurement

In the laboratory, the ropes were washed with running seawater and the mussels were individually removed. Morphometric measurements including length, thickness and weight were measured according to the methods described by Vakily *et al.* (1988) using a caliper at 0.1 mm accuracy. The total weight was weighed by using analytical balance (Sartorius) of 0.001 gram accuracy. In total 1,308 mussel samples were then grouped into shell length classes of 5 mm interval following Al-Barwani *et al.* (2007).

2.4 Water physiochemical parameters

Physiochemical parameters of water such as dissolve oxygen (DO), pH, salinity and temperature was measured

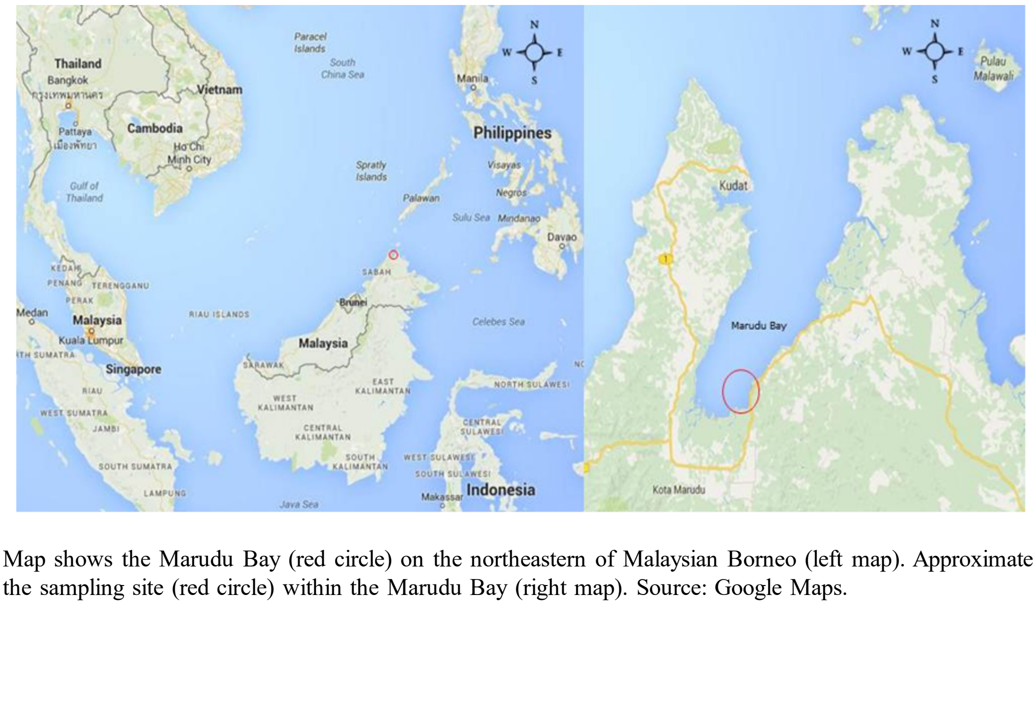

Figure 1. Map shows the Marudu Bay (red circle) on the northeastern of Malaysian Borneo (left map). Approximate location of the sampling site (red circle) within the Marudu Bay (right map). Source: Google Maps.

once every month by using a HI 9829 Multi-parameters water quality checker (Hanna Instrument). Current speed was measured by using the Direct Reading Electromagnetic Current Meter (AEM-213D).

2.5 Water nutrients and chlorophyll-α analysis

In total of 500 ml of water samples were filtered through membrane filter (0.45µm) with the help of a vacuum pump. The filtered membranes were subjected to chlorophyll-α analysis following the method of Strickland and Parsons (1972). Chlorophyll-α was then extracted with 10 ml of 90% acetone. The absorbance of the extracts was determined at 664, 647, and 630 nm. The chlorophyll-α concentration was calculated by following equation (Talling and Driver, 1963):

Chlorophyll-α (µg/L) =

$$(11.85\,A_{664} - 1.54\,A_{647} - 0.08\,A_{630}) \times \frac{V}{S} \times 1000$$

A_{664} = Absorbance at 664 nm
A_{647} = Absorbance at 647 nm
A_{630} = Absorbance at 630 nm
V = Volume of acetone used (mL)
S = Volume of sampled filter (mL)

In the other hand, 25 ml each of the filtered seawater was analyzed for water nutrients (ammonia, nitrate, nitrite and phosphate) following the method of Parsons *et al.* (1984). The concentration of each water nutrient was determined spectrophotometric at 640 nm (ammonia), 543 nm (nitrate and nitrite), and 882 nm (phosphate).

2.6 Statistical analysis

Growth and recruitment of farmed Asian green mussel at different months were analyzed by ONE-way ANOVA followed by Turkey multiple comparison test (Turkey HSD). Data were also subjected to correlation and bivariate tests to find significant relationship between the mussel density and water parameters. Tests were judged to be significant at $p < 0.05$ level. Prior to analyses, all the variables were tested for normality and homogeneity of variances using One-way ANOVA. All the parametric tests were performed by using SPSS Windows Statistical Package (version 18).

Von Bertalanffy growth function (VBGF) and recruitment pattern of green mussel was estimated based on frequency distribution of each length class sizes every month for one year period. The estimation were performed in ELEFAN-1 (Pauly and David, 1981) using the FiSAT software as explained in detail by Gayanilo et al. (1996). Asymptotic length (L_∞) and growth coefcient (*K*) of the *K*-scan routine was conducted to assess a reliable estimate of the *K* value. The parameters L_∞ and K were then used to calculate the growth performance index (φ') of the farmed Asian green mussel using the equation $\varphi' = 2\,\mathrm{Log}_{10} L_\infty + \mathrm{Log}_{10} K$ (Pauly and Munro, 1984). Normal distribution of the recruitment pattern was determined by using the NORMSEP, FiSAT (Pauly and Caddy, 1985). On the other hand, the density of the mussel in 1 m^2 surface area of the substrate (rope) was estimated using the following formula.

Density (per m^2) =

$$\frac{\text{Number of enumerated individuals (N)}}{\text{Surface area of substrate (rope) } (2\Pi r^2 + 2\Pi r(h))}$$

where N = number of green mussel in 1 rope (total individual found /9 ropes taken every month), h = 1 m (height of rope), and r = 0.01m (radius of rope).

3. Results

3.1 Mussel density

The monthly density of the Asian green mussels attached to substrate (ropes) is illustrated in Figure 2. Highest number of the mussels (about 500 ind m^{-2}) was recorded in December 2013. This value was significantly higher ($p<0.05$) than the density of the mussels recorded in any other months (150 to 250 ind m^{-2}).

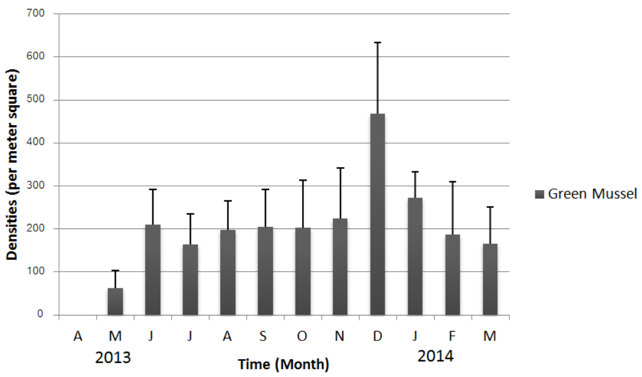

Figure 2. Density of farmed Asian green mussel attached on the experimental substrate (ropes) in Marudu Bay recorded from April 2013 to March 2014. Standard error (SE) is indicated by the bar line on top of each bar.

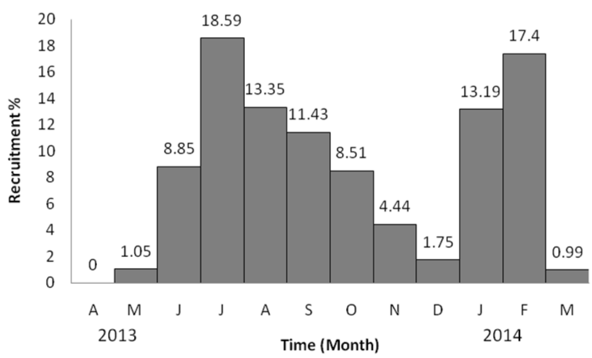

Figure 3. Recruitment percentage of farmed Asian green mussel recorded in Marudu Bay between April 2013 and March 2014. The recruitment was estimated based on the time projection of length frequencies.

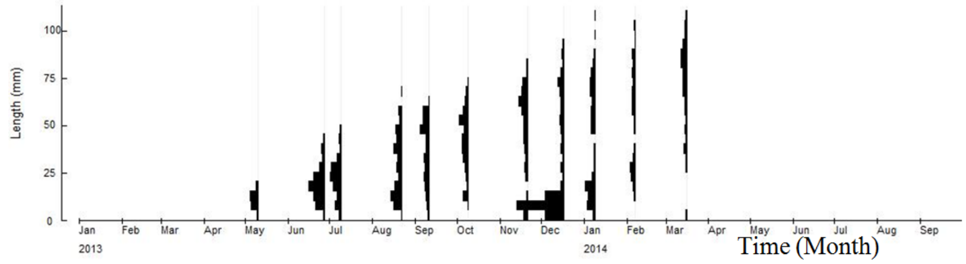

Figure 4. The length frequency data of farmed Asian green mussel in Marudu Bay. Note that the class sizes spread widely and oscillate every month.

3.2 Mussel recruitment

Recruitment of Asian green mussel occurred throughout the year with two seasonal peaks (Figure 3). The peaks recruitment of 18% and 17% were recorded in July 2013 and February 2014, respectively. The cohorts arising from the July recruitment were found to reach a marketable size (50-60 mm) in 5 to 6 months (Figure 4).

3.3 Growth performance

The asymptotic length (L_∞) of the farmed Asian green mussel was estimated at 113.4 mm and the growth coefficient (K) was at 1.7 year^{1} (Figure 5). The calculated growth performance index (φ') was 4.34. The maximum length observed in 12 months monitoring was 110 mm, and the predicted extreme length of the mussel was 129.03mm. The maximum length at 95% confidence range was 102.91-155.15 mm (Figure 6). The monthly average length and weight of the mussels was relatively increased in tandem with the study period. In December 2013, the average length and weight dropped more than 50% compared to November 2013. The highest average length and weight recorded in the culture area was in March 2014 with 82% (74.6mm) increase in length and 99% (30.6g) increase in weight compared to that in May 2013.

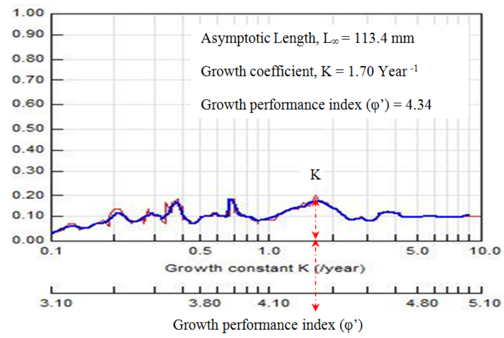

Figure 5. Von Bertalanffy growth function of farmed Asian green mussel in Marudu Bay estimated between April 2013 and March 2014.

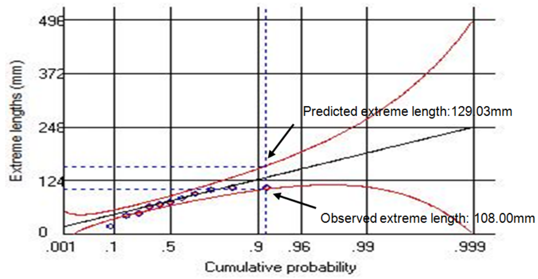

Figure 6. Maximum length of farmed Asian green mussel in Marudu Bay predicted from extreme values, with range at 95% confidence interval: 102.91mm - 155.15mm.

3.4 Relationship between mussel density and water parameters

The water parameters recorded throughout the one-year sampling period are shown in Figure 7. The highest surface water temperature was recorded in April 2013, while the lowest water temperature recorded in October 2013. The salinity varied from 25 ppt to 31 ppt. Meanwhile, water pH recorded during the sampling period was within the normal range (7.6-8.2). Dissolved oxygen fluctuated from 3.5 mg/L (May 2013) to 5.9 mg/L (January 2014). Water velocity (current speed) exhibited variation from 5.85 cm/s to 19.96 cm/s. Water nutrients levels were relatively high between April 2013, and December 2014 to March 2014. However, low averages of nitrate and nitrite concentrations (below 1 µg/L) were recorded throughout the sampling period. Chlorophyll-α concentration was recorded slightly higher in June and October but low between December and April. The bivariate test illustrated that there was a significantly negative relationship between the mussel density with water temperature, velocity and phosphate concentrations ($p<0.01$) as shown in Table 1. However, the mussel density was found to have a significant positive correlation with dissolved oxygen.

4. Discussion

The highest density of *Perna viridis* was only observed in certain months (December 2013 and January 2014). Similar finding was also reported in India and Thailand during the first year of substrate deployment (Chaitanawisuti and Menasveta, 1987; Rajagopal *et al.*, 1998). However, the density of green mussel in study area was extremely lower than reported in Hong Kong, Thailand, New Zealand and

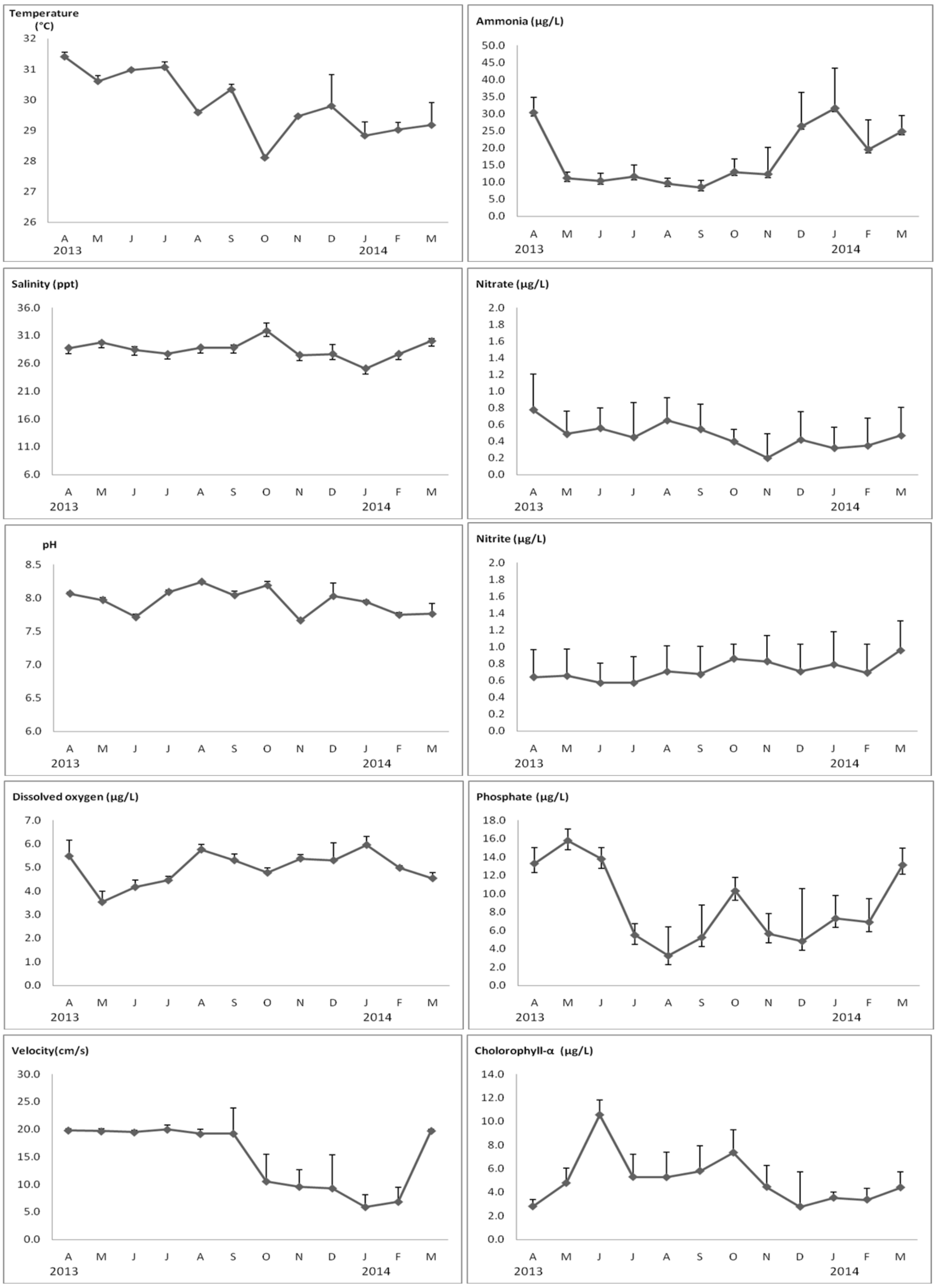

Figure 7. Water parameters in Marudu Bay recorded from April 2013 to March 2014.

India mussel farms with settlement density more than 1,000 ind m^{-2} (Lee, 1985; Chaitanawisuti and Menasveta, 1987; Rajagopal *et al.*, 1998; Alfaro, 2006). Despite the poor density, the recruitment was found to occur all-year round, similar to that in other tropical Asian countries (Al-Barwani *et al.*, 2007; Khan *et al.*, 2010; Laxmilatha, 2013). The major recruitment of the green mussel in Marudu Bay occurred twice a year; first in July (Southwest Monsoon) and second in February (Northeast Monsoon) which coincided with the monsoon seasons in Malaysia. Although recruitment of the mussel occurs throughout the year, its peaks of recruitment vary according to places (Khan *et al.*, 2010). Such phenomenon may be influenced by the complicated interactions between biological, chemical and physical factors (Broitman *et al.*, 2005; Smith *et al.*, 2009).

In the present study, the asymptotic length (L_∞= 113.4mm) of the farmed green mussel in Marudu Bay was recorded higher than those reported in other places in Malaysia including Malacca (102.38) and Penang (89.4mm) (Al-Barwani *et al.*, 2007). This value is resembled to those

Table 1. Pearson correlation coefficient (r) between farmed Asian green mussel density and water parameters

	Density	Temperature	pH	Salinity	Dissolved oxygen	Velocity	Ammonia	Nitrate	Nitrite	Phosphate	Chlorophyll-α
Density	1	**-0.455****	-0.179	-0.285	**0.367***	**-0.507****	-0.082	-0.287	0.158	**-0.336***	-0.060
Temperature	**-0.455****	1	0.106	0.013	-0.362*	0.759**	-0.007	0.410*	-0.149	0.237	-0.050
pH	-0.179	0.106	1	0.306	0.075	0.329	-0.173	0.351*	-0.069	-0.281	0.330*
Salinity	-0.285	0.013	0.306	1	-0.682**	0.468**	-0.267	0.091	0.110	0.333*	0.467**
Dissolved oxygen	**0.367***	-0.362*	0.075	-0.682**	1	-0.471**	0.316	0.041	-0.077	-0.588**	-0.335*
velocity	**-0.507****	0.759**	0.329	0.468**	-0.471**	1	-0.227	0.446**	-0.145	0.210	0.320
Ammonia	-0.082	-0.007	-0.173	-0.267	0.316	-0.227	1	0.307	0.345*	0.225	-0.627**
Nitrate	-0.287	0.410*	0.351*	0.091	0.041	0.446**	0.307	1	0.149	0.185	0.012
Nitrite	0.158	-0.149	-0.069	0.110	-0.077	-0.145	0.345*	0.149	1	0.304	-0.148
Phosphate	**-0.336***	0.237	-0.281	0.333*	-0.588**	0.210	0.225	0.185	0.304	1	-0.052
Chlorophyll-α	-0.060	-0.050	0.330*	0.467**	-0.335*	0.320	-0.627**	0.012	-0.148	-0.052	1

**correlation is significant at the 0.01 level (2-tailed); *correlation is significant at the 0.05 level (2-tailed).

reported in other Asian countries particularly in Hong Kong and Thailand at 101.9 mm and 112 mm, respectively (Lee, 1985; Tuaycharden *et al.*, 1988). The *K* value of the mussel in Marudu Bay was higher compared to those in Malacca (Al-barwani *et al.*, 2007), Bangladesh (Khan *et al.*, 2010), Hong Kong (Lee, 1985), India (Narasimham, 1981) and Thailand (Tuaycharden *et al.*, 1988). The high K value and the excellent asymptotic length ($L\infty$) of the mussel in Marudu Bay may be explained by the rapid growth of mussels in small clump with less density (~9 ind), than the mussels in larger clumps with high density (< 20 ind) (Seed and Suchanek, 1992).

The physiochemical properties of seawater in Marudu Bay can be characterized as suitable site for mussel farming because they ranged within the suggested good conditions for mussels (Shamsudin, 1992; Kingzett and Salmon, 2002; Tan and Ransangan, 2014). Massive mortality event in late 2009 occurred approximately after 10 years since the introduction of green mussel aquaculture in Marudu Bay has caused significant reduction in commercial production. After three years of the mortality event, density of the green mussel on suspended ropes (substrate) is still low. Such situation might be best explained by the ecological effects of the long term suspension of the ropes used for mussel farming (Keeley *et al.*, 2009; Yap, 2012) and adaptation of the introduced green mussels to the ambient environment in the bay (Riisgard *et al.*, 2013; Zbawicka *et al.*, 2014). Ecological effects create novel habitats for micro and macro fouling organism such as algae (focus) and barnacles to outcompete for space and food (Bendell-Young, 2006; Yap, 2012). Colonization of other biofouling and mussel epibionts on suspension ropes as well as on mussel shell influences the mussel growth and may cause poor mussel settlement (Garner and Litvaitis, 2013a). Weak settlement increases the risk of dislodgement and detachment from the substratum and may lead to high mortality (Harger and Landenberger, 1971; Yap, 2012).

Increase in temperature and water velocity has been reported to cause disturbances to mussel settlement on rope substrates by means of byssogenesis (Tamarin *et al.*, 1974). Strength of byssal threads is important for green mussel to remain anchored on the suspension substrate (Garner and Litvaitis, 2013b). *Mytilus edulis* (Carrington, 2002) and *Mytilus galloprovincialis* (Zardi *et al.*, 2007) were found to produce less byssal threads at high temperature and during reproductive period. Such occurrence is influenced by the high decaying rate and least energy provided for byssogenesis as mussels are allocating more energy for gamete production (Rajagopal *et al.*, 1998). In addition, at higher temperature (> 29°C) time taken by mussel larvae to settle on substrate becomes longer than at lower temperature (26°C) (Siddall, 1979; Monaj and Appukutan, 2003). Delay in settlement elevates the changes of mussel larvae and even adults to predation and mortality (Harger and Landenberger, 1971; Smale and Buchan, 1981).

Strong mechanical forces as the result of high water flow also suppressed the byssal threads production by weakening the mussel foot ability to produce strong byssal threads (Tamarin *et al.*, 1974; Carrington, 2002; Moeser *et al.*, 2006; Carrington *et al.*, 2008; Garner and Litvaitis, 2013a). This causes the mussels to experience difficulty in settling down on substrate. This was evident in the current study where the density of green mussels was higher (400 ind m^{-2}) in December 2013 compared to that in March 2014 (>200 ind m^{-2}) with different water current recorded at 9.7cm/s and 19.8 cm/s, respectively. According to Moeser *et al.* (2006), ambient water velocity for stronger byssal thread attachment of blue mussel was at 11 cm/s but reduced at above 15 cm/s. Combination of high water temperature and water velocities (r = 0.759) in Marudu Bay could have influenced the mussel attachment resulting lower settlement density.

The low dissolved oxygen in Marudu Bay (r = 0.588) could have been influenced by the high phosphorus content in the water column (McCormik and Laing, 2003). The high loading of phosphorus in the bay is originated from the intense anthropogenic activities which are recently taking place along the major rivers (Aris *et al.*, 2014) and the runoffs from palm oil plantations (Zakaria and Rajpar, 2015) surrounding the bay. In this study, reduced dissolved oxygen and high phosphorus in water column correlated with reduced mussel settlement density. Low mussel population at high phosphorus content and low dissolved oxygen has also been reported by Sarnelle *et al.* (2012) and Clarke and McMahon (1996). There, mussel attachment strength on substrate surface may weaken under low dissolved oxygen (Clarke and McMahon, 1996), whereas high phosphate level reduces the mussel movement, ventilation and siphons closure. Such conditions can cause physiological stress to mussels (Jenner *et al.*, 1992; Reynolds and Guillaume, 1998).

Researchers have shown that there is a strong relationship between food availability and green mussel density (Rajagopal *et al.*, 1998; Alfaro, 2006). However, the present study did not find any correlation between monthly chlorophyll-α measurement and the settlement density of green mussel in the bay. This shows that the chlorophyll-α measurement alone is not able to justify the food sufficiency for bivalve due to their selective feeding behavior (Ren *et al.*, 2000; Rouillon and Navarro, 2003).

5. Conclusions

The high asymptotic length of the green mussel in Marudu Bay, although is influenced by the low density, it also indicates that the bay is good for mussel farming. Recruitment of green mussel in Marudu Bay occurs throughout the year with two seasonal peaks (July and February), one in the Southeast Monsoon and another one in Northeast Monsoon. The low density of the green mussel in the bay may have been influenced by the ecological effects due to long term suspension of the culture substrates. Increase in water temperature and water velocity may disturb or delay the attachment of green mussel to settle on substrates, hence resulting in low settlement density. The low dissolved oxygen and high amount of phosphorus in the bay may also affect the settlement density. Studies on the feeding behaviors and food preference of green mussel are necessary to determine if the high mortality of the green mussel in the bay is related to food scarcity.

Acknowledgements

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