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Original Article

Food availability estimation of the blood cockle, *Anadara granosa* (Linnaeus, 1758), from the aquaculture grounds of the Selangor coast, Malaysia

Tatsuya Yurimoto*[1], Faizul Mohd Kassim[1], Reiko Fuseya[2], Kazumi Matsuoka[3], Alias Man[4]

[1]Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba-shi, Ibaraki, Japan.
[2]Fisheries Technology Institute, Japan Fisheries Research and Education Agency, Yokohama-shi, Kanagawa, Japan.
[3]Institute for East China Sea Research, Nagasaki University, Nagasaki-shi, Nagasaki, Japan.
[4]Fisheries Research Institute (FRI), Department of Fisheries (DOF), Kampung Acheh, Sitiawan, Perak, Malaysia.

Abstract: Blood cockles, *Anadara granosa* (Linnaeus, 1758), were collected from the aquaculture grounds (4 stations) of the Selangor coast, Malaysia, and the water quality (water temperature, salinity, dissolved oxygen, turbidity, and chlorophyll *a*) was measured from September 2011 to June 2013. At all stations, the water temperature fluctuated around 30℃. At station C, located at the mouth of the Selangor River, the salinity was occasionally lower than 20 PSU. However, the salinity of the other stations fluctuated around 30 PSU. In addition, at all stations, the content of dissolved oxygen generally fluctuated around 3 mg.L^{-1} or above, and the turbidity changed irregularly, sometimes exceeding 300 FTU. The chlorophyll *a* content fluctuated mainly ranging 4-20 µg.L^{-1} at all stations, and values above 20 µg.L^{-1} were occasionally observed. The phytopigment content, a food availability indicator, in the digestive gland tissue of the blood cockles collected from all stations fluctuated ranging 30-770 µg.g^{-1}. However, there was no proportional correlation between phytopigment content in the digestive gland and chlorophyll *a* content at all stations. Therefore, even in a high chlorophyll *a* content (over 20 µg.L^{-1}) environment, the accumulated phytopigment in the digestive gland was around 290 µg.g^{-1}. In general, these results indicated the cockles were eating a sufficient amount of foods (organic materials including phytoplankton) all year round during the study period. And, the food availability environment in the aquaculture grounds of the Selangor coast was estimated sufficient to grow the blood cockle.

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Introduction

The blood cockle, *Anadara granosa* (Linnaeus, 1758), inhabits a wide coastal area, ranging from tropical to temperate environments (Narasimham, 1988; Nakamura and Shinotsuka, 2007; Soegianto and Supriyanto, 2008). In Southeast Asian countries, especially Malaysia, Thailand, and Vietnam, the blood cockle is cultured in mudflats of the mangrove coastal areas (Broom, 1985; Tri and Lin, 1999; Chalermwat et al., 2003; Phuong and Minh, 2005; Watanabe, 2009; Yurimoto et al., 2013, 2014a, b, c, d), because it is an important protein source for the residents of these countries (Pathansali and Soong, 1958; Broom, 1983; Watanabe, 2009). The Selangor coast, Peninsular Malaysia, has a broad coastline of approximately 100 km, with many inflowing rivers accompanied by huge mudflats and mangrove areas around the river estuaries. In addition, along these shorelines, a total of 654 hectares of blood cockle farming plots area of approximately 50 ha per plot are arranged in a line (Ramli et al., 2013; Harith et al., 2016). Moreover, this region is famous for supplying blood cockle spats to other regions in Malaysia, because of the large number of cockle spats that occur on the mudflats every year (Yurimoto, 2013; Yurimoto et al., 2014c, d).

Production of blood cockle culture along the Selangor coast has been decreasing since 2011, after peaking at approximately 40,000 tons in 2010 (Ramli et al., 2013; Shimoda et al., 2016). The temporary peak in production was caused by an increase in blood cockle farmers due to an incentive policy outlined by the Malaysian government. However, it has been

*Correspondence: Tatsuya Yurimoto
E-mail: yurimoto@outlook.com
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suggested that the decrease in production was due to mass mortalities (Ramli et al., 2013; Yurimoto et al., 2014d) and a reduction in suitable culture grounds caused by coastal erosion (Jeyanny et al., 2009, 2012; Asmawi and Ibrahim, 2013). Therefore, environmental evaluations around the estuary are important for the protection of suitable aquaculture grounds. In this study, four monitoring stations, located at the main areas for blood cockle aquaculture, were established along the Selangor coast from September 2011 to June 2013. Then, we sampled blood cockles to evaluate the food availability conditions (phytopigment content in the digestive gland). Furthermore, the water quality (water temperature, salinity, dissolved oxygen, turbidity, and chlorophyll *a*) was monitored at all stations at the time of sampling.

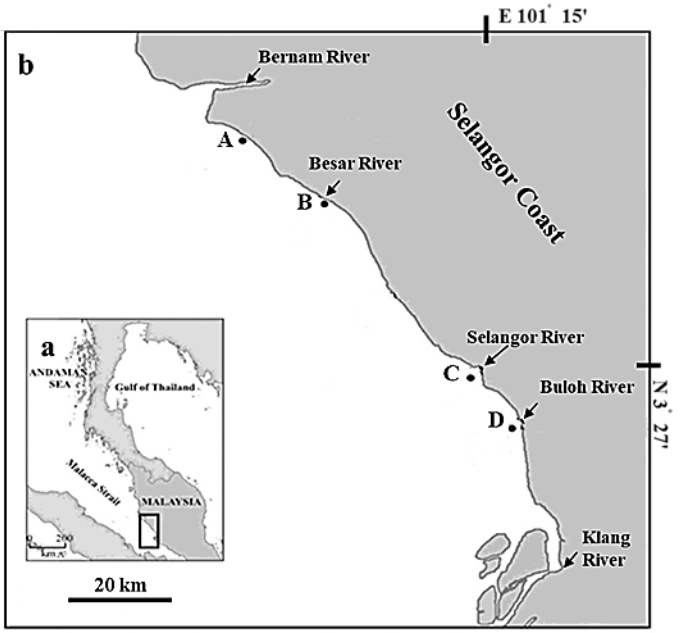

Figure 1. Location of the Selangor coast in Peninsular Malaysia (square in map a) and four sampling stations in the main aquaculture grounds of the area (map b). St. A: Bagan Nakhoda Omar, St. B: Sungai Besar, St. C: Kuala Selangor, and St. D: Sungai Buloh.

Materials and Methods

From September 2011 to June 2013, sampling stations (St. A: Bagan Nakhoda Omar, St. B: Sungai Besar, St. C: Kuala Selangor, and St. D: Sungai Buloh) were set in four sea areas along the Selangor coast (Fig. 1). These areas are known as main sites for blood cockle, *A. granosa*, aquaculture and the cockle landing. Environmental survey and the blood cockle sampling were carried out with a fishing boat at the four stations mainly from medium to low tide period on almost every month. The cockles were periodically collected with a hand dredge (basket size: 60 cm wide, 15 cm high, 30 cm deep) and ten individuals for this study were randomly taken from the collected cockles. However, when enough the cockles could not be collected, possible number of the cockles were used for this study sample. The shell lengths (mean± standard deviation) of the collected cockles during the survey period were 27±3 (n=195), 28±5 (n=176), 40±9 (n=128), and 30±4 (n=191) mm for stations A, B, C, and D, respectively. Additionally, the water quality (water temperature, salinity, dissolved oxygen, turbidity, and chlorophyll *a* content) of the bottom layer (from bottom surface to 50 cm above the bottom) of each station at the time of sampling was measured every 10 cm using a throw-in-type water quality sensor (AAQ-RINKO, JFE Advantech Co. Ltd., Japan), and the measured data were calculated as the average value±standard deviation. In addition, due to a failure in the turbidity sensor, the turbidity data were not collected after October 2012.

The collected blood cockles were transported alive to a temporary laboratory, located near the monitoring site, where the shell lengths were measured, and the shells were opened to collect the soft tissues for phytopigment analysis. The phytopigment analysis was performed using a modified method of Numaguchi et al. (1985, 2001). The soft tissue was stored in a freezer (lower than -20℃) until analysis, and a tissue block of the digestive gland was excised from the soft tissue at the time of analysis. The tissue block was immersed in 90% acetone solution under shading overnight for the extraction of the phytopigment. The solution was centrifuged (3,000 rpm, 10 min) to obtain the supernatant, and a small amount of 4N hydrochloric acid solution was added to completely convert the pigments to phytopigments. Two wavelengths, 665 and 750 nm, were measured with a spectrophotometer to calculate the value of phytopigments (absorption wavelength 665 nm)

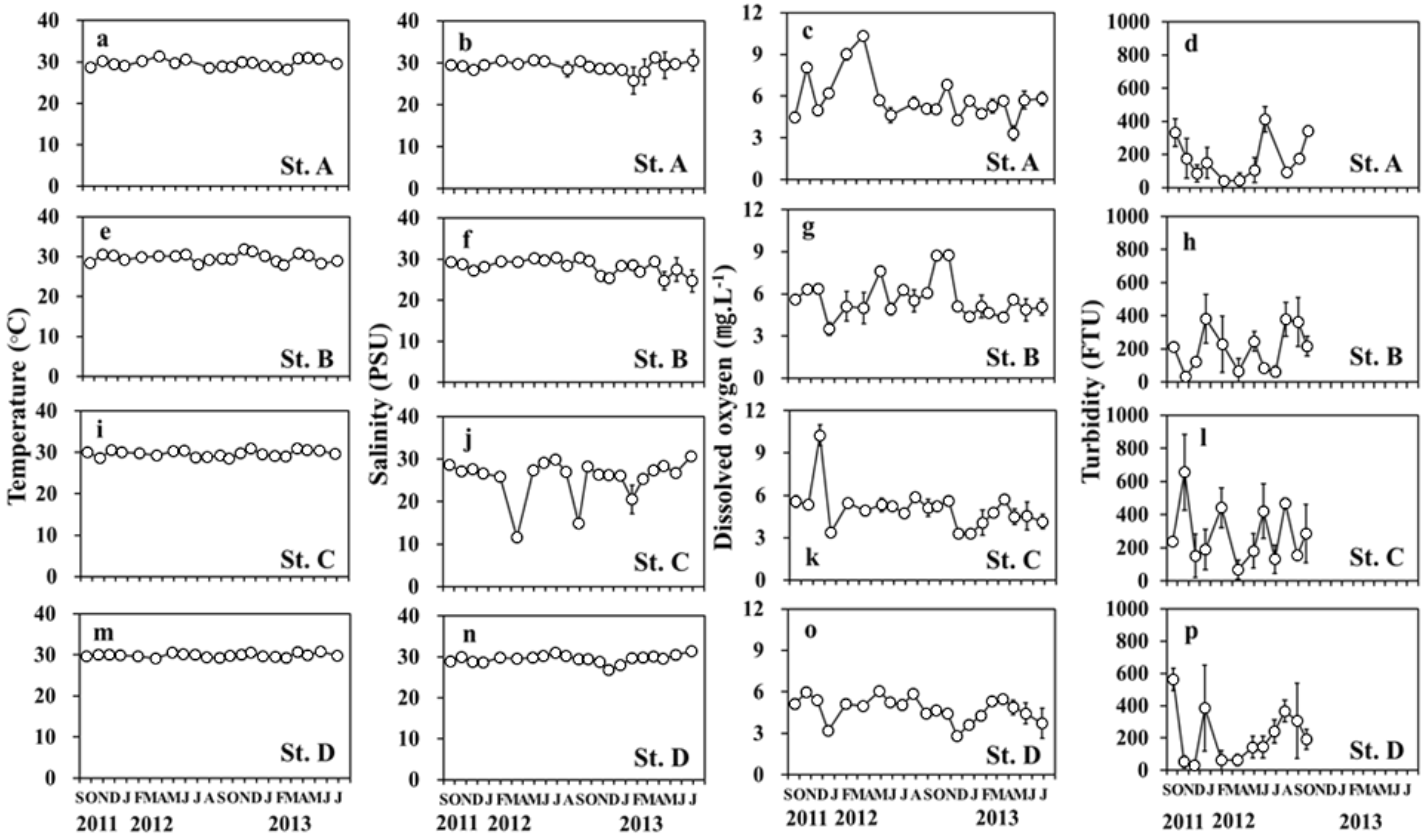

Figure 2. Water quality monitoring in the bottom layer (bottom-50 cm) at the four stations (St. A-D) along the Selangor coast. a, e, i, m: water temperature; b, f, j, n: salinity; c, g, k, o: dissolved oxygen and d, h, l, p: turbidity. a-d: St. A; e-h: St. B; i-l: St. C and m-p: St. D. Mean±SD.

minus the value of impurities (absorption wavelength 750 nm) to obtain an actual value of the phytopigments. Finally, the phytopigment content in 1 g of the midgut gland tissue (wet weight) was calculated from the measured data using a modified formula from Strickland and Parsons (1968).

Results

Environment: Annually, the water temperature fluctuated around 30℃ at all stations (Fig. 2a, e, i, m). At station C, a low salinity of below 25 Practical Salinity Units (PSU) was observed in March, August, and January 2013, while the salinity in the other months was higher than 25 PSU (Fig. 2j). The salinity at the other stations fluctuated around 30 PSU during the survey period (Fig. 2b, f, n). At station D, the dissolved oxygen content decreased to 2.8 $mg.L^{-1}$ in November 2012, whereas that of the stations A, B and C changed higher than 3 $mg.L^{-1}$ during the monitoring (Fig. 2c, g, k, o). The turbidity fluctuated irregularly at all stations, and frequently exceeded 300 Forumajin Turbidity Units (FTU) (Fig. 2d, h, l, p). Notably, the highest turbidity reached 655 FTU, which was at station C in October 2011.

The chlorophyll *a* content fluctuated mainly ranging from 4 to 20 $\mu g.L^{-1}$ at stations A and B during the survey period. In addition, the content at the both stations exceeded 20 $\mu g.L^{-1}$ in May and October 2012, which was also observed in January (St. B) and May (St. A) 2013 (Fig. 3a, c). Furthermore, at station C, the chlorophyll *a* content exceeding 20 $\mu g.L^{-1}$ was observed on October, November in 2012 and April, May, July, September in 2013 (Fig. 3e, g). In contrast, at station D, the chlorophyll *a* content fluctuated mainly ranging from 4 to 19 $\mu g.L^{-1}$. And the high content exceeding 20 $\mu g.L^{-1}$ observed only on August 2012.

Phytopigment: The phytopigment content in the midgut gland of the blood cockle, *A. granosa*, collected from stations A and B was less than 160 $\mu g.L^{-1}$ from September to November 2011, and has been fluctuated ranging from 170 to 370 $\mu g.L^{-1}$

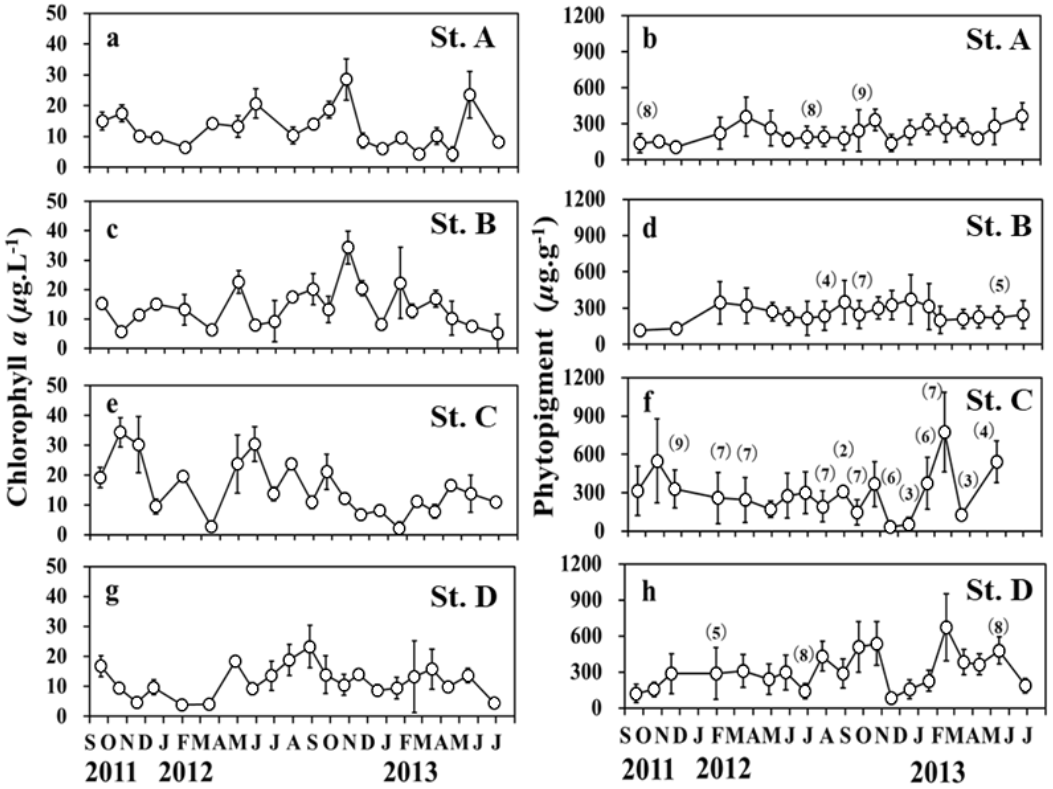

Figure 3. Changes in the chlorophyll *a* content of the bottom layer (bottom-50 cm) at the four stations (St. A-D) and phytopigment content in the blood cockle, *Anadara granosa*, collected from the same stations along the Selangor coast. a, c, e, g: chlorophyll *a* and b, d, f, h: phytopigment contents. a, b: St. A; c, d: St. B; e, f: St. C and g, h: St. D. The numbers in parentheses in the b, d, f, h graphs indicate the number of analysed samples from each plot, while plots with no parentheses indicate that 10 samples were analysed. Mean±SD.

(except 140 μg.L^{-1} at St. A in November 2012) since February 2012 (Fig. 3b, d). And the content at station B has decreased to 200 μg.L^{-1} on February 2013. The phytopigment content at station C remained ranging from 150 to 370 μg.L^{-1} from September 2011 to October 2012, and then decreased to lower than 100 μg.L^{-1} from November to December 2012 (Fig. 3f), after which it increased to over 500 μg.L^{-1} in February and May 2013. In contrast, the content at station D was less than 160 μg.L^{-1} in September-October 2011, and then increased ranging from 240 to 300 μg.L^{-1} from November 2011 to May 2012, before further increasing to over 500 μg.L^{-1} from September to October 2012 (Fig. 3h). After that, the value decreased again to lower than 100 μg.L^{-1} in November 2012 and increased again to over 600 μg.L^{-1} in February 2013, and fluctuated ranging from 360 and 480 μg.L^{-1} from March to May 2013. Additionally, to clarify the relationship between the chlorophyll *a* in the fishing ground and the phytopigment content in the digestive gland, we set the chlorophyll *a* on the X-axis and the phytopigment on the Y-axis for both values obtained at each sampling station on each month and the mean value±SD was plotted to examine the both correlation (Fig. 4). As the results, y=1.6887x+208.69, R^2=0.0215, n=19 at station A, y=2.1762x+225.27, R^2=0.0502, n=19 at station B, y=2.7869x+252.39, R^2=0.0212, n=18 at station C, and y=3.3719x+267.58, R^2=0.0132, n=20 at station D, and no clear correlation was observed at the all stations.

Discussions

Environment: At each station, the water temperature of the blood cockle aquaculture ground was almost stable at approximately 30℃ (Fig. 2a, e, i, m). A low salinity, below 25 PSU, was occasionally observed at station C, which is near the mouth of the Selangor River (Fig. 2j), while the salinity of the other stations fluctuated around 30 PSU (Fig. 2b, f, n). This result indicates that station C is the most susceptible to river water among the stations. In addition, the dissolved oxygen content was ordinally higher than 3 mg.L^{-1} at

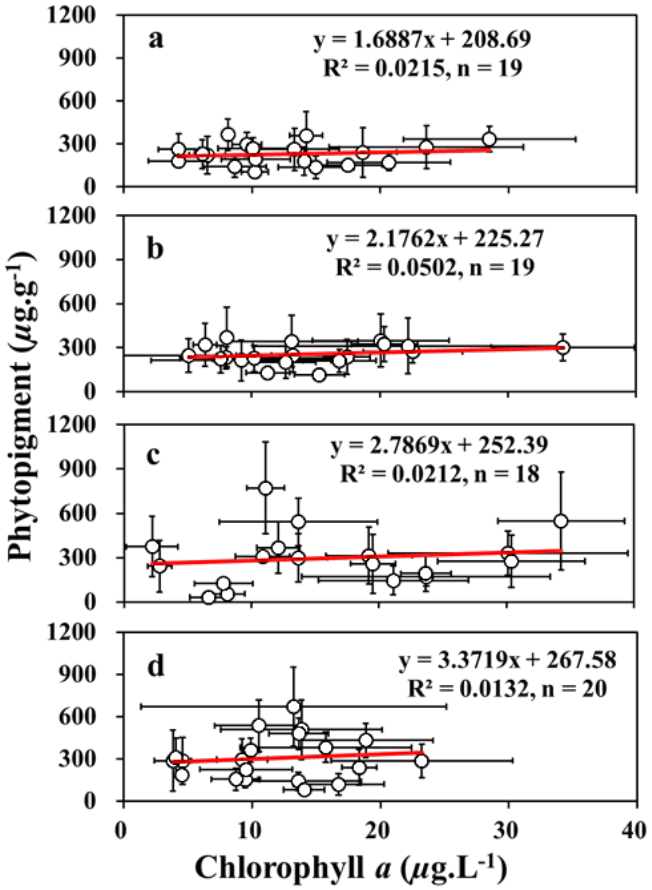

Figure 4. Relationships between the chlorophyll *a* content of the bottom layer (bottom-50 cm) and phytopigment content in the blood cockle, *Anadara granosa*, at the four stations (St. A-D) along the Selangor coast. a: St. A; b: St. B; c: St. C and d: St. D. A thick red straight line represents a linear approximation. Mean±SD.

each station (Fig. 2c, g, k, o). Therefore, it is considered that the oxygen level was not serious hypoxic environment to the blood cockle, because it is known that the hypoxic level for many benthic organisms, including bivalves, is around 2 mg.L^{-1} or less, and organism survival is affected if low oxygen levels continue for several days (Vaquer-Sunyer and Duarte, 2008). Additionally, in case of the blood cockle, it inhabits in surface layer of sea bottom (Sato, 2006) and it is known also an oxygen regulator adapted to hypoxic environment. Davenport and Wong (1986) investigated the blood cockles exposed in anoxia condition for 18 hours and they found the cockle could increase oxygen absorption ability to 2.8 times than the normal. On the other hand, in case of manila clam, *Ruditapes philippinarum*, the feeding ability does not affect even if it is exposed under anoxic condition for 24 hours (Nagasoe et al., 2011). Therefore, the food availability of the blood cockle was suggested almost little or no affected by the dissolved oxygen condition, because serious low oxygen level was not observed on the all stations during the study period.

On the other hand, high turbidity values were irregularly observed. The influence of high turbidity on the blood cockle is unknown. However, the blood cockle commonly inhabits muddy tidal flats around mangrove estuaries (Lai et al., 2020). And, in case of the mangrove estuaries, the high turbidity contains high amount of organic matter as a food source for bivalve not only clay silt and it is a low negative affect on the cockle habitat such as their pseudo-feces production relating their debilitation (Velasco and Navarro, 2002; Sroczyńska et al., 2012; de Alencar Leite et al., 2020). Tanaka et al. (1982) highlighted that the resuspended mud in the mudflats of inner bays contain a large amount of particle matter, such as phytoplankton and organic particles, which are food sources for benthic organisms. In addition, Yurimoto et al. (2008) conducted an indoor experiment to evaluate the effects of resuspended mud in a case of the pen shell, *Atrina pectinata*, reporting resuspended mud from an inner bay contains organic matter and has a food source value for the bivalve in comparison with inorganic clay. Normally, in mangrove areas large amounts of organic matter accumulate (Sanders et al., 2010), especially suspension matter in the coastal waters, which also contains significant organic matter (Williams and Benson, 2010; Saifullah et al., 2016). Additionally, Yurimoto et al. (2014b) reported that phytoplankton cells were mainly found in the digestive tube of the blood cockle in the Matang mangrove estuary of Malaysia, but various suspended particles were also observed such as mangrove litter. Therefore, it is considered the suspension matter in the four stations had a food value and low negative effects on food availability and habitat of the blood cockle.

The chlorophyll *a* content, which is the main food environment indicator for bivalves, generally fluctuated mainly ranging from 4 to 20 μg.L^{-1} at all stations, and the occasionally increased to over 20 μg.L^{-1} during the survey period. Then, it was estimated

that the food environment had enough content levels for bivalve feeding at all stations. In many kinds of cockle, *Anadara* (*Scapharca*) *kagoshimensis*, *A. inaequivalvis*, *Cerastoderma edule* and *Clinocardium nuttallii*, those are known to grow well or to develop the gonad in environment of the chlorophyll *a* content around 3 $\mu g.L^{-1}$ (Yurimoto et al., 2007; Acarli et al., 2012; Martínez-Castro and Vázquez, 2012; Dunham et al., 2013). Moreover, the coastal waters around tropical mangroves commonly have a high chlorophyll *a* content of over 20 $\mu g.L^{-1}$ in wet, dry or whole seasons depend on region (Senthilkumar et al., 2008; Rivera-Monroy et al., 2011; Teoh et al., 2016). Especially, Teoh et al. (2016) surveyed primary production including chlorophyll *a* content in water column of Selangor coast and reported southern coast from Kuala Selangor to Sungai Buloh was high chlorophyll *a* content over 20 $\mu g.L^{-1}$ in dry season (February-March 2015). Therefore, the chlorophyll *a* content observed along the Selangor coast in this study reconfirmed that the waters have a high basic productivity similarly with those around other tropical mangrove coasts.

Food availability: Phytopigment is a generic name that encompasses decomposition products of pigments, such as chlorophyll and pheophytin (Numaguchi, 1985). Therefore, the pigments extracted from the digestive gland of a bivalve is a useful indicator for the food availability conditions of the individual. Numaguchi (1985, 2001, 2002) focused on this index, evaluating the phytopigment (sum of chlorophyll *a* and pheophytin contents) content in the digestive gland of bivalves. The phytopigment content in the digestive gland of pearl oysters from the coastal waters of Japan is as follows: the phytopigment content in the digestive gland was approximately 150 $\mu g.g^{-1}$ when the chlorophyll *a* + pheophytin in the water column was increased to approximately 15 $\mu g.L^{-1}$ (Numaguchi, 1985). In addition, the phytopigment content in the digestive gland of *A.* (*S.*) *kagoshimensis*, fluctuated around several tens of $\mu g.g^{-1}$ during winter (Yurimoto et al., 2007). On the contrary, when the water temperature rose to more than 25°C and the chlorophyll *a* content was detected at 10 $\mu g.L^{-1}$ or more during summer, the phytopigment content increased to approximately 160 $\mu g.g^{-1}$ (Yurimoto et al., 2007). In contrast, the phytopigment content of the blood cockle along the Selangor coast was rarely lower than 100 $\mu g.g^{-1}$, but mostly fluctuated ranging from 150 to 350 $\mu g.g^{-1}$. This value is almost twice that measured for other bivalves. Therefore, it was considered that the food environment of the Selangor coast was sufficient to grow the blood cockle. In addition, unlike blood cockles living in the temperate zone, this result showed that seasonal changes to the phytopigment content in the cockle were unclear. In the temperate zone, the water temperature shows huge variabilities depending upon the season, and the spawning period is short compared with that of the tropical zone. Therefore, it is suggested that the food availability for the blood cockle in the temperate zone has clear seasonality compared with that in the tropical zone. Additionally, there was no correlation between the phytopigment content in the blood cockle and the chlorophyll *a* content in the bottom layer of the coastal water, as the coefficient of determination (R^2) was less than 0.06 at all stations. This suggests that the phytopigment content that can accumulate in the digestive gland of the blood cockle is around 290 $\mu g.g^{-1}$, even when the cockle is placed in an environment with an excessive chlorophyll *a* content. Therefore, the above values were considered to be one of the indicator criteria to show that the cockles were feeding sufficiently. On the contrary, at stations C and D, in some cases when the chlorophyll *a* content in the sea bottom layer was approximately 12 $\mu g.L^{-1}$, a high phytopigment content of above 500 $\mu g.g^{-1}$ was detected in the digestive gland of the blood cockle. Factors influencing the high accumulation of phytopigment by the blood cockle are currently unknown. However, these high levels were mainly detected in September, October, February, and May, which are periods when there is not necessarily a high chlorophyll *a* content in the sea bottom layer.

According to Yurimoto et al. (2014a, c), the sexual maturation of the blood cockle on the west coast of Peninsular Malaysia is less clear than that of a kind of blood cockle in the temperate zone. However, the

stages are roughly divided into development and mature stages in September-October (early rainy season), and spawning and spent stages in February-May (post rainy season). Therefore, this suggests that the cockles may engage in aggressive feeding to develop their gonads or to restore their own strength after spawning. The environmental conditions were suitable for such excessive feeding, as the water temperature was almost stable at stations C and D, while the chlorophyll *a* content in the bottom layer was mainly around 12 $\mu g.L^{-1}$. Thus, it was suggested that a chlorophyll *a* content around 12 $\mu g.L^{-1}$ enhanced the feeding activity of the blood cockle. Marescaux et al. (2016) found that rearing experiments with freshwater bivalves revealed a higher filtration rate at a chlorophyll *a* content of 10-20 $\mu g.L^{-1}$. Thus, it is likely that the blood cockle has the same favourable concentration.

Conclusion

The chlorophyll *a* content in the bottom layer of the main blood cockle aquaculture ground of the Selangor coast fluctuated mainly ranging from 4 to 20 $\mu g.L^{-1}$, and occasionally increased to over 20 $\mu g.L^{-1}$. At all stations, the phytopigment content in the digestive gland of the blood cockle fluctuated ranging from 30 to 770 $\mu g.g^{-1}$. However, there was no correlation between the contents of phytopigment in the blood cockle and chlorophyll *a* in environmental water. Therefore, even in a high chlorophyll *a* content (over 20 $\mu g.L^{-1}$) environment, the accumulated phytopigment in the digestive gland was around 290 $\mu g.g^{-1}$. In general, these results indicated the cockles were eating a sufficient amount of foods (organic materials including phytoplankton) all year round during the study period. And, the food availability environment in the aquaculture grounds of the Selangor coast was estimated sufficient to grow the blood cockle.

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