Influence of Seasonal Variation and Anthropogenic Stress on Blood Cockle (*Tegillarca granosa*) Production Potential

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ABSTRACT

This study attempts to investigate the effects of seasonal variation and anthropogenic stress on blood cockle production by using a model simulation. Seawater, sediment and cockles were collected from Klong Khon district, Samut Songkhram Province, Thailand. Results from field observations were employed to develop a blood cockle production model using multiple linear regressions. Then, anthropogenic stress scenarios were simulated using varying seawater and sediment qualities in the developed model. Results demonstrated that seawater quality and blood cockle density varied among seasons, unlike sediment. The cockle density and production potential were 0.87±0.78 indv·m^{-2} and -630±857 kg·km^{-2}·month^{-1}, respectively. Statistical analysis by using Pearson correlation coefficient demonstrated that decrease in cockle production was related to reductions in salinity and dissolved oxygen, coupled with increases in temperature and ammonium concentration with r_{xy} values of 0.56, 0.53, -0.73, and -0.57, respectively. The model developed in this study effectively estimates the cockle's condition index, which reflects cockle health and possibly readiness for reproduction, under environmental stress caused by seasons and anthropogenic activities such as discharge of domestic wastewater and contamination by strong acid in sediment (dumping chemical waste). The model reveals that blood cockles tend to die off when salinity, seawater temperature, dissolved oxygen and ammonium nitrogen reach 3 ‰, 32.4 ºC, 1 mg·L^{-1} and 4.14 µM, respectively. Results can raise awareness of the effects of water pollution in blood cockle cultivation areas and assist in preventing these effects.

Keywords: Anthropogenic stress, Environmental factors, Production potential, Seasonal variation, *Tegillarca granosa*

INTRODUCTION

Blood cockle (*Tegillarca granosa* or the former specific name *Anadara granosa*) is one of the most valuable seafood species in the Indo-Pacific region (Khalil, 2013; Jahangir *et al*., 2014; Harith *et al*., 2016). It is a marine bivalve which belongs to the family Arcidae. This species is indigenous to the intertidal mudflats bordering the coastal regions of many Southeast Asian countries, particularly Thailand, Malaysia and Indonesia. The cockle is valuable to this region and important to the communities where it is harvested. It is not only significant in terms of its commercial value as food for human consumption, but also remarkable as an edible species in the marine ecological food web. Cockles are considered as an important source of supplemental income for local communities, whether they are obtained through wild catch or culture. Moreover, it is also a major food source for wading birds, intertidal-feeding fish and crustaceans such as shore crabs and shrimps

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(Burdon *et al*., 2014). In addition, the cockle has the ability to act as a sentinel species and as a bioindicator for marine pollutants such as heavy metals (Ishak *et al*., 2016) and polycyclic aromatic hydrocarbons (PAHs) (Sany *et al*., 2014). Furthermore, cockle shells can be utilized as a source of calcium oxide to produce a low cost catalyst for biodiesel production (Boey *et al*., 2011). These studies provide evidence to confirm that the cockle is not only essential to the marine ecosystem, but it is also valuable commercially.

Currently, Thailand is facing a continuous decline in blood cockle production. According to fisheries statistics from Thailand during the years 2006-2016, the cockle production potential in Samut Songkhram Province, one of the largest blood cockle cultivation areas in Thailand, decreased from 0.78 to 0.15 $kg \cdot m^{-2} \cdot year^{-1}$, or by 80.80 % (Department of Fisheries, 2008; 2018). The causes of this crisis have been the subject of considerable debate; however, the main possibilities involve environmental disturbance by seasonal patterns and anthropogenic stress.

Seasons can cause variation in seawater salinity, temperature and food availability (Khalil, 2013; Bhadja *et al*., 2014; Jahangir *et al*., 2014), and subsequently limit the blood cockle's growth and ability to survive. Normally, the blood cockle burrows itself in muddy and sandy sediments near mangrove forest or intertidal mudflats. Even though the cockle can withstand wide ranges in water quality parameters, it has been reported that environmental conditions are exogenous factors related to the cockle's growth and survival (Broom, 1982; Din and Ahamad, 1995; Nakamura and Shinotsuka, 2007; Khalil, 2013; Harith *et al*., 2016). During the rainy season, extreme fluctuations of seawater salinity and temperature can cause changes in the blood cockle's physiology such as deleterious biochemical components, cellular damage, reduction in condition index and increase in mortality (Broom, 1982; Taylor *et al*., 2017). In addition, these parameters act together as the main trigger to induce a cockle to perform a reproductive cycle (Khalil, 2013; Harith *et al*., 2016).

Anthropogenic activities such as industry, residential developments, harbors, and intensive aquaculture can cause marine pollution, and subsequently influence physiological responses in bivalves including the blood cockle (Broom, 1982; Nakamura and Shinotsuka, 2007). Human activities, both in domestic and industrial sectors, can cause reduction in dissolved oxygen and high loading of nutrients and heavy metals (Mat *et al*., 1994; Din and Ahamad, 1995). Research by Din and Ahamad (1995) found that water pollution was causing blood cockles to die off. Individuals nearest to an industrial discharge point had the lowest growth rates and they all died after four weeks of exposure. In case of the study area, Klong Khon district, Samut Songkhram Province, in the past, there was tremendous mangrove deforestation in this area in order to build shrimp farms. Moreover, during rainy season, high loading of organic waste from municipalities, intensive shrimp farming and livestock activities in the upstream area brought wastewater pollution in seawater and mudflat areas. Consequently, it led to mangrove forest deterioration, decline of nursery areas for aquatic animals and a decrease in biodiversity.

According to the previously mentioned research and the background of the study area, there is consensus that blood cockles respond physiologically to seasonal fluctuations and anthropogenic disturbances in coastal oceanographic processes. However, none of research has investigated the correlation between blood cockle production and environmental factors, which include both seawater and sediment characteristics. In addition, predictions regarding anthropogenic stress effects on blood cockle productivity have not been thoroughly illustrated in any previous research. Therefore, the aims of this study are to; 1) investigate seasonal variation in seawater, sediment and blood cockle production potential; 2) evaluate the correlation between blood cockle density and environmental parameters; and 3) estimate anthropogenic stress on blood cockle production using a model simulation. Our hypothesis is that blood cockle production varies due to environmental fluctuation and this variation can be predicted by a model equation.

MATERIALS AND METHODS

Study area and sample collection

The research was conducted in Klong Khon, Samut Songkhram Province, Thailand (UTM 605276 E, 1473762 N). The study area is located approximately 15 km from Samut Songkhram city center. The total area is 31.96 km^2 and the land featured in the study area consists of 24.50 km^2 of shrimp farms (76.67 %), 5.19 km^2 of mangrove forest (16.23 %), 2.20 km^2 of agricultural area (6.89 %) and 0.07 km^2 of other uses (0.21 %). Most of the residents are fishermen. They participate in inshore fisheries and aquaculture of species such as shrimp, blood cockle and green mussel.

As shown in Figure 1, the sampling site is a mudflat in front of mangrove forest located in Klong Khon district in the Inner Gulf of Thailand. Next to the mangrove forest, there are shrimp farms, resorts, homestays and residences. The average depth of seawater in the study area is 2.5±0.7 m. There were 12 sampling stations (KK 1-12) covering blood cockle distribution in the study area (Figure 1). Blood cockles are found and collected in their natural habitat without having to seed the juvenile cockles.

In order to investigate seasonal variation in seawater, sediment and blood cockle production, data were collected in December 2016, April 2017 and September 2017, which represent winter, summer and rainy season, respectively. In each sampling period, blood cockles were sampled during the low tide in the spring tide period in order to evaluate anthropogenic stress, which refers to the loading amounts of pollution from land, seawater and sediment.

At each station, in-situ seawater quality was investigated. Salinity and pH of water were measured using refractometer and Cybercan PC 300 (EUTECH Instruments, Singapore), respectively. Temperature and dissolved oxygen (DO) were analyzed in-situ by Cybercan DO 110 (EUTECH Instruments, Singapore). Then, seawater was sampled for analysis of chlorophyll *a*, total suspended solids (TSS), and nutrient concentration in the laboratory. Sediment at 0-1 cm depth from the sediment surface was collected using a gravity corer. Then, the sediment sample was stored in a plastic bag to determine salinity, pH, grain size, water content, organic matter and total nitrogen in the laboratory. All fresh blood cockles were collected within a 5x5 m grid. The cockles were removed from

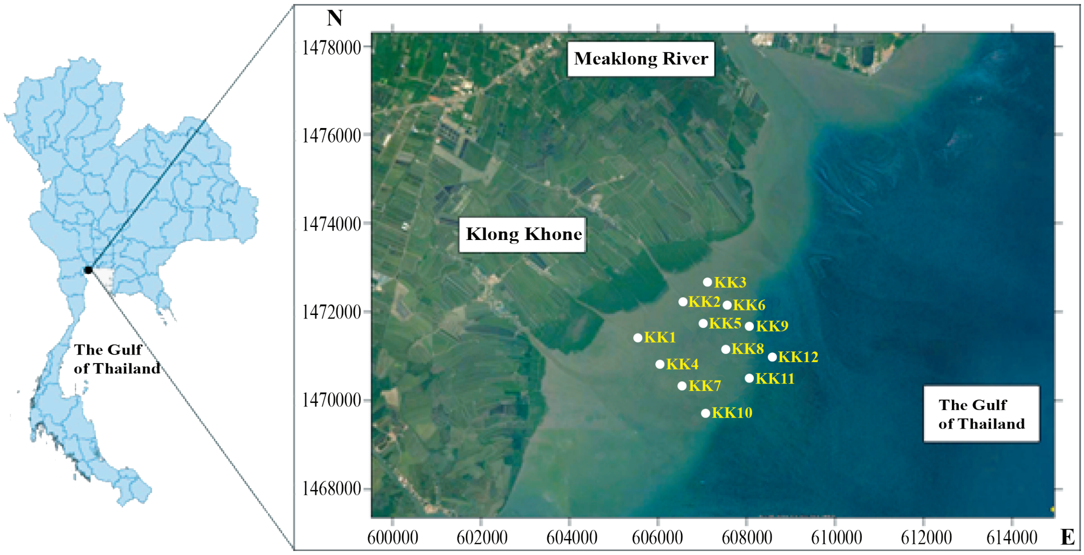

Figure 1. Map of sampling stations at Klong Khon, Samut Songkhram Province, Thailand.

sediments using a rake and by hand picking. Their valves were cleaned in-situ and the samples were stored in a plastic bag. All seawater, sediments and blood cockle samples were transported to the laboratory at Bansomdejchaopraya Rajabhat University, Bangkok, and stored in a refrigerator (-4 ºC) for further analysis as described in the following sections.

Seawater analysis

Each seawater sample was determined for chlorophyll *a* concentration as described in Parsons *et al*. (1984). Total suspended solids (TSS) was determined by filtering the seawater through filter paper (GF/C) and drying it in a hot air oven at 105 ºC for three days. Next, the sample was weighed using a 4-digit analytical balance. Concentrations of ammonium nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), total Kjeldahl nitrogen (TKN) and phosphate phosphorus (PO_4^{3-}-P) were determined following standard methods (APHA, 2012).

Sediment analysis

Sediment characteristics such as salinity and pH were determined as described in Beck (1999). Grain size composition was analyzed using sieves with pore size of 1, 0.125 and 0.06 mm to filter sand, silt and clay, respectively. Moisture content, or the percentage of water in the sediment, was determined by subtracting dry weight (after drying in hot air oven at 105 ºC for three days) from wet weight. All dried sediment samples were milled into fine particles using mortar and pestle. Finally, the samples were analyzed for organic matter (OM) using the wet oxidation method (Allison, 1965) and total nitrogen (TN) using the Kjeldahl method (Bremner and Mulvaney, 1982).

Blood cockle analysis

All blood cockle samples were analyzed for density, condition index, growth rate and production potential. The cockle density was determined by counting the amount of cockles found in each grid, and the results were calculated as individuals per area ($indv \cdot m^{-2}$).

In order to verify blood cockle condition index, each cockle's body was measured for wet weight, shell length, height and width. After the measurements, the cockle was oven dried at 65 ºC for two days, and dry weights of tissue and shell were recorded. Then, the cockle's condition index (CI) was calculated by the following equation (Marsden *et al*., 2014):

$$CI = \frac{\text{Dry weight of tissue (g)} \times 100}{\text{Dry weight of shell (g)}}$$

Blood cockle growth rates were calculated from the differences in the average shell length, height, width and whole body wet weight between months during the study period. Finally, the production potential of cockles was calculated by the following equation:

$$CP = \frac{(D_t - D_0) \times 1000}{t}$$

where CP is the cockle production potential ($kg \cdot km^{-2} \cdot month^{-1}$), D_t is the cockle density at time t ($g\ WW \cdot m^{-2}$), D_0 is initial cockle density ($g\ WW \cdot m^{-2}$), and t is time.

Statistical analysis

In order to determine the significance of spatial and seasonal variation in seawater quality, sediment quality and blood cockle density, two-way ANOVA was employed. The relationship between environmental factors and cockle density was illustrated using the Pearson correlation coefficient (r_{xy}). Finally, the results from the correlation analysis were employed to develop the blood cockle potential model using multiple linear regressions. The model was validated by making a comparison between simulation data and field observation data. Then, the data were used to calculate a coefficient of determination (R^2) to evaluate the model accuracy. These data and statistical analyses were completed using Microsoft Excel (2010).

Model simulation of anthropogenic stress

The influence of anthropogenic stress on blood cockle production potential was simulated by using the blood cockle potential model as described in the previous section. The anthropogenic scenarios included variation in seawater salinity, temperature, dissolved oxygen and ammonia concentrations. These model parameters correspond to those used in previous studies of wastewater from municipal discharges, intensive aquaculture farming and livestock activities, which may contaminate estuarine and coastal areas through river runoff (Din and Ahamad, 1995; Jackson *et al*., 2003; Liu *et al*., 2010).

RESULTS

Environmental factors

Seawater and sediment parameters that were investigated during the study period are shown in Figure 2-4. The statistical analysis revealed that there were no differences in seawater parameters among sampling stations. In addition, the results demonstrate that there were significant differences among months ($p<0.01$), except for total Kjeldahl nitrogen and chlorophyll *a*. As shown in Figure 2 and 3, most of the seawater parameters were found to be highest in September 2017 (rainy season), while salinity and dissolved oxygen were found to be lower than in the other months. This may be due to high loading of freshwater from heavy rainfall during the season.

The statistical analysis also revealed that apart from water content, organic matter and total nitrogen, there were no differences in sediment characteristics among sampling stations (Figure 4).

Blood cockle density

The densities of blood cockle found in this study are shown in Figure 5. The cockle was found to be higher density at near shore where was influenced by river runoff and absent at 3-4 km away from the shore. During the study period, cockle density was in the range of 0.04-2.28 indv·m^{-2}. The statistical analysis revealed that there was no significant difference in the cockle density among sampling stations ($p>0.05$) but on the other hand, there was significant difference among months ($p=0.02$). The cockle density in the study area averaged 1.63±0.33, 0.91±0.90 and 0.31±0.30 indv·m^{-2} in December 2016, April 2017 and September 2017, respectively.

The condition index of blood cockles was found to be in the range of 4.02-7.87. Similar to the cockle density, no significant difference in the condition index was found among the sampling stations, but there was significant difference among months ($p<0.05$). The average condition indices in December 2016, April 2017 and September 2017 were 7.23±0.67, 5.06±1.18, and 4.63±0.53, respectively.

As shown in Figure 6, the largest cockle was found in September 2017, which was the rainy season, and the size of cockles differed by month. Average cockle shell lengths were found to be 28.23±3.03, 25.10±3.39 and 30.08±3.22 mm in December 2016, April 2017 and September 2017, respectively. The averages of shell length, height and body weight in April 2017 were less than in December 2016, because the proportion of large cockles (shell length>30 mm) was smaller than in other months (Figure 7). During December 2016 to April 2017, some large cockles were lost, possibly due to hand picking or die-off. Meanwhile, the small cockles (shell length<20 mm) had been growing. Therefore, the cockle growth rates were calculated from the difference in cockle size between April and September 2017. Results demonstrated that blood cockles in Klong Khon increased in shell length, height and width by 1.24, 0.91, and 0.89 mm·month^{-1}, respectively. In addition, it was found that the cockles gained weight by 0.75 g WW·month^{-1}.

In this study the cockle production potentials were calculated from difference of the cockle density between months. Even though the data for cockle growth rates from the study area showed the possible effect of harvest, our results showed that blood cockle production in Klong Khon is decreasing at a very high rate and may

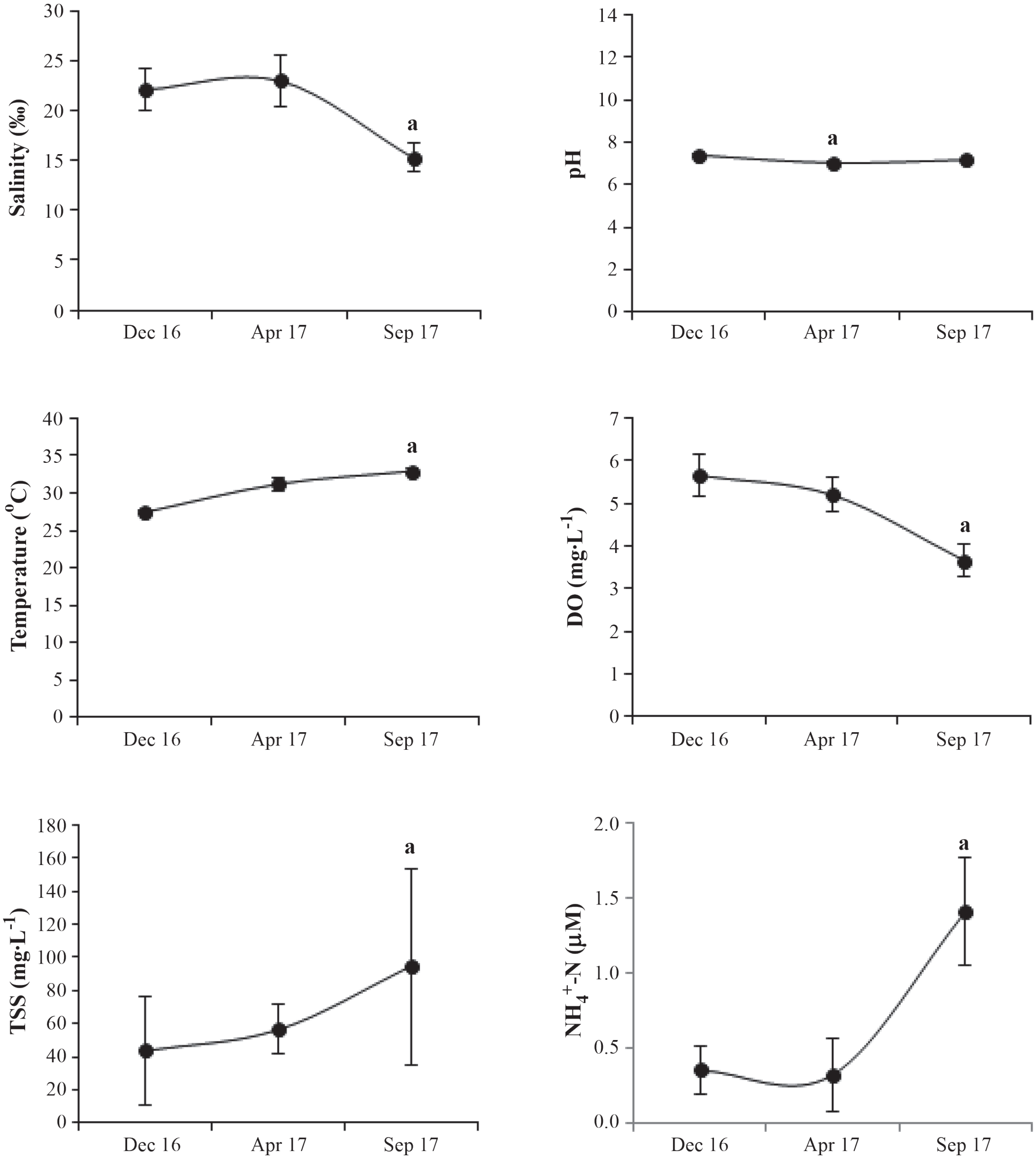

Figure 2. Seasonal variation in seawater parameters including salinity, pH, temperature, dissolved oxygen (DO), total suspended solids (TSS) and ammonium nitrogen (NH_4^+-N) at Klong Khon, Samut Songkhram Province. December 2016, April 2017 and September 2017 represent winter, summer and rainy season, respectively. Letter a represent statistical difference among months ($p<0.01$). Error bars represent SD.

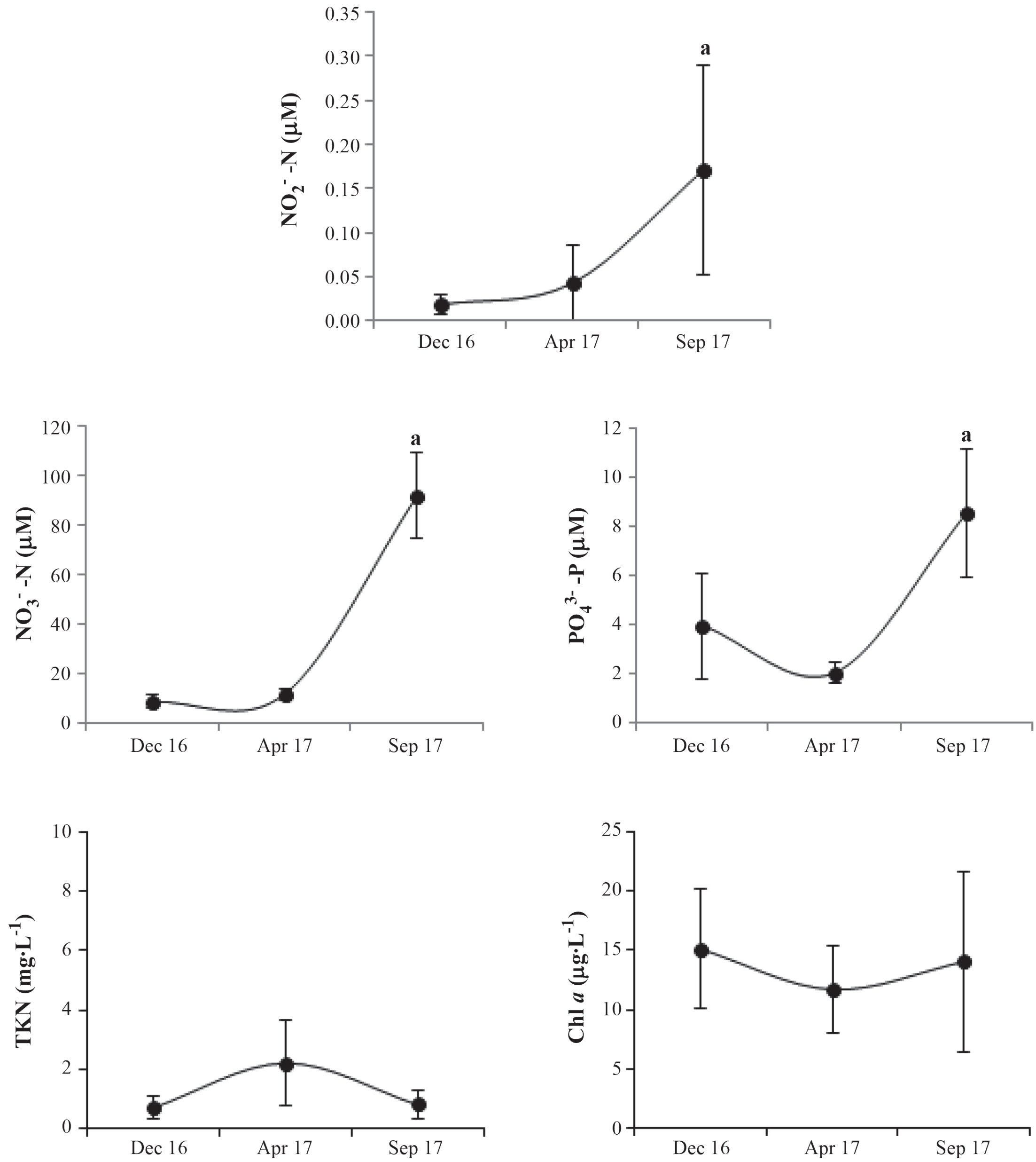

Figure 3. Seasonal variation in seawater parameters including nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), total Kjeldahl nitrogen (TKN), phosphate phosphorus (PO_4^{3-}-P) and chlorophyll *a* (Chl *a*) at Klong Khon, Samut Songkhram Province. December 2016, April 2017 and September 2017 represent winter, summer and rainy season, respectively. Letter a represent statistical difference among months ($p<0.01$). Error bars represent SD.

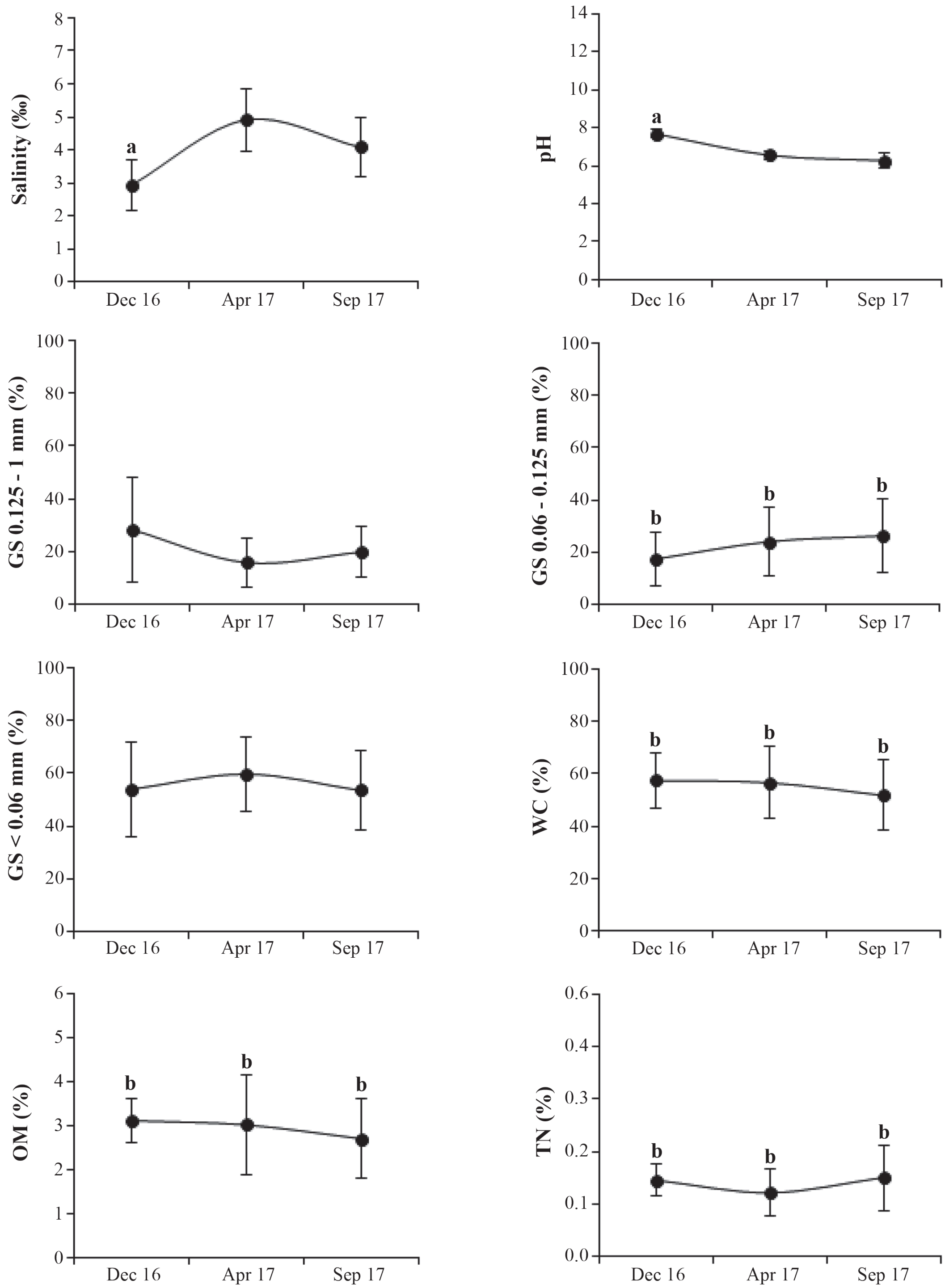

Figure 4. Seasonal variation in sediment characteristics including salinity, pH, grain size (GS) composition (0.125-1 mm, 0.06-0.125 mm and <0.06 mm), water content (WC), organic matter (OM) and total nitrogen (TN) in Klong Khon, Samut Songkhram Province. December 2016, April 2017 and September 2017 represent winter, summer and rainy season, respectively. Different letters at time points represent statistical difference ($p<0.01$); a differences among months and b differences among stations. Error bars represent SD.

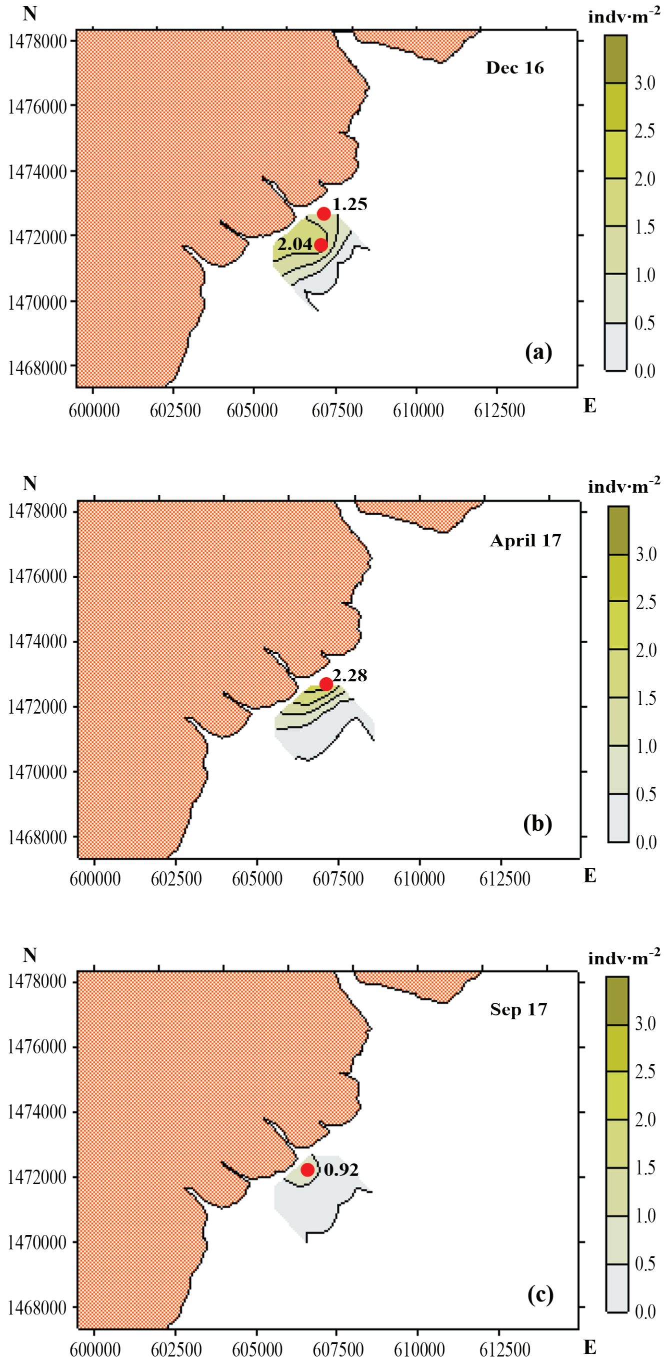

Figure 5. Contour map showing distribution of blood cockle density in Klong Khon, Samut Songkhram, Thailand in December 2016 (a), April 2017 (b), and September 2017 (c).

lead to a collapse of the local population in the coming years. This is because during the study period, blood cockles increased their shell size and gained weight; however, most of the cockles either died or were captured by local fishermen. As shown in Figure 8, only stations KK 4 and 6, where there was very low cockle hand picking, had positive production potential. The average of the cockle potential in the study area was -630±857 $kg{\cdot}km^{-2}{\cdot}month^{-1}$. This shows that in Klong Khon the production potential of blood cockle was influenced by environmental status and fishing effort.

Correlation between environmental factors and blood cockle density

The correlation coefficient analysis revealed that blood cockle density was related to the seawater parameters salinity, temperature, DO, NH_4^+-N, NO_2^--N, and NO_3^--N, with rxy values of 0.56 -0.73, 0.53, -0.57, -0.41 and -0.62, respectively. Of the sediment characteristics, cockle density was related to salinity and pH, with rxy of -0.54 and 0.62, respectively. T hese results demonstrate that the cockle density tends to increase when salinity and DO of the water are higher, and when pH of the sediment is higher. On the other hand, the cockle density tends to decrease when temperature, NH_4^+-N, NO_2^--N and NO_3^--N of the water increase, and when sediment salinity increases.

Blood cockle production potential model

According to the results presented above, environmental factors that were found to be correlated with blood cockle density were chosen as the model parameters. The cockle production potential model was developed by using multiple linear regressions. The models can be expressed as the flowing equations:

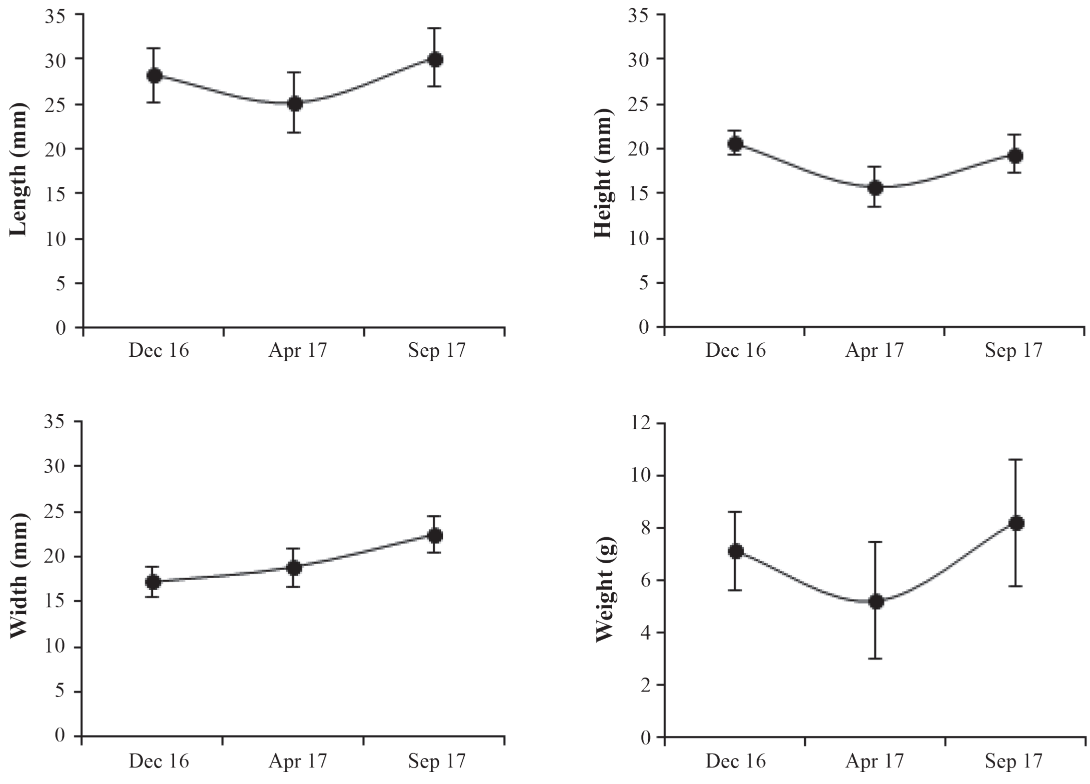

Figure 6. Mean of shell length, height, width and weight of blood cockles in Klong Khon, Samut Songkhram Province during the study period. Error bars represent SD.

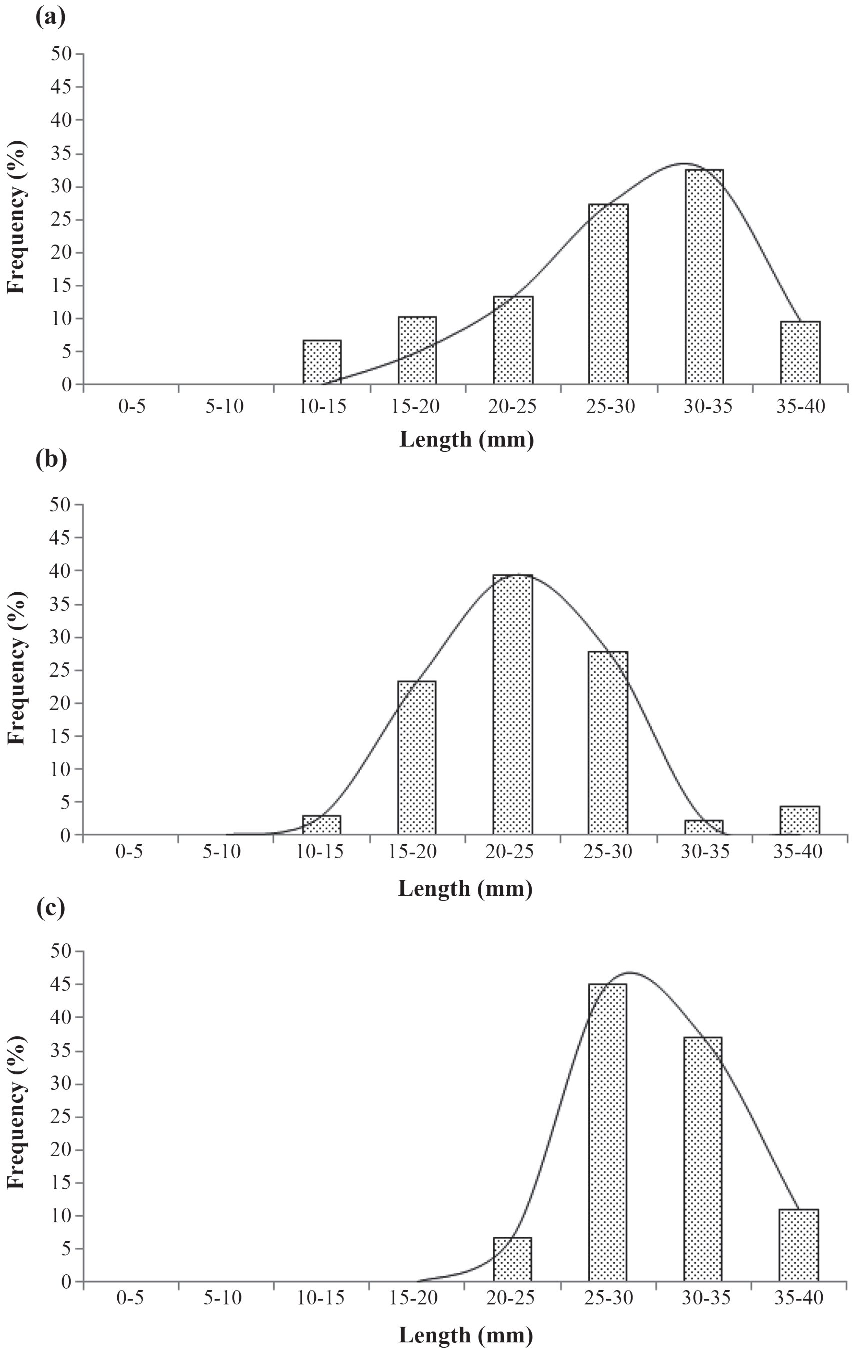

Figure 7. Length frequency of blood cockle in the study area in December 2016 (a), April 2017 (b), and September 2017 (c).

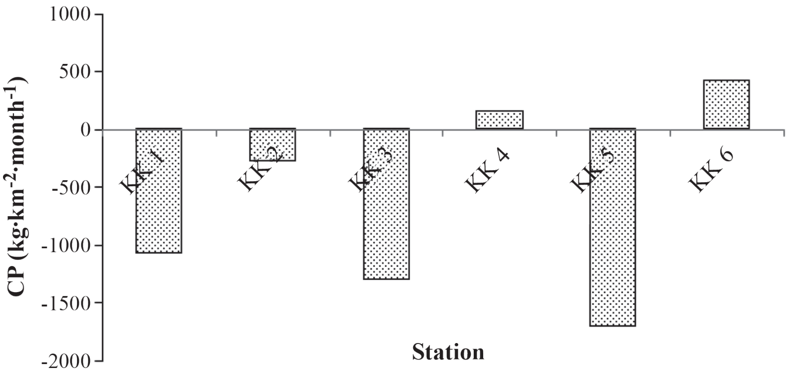

Figure 8. Blood cockle production potential in Klong Khon, Samut Songkhram Province.

$$CD_i = -0.10(S_w)+7.78(P_w)-0.21(T)-0.10(D)+0.91(N_1)-7.34(N_2)-0.07(N_3)-0.72(S_s)-2.95(P_s)-16.74$$

$$CD_g = -0.35(S_w)+36.29(P_w)-0.65(T)-5.29(D)+3.53(N_1)-29.33(N_2)-0.29(N_3)-3.17(S_s)-8.55(P_s)-123.64$$

$$CI = 0.31(S_w)-8.78(P_w)-1.42(T)+1.41(D)-2.12(N_1)+3.79(N_2)+0.13(N_3)+1.27(S_s)+1.09(P_s)+83.38$$

where CD_i is blood cockle density (indv $\cdot m^{-2}$), CD_g is blood cockle density (g $WW \cdot m^{-2}$), C_I is condition index, S_w is salinity of seawater (‰), P_w is pH in seawater, T is temperature (ºC), D is dissolved oxygen ($mg \cdot L^{-1}$), N_1 is concentration of ammonium nitrogen (μM), N_2 is concentration of nitrite nitrogen (μM), N_3 is concentration of nitrate nitrogen (μM), S_s is salinity of sediment (‰), P_s is pH in sediment.

Finally, the results from the model simulations were validated by comparison between simulated data and the data from the field observations. The values of CD_i, CD_g, and CI from the model simulation were close to the data from field observations, with high coefficients of determination (R^2) 0.8949, 0.9041 and 0.8556, respectively. These results demonstrate that all models developed in this study can perform effectively.

Simulation of anthropogenic stress

In order to evaluate the influence of anthropogenic stress, the model of condition index (CI) was employed. The cockles' density, in terms of both indv$\cdot m^{-2}$ and g $WW \cdot m^{-2}$, is involved in their reproductive state, which was not assessed in this study. However, the condition index can effectively reveal the integrity of blood cockles which are healthy and ready to reproduce (Khalil *et al*., 2017). Thus, high CI can represent favorable blood cockle production potential, and zero CI can indicate the death of cockles, which possess no remaining tissue in the shell.

Blood cockle's condition index (CI) was calculated from the model simulation by varying seawater salinity, temperature, dissolved oxygen, ammonia concentration and pH of sediment. Meanwhile, other parameters were fixed at the average value in each month. Seawater salinity varied from 0-40 ‰ in order to simulate an extreme freshwater discharge from construction of reservoirs and hydroelectric power stations. As shown in Figure 9, CI tends to increase in higher salinity. Mortality of the cockle can be found when salinity in seawater reaches 3 ‰, reflected by CI's decrease to zero. Unlike with salinity, CI tends to increase in cooler seawater (Figure 9). Seawater temperature in the model varied from 25-40 ºC, based on temperatures normally found in wastewater pollution in estuarine and coastal areas in tropical countries (Din and Ahamad, 1995; Liu *et al*., 2010). Another simulation included variation of dissolved oxygen

(DO) from 0 (the direct discharge point) to 8 mg·L^{-1} (DO saturation at seawater temperature 25 ºC). Concordant with theory, reduction of DO results in lower CI. Based on the seawater standards of Thailand implemented by the pollution control department (PCD) for class 3 aquaculture areas, concentration of ammonium nitrogen (NH_4^+-N) in the model varied from 0 (non-polluted seawater) to 50 µM. Moreover, in the simulation pH of seawater was constant at 7.2 based on the average of pH value found in this study. As shown in Figure 9, an increase in NH_4^+-N concentration lowers the level of CI, and the cockles may die off at NH_4^+-N 4.14 µM. Finally, pH of sediment varied from 0-14 in the model to simulate direct contamination by strong acid or base. The simulation shows that CI reaches zero when pH of sediment reaches 1.30, which indicates strong acidity such as dumping chemical waste, and CI tends to increase at higher pH (Figure 9). These simulation results demonstrate the critical environmental conditions that cause extreme mortality of blood cockles.

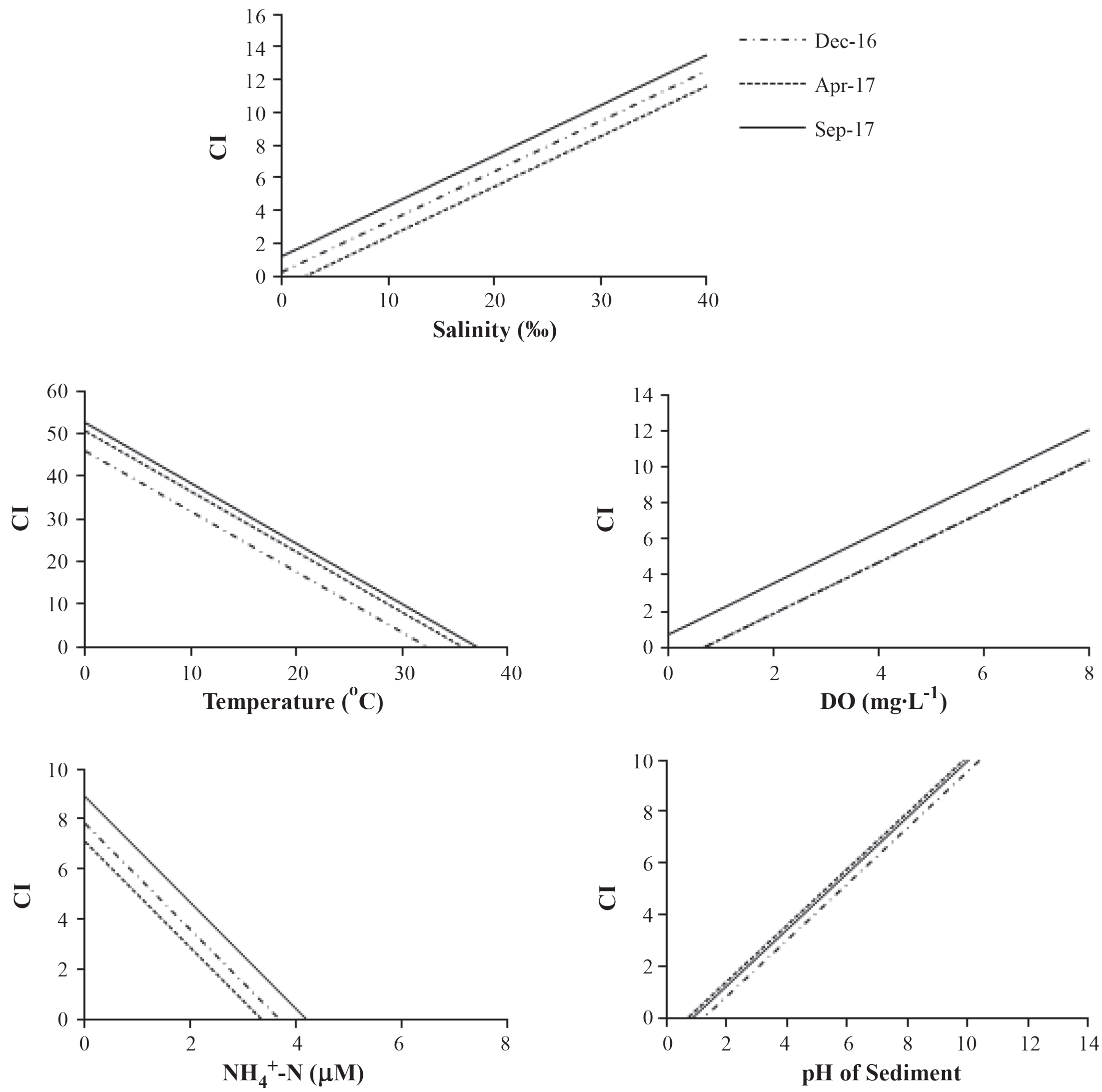

Figure 9. Simulation results of condition index (CI) from the anthropogenic stress model simulation.

DISCUSSION

Seasonal variation in environmental factors

Most of the seawater parameters were found to be significantly different among months, except for total Kjeldahl nitrogen (TKN) and chlorophyll *a*. These results illustrate the variation of seawater quality in different seasons. The cold weather in December (winter) caused low seawater temperature, while the hot weather in April (summer) caused high water evaporation and led to an increase in seawater salinity. In September (rainy season), water quality parameters frequently fluctuated due to the long monsoon season. Heavy rainfall and high loading of river runoff caused tremendous quantities of fresh water to enter estuarine and coastline areas. This phenomenon results in lower seawater salinity, decline of DO, increase in water turbidity, and excessive nutrients (Parekh and Gadhvi, 2015, Sangeeta and Neha, 2015). Moreover, our research found that seawater temperature reached the highest value in September. This may be due to higher amounts of suspended particles in seawater, which can absorb and store more heat, and thus heat the surrounding water (Chagas and Suzuki, 2005). Even though statistical analysis demonstrated that chlorophyll *a* did not differ among months, the highest concentration was found in September. This may be due to a greater supply of nutrients, which is favorable for phytoplankton growth.

Unlike seawater, there were no differences found in sediment characteristics among months except salinity and pH. Salinity reached the lowest value in December, and highest in April. This may be due to the difference in cohesive sediment and cation exchange capacity. In December, increases in salinity from the previous season (rainy season) may enhance the cohesion between sediment particles (Kim *et al*., 2016); subsequently, salt ions in seawater may not diffuse into the sediment pore water. Increased seawater temperature in April may cause increased cation exchange capacity (CEC) in sediment, which is a measure of the sediment's ability to hold positively charged ions (Hazelton and Murphy, 2007). Then, cations in seawater such as Na^+, Ca^{2+} and Mg^{2+} can accumulate in sediment. In the case of pH, the lowest value was found in September. This may be due to excessive fresh water from river runoff. Moreover, domestic waste polluting the runoff may be accumulated in the sediment and enhance microbial activity. Organic matter degradation by microbial metabolism produces acetic acid in the sediment, and it causes pH reduction (Salaenoi *et al*., 2015).

Seasonal variation in blood cockle density

Blood cockle densities were found to differ significantly by season during the study period. The highest density was found in December 2016, followed by April and September 2017. This demonstrates that in winter, there was a higher assemblage of blood cockles than in summer and the rainy season, respectively. Our results are in agreement with the research on community structure and distribution patterns of intertidal invertebrates along the coast of Kathaiwar Peninsula, India by Bhadja *et al*. (2014). They reported that during winter, when seawater temperature was lower than in other seasons, the diversity, richness and evenness of invertebrate species were found to be at higher levels. The decrease in climate temperature corresponding to decreasing seawater temperature is a favorable condition for blood cockles to grow (Khalil, 2013; Harith *et al*., 2016). A strong negative correlation between cockle density and seawater temperature may be explained by a reduction of metabolic rate which lengthens their life span and results in an increase in the assemblage of bivalves (Abele *et al*., 2009).

Another reason which may explain the high blood cockle density during winter is that there was higher salinity and dissolved oxygen (DO) than in other seasons. Blood cockles are marine bivalves, so they prefer higher salinity. They were found to be generally growing in salinity of 24-33 ‰ (Din and Ahamad, 1995; Jahangir *et al*., 2014). When exposed to low salinity, they respond behaviorally by tightening and effectively closing their shell valves in order to adjust osmoregulation in their body (Davenport and Wong, 1986). In addition, blood cockles prefer DO higher than 3.8 $mg \cdot L^{-1}$ (Nabhitabhata *et al*., 1990). Lower oxygen levels will reduce abundance and viability of haemocytes and decrease the phagocytosis process, which is involved in immune responses (Ellis *et al*., 2011).

Moreover, in this study area, during winter season there was low tide at night. This means that blood cockles were completely submerged during daytime when there was a higher phytoplankton assemblage. Hence, cockles could continuously filter phytoplankton from the water on the mudflats. This provides more feeding time for the cockles, which subsequently results in a higher growth rate (Broom, 1982; Jahangir *et al*., 2014). Research on microgrowth line patterns during spring tide and neap tide also provides evidence that wider line spacing (indicating faster growth) can occur due to longer duration of immersion (Mirzaei *et al*., 2014). The microgrowth lines are typically formed in accordance with tidal emersion, providing a calendar baseline for high-resolution environmental reconstructions. The banding pattern in shells from the subtidal environments showed narrow increments during spring tides alternating with a few wider increments during neap tides, which provide longer submersion time. The growth increments can be used to record growth rate and estimate the age of shells. Therefore, they demonstrated that the cockle growth rate increases corresponding to higher submersion time and thus more time to feed on phytoplankton.

During summer (April), environmental factors in the study area remained similar to the winter season, except for seawater temperature. In this period, seawater temperature rose in concordance with increasing air temperature. Higher temperature can increase the blood cockle's feeding activity and gonad development; subsequently, it enhances the growth rate. A study on suspension feeding and growth of blood cockles (*Anadara granosa*) by Nakamura and Shinotsuka (2007) reported that when the temperature increased from 20 to 29 ºC, the cockles showed higher feeding activity. In addition, high fluctuation of daily temperature can induce the development of gonads in the cockle (Khalil, 2013). This finding was in agreement with research on the cockle *Anadara antiquate* by Jahangir *et al*. (2014), who reported that gonad maturation and spawning seemed to be associated with rising temperature. Moreover, Nieves-Soto *et al*. (2011) also found that higher temperature can enhance food absorption efficiency of the blood cockle, *Anadara tuberculosa*. These previous studies demonstrate that during the summer season, environmental conditions support blood cockle growth, and that the cockles also develop gonads and increase shell size. In our results, during this period the average length of cockle shells was 25.10±3.39 mm, which could be extrapolated to an asymptotic length 44.4 mm (Broom, 1982; Harith *et al*., 2016).

During the rainy season (September), seawater salinity was significantly influenced by the southwest monsoon. Heavy rainfall results in a tremendous addition of freshwater; subsequently, it causes an extreme salinity reduction. Moreover, a high amount of total suspended solids (TSS) causes water to heat up more rapidly and hold more heat (Chagas and Suzuki, 2005). The combined effects of salinity and temperature become the stimulus in the spawning activities of the cockle population (Khalil, 2013). Based on previous studies, seawater quality disturbance caused by seasonal variation can influence the blood cockle's reproductive cycle. According to the analysis of blood cockle reproductive pattern in Malaysia by Broom (1982), there was evidence that seasonality in mudflats, which induces spawning in certain species, was triggered by the major annual salinity depression at the time of the onset of the northeast monsoon in October - November. In our study mature-sized cockles were found in September (rainy season). Hence, by fluctuation of salinity and temperature, the cockles tend to release reproductive cells. Unfortunately, they may not live through the season. Our results revealed that during the monsoon season, nutrients, especially ammonium, nitrite and nitrate, are extremely high as a consequence of the river runoff. High amounts of these nutrients results in lower pH and dissolved oxygen (DO) through biodegradation of organic waste. This water pollution may be caused by anthropogenic activities and it may consequently reach the threshold limit for cockle survival.

Blood cockle production potential

The results showed that the production potential in the study area was less than zero which caused by unsuitable seawater quality and fishing effort. It means that the production ability was extremely low; subsequently, this would lead to an extinction of blood cockles in the future. The average cockle growth rate found in this research was shell length increase 1.24 mm·month^{-1} which is lower than former recorded by Jongpepean *et al.* (1985), who found that in year 1985, blood cockles in Klong Khon had the ability to increase their shell length by 1.67 mm·month^{-1}. This because in the past (>30 years), the study area, Klong Khon, once had abundant mangrove forests. Unfortunately, it has since been deforested for charcoal production, residences and shrimp farms. These anthropogenic activities produce domestic waste which pollutes seawater; subsequently, the water quality is not suitable for blood cockle growth. Moreover, the cockle growth rate found in this study is much lower than cockles in other cultivation areas (Harith *et al*., 2016). This because lower chlorophyll *a* concentrations in seawater, which represents abundance of phytoplankton. In addition, the study area has been facing urban sprawl and mass tourism (Lohasarn *et al*., 2013). These can lead to tremendous amounts of wastewater into intertidal mudflats, the blood cockle's habitat. Taken together, these factors can explain the reduction of blood cockle production potential.

Anthropogenic stress on environmental factors and blood cockle

In the study area, there is anthropogenic stress caused by land use change. In the past, Klong Khon had plentiful mangrove forest cover. However, most of the mangrove trees were replaced by shrimp farms (Lohasarn *et al*., 2013). Moreover, human activities upstream and around the study area have been expanding. Litter and wastewater is discharged into Klong Khon through the river runoff, especially during rainy seasons. Meanwhile, blood cockle production in the study area has been decreasing sharply (Department of Fisheries, 2008; 2018), possibly due to anthropogenic stress from sources on land.

It has been recognized that anthropogenic stressors can influence environmental factors and the physiology of more vulnerable species, thus differentially affecting populations and communities (Khalil, 2013; Bhadja *et al*., 2014). Previous studies using growth models for the blood cockle mainly focused on food quantity and quality, feeding activity and absorption efficiency, but none of them included the effects of anthropogenic stress (Broom, 1982; Abele *et al*., 2009; Harith *et al*., 2016; Khalil *et al*., 2017). For this reason, our study proposes a more understandable prediction model of blood cockle production potential, which has been negatively affected by environmental fluctuations caused by anthropogenic stresses.

In this research, heavy metals in seawater are not taken into account in the developed model; instead, the water quality component mainly focuses on discharged wastewater from municipalities, intensive aquaculture farming and livestock activities, which are distributed to the study area through the river runoff. Moreover, regarding physiological responses, blood cockles are more sensitive to environmental factors other than heavy metals (Din and Ahamad, 1995). Environmental stress leads to weakness in blood cockles and an easier intake of heavy metals into their bodies. Thus, in this study only variation in environmental factors due to human activities was emphasized . The model scenarios were varied seawater salinity, temperature, dissolved oxygen, nutrient concentrations and pH of sediment, based on characteristics of municipal, aquaculture and livestock wastewater which could be dispersed into estuarine and coastal areas through the river runoff (Din and Ahamad, 1995; Jackson *et al*., 2003; Liu *et al*., 2010).

This current study provides a model simulation on blood cockle condition index (CI). Without Gonadal Index (GI), CI alone does not measure the ability of cockles to reproduce, but it can demonstrate the cockle's health, which impacts a cockle's reproductive success (Khalil *et al*., 2017). The model simulations show that CI increases with increasing salinity. Blood cockle is marine bivalves so it prefers salt water. Higher growth rates can be found at higher salinity (Din and Ahamad, 1995). The model simulation results also demonstrated that a CI value of less than 2 when salinity is lower than 9 ‰. Concordance with the research by Nudee and Mahasawat (2007) who found that at extremely low salinity (less than 5 ‰), the cockle is unable to survive. This can explain by suppress haemocyte production and integrity of lysosomes in bivalves when salinity is reduced (Ellis *et al*., 2011). Consequently, the immune response becomes disabled. The variation in salinity in the study area can be caused by human activities such as domestic wastewater from residences and discharge from livestock activities (cattle and pig farms) through the river runoff expecially during rainy season. Therefore, the results from the developed model in this study are reasonable and are able to predict the influence of altered salinity on blood cockle integrity, which reflects the reproductive ability.

Increase of seawater temperature can cause by both seasonal variation and anthropogenic activity such as excessive quantities of wastewater from domestic and industrial sectors (Din and Ahamad, 1995; Liu *et al*., 2010). According to the model developed in this study, high seawater temperature results in CI reduction and cockles may die off in large numbers at 32.4 ºC. This can be explained by physiological responses of blood cockle when expose to extremely high seawater temperature. For instance, the cockle's condition index was lowered together with increased lysosomal destabilization when the temperature reached 30 ºC (Taylor *et al*., 2017). Increase in seawater temperature can reduce heamocytosis, lysozyme activity, and inhibit phenoloxidase activity (Ellis *et al*., 2011). These can cause illness in the cockle. Moreover, rising water temperature causes increase the cockle's clearance rate (Nakamura and Shinotsuka, 2007). The higher clearance rate can allow the cockle to filter more food; however, if the water is contaminated with heavy metals or carcinogens, this will increase the rate of accumulation of these pollutants into the cockle body. Furthermore, increased filtration rate results in more energy consumption, and a condition index (CI) decrease may occur.

Another anthropogenic stress on blood cockle which simulated in this study was depletion of dissolved oxygen (DO) caused by contamination of wastewater. The model simulation illustrated that a reduction of oxygen resulted in a low condition index. The model also revealed that when DO concentration went down to zero, the cockle could still survive, with a condition index lower than 1. However, it may die off shortly afterwards. These results are in accordance with previous research. Nabhitabhata *et al*. (1990) found that the lethal DO level was 0.8 $mg{\cdot}L^{-1}$. Under this condition, the cockle was found to be alive for a very short period of time (Davenport and Wong, 1986; Din and Ahamad, 1995). It will reduce haemocyte production and phagocytic activity (Ellis *et al*., 2011). As a result, they may reduce food intake, get easily infected by pathogens and finally die off.

A high degree of anthropogenic pressure such as discharge of wastewater from municipal, aquaculture and livestock activities can significantly alter nutrient concentrations in an intertidal zone (Din and Ahamad, 1995; Jackson *et al*., 2003; Liu *et al*., 2010). In our model simulation varying loads of ammonium represent influence of anthropogenic stress on blood cockle. Results revealed that high ammonium concentration results in low condition index (CI). When ammonium reaches 2.36 µM, CI is less than 2, and at 4.14 µM, CI is zero. Even though there is no published research on the effects of ammonium on blood cockle growth and survival, ammonia seems to be the most dangerous toxicant to mollusc such as Asian clams (*Corbicula fluminea*) (Cherry *et al*., 2005; Cooper *et al*., 2005) and Taiwan abalone (*Haliotis diversicolor supertexta*) (Cheng *et al*., 2004). These research evident that high loading of ammonium can cause a reduction in blood cockles' filtration and immune responses; subsequently, a decrease in condition index may occur.

Similar to the influence of anthropogenic activities on seawater quality, human activities such as sanitation waste and intensive aquaculture through the river runoff can significantly reduce pH of sediment (Salaenoi *et al*., 2015). The model simulation in this study showed that lower blood cockle condition index (CI) at lower pH of sediment. The CI reached zero at pH level was 1.30. This extreme low pH of sediment can cause by directly dumping chemical waste into the study area. Lowered pH influences on blood cockle growth and survival by decrease its metabolism and calcification (Zhao *et al*., 2017). Moreover, when blood cockles expose to higher acidity of sediment, they tend to be easily infected and more easily accumulate any toxicants that are present in the sediment (Mat *et al*., 1994). This cause weakness and lowered CI in blood cockles.

Model limitations

The blood cockle condition index which represents its production potential can be evaluated using the developed model in this study; however, there are some limitations. First, in natural condition, beside environmental factors both seawater and sediment quality, there are other factors affecting blood cockle. Other predators on food web can cause variation of blood cockle density in the study area. Wading birds, intertidal-feeding fish and crustaceans such as shore crabs and shrimps are remarkable predator on the cockle (Burdon *et al*., 2014). In this study, interaction between cockle and its predator was absence. The authors emphasize only interaction between the cockle and environmental factors. Fishing effort is another factor that can influence on blood cockle density. However, it does not influence on cockle condition index. Therefore, it was ignore in the model simulation. Land-use change and human activity on land can affect blood cockle production potential. Increase of urban, industry and agriculture area can cause discharge of wastewater and disperse into intertidal zone through the river run off. Consequently, this phenomenon influence on blood cockle condition index by changing in characteristic of seawater and sediment. These parameters were omitted because they are indirect effect on the cockles. Anthropogenic activity caused fluctuation of environmental factors (seawater and sediment); then influence on the distribution pattern and reproductive ability of intertidal marine bivalves. In case of model scenarios, the developed model may be site-specific to the study area; however, this model can assist in model simulations in other areas nearby which share similar characteristics to the area studied. Moreover, in this study, all data were collected only three times (December 2016, April 2017 and September 2017) in order to represent seasonal variation in Thailand, and only for a single annual cycle. This may be a small data set. Therefore, it is recommended that in further study, a set of time series data stretching from January to December would make the model equation more accurate. Lastly, all model parameters require real-time data in order to reach high performance of the model. Therefore, a substantial data system should be developed to support this model.

CONCLUSION

In the study area, seasonal variation causes seawater quality disturbance by cool weather in winter, high evaporation in summer, and large river runoff in the rainy season. Sediment characteristics were not found to be different among seasons, but significantly different among the sampling stations. The highest cockle density and condition index (CI) were found in the winter season followed by summer and rainy season, respectively. Our results showed that during the study period, average cockle production potential was less than zero. A very high decreasing rate was found in rainy season when seawater quality was unsuitable for blood cockle. Statistical analysis results revealed that the cockle density tend to increase when seawater salinity, DO, and pH of sediment increase. Extreme rising of seawater temperature, ammonia, nitrite, nitrate and salinity of sediment cause reduction of the cockle density. The model scenarios on anthropogenic stress demonstrated that the complete decline of cockle condition index occurred when salinity, temperature, dissolved oxygen, ammonia and pH of sediment reached 3 ‰, 32.4 °C, 0 $mg{\cdot}L^{-1}$, 4.14 µM, and 1.30, respectively. These results indicate the threshold levels of blood cockle and can be used to effectively assist in blood cockle cultivation management, both in commercial and ecological conservation aspects.

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LITERATURE CITED

Abele, D., T. Brey and E. Philipp. 2009. Bivalve models of aging and the determination of molluscan lifespans. **Experimental Gerontology** 44: 307–315.

Allison, L.F. 1965. **Organic carbon.** In: method of soil analysis part 2 agronomy no.9 (ed. C.A. Black), pp. 1372–1375. American Society of Agronomy, USA.

American Public Health Association (APHA). 2012. **Standard Methods for The Examination of Water and Wastewater,** 22nd ed. American Public Health Association (APHA), American Water Works Association (AWWA) and Water Pollution Control Federation and Water Environment Federation (WPCF), Port City Press, USA. 1496 pp.

Beck, R. 1999. **Soil Analysis Handbook of Reference Methods.** CRC Press, USA. 264 pp.

Bhadja, P., P. Poriya and R. Kundu. 2014. Community structure and distribution pattern of intertidal invertebrate macrofauna at some anthropogenically influenced coasts of Kathiawar Peninsula (India). **Advances in Ecology** 2014: 1–12.

Boey, P.L., G.P. Maniam, S.A. Hamid and D.M.H. Ali. 2011. Utilization of waste cockle shell (*Anadara granosa*) in biodiesel production from palm oil: Optimization using response surface methodology. **Fuel** 90: 2353–2358.

Bremner, J.M. and C.S. Mulvaney. 1982. **Nitrogen-total.** In: methods of soil analysis part 2 agronomy no.9 (eds. A.L. Page, R.H. Miller and D.R. Keeney), pp. 595–624. Madison, Wisconsin, USA.

Broom, M.J. 1982. Analysis of the growth of *Anadara granosa* (Bivalvia: Arcidae) in natural, artificially seeded and experimental populations. **Marine Ecology-Progress Series** 9: 69-79.

Burdon, D., R. Callaway, M. Elliott, T. Smith and A. Wither. 2014. Mass mortalities in bivalve populations: A review of the edible cockle *Cerastoderma edule* (L.). **Estuarine, Coastal and Shelf Science** 150: 271–280.

Chagas, G.G. and M.S. Suzuki. 2005. Seasonal hydrochemical variation in tropical coastal lagoon (Acu Lagoon, Brazil). **Brazilian Journal of Biology** 65: 597–607.

Cheng, W., I.S. Hsiao and J.C. Chen. 2004. Effect of ammonia on the immune response of Taiwan alone *Haliotis diversicolor supertexta* and its susceptibility to *Vibrio parahaemolyticus*. **Fish and Shellfish Immunology** 17: 193–202.

Cherry, D.S., J.L. Scheller, N.L. Cooper and J.R. Bidwell. 2005. Potential effects of Asian clam (*Corbicula fluminea*) die-offs on native freshwater mussels (Unionidae) I: Water-column ammonia levels and ammonia toxicity. **Journal of the North American Benthological Society** 24(2): 369–380.

Cooper, N.L., J.R. Bidwell and D.S. Cherry. 2005. Potential effects of Asian clam (*Corbicula fluminea*) die-offs on native freshwater mussels (Unionidae) II: Porewater ammonia. **Journal of the North American Benthological Society** 24(2): 381–394.

Davenport, J. and T.M. Wong. 1986. Responses of the blood cockle *Anadara granosa* (L.) (Bivalvia: Arcidae) to salinity, hypoxia and aerial exposure. **Aquaculture** 56: 151–162.

Department of Fisheries. 2008. **Fisheries Statistics of Thailand 2006.** Department of Fisheries, Ministry of Agriculture and Cooperatives, Thailand. 91 pp.

Department of Fisheries. 2018. **Fisheries Statistics of Thailand 2016.** Department of Fisheries, Ministry of Agriculture and Cooperatives, Thailand. 82 pp.

Din, Z.B. and A. Ahamad. 1995. Change in the scope for growth of blood cockles (*Anadara granosa*) exposed to industrial discharge. **Marine Pollution Bulletin** 31: 406–410.

Ellis, R.P., H. Parry, J.I. Spicer, T.H. Hutchinson, R.K. Pipe and S. Widdicombe. 2011. Immunological function in marine invertebrates: Responses to environmental perturbation. **Fish and Shellfish Immunology** 30: 1209–1222.

Harith, H., M.L. Husain and M.F.M. Akhir. 2016. Coastal oceanographic processes associated with blood cockle (*Anadara granosa*) induce spawning season in Kapar, Selangor, Malaysia. **Journal of Ocean Engineering and Science** 1: 289–299.

Hazelton, P.A. and B.W. Murphy. 2007. **Interpreting Soil Test Results: What Do All The Numbers Mean?** CSIRO Publishing; Australia. 152 pp.

Ishak, A.R., S. Mohamad, T.K. Soo and F.S. Hamid. 2016. Leachate and surface water characterization and heavy metal health risk on cockles in Kuala Selangor. **Procedia - Social and Behavioral Sciences** 222: 263–271.

Jackson, C., N. Preston, P.J. Thompson and M. Burford. 2003. Nitrogen budget and effluent nitrogen components at an intensive shrimp farm. **Aquaculture** 218: 397–411.

Jahangir, S., G. Siddiqui and Z. Ayub. 2014. Temporal variation in the reproductive pattern of blood cockle *Anadara antiquate* from Pakistan (northern Arabian Sea). **Turkish Journal of Zoology** 38: 263–272.

Jongpepean, T., W. Phujaroen and P. Prompye. 1985. **Seasonal Distribution of Blood Cockles (*Anadara granosa* L.) and Razor Clams (*Solen grandis* (Dunker)) at Samut Songkarm Province.** Department of Fisheries, Ministry of Agriculture and Cooperatives, Thailand. 23 pp.

Khalil, M. 2013. The effect of environmental condition on the spawning period of blood cockle *Anadara granosa* (bivalvia: arcidae) in Lhokseumawe, the northern straits of Malacca. **Journal Agrium** 10: 69–76.

Khalil, M., Z. Yasin and T.S. Hwai. 2017. Reproductive biology of blood cockle *Anadara granosa* (Bivalvia: Arcidae) in the northern region of the Strait of Malacca. **Ocean Science Journal** 52(1): 75–89.

Kim, K., H.S. Yoon, I.C. Lee and T. Hibino. 2016. An influence of salinity on resuspension of cohesive sediment. **Journal of Coastal Research** (Special Issue 75): 68–72.

Liu, Y.X., T.O. Yang, D.X. Yuan and X.Y. Wu. 2010. Study of municipal wastewater treatment with oyster shell as biological aerated filtermedium. **Desalination** 254: 149–153.

Lohasarn, O., S. Worachananant and V. Jintana. 2013. Land use on the newly formed mudflat for blood clam culture at Khlong Khon sub-district, Mueang district, Samut Songkhram province. **Thai Journal of Forestry** 32: 161–172.

Marsden, I.D., B.D. Smith and P.S. Rainbow. 2014. Effects of environmental and physiological variables on the accumulated concentrations of trace metals in the New Zealand cockle *Austrovenus stutchburyi*. **Science of Total Environment** 470–471: 324–339.

Mat, I., M.J. Maah and A. Johari. 1994. Trace metal geochemical associations in sediments from the culture-bed of *Anadara granosa*. **Marine Pollution Bulletin** 28: 319–323.

Mirzaei, M.R., Z. Yasin and A.T.S. Hwai. 2014. Periodicity and shell microgrowth pattern formation in intertidal and subtidal areas using shell cross sections of the blood cockle, *Anadara granosa*. **Egyptian Journal of Aquatic Research** 40: 459–468.

Nabhitabhata, J., S. Kbinrum and Y. Soodmee. 1990. **Oxygen Consumption of Blood Cockle, *Anadara granosa* Linn.** Department of Fisheries, Ministry of Agriculture and Cooperatives, Thailand. 18 pp.

Nakamura, Y. and Y. Shinotsuka. 2007. Suspension feeding and growth of ark shell *Anadara granosa*: comparison with ubiquitous species *Scapharca subcrenata*. **Fisheries Science** 73: 889–896.

Nieves-Soto, M., F. Enriquez-Ocaña, P. Piña-Valdez, A.N. Maeda-Martínez, J.R. Almodóvar-Cebreros and H. Acosta-Salmón. 2011. Is the mangrove cockle *Anadara tuberculosa* a candidate for effluent bioremediation? Energy budgets under combined conditions of temperature and salinity. **Aquaculture** 318: 434–438.

Nudee, V. and J. Mahasawat. 2007. **Relationship between Water and Soil Qualities with Condition Index of Cockle *Anadara granosa* in Bandon Bay, Suratthani province.** Department of Fisheries, Ministry of Agriculture and Cooperatives, Thailand. 19 pp.

Parsons, T.R., Y. Maita and C.M. Lalli. 1984. **A Manual of Chemical and Biological Methods for Seawater Analysis.** Pergamon Press, Oxford. 173 pp.

Salaenoi, J., C. Sukudom, T. Wongsin and S. Sirisuay. 2015. **Sediment quality in cockle culture and non-cultured area at Bandon Bay, Thailand.** Proceedings of the International Conference on Plant, Marine and Environmental Sciences 2015: 110–114.

Sangeeta, P. and P. Neha. 2015. Monitoring of seasonal variation in physicochemical water parameters in Nalasopara Region. **Journal of Ecosystem and Ecography** 5: 1–4.

Sany, S.B.T., R. Hashim, M. Rezayi, A. Salleh, M.A. Rahman, O. Safari and A. Sasekumar. 2014. Human health risk of polycyclic aromatic hydrocarbons from consumption of blood cockle and exposure to contaminated sediments and water along the Klang Strait, Malaysia. **Marine Pollution Bulletin** 84: 268–279.

Taylor, A.M., W.A. Maher and R.P. Ubrihien. 2017. Mortality, condition index and cellular responses of *Anadara trapezia* to combined salinity and temperature stress. **Journal of Experimental Marine Biology and Ecology** 497: 172–179.

Zhao, X., W. Shi, Y. Han, S. Liu, C. Guo, W. Fu, X. Chai and G. Liu. 2017. Ocean acidification adversely influences metabolism, extracellular pH and calcification of an economically important marine bivalve, *Tegillarca granosa*. **Marine Environmental Research** 125: 82–89.