

## Research article

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### Seawater Characteristics and Their Influence on Green Mussel (*Perna viridis*) Production Potential

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#### Abstract

Seawater fluctuation caused by climate change and domestic wastewater can affect the cultivation of green mussel (*Perna viridis*), which is a valuable marine food in the Indo-Pacific region. Hence, this research aimed to investigate the variation of seawater parameters in green mussel cultivation areas and the impact of changes in those parameters on mussel production. Three cultivation sites, pond, coastal line, and estuarine, were chosen to represent three cultivation methods. At each site, seawater variables and green mussels were collected monthly from January to December 2019. The results showed that seawater parameters varied significantly across the cultivation sites, and seasonal variations were found to be related to intense rainfall. The highest mussel production was found in estuarine areas followed by coastal line areas and ponds, which were  $0.25 \pm 0.05$ ,  $0.01 \pm 0.003$ , and  $-0.01 \pm 0.02$  kg/m<sup>2</sup>/month, respectively. Statistical analysis revealed that salinity and conductivity were factors that positively influenced mussel production potential. In estuarine areas, the salinity was suitable for mussel growth and there was abundant food for mussels; subsequently, estuarine areas had the highest mussel production. Meanwhile, ponds were not suitable for mussel cultivation due to low salinity. Although coastal line areas had the highest salinity, chlorophyll a levels were low, indicating a lack of food available for mussels. Consequently, the production potential was low. These results can assist farmers to select the best available areas for green mussel culture. Finally, in time of unstable seawater quality caused by environmental crises, our results can be useful for identifying the impact of seawater quality on green mussel production in each cultivation area.

**Keywords:** water quality; *Perna viridis*; marine bivalves; mariculture; production potential

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## 1. Introduction

Global climate change and domestic wastewater discharge can cause tremendous variations in marine environmental factors (Doney et al., 2012). These robust phenomena have resulted in the degradation of marine living organisms, especially green mussels (*Perna viridis*), a significant commercial food industry in Indo-Pacific regions such as India, Indonesia, Philippines, Malaysia, and Thailand (FAO, 2021).

Green mussels are well known as economically profitable for aquaculture systems due to their high tolerance to a wide range of environmental factors, quick growth, short cultivation period to reach market size, and availability of spat year-round without the need for production (Rajagopal et al., 2006). Their cultivation constitutes the largest fraction of shellfish production in Thailand; however, it was reported that the mussel production trend decreased over time (Department of Fisheries, 2021). This shows that there was an unfavorable environmental condition for the mussel.

It is well known that many environmental variables influence mussel growth and survival. For instance, it was documented that heat stress and food limitation cause damage and decrease mussel physiological response and limit energy reservation (Sasikumar & Krishnakumar, 2011; Delorme et al., 2020). Lowered pH in seawater affects the physiological and immune response in mussels (Sivalingam, 1977). Decreased salinity and dissolved oxygen result in the degradation of mussel hemocytes (Wang et al., 2012). Water turbidity or extremely high suspended solids have long-term effects on mussel feeding ability due to gill damage in mussels and lowering of quality of food (Cheung & Shin, 2005). Moreover, mussel integrity was shown to be affected by stock origin and seasonality (Noor et al., 2011; Ren et al., 2019). Mussels that are cultured in estuarine areas may experience rapid salinity reduction during intense rainfall events due to the high levels of discharge of freshwater through river runoff (Alosairi et al., 2019). Temporal patterns of storms and turbulence weather can cause variations in salinity, nutrient concentration, and quantity of phytoplankton (Alfaro et al., 2010), all of which affect mussel growth and survival (Noor et al., 2011; Isnain et al., 2020). From the above-mentioned literature, it can be concluded that mussel health is influenced by the fluctuation of environmental variables that occur due to anthropogenic stress and climate change phenomenon. Nevertheless, the impact of seawater parameters on the productivity of green mussels is still not clearly understood.

Green mussel production refers to the quantity of the mussels that can be produced, and mussel production potential refers to the capacity to efficiently produce mussels over time. Although mussel production had been evaluated in the case of different cultivation techniques (Sagita et al., 2017; Rejeki et al., 2021) and seeding density (Skelton & Jeffs, 2022), the exact environmental factors that cause variations in mussel production remain unclear. Hence, this research aimed to: 1) investigate the spatial and seasonal variation of seawater parameters in green mussel cultivation areas; 2) determine the trends of mussel production and production potential; and 3) evaluate the impact of seawater parameters on mussel production potential. Our results can assist farmers to assess the feasibility of sites for green mussel cultivation during disturbance of seawater quality caused by pollution discharge and climate change events. Furthermore, the results also provide significant information for policymakers and other scientists who are interested in sustainable marine culture.

## 2. Materials and Methods

### 2.1 Study area

The Inner Gulf of Thailand is a significant green mussel cultivation area in Southeast Asia and three sampling sites with productive green mussel farms were selected for this study (Figure 1). The first sampling location was in Bangkhuntein district (BKT), Bangkok (UTM 47P 655299 m E, 1497465 m N). The mussels were cultured inside ponds that formerly were shrimp farms. Each pond was rectangular with dimensions of 370 m x 80 m and 2 m in depth. The juvenile mussels were attached with cultivation rope and hung on floating rafts. Seawater was circulated by opening the gate at high tide and closing the gate during low tide each day. The second sampling location was Sriracha (SRI), Chonburi (UTM 47P 708208 m E, 1458524 m N). The mussel cultivation was in a coastal line area. Similar to BKT, in this area, the mussels were cultured in floating rafts. Seawater could easily flow through the rafts because they formed an open water circulation system. The last location was the Maeklong River mouth (MK), in Samut Songkhram (612675 m E, 1475015 m N). The mussels were cultured in horizontal racks made from rope. The seawater characteristic in this area was influenced by river runoff. These sampling locations covered all green mussel cultivation methods used in Thailand and the seawater types were representative of the types of seawater that were employed to grow the mussels.

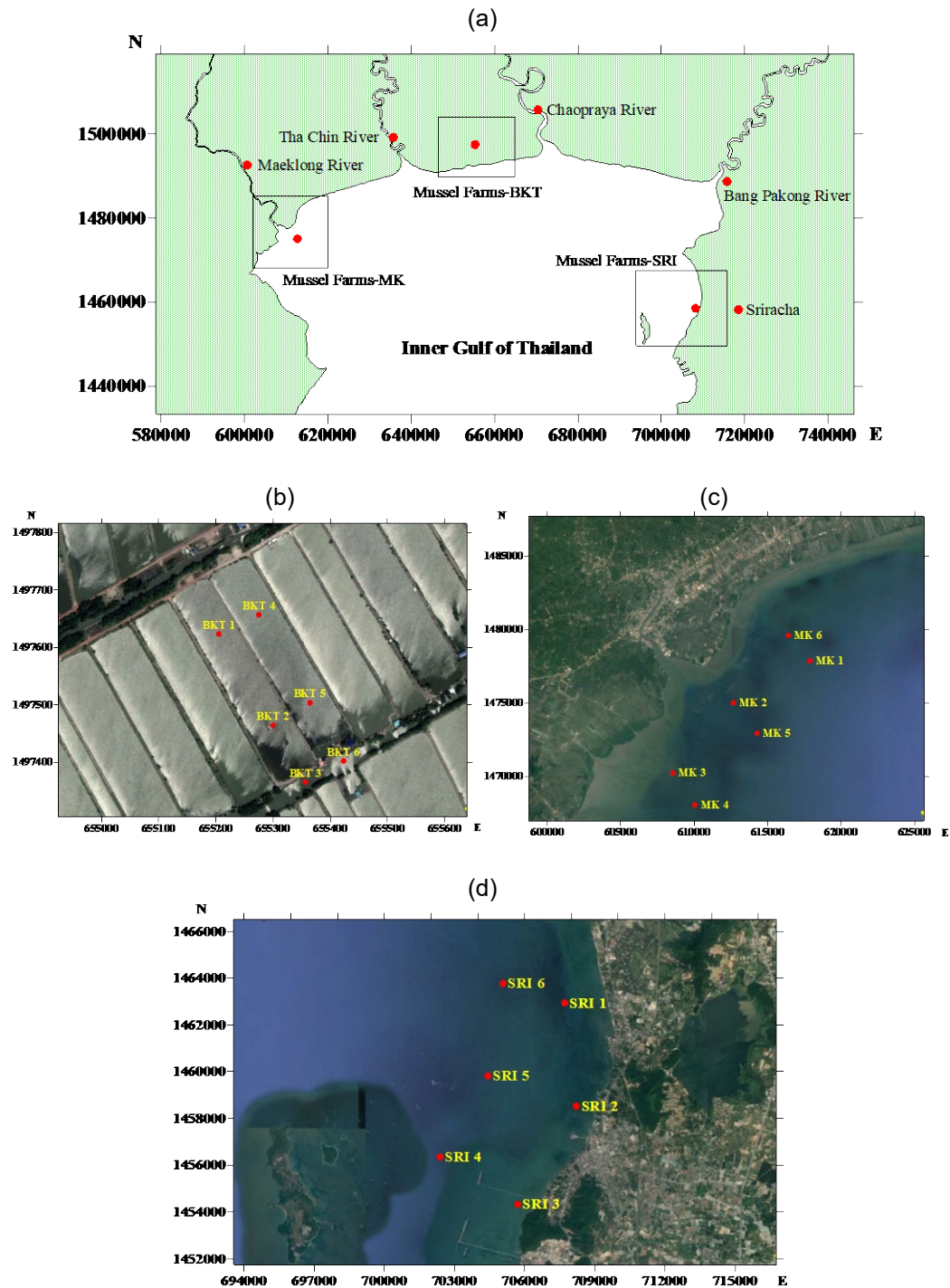
### 2.2 Seawater sampling

To investigate the seawater characteristics at each green mussel growing site, seawater samples were collected monthly during low tide in the spring tide period for a full year (January to December 2019). There were 6 sampling stations in each location (Figure 1). At each station, seawater temperature, pH, salinity, conductivity, and dissolved oxygen (DO) were measured in situ using a handheld multi-parameter probe (YSI 600QS, USA). Transparency was analyzed in situ using a Secchi disk. Seawater was sampled, stored in opaque bottles, and transported to the laboratory. Each sample was determined for chlorophyll *a* (Chl *a*) and total suspended solids (TSS) using the procedure described in Parsons et al. (1984). Then, the seawater sample was filtered through filter paper GF/F (pore size 0.7 µm) and frozen at -20°C for nutrient analysis. The concentrations of ammonium-nitrogen (NH<sub>4</sub><sup>+</sup>-N), nitrite and nitrate-nitrogen (NO<sub>2</sub><sup>-</sup>+NO<sub>3</sub><sup>-</sup>-N), phosphate-phosphorus (PO<sub>4</sub><sup>3-</sup>-P), and silicate-silicon (Si(OH)<sub>4</sub>-Si) were determined using a segment flow TrAacs 2000 Auto Analyzer (Seal Analytical, USA) (sensitivity±0.01 µg/L).

### 2.3 Green mussel samples

After the seawater sample was conducted, all green mussels that were attached to one cultivation rope were immediately collected from each sampling station. All epifauna and attachments were removed from the mussels. Each mussel's whole body was measured for wet weight using a 4-digit analytical balance. Then, the mussel production (kg/m<sup>2</sup>) in each mussel farm was determined by multiplying the fresh mussels' weight by the number of cultivations ropes and dividing by the farm area. Finally, the production potential of the mussel (GPP, kg/m<sup>2</sup>/month) was calculated by equation (1).

$$GPP = \frac{(GMPt - GMP0)}{t} \quad (1)$$



**Figure 1.** Location of green mussel cultivation areas in (a) the Inner Gulf of Thailand: (b) Bangkhuntein (BKT), (c) the Maeklong River mouth (MK), and (d) Sriracha (SRI)

Where,  $GMP_t$  is the mussel production at time  $t$  ( $\text{kg}/\text{m}^2$ ),  $GMP_0$  is initial mussel production ( $\text{kg}/\text{m}^2$ ), and  $t$  is time.

The research procedure was performed in accordance with animal ethics protocol. All mussels were collected from their natural condition with the minimum requirement taken for statistical analysis and were not employed for any experiment, tissue sample, or blood collection.

## 2.4 Statistical analysis

The means and standard deviation of all seawater parameters and the mussel productions were analyzed using Microsoft Excel 365. Then, analysis of variance (ANOVA) and Tukey-HSD were employed to determine spatial and temporal variations of all variables. Finally, to determine the influence of seawater parameters on mussel production potential, the correlation between monthly mussel production and the average of seawater variables during the cultivation period was determined using the Pearson correlation coefficient ( $r_{xy}$ ). All statistical tests were considered at the significant level of  $P < 0.05$  and  $P < 0.01$ .

## 3. Results and Discussion

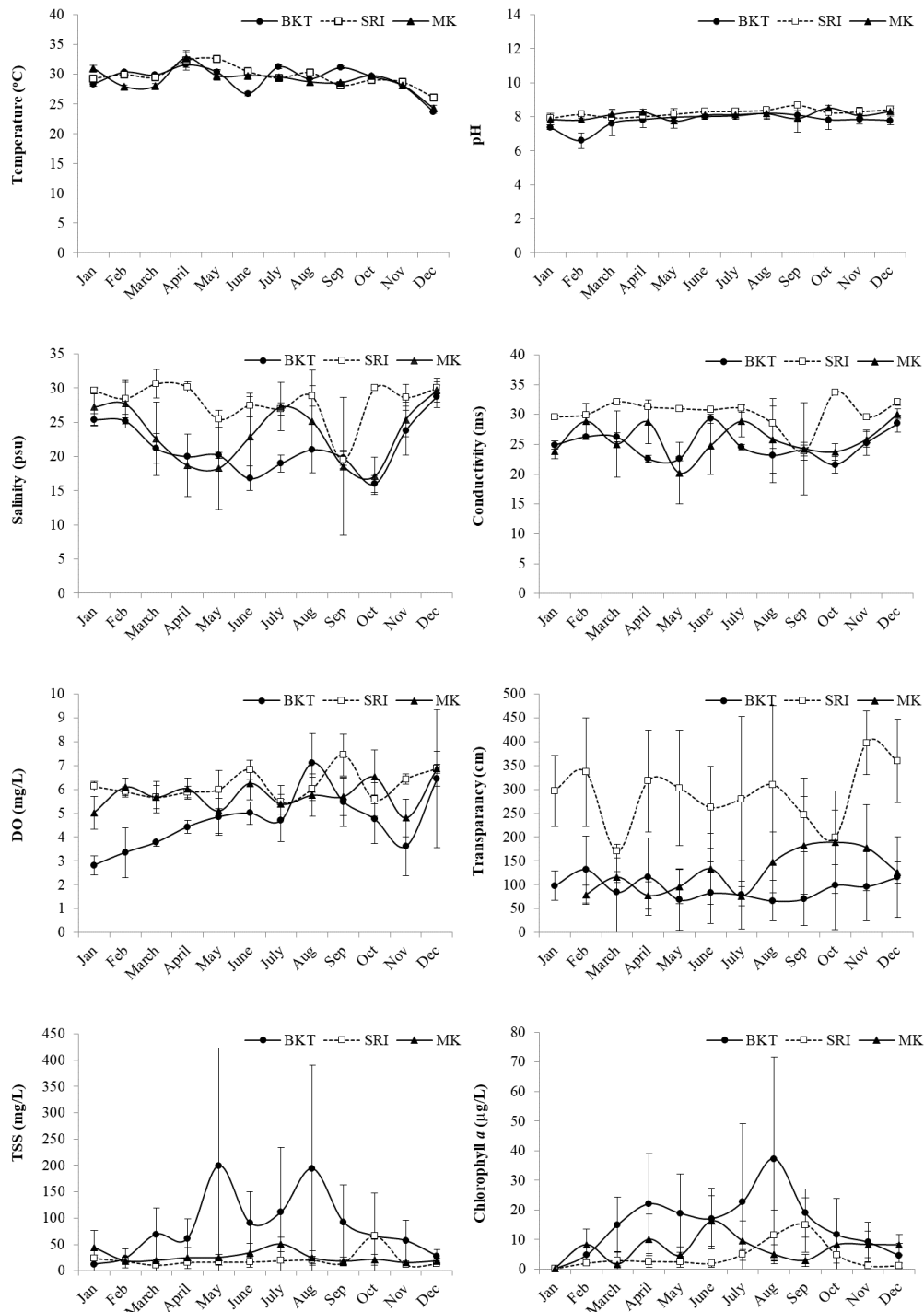
### 3.1 Seawater characteristic in green mussel cultivation areas

Seawater characteristic trends during the study period in each cultivation area are shown in Figures 2 and 3. The results of the statistical analysis revealed that there was spatial and temporal variation in seawater parameters (Table 1). There were significant differences in seawater parameters across the cultivation areas except for temperature parameter. In all areas, seawater temperature was in the range of  $23.68$ – $32.69^\circ\text{C}$ , which was suitable for green mussel growth and survival (Wong & Cheung, 2003). The lowest temperature was found in December and the highest was found in April which were the winter and summer seasons in Thailand, respectively. The pH level in BKT was found to be lower than pH in SRI and MK. This was because green mussels were cultured in ponds that were influenced by freshwater input during the low tide period.

The highest values of salinity and conductivity were found in the SRI, which was coastal line areas, followed by MK and BKT, respectively. In September and October, salinity was found to be lower than 24 psu in all cultivation areas which was not suitable for green mussel growth and could cause 50% die-off (Sivalingam, 1977). This low level of salinity was attributable to the intense rainfall during these months. Consequently, freshwater from rain was discharged from land into the sea through river runoff. This resulted in a rapid reduction of salinity in the study areas.

Dissolved oxygen (DO) in this study varied across the cultivation areas and months (Table 1). However, the DO level was always higher than  $3 \text{ mg}/\text{L}$  which is a level that does not affect any marine organisms including green mussels (Wang et al., 2009).

The results showed that farms in the BKT area had the lowest transparency and the highest TSS (Figure 2). This was because green mussels in BKT were cultured in ponds that were only 2 m deep. Sediment on the seafloor can be easily dispersed into the seawater column due to water movement during high tide and low tide. Moreover, there was a seasonal variation of these parameters. During the rainy season (August–October), low transparency and high TSS were found. This was because heavy rainfall can cause sediment erosion. The eroded sediments subsequently become suspended particles in the water column.

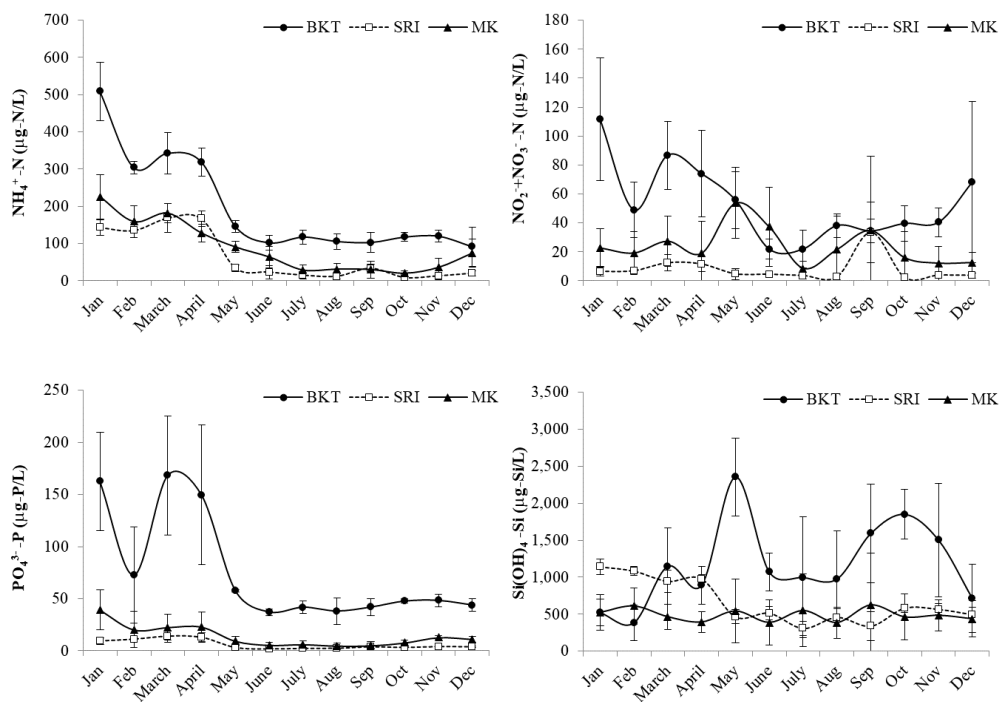


**Figure 2.** Seasonal variation of seawater parameters in green mussel cultivation areas: Bangkhuntein (BKT), Sriracha (SRI), and the Maeklong River mouth (MK) in year 2019

Chlorophyll *a* concentrations in MK and SRI were similar, and both were lower than chlorophyll *a* concentrations in BKT (Figure 2). This demonstrated that BKT ponds provided better food availability than the other areas. Chlorophyll *a* is a parameter representing the amount of phytoplankton which is the main food source for green mussels. High concentrations of chlorophyll *a* indicate an abundance of phytoplankton.

Similar to chlorophyll *a*, nutrient concentrations in BKT were significantly higher than MK and SRI, respectively (Figure 3). This was because BKT received nutrient loading from river runoff to a greater degree than other cultivation areas. These nutrients can enrich phytoplankton quantity which is essential for green mussel growth and survival.

These spatial and temporal variations of seawater parameters show that each cultivation area had unique seawater characteristics which corresponded to different green mussel production levels.



**Figure 3.** Seasonal variation of nutrients concentrations in green mussel cultivation areas: Bangkhuntein (BKT), Sriracha (SRI), and the Maeklong River mouth (MK) in year 2019

### 3.2 Green mussel production

In this study, green mussel production was calculated from the fresh mussel weight per farm area. Then, green mussel production potential was estimated from differences in the production through time during the cultivation period. The results from the statistical analysis showed that green mussel production in the three cultivation areas (BKT, MK, and SRI) varied significantly (Table 1). The highest production was found in MK (estuarine areas) followed by SRI (coastal line areas) and BKT (ponds), respectively. The average

production was  $12.33 \pm 2.92$ ,  $1.03 \pm 0.33$ , and  $0.09 \pm 0.05$  kg/m<sup>2</sup>, respectively. As shown in Figure 4, during the cultivation period (January to December), the mussel production in BKT tended to decrease over time. The mussel production potential was  $-0.01 \pm 0.02$  kg/m<sup>2</sup>/month. This was because for most of the time, the salinity of seawater in this area was lower than 24 psu (Figure 2) which can inhibit mussel growth by reducing hemocytes and immune responses in green mussels (Sivalingam, 1977; Wang et al., 2012).

Green mussel production in SRI tended to increase from January to July (Figure 4), which was in the middle of the cultivation period. In this area, the mussel seeds were attached to the cultivation ropes in October and grew naturally throughout the year. Then, the mussels were harvested in September of the next coming year. In this study, from January to July, green mussels had been generally growing. Then, the mussel production rapidly decreased in September (Figure 4) which was in the rainy season in Thailand. Intense rainfalls during this season cause a high discharge of freshwater through river runoff, and subsequently rapid salinity reduction can occur (Alosairi et al., 2019). In this study, the seawater salinity in September was only 19.50 psu (Figure 2) which inhibited mussel growth (Rowchai, 2004) and led to high mussel mortality. Hence, due to the tremendous loss of mussel production during the rainy season, the overall mussel production potential in this area was only  $0.01 \pm 0.003$  kg/m<sup>2</sup>/month.

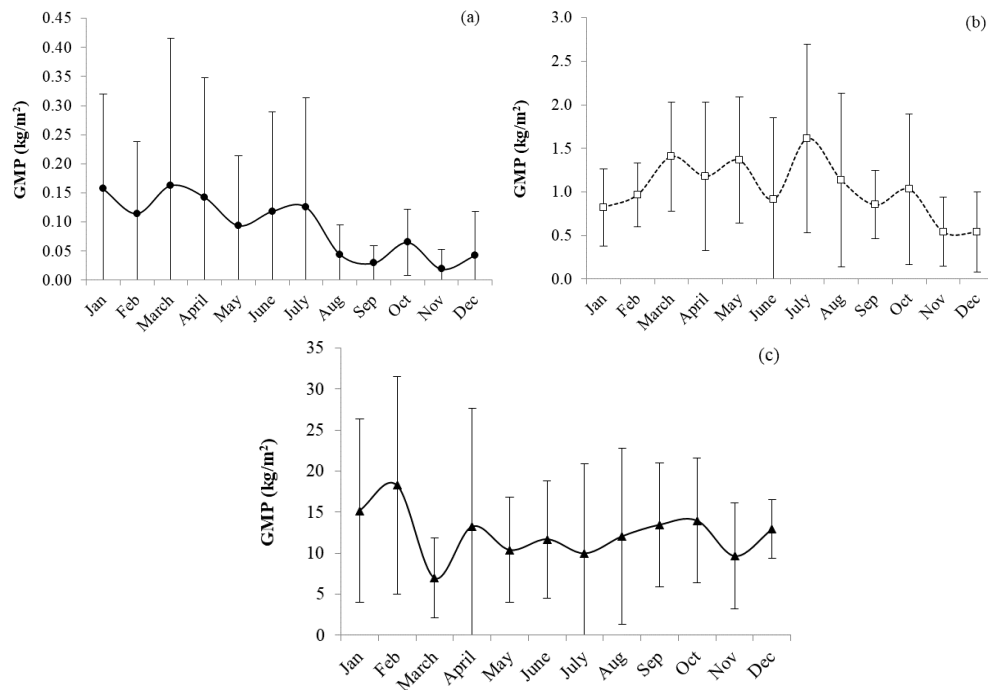
As shown in Figure 4, during the cultivation period (March to December), green mussel production in MK tended to increase over time. The mussel production potential was  $0.25 \pm 0.05$  kg/m<sup>2</sup>/month which was much higher than BKT and SRI (Figure 5). This was because there were more favorable environmental conditions for mussel growth in MK than in other areas. The mussels in MK were cultured in estuarine areas. Similar to pond conditions (BKT), there was seawater salinity fluctuation in estuarine areas (Figure 2). However, for most of the time, salinity in this area was higher than 25 psu which was suitable for green mussel growth (Gao et al., 2008), unlike pond conditions. In addition, there was more food availability (higher chlorophyll *a*) in MK (estuarine areas) than in SRI (coastal line areas) (Figure 2). This influenced the feeding behavior of the green mussels.

**Table 1.** Spatial and temporal variations of green mussel production (GMP) and seawater parameters at Bangkhuntein (BKT), Sriracha (SRI), and the Meaklong River Mouth (MK) in year 2019 using one-way ANOVA and Tukey-HSD

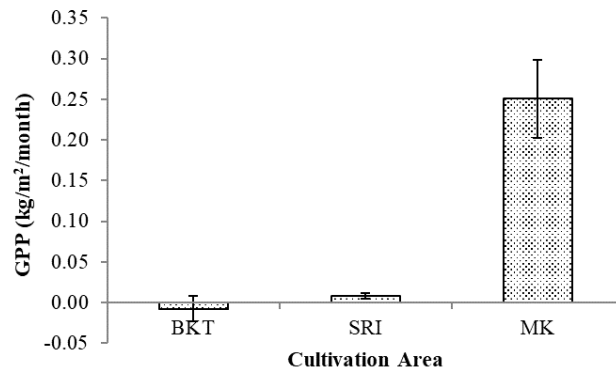
Parameter	Spatial Variation				Temporal Variation		
	One-way ANOVA	Tukey-HSD			One-way ANOVA		
	All Areas	BKT	SRI	MK	BKT	SRI	MK
GMP (kg/m <sup>2</sup> )	**	a	a	b	ns	ns	ns
Temp (°C)	ns	-	-	-	**	**	**
pH	**	a	b	b	**	**	**
Sal (psu)	**	a	b	c	**	**	**
Conduct (ms)	**	a	b	a	**	**	**
DO (mg/L)	**	a	b	c	**	**	**
Trans (cm)	**	a	b	a	ns	*	**
TSS (mg/L)	**	a	b	b	ns	*	**
Chl <i>a</i> (mg/L)	**	a	b	b	*	**	**
NH <sub>4</sub> <sup>+</sup> -N (mg/L)	**	a	b	b	**	**	**
NO <sub>2</sub> <sup>-</sup> +NO <sub>3</sub> <sup>-</sup> -N (mg/L)	**	a	b	c	**	**	*
PO <sub>4</sub> <sup>3-</sup> -P (mg/L)	**	a	b	b	**	**	**
Si(OH) <sub>4</sub> -Si (mg/L)	**	a	b	b	**	**	ns

**Note:** Significance level of one-way ANOVA: \*\* =  $P < 0.01$ ; \* =  $P < 0.05$ ; ns = not significant





**Figure 4.** Change in green mussel production (GMP) at Bangkhuntein (a), Sriracha (b), and the Maeklong River mouth (c) in year 2019



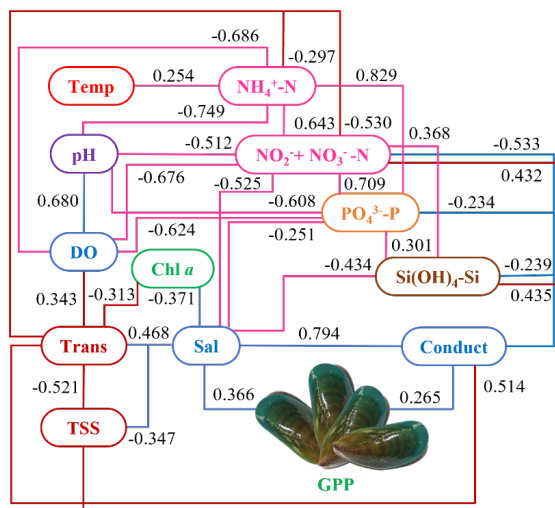
**Figure 5.** Green mussel production potential (GPP) during cultivation period at Bangkhuntein (BKT), Sriracha (SRI) and the Meaklong River Mouth (MK) in year 2019

Mussels are dependent on the amount of phytoplankton suspended in seawater for their nutrition requirements (Rajagopal et al., 1998). Therefore, due to the suitable range of seawater salinity and abundance of food for mussel growth, MK was the region with the highest green mussel production potential in this study.

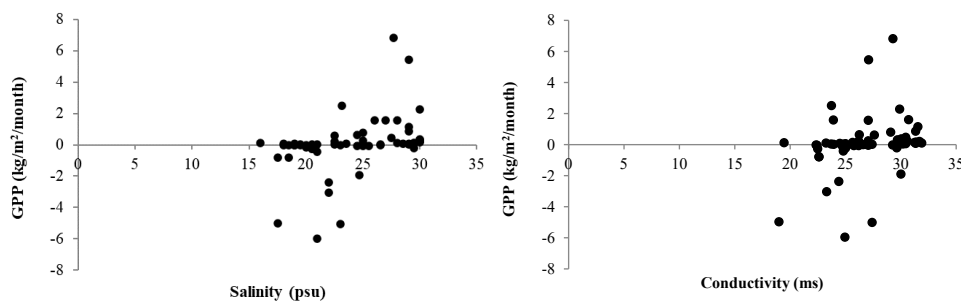
### 3.3 Effect of seawater variables on green mussel production potential

The results from the statistical analysis revealed that the potential of green mussel production in all three cultivation areas (BKT, SRI, and MK) depended on seawater salinity and conductivity. As shown in Figure 6, the mussel production potential correlated to seawater variables, and seawater variables also related to each other. This showed that the mussels could receive both direct and indirect effects from seawater.

Salinity and conductivity of seawater in this study significantly influenced green mussel production potential with Pearson correlation coefficients ( $r_{xy}$ ) of 0.366 and 0.265, respectively. High salinity and conductivity resulted in high production potential (Figure 7). This was because green mussels are marine bivalves. They generally grow with salinity in the range of 25-35 psu (Rajagopal et al., 1998; Gao et al., 2008). When salinity is low, the green mussels exhibit their metabolism by closing the valve, reducing respiratory rate (Wendling et al., 2013) and decreasing immune functions (Wang et al., 2012). However,



**Figure 6.** Relationship between green mussel production potential (GPP) and seawater parameters described by Pearson correlation coefficient ( $r_{xy}$ )



**Figure 7.** Relationship between green mussel production potential (GPP), salinity, and conductivity

they cannot withstand it for a long period. Acute death can be found when the mussel was exposed to seawater salinity lower than 15 psu (Rowchai, 2004). In this study, the lowest seawater salinity was found in BKT (pond condition) followed by MK (estuarine areas) and SRI (coastal line areas), respectively. The salinity was  $21.43 \pm 3.73$ ,  $23.39 \pm 4.34$ , and  $28.00 \pm 3.07$  psu. These results showed that salinity in BKT was not in the optimum concentration range for mussel growth and this caused low mussel production in this area.

In this study, nutrients were an indirect influencing factor affecting green mussel production potential. As shown in Figure 6, statistical analysis revealed that increased nutrient concentrations as  $\text{NO}_2^- + \text{NO}_3^- - \text{N}$ ,  $\text{PO}_4^{3-} - \text{P}$ , and  $\text{Si}(\text{OH})_4 - \text{Si}$  were related to decreased salinity, and low salinity led to a decline in green mussel production potential. This may have been due to the high loading of domestic waste from river runoff during the rainy season. In this study, salinity tended to decrease in September and October when intense rainfall occurred. This phenomenon can cause input of freshwater along with domestic wastes including nutrients, chemical substances, and suspended solids into the cultivation areas through river runoff. Although high nutrient concentrations can enhance phytoplankton growth and thus provide an available food source for green mussels, low salinity can deteriorate the mussel health. In this study, BKT had the most turbid seawater with high chlorophyll *a* and nutrients concentrations. However, the region also showed very low salinity which stifled mussel growth and survival. Therefore, a decline in the mussel production potential was found.

Based on the results from this study, the MK (estuarine area) region was the most suitable for green mussel cultivation followed by the SRI (coastal line areas) and the BKT (pond condition) regions, respectively. Although statistical analysis demonstrated that high salinity could provide high mussel production, the production potential in SRI was less than MK (Figure 5). This was because there were higher levels of nutrients in MK. When salinity in a cultivation area reaches the optimum range for green mussel growth and survival, nutrients become another key factor influencing the production potential of the marine bivalves (Srisunont & Srisunont, 2022). Nutrients may not directly affect green mussel health, but they influence the quantity of phytoplankton which is the main food source for green mussels. Hence, although salinity was high in SRI, a very low nutrient level could cause a lack of food available for the mussels. The results can be part of a database for the implementation of green mussel cultivation model that can better interpret the multiple factors present in natural conditions.

Even though our results suggest that the estuary (MK) was a suitable location for productive green mussel cultivation, a huge mortality rate of the mussel can be found at locations close to the river mouth due to extreme depletion of salinity. However, the further distance from the river mouth, where seawater salinity is higher than 24 psu and not lower than 15 psu during the year, can promote the mussel production potential. In addition, cultivation periods should be a concern as an important factor for effective green mussel production. The results showed that in estuarine areas (MK), salinity decreased extremely in September and October (Figure 2). This phenomenon can cause the death of green mussels. To avoid tremendous production loss, farmers should not culture green mussels during these months. The suitable period is November to August in the next year, which is when salinity is higher than 24 psu. Moreover, the mussels should be harvested just before the extreme salinity reduction in September. This information can be useful in the formulation of cultivation plans and can provide guidelines for farmers to enhance green mussel production in estuarine areas.

#### 4. Conclusions

The results from this study demonstrated that each cultivation area had unique seawater characteristics, and as a result, a significant difference in green mussel production potential. Seawater in BKT (pond condition) areas had higher suspended solids, chlorophyll *a*, and nutrients concentration than in other areas. However, salinity in the pond was very low. This caused a reduction in green mussel production over time. The seawater in SRI (coastal line areas) was very clear and had the highest transparency and salinity. These conditions were suitable for mussel growth. However, the lowest chlorophyll *a*, and nutrients concentration corresponded to low food availability. Therefore, even though mussel production in the SRI tended to increase, it was of low potential. Seawater in the MK (estuarine areas) was different from BKT and SRI. There was salinity fluctuation, but the salinity was still in the optimum range for mussel growth. The concentration of chlorophyll *a* and nutrients were higher than SRI. This demonstrated that there was more food available in this area. Consequently, the highest green mussel production potential was found in MK. These results explain the influence of seawater variables on green mussel production. This knowledge leads to better understanding of the effects of environmental change on natural resources, especially on marine bivalves. Finally, the data can be employed to describe what takes place when there are unstable seawater parameters and where is the best feasible location for mussel cultivation.

#### 5. Acknowledgements

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#### 6. Conflicts of Interest

There is no conflict of interest.

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