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Research Article

Effect of water quality, sediment quality and microbial community on the growth of green mud crab (*Scylla paramamosian*) at different stocking densities

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Abstract - The objective of this study was to examine the influence of water quality, sediment quality, and microorganisms on the growth of green mud crabs (*Scylla paramamosian*) at different stocking densities. The experiment involved stocking mud crab culture at four densities: (T-1) 1 crab/m^2, (T-2) 1.5 crab/m^2, (T-3) 2 crab/m^2, and (T-4) 3 crab/m^2, with triplicates for each density. The growth performance of the mud crab over a 101-day culture period was monitored. The survival rate at 1 crab/ m^2 was significantly higher than at 2 and 3 crabs/m^2 ($P < 0.05$). Additionally, the average production of 1 and 1.5 crabs/m^2 was significantly higher than at 3 crabs/m^2 ($P < 0.05$). The four stocking densities all had a clay texture, which is not ideal for optimal green mud crab growth. The clay texture can impede the crabs' ability to conceal themselves in clay and dig burrows. The dissolved oxygen levels tended to decrease below 4 mg/L towards the end of the culture period. Alkalinity levels were higher than standard specifications. Nitrite levels in sediment at the 3 crabs/m^2 stocking density were significantly higher than at the 1 crab/m^2 stocking density during the culture period. The dominant phyla found in the ponds were Phylum Cyanobacteria, Actinobacteria, and Bacteroidota, with no microbial pathogens detected. The recommended stocking densities for green mud

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crab culture range between 1 crab/m^2 and 1.5 crab/m^2, as these densities are deemed suitable for maximizing productivity in green mud crab farming.

Keywords: Mud crab, water quality, sediment quality, microbial community, stocking density

1. Introduction

The green mud crab (*Scylla paramamosian*) can be found across the Indo-Pacific region. These crabs typically inhabit in intertidal areas within mangrove forests and estuaries (Chen & Wang, 2019; Mondal et al., 2020). Green mud crab is abundant along the coasts of Gulf of Thailand (Nooseng, 2015). Green mud crabs hold significant economic value due to their high market demand, particularly for females with mature ovaries and large sizes (Fazhan et al., 2017). Green mud crab juveniles, also known as seeds, are typically cultivated through extensive culture methods in earthen ponds. The stocking rate is commonly 1-3 crabs/m^2 (Keenan, 1999). The global demand for mud crabs is steadily rising with each passing day, especially in countries such as China, USA, Australia, Canada, Thailand, and India (Islam et al., 2017). The mud crab farming in Thailand increased by 51.35 percent in 2020. The total mud crab (*Scylla* sp.) cultivation during that year amounted to 2,555.81 tons, with a market value of 876.56 million Thai baht (or equivalent to US$ 230,388) (Department of Fisheries Thailand, 2018).

Normally, the cultivation of mud crabs is carried out in earthen ponds (Nooseng, 2015). Adequate water quality plays a crucial role in the success of aquaculture. Maintaining the right combination of physical, chemical, and biological characteristics in pond water is essential for the successful production of aquaculture (Chowdhury et al., 2012).

The excessive accumulation of feces and uneaten feed can result in the deterioration of ponds and a decline in water quality. It is therefore crucial to maintain optimal water quality in order to ensure the health and well-being of green mud crabs in aquaculture (Munni et al., 2015). In addition, sediment quality in earthen ponds used for aquaculture is a vital factor for successful rearing (Hussenot & Martin, 1995). Green mud crabs, as benthic organisms, primarily inhabit the bottom of surface ponds. The interplay between sediment quality, water quality, and green mud crab production influences the chemical and biological processes taking place in the soil of these ponds (Boyd, 1998). The consumption of oxygen by microbes in the decomposition of organic matter leads to oxygen depletion. This process produces carbon dioxide, ammonia, nitrite, and nitrate through mineralization, which can reduce sediment quality. The presence of toxic material poses a risk to mud crab culture, affecting growth, morality, and disease susceptibility (Hussenot & Martin, 1995; Avnimelech & Ritvo, 2003).

Nutrients from feed such as carbon, nitrogen, and phosphorus can accumulate in pond soil ranging from 5% to 40%. It was observed that a significant portion of nitrogen (75%) and phosphorus (80%) that was not recovered from harvested aquatic animals ended up in the pond bottom (Avnimelech & Ritvo, 2003). Moreover, the excessive accumulation of nutrients leads to a significant algae growth at the bottom of the pond, potentially causing a depletion of oxygen during nighttime (Bai et al., 2023). In order to enhance the soil properties of the pond and make it more conducive for aquaculture in the long run, the implementation of effective pond management practices is crucial.

Hence, microbial communities in aquaculture ponds are essential for

nutrient cycling (Deng et al., 2020). Excessive accumulation of organic matter during the later stages of cultivation can lead to uncontrollable water quality due to microbial degradation (Fan & Li, 2019; Rao et al., 2000). This process may decrease beneficial probiotic species and promote the growth of pathogens in the pond, ultimately resulting in an unhealthy ecosystems and environmental stress in mud crab ponds (Huang et al., 2018). In addition, alterations in the aquaculture pond environment are linked to changes in microbial composition. These changes also have an influence on disease outbreaks that impact mud crabs (Deng et al., 2020; Bentzon-Tilia et al., 2016). *Vibrio parahaemolyticus*, for instance, has been found to cause the death of green mud crabs (*Scylla paramamosian*) by affecting the metabolites involved in energy biosynthesis and the innate immune system (Zhang et al., 2019). Consequently, the cultivation of mud crabs in earthen ponds is affected. The regulation of environmental conditions within ponds plays a crucial role in green mud crab aquaculture. This research aimed to examine variations in water quality, the change in pond sediment under various stocking densities, as well as the analysis of bacterial composition in the pond water, focusing on the impacts of these variables on the growth of green mud crabs raised in the earthen ponds. This study can be a guideline for managing aquaculture, water quality, soil quality, and microbial composition including enhancement of longevity of green mud crab culture in earthen ponds.

2. Materials and methods

2.1 Animals and experimental design

The green mud crab (*Scylla paramamosian*) was subjected to four different densities in a culture that took place from May to August, 2021. The green mud crab seed was obtained from the Samutsongkram Fisheries Research Station at Kasetsart University in Samutsongkram Province. Three ponds were renovated for this purpose, with varying water surface areas of 80,000 m^2 and an average water depth of 1.0 m. The salinity for mud crab culture was 25 ppt. The water used for filling the ponds and exchanging water was sourced from the canal and groundwater. To maintain water quality, exchanges were conducted every two weeks. The mud crabs were fed twice a day with chopped fish (*Sardinella sp*.), with the amount equivalent to 10% of their body weight. The amount of was adjusted every two weeks. Feeding trays were placed in each cell, with two trays per cell. Additionally, each cell was equipped with 40 box shelters, providing approximately one box per 5 m^2. Mechanical aerators were employed to ensure proper aeration. Furthermore, a plastic box shelter measuring 15x40x10 cm was placed in the pond after the application of dried pond lime.

The experimental mud crab culture compared four different treatments namely: T-1, T-2, T-3, and T-4. The ponds used in the experiment had a size of 800 m^2. The mud crabs, with an average carapace width of 2 centimeters, were stocked at different densities for each treatment: 1 crab/s (200 crabs) for T-1, 1.5 crab/s (300 crabs) for T-2, 2 crab/s (400 crabs) for T-3, and 3 carb/s (600 crabs) for T-4. Each pond was divided into four cells of 200 m^2 each, with 3 replicates separated by PVC plastic netting. The experimental design in this study was a randomized complete block design (RCBD).

2.2 Water and sediment quality

Water samples were collected from three mud crab ponds from 4 site surface water at 30 cm of 1L monthly by water sampler.

Water quality parameters including dissolved oxygen (DO), water temperature, and conductivity were measured using a hand-held multiparameter model YSI 550A; the pH level was determined using a pH meter YSI 60/10 FT; salinity was measured

using Salinometer; and transparency was assessed using a Secchi disc. These measurements were taken daily at 07.30 in each cell. In addition, the alkalinity was determined using the titrimetric method; the total ammonia was quantified using the phenol-hypochlorite method; the nitrite levels were assessed using the colorimetric method; the orthophosphate concentrations were measured using ascorbic acid; the BOD 5 days was determined through the 5-day BOD test; and hardness and calcium levels were analyzed using the EDTA titrimetric method. These measurements were conducted on a biweekly basis. The sediment depth was measured with a transparent plastic pipe with a diameter of 5 cm (Steeby et al., 2004).

Meanwhile, sediment samples were collected from both the top and bottom areas of each cell monthly, at a depth of 5 cm by core sediment, and were then combined to create composite samples.

Samples of sediment were dried at 102 ˚C for analysis of dry bulk density (Blake & Harte, 1986). The soil pH was measured by the glass electrode technique (Thunjai et al., 2004). Dried sediment samples at 60 ˚C were prepared for organic matter analysis using the Walkley Black method (Nelson & Sommers, 1982). Analysis of soil particle size distribution was conducted using the hydrometer method (Weber, 1977). The method described by Chuan and Sugajara (1984) was used to determine soil nutrients. Nikhom (2019) further modified the method by incorporating the Azide modification as recommended by APHA, AWWA, and WEF (1998). The measurement of milligrams of carbon dioxide evolved per gram of soil incubated in the dark at 20 ˚C was used to assess soil oxygen demand (SOD). The concentration of soil SOD ($mg\text{-}O_2/g^{-1}hr^{-1}$), was then calculated as follows Nikhom (2019): SOD = ((DO_0-DO_1) s - (DO_0-DO_1) b)/W×T. When DO_0 is the initial DO concentration or DO concentration after each re-aeration, DO1 is DO concentration after incubation of 5 hours, (DO_0-DO_1) s is the DO concentration of soil sample, (DO_0-DO_1) b is DO concentration of blank, T is incubated time (hour), W is soil volume (g).

2.3 Microbial community

2.3.1 Preparation and Sequencing

Water samples were collected at 30 cm from three ponds at the end of crop of 1.5 L by water sampler. Three water samples collected from mud crab ponds were sent to the Pilot Plant Development and Training Institute at King Mongkut's University of Technology Thonburi for further analysis. Subsequently, genomic DNA was extracted from these water samples and subjected to a sequence of analytical procedures such as PCR amplification, PCR purification, library preparation, and sequencing.

2.3.2 16S rRNA amplification, Sequencing data processing, OTU cluster and Taxonomic annotation

The analysis of the 16S rRNA amplicon was conducted by Novogene Co., Ltd., in accordance with the specified protocol detailed in the Novogene Analysis Report of X401SC21123892-Z01-F002. The V4-V5 region of the 16S rRNA gene was amplified using the primers 515F (GTGCCAGCMGCCGGTAA) and 907R (CCGTCAATTCCTTTGAGTTT) (Youssef et al, 2009). Additionally, a unique barcode was incorporated into the reverse primer. The PCR products underwent size selection assessment through 2% agarose gel electrophoresis. Subsequently, the PCR results from each sample were combined in equal amounts, underwent end-repair and A-tailing, and were then ligated with Illumina adapters.

The libraries that were produced underwent sequencing on an Illumina platform using paired-end sequencing, resulting in 250bp raw reads. Quality control of the library was conducted through Qubit, real-time PCR, and bio-analytical methods to assess size distribution and quantify

the samples. Following quantification, the libraries were sequenced on Illumina platforms based on the necessary data volume and optimal library concentration.

After obtaining paired-end reads with unique barcodes for each sample, the barcode and primer sequences were subsequently removed. FLASH (V1.2.7) was employed to merge paired-end reads whenever there was an overlap between reads originating from opposite ends of the same DNA fragment or when raw tags were present. Subsequently, the raw tags underwent quality filtering using specific filtering parameters as outlined by Qiime (Version 1.7.0), with the aim of producing a set of high-quality clean tags.

The Uparse software (Uparse v7.0.1090) was employed for sequence analysis with the incorporation of various informative tags. Sequences that were grouped into the same Operational Taxonomic Units (OTUs) exhibited 97% similarity threshold, leading to their classification within the same OUT. Taxonomic classification was carried out through the Mothur method utilizing the SILVA138 Database. To assess the biodiversity complexity within a sample, six diversity indices – Observed-species, Chao1, Shannon, Simpson, ACE, and Good-coverage – were used for alpha diversity analysis. The Qiime software (Version 1.7.0) and R software (Version 2.15.3) were used to compute and visualize each of these diversity indices within the samples.

2.4 Growth performances

The development of mud crabs was observed biweekly using crab traps. Carapace dimensions were assessed with slide calipers, while weight was recorded using a balance. The duration of the culture phase lasted approximately three months. Following the complete drainage of the pond, all mud crabs were manually harvested once the pond had dried up. Key production metrics included survival rate (SR) which followed formula by Chen et al. (2020), average daily gain (ADG) which followed formula by Hossain (2017), specific growth rate (SGR) which followed formula by Chen et al. (2020), food conversion rate (FCR) which followed formula by Hedayati et al. (2020), carapace width, carapace length, and total production were calculated with the following formula.

SR (%) = (final number of crab/ initial number of crab) × 100

FCR = Feed intake (g)/ Weight gain (g)

SGR (% per day^{-1}) = 100× (ln W_f – ln W_i)/T

When W_{fis} the final weight and W_i is the initial weight While T is the duration of culture

ADG (g/crab/day) = (Average final crab weight - Average initial crab weight) /Time (culture period)

2.5 Data analysis

The water quality indicators were analyzed descriptively. Growth performance and sediment indices underwent One-way ANOVA analysis. Treatments were compared using the Duncan multiple range test, with a significance level set at 5% ($P > 0.05$) in SPSS version 27 (Corp, 2020).

3. Result

(Table 1) displays the growth performance of the green mud crab culture over a period of 101 days under different stocking densities. The results indicate that there were no significant differences ($P > 0.05$) in initial weight, final weight, carapace width, carapace length, ADG, and SGR among the four stocking densities. However, the survival rate of 1 crab/m^2 showed a significant difference compared to 2 and 3 crabs/m^2 ($P < 0.05$). Additionally, the average production of 1 and 1.5 crabs/m^2 exhibited a significant difference compared to 3 crabs/m^2 ($P < 0.05$).

Table 1. The growth performance of green mud crab in ponds with 4 stocking densities for 101days

Parameter	Treatment			
	1 crab/m²	**1.5 crab/m²**	**2 crab/m²**	**3 crab/m²**
Initial weight (g)	2.08±0.05[a] (2.02-2.13)	2.01±0.01[a] (2.00-2.02)	2.06±0.01[a] (2.05-2.07)	2.01±0.07[a] (1.96-2.09)
Final weight (g)	128.77±11.04[a] (121.0-141.4)	132.7±5.81[a] (128.1-139.2)	126.80±6.16[a] (119.71-130.80)	139.73±6.92[a] (132.6-146.4)
Carapace width	8.52±1.52 [a] (8.42-8.70)	8.45±1.02[a] (8.34-8.53)	8.41±1.27[a] (8.32-8.56)	8.53±1.52[a] (8.37-8.66)
Carapace length (cm.)	6.10±1.86 [a] (5.9-6.26)	6.13±1.24 [a] (5.99-6.21)	6.13±0.19 [a] (6.05-6.23)	6.21±0.41 [a] (6.19-6.26)
Feed conversion ratio	5.2±1.40[a] (4.0-6.7)	7.2±1.35[a] (6.2-8.8)	13.7±2.12[b] (11.4-15.6)	21.7±1.80[c] (20.6-23.8)
Specific growth Rate (%/day)	4.39±0.15[a] (4.28-4.56)	4.51±0.09[a] (4.43-4.60)	4.38±0.08[a] (4.29-4.44)	4.51±0.07[a] (4.46-4.59
Average daily gain (g/crab/day)	1.04±0.17[a] (0.88-1.21)	1.09±0.16[a] (0.91-1.19)	1.15±0.15[a] (0.98-1.28)	1.23±0.18[a] (1.02-1.34)
Survival rate (%)	12.17±1.26[a] (11-13.5)	7.43±1.12[ab] (6.6-8.7)	4.75±0.90[bc] (3.75-5.5)	2.61±0.19[c] (2.5-2.83)
Average yield	3.2±0.37[a] (2.9-3.6)	3.0±0.41[ab] (2.7-3.5)	2.5±0.43[bc] (2.0-2.7)	2.2±0.11[c] (2.1-2.3)
Total yield (kg.)	9.62	9.10	7.39	6.68

Note: Different letters in the same row indicate significant differences ($P < 0.05$)

3.1. Water quality indices in green mud crab ponds

Water quality parameters were analyzed in three 800 m^2 ponds used for green mud crab culture and compared with water quality standards for green mud crab culture (ACFS, 2012). Salinity remained within the recommended range at 23-28 parts per thousand. Dissolved oxygen values tended to decrease below 4 mg/L towards the end of the culture period, while BOD levels exceeded 2 mg/L consistently. Total ammonia nitrogen concentrations were generally below 0.4 mg/L but exhibited an increasing trend towards the end of the culture cycle. Water nitrite levels remained below 0.1 mg/L until the final month, following the introduction of aerators in the initial rearing phase. Although the aerators improved surface water oxygen levels, they were insufficient to maintain adequate dissolved oxygen levels at the pond bottom. Alkalinity levels were higher than that standard specifications, likely due to the addition of lime during pond preparation.

Table 2. Water qualities (mean ± SD) in different stocking densities for 101 days

Parameter	Period			
	Before culture	4th week	8th week	The end of culture
pH	8.28±0.04 (8.20-8.34)	8.62±0.08 (8.43-8.70)	8.22±0.02 (8.17-8.24)	8.29±±0.03 (8.22-8.35)
Salinity (ppt)	26.45±0.86 (25.00-27.70)	25.80±0.46 (25.30-26.50)	24.40±0.50 (23.60±25.00)	25.40±0.23 (25.10-25.70)
Conductivity (μm/s)	53.01±1.62 (50.37-55.50)	51.60±1.01 (50.58-53.02)	48.99±0.32 (48.38-49.49)	51.41±0.35 (50.89-51.72)
Dissolved oxygen (mg/L)	4.19±0.30 (3.67-4.70)	3.53±0.08 (3.38-3.64)	3.17±0.03 (3.12-3.22)	2.76±0.03 (2.73-2.81)
Temperature (°C)	32.42±0.50 (31.73-33.10)	30.14±0.03 (30.10-30.21)	30.49±0.03 (30.42-30.52)	30.47±0.04 (30.44-30.53)
Transparency	45±.14 (40-45)	45±0.98 (44-46)	44±2.26 (41-46)	41±0.85 (40-42)
Alkalinity (mg/L as $CaCO_3$)	203.13±21.45 (171-218)	165.83±8.77 (155-181)	186.25±4.83 (179 -194)	262.63±6.53 (251-271)
Hardness (mg/L as $CaCO_3$)	6,520.50±210.94 (5,865-6,570)	6,526.67±96.89 (6,395-6,690)	6,223.75±94.68 (6,000-6,395)	5,591.25±89.27 (5,475-5,715)
Calcium (mg/L as $CaCO_3$)	496.99±12.08 (476-517)	506.34±10.99 (492-528)	690.04±43.60 (597-745)	794.92±20.46 (769-829)
Total ammonia (mg/L)	0.12±0.08 (0.03-0.25)	0.04±0.01 (0.03-0.07)	0.12±0.05 (0.05-0.18)	0.38±0.09 (0.29-0.55)
Nitrite (mg/L)	0.14±0.07 (0.061-0.261)	0.057±0.014 (0.039-0.074)	0.065±0.016 (0.045-0.088)	0.060±0.011 (0.041-0.074)
BOD (mg/L)	4.23±1.02 (2.60-6.00)	(6.52±0.50) (5.80-7.40)	10.53±0.83 (8.80-11.60)	8.20±0.72 (7.40-9.60)

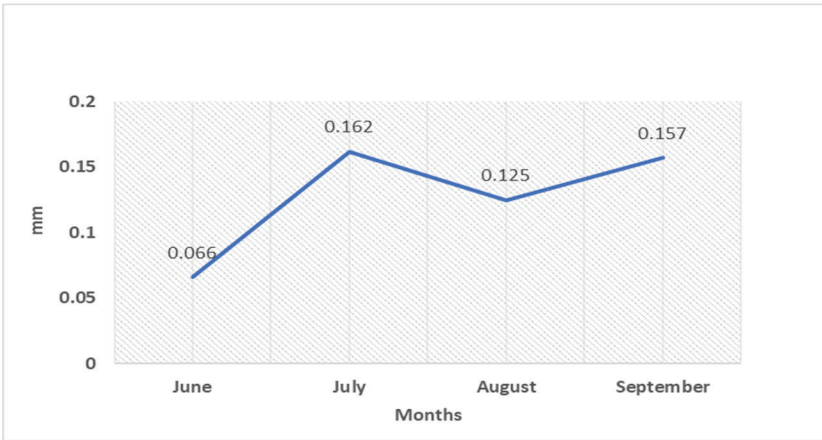

Figure 1. Rainfall collection using the HOBO U30_Station for the period of June to September in 2021 at the Samutsongkram Fisheries Research Station, Kasertsart University in Samutsongkram Province

Based on the monthly rainfall data from the Samut Songkhram Fisheries Research Station, as shown in Figure 1, it indicates that the average rainfall in June was relatively low. However, during July to September, the average rainfall was higher than in June. This resulted in an increased inflow of freshwater into the pond, affecting water quality by reducing salinity and increasing sediment levels due to soil erosion. The water temperature in the pond also dropped, impacting the feeding behavior of the sea crabs.

3.2 Sediment qualities in mud crab ponds

All dry pH sediment samples did not exhibit significant differences ($P > 0.05$) among the four stocking densities. The average dry sediment pH values for the four stocking densities were 7.59 ± 0.81, 7.64 ± 0.81, 7.59 ± 0.89, and 7.60 ± 0.70, respectively. Similarly, the organic matter concentration did not show any significant differences ($P > 0.05$) among the four stocking densities. The average organic matter concentration for the four stocking densities were 4.22 ± 0.35, 4.00 ± 0.33, 4.06 ± 0.23, and 4.03 ± 0.31%, respectively. In addition, soil oxygen demand did not differ significantly ($P > 0.05$) among the four stocking densities, with average values of 2.28 ± 1.30, 2.21 ± 1.45, 2.27 ± 1.42, and 2.36 ± 1.57 mg-$O_2/g^{-1}hr^{-1}$, respectively. The total ammonia sediment did not show significant differences ($P > 0.05$) among the four stocking densities, with average values of 10.76 ± 4.45, 14.80 ± 8.41, 13.73 ± 7.24, and 13.12 ± 7.38 mg/L, respectively. Furthermore, the orthophosphorus sediment did not exhibit significant differences ($P > 0.05$) among the four stocking densities, with average values of 1.94 ± 0.67, 2.56 ± 1.39, 2.73 ± 1.50, and 2.20 ± 0.85 mg/L, respectively. Notably, there was a significant difference ($P < 0.05$) in nitrite sediment between 1 crab/m^2 and 3 crab/m^2, with average values of 5.71 ± 2.22 and 8.68 ± 3.69 mg/L, respectively. The nitrite sediment at 3 crab/m^2 was observed to be higher compared to other stocking densities. The soil texture for all four stocking densities was clay, and the particle size distribution analysis indicated that the percentages of sand, silt, and clay did not differ significantly ($P > 0.05$) among the four stocking densities. Sand particles exhibited a range of 23.32 ± 5.79, 21.65 ± 8.22,19.12 ± 9.22, and 22.53 ± 6.21%. While silt particles displayed values of 32.46 ± 4.82, 31.96 ± 7.30, 31.66 ± 8.73, and 32.16 ± 6.95%. Lastly, the clay particle demonstrated percentages of 42.53 ± 4.46, 42.66 ± 4.82, 44.89 ± 4.22, and 43.33 ± 2.95%.

Table 3. Sediment qualities (mean ± SD) among different stocking densities during the period of June 2021 to September 2021

Parameter	Treatment			
	1 crab/m²	**1.5 crab/m²**	**2 crab/m²**	**3 crab/m²**
Dry sediment pH	7.59±0.81[a] (7.50-7.72)	7.64±0.81[a] (7.53-7.77)	7.59±0.89[a] (7.47-7.77)	7.60±0.70[a] (7.50-7.71)
Organic matter (%)	4.22±0.35[a] (3.46-4.73)	4.00±0.33[a] (3.42-4.37)	4.06±0.23[a] (3.64-4.31)	4.03±0.31[a] (3.49-4.59)
Soil oxygen demand ($mg\text{-}O_2/g^{-1}hr^{-1}$)	2.28±1.30[a] (0.50-4.00)	2.21±1.45[a] (0.70-5.00)	2.27±1.42[a] (0.60-5.10)	2.36±1.57[a] (0.20-5.70)
Total ammonia sediment (mg/L)	10.76±4.45[a] (4.04-16.78)	14.80±8.41[a] (3.36-33.12)	13.73±7.24[a] (4.24-26.16)	13.12±7.38[a] (3.94-28.15)
Nitrite sediment (mg/L)	5.71±2.22[ab] (2.95-9.86)	6.27±3.05[bc] (2.07-14.15)	8.02±3.61[bc] (2.57-15.56)	8.68±3.69[c] (4.89-16.70)
Orthophosphorus sediment (mg/L)	1.94±0.67[a] (1.11-2.86)	2.56±1.39[a] (1.15-6.08)	2.73±1.50[a] (0.87-5.51)	2.20±0.85[a] (0.76-3.71)
Sand particle (%)	23.32±5.79[a] (9.16-28.08)	21.65±8.22[a] (7.72-32.08)	19.12±9.22[a] (5.72-33.16)	22.53±6.21[a] (8.80-30.08)
Silt particle (%)	32.46±4.82[a] (23.36-40.20)	31.96±7.30[a] (17.32-43.64)	31.66±8.73[a] (11.36-44.00)	32.16±6.95[a] (21.36-44.20)
Clay particle (%)	42.53±4.46[a] (36.00-50.64)	42.66±4.82[a] (36.64-49.36)	44.89±4.22[a] (39.36-52.64)	43.33±2.95[a] (40.6448.644)

Note: Different letters in the same row indicate significant differences ($P < 0.05$)

3.3 Sequencing results

The sequencing results of the 16S rRNA analysis using the Illumina paired-end platform produced 250 bp paired-end raw reads (Raw PE), which were subsequently merged and pretreated to obtain clean tags. The identification and elimination of chimeric sequences from clean tags resulted in the generation of Effective Tags suitable for further analysis. The data pertaining to water samples collected from 3 separate mud crab ponds are presented in (Table 4).

Table 4. The results were sequenced on the Illumina paired-end platform of the V4-V5 segment of the 16S rRNA sequencing in water samples collected from 3 separate mud crab ponds

Sample name	Raw PE	Raw tags	Clean tags	Effective tags	Base (nt)
Pond A	187,919	184,002	182,298	136,267	51,239,683
Pond B	177,956	174,284	172,887	120,019	45,094,570
Pond C	179,425	175,732	174,294	135,630	51,024,995

3.4 Alpha diversity indices in mud crab ponds

The microbial diversity in crab culture ponds was assessed using three distinct alpha diversity indices shown in (Table 5). The number of observed species ranged from 750-832. In addition, the individual ponds exhibited a consistent pattern in terms of species richness within the microbial community, as indicated by the similar trends observed in the individual Shannon index, Simpson index, Chao1, and ACE. The alpha diversity indices for the Shanon index, Simson index, Chao1, and ACE were recorded as 4.88 + 0.21, 0.91 + 0.01, 844.14 + 27.74, and 863.48 + 32.10, respectively.

Table 5. The number of observed microbial species in green mud crab ponds

Sample name	Raw PE	Raw tags	Clean tags	Effective tags	Base (nt)
Pond A	820.00	5.10	0.91	874.94	899.52
Pond B	832.00	4.85	0.90	836.37	852.97
Pond C	750.00	4.69	0.91	821.12	837.96

3.5 Microbiome in mud crab ponds

The classification of abundant microbiota had been classified into 2 kingdoms namely Archaea and Bacteria, with a total of 44 phyla, 87 classes, 204 orders, 277 families, and 356 genera. The data on microbial communities in water from three ponds were collected and averaged for reporting purposes. The microbiota contents consisted of 130,639 OTUs with numbers ranging from 814-860 and an average OTU count of 852. Bacteria were found to be the dominant kingdom, accounting for 78.498% of the microbiota in the crab ponds. Within this kingdom, the relative abundance of phyla was classified into four main categories: Cyanobacteria (46.668%), Actinobacteria (23.486%), Bacteroidota (6.155%) and Proteobacteria (2.189%). The three most dominant genera identified were Cyanibuim_PCC-6307 (41.878%), PeM15 (15.419%), and Synechococcus_CC9902 (4.790%). The most common microbial taxa species found in all ponds belonged to the phylum Cyanobacteria, specifically *Synechococcus* spp. (4.683%). The percentages mentioned above represent the relative abundance within the entire taxonomic group. A Venn diagram was used to illustrate the shared and unique OTUs in the culture ponds. It was observed that there were 416 overlapping OTUS that were present in all ponds, while the unique OTUs selectively found in separate ponds ranged from 182-196.

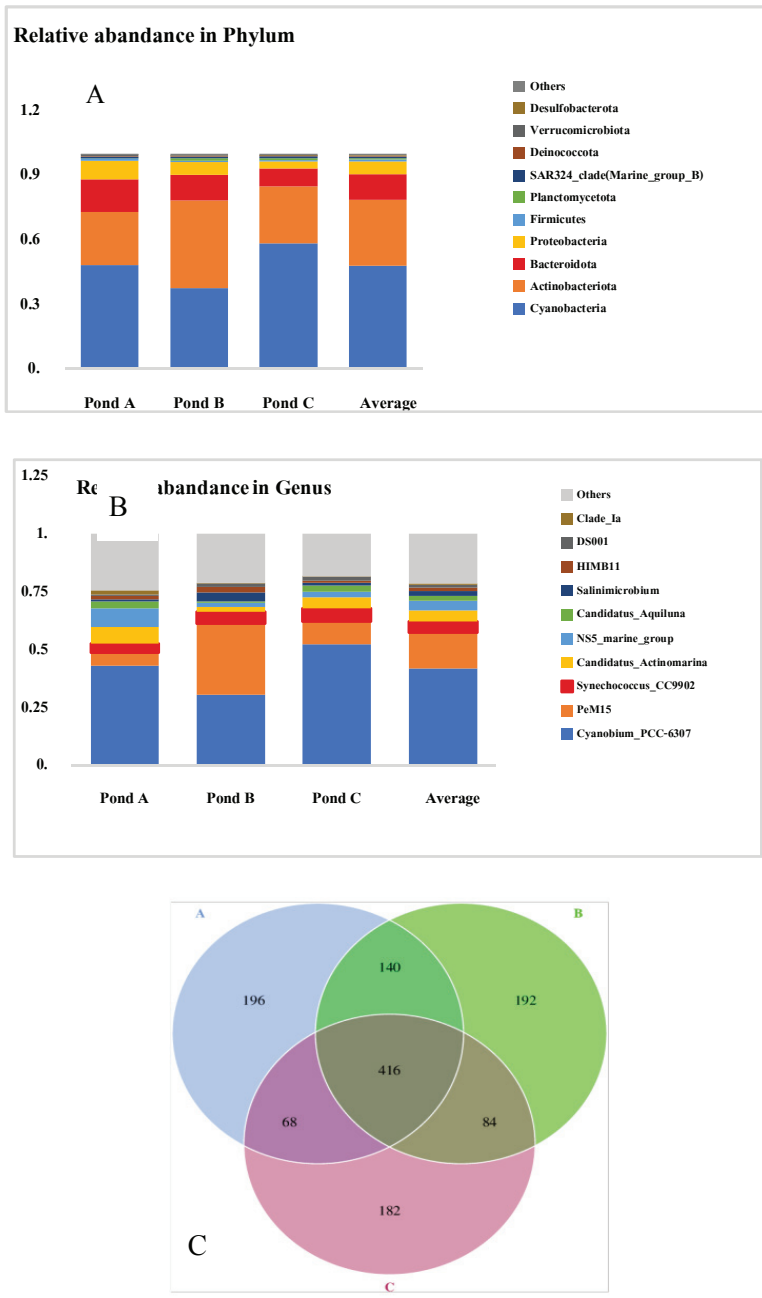

Figure 2. Top ten phyla and genera of microbiota discovered in mud crab ponds (A), top ten genus of microbiota discovered in mud crab ponds (B), and Venn diagram based on OTUs from 3 mud crab culture ponds (C)

4. Discussion

Fresh fish were used as feed for the crabs, leading to potential risk of leftover feed impacting water and soil quality in the ponds, consequently affecting aquatic animal production. This study aimed to examine the effect of changing water quality and the presence of bacterial composition in water. Specifically, Comparing sediment indicators among four different stocking densities. The results indicated that lower stocking densities generally resulted in higher survival rates and more favorable feed conversion ratios (FCR). Previous

studies, Baliao et al. (1981), Mia et al. (2007), and Washim et al. (2022), suggest that lower stocking densities, particularly 5,000 individuals/ha, lead to higher survival rates and more efficient FCR in mud crab culture. The feed conversion ratio (FCR) of 1 and 1.5 crabs/m^2 showed a significant difference compared to 2 and 3 crabs/m^2 ($P < 0.05$). When analyzing the growth data presented in (Table 1), the primary factors taken into consideration to determine the optimal stocking density for mud crabs were the survival rate, feed conversion ratio, and production yield. Based on these factors, it is recommended that stocking densities of 1 crab/m^2 (200 crabs) and 1.5 crabs /m^2 (300 crabs) represent the ideal range for mud crab cultivation. The experimental results align with the previously mentioned research, with one of the contributing factors being the cannibalistic behavior observed in mud crab culture at high stocking densities (Hubatsch et al., 2016). Cannibalistic behaviors have been observed in various crustacean decapod groups. This phenomenon typically manifests in two main scenarios: firstly, mud crabs exhibit cannibalistic tendencies during the molting process; following a successful molt, the mud crab will experience a growth in size and may become aggressive towards smaller crabs, ultimately impacting their survival rates (Zhang et al., 2019; Romano & Zeng, 2016). During the molting period, mud crabs are particularly vulnerable as they are weak, and sediment may easily attach to their gills, making them susceptible to infections that can further affect their survival rates. A comparison of water quality in crab culture with previous reports indicates that an optimal dissolved oxygen content exceeding 4 mg/L is necessary for the well-being of crabs throughout all stages of culture. However, inadequate levels of dissolved oxygen, as observed in this study, can have detrimental effects on the survival rates of crabs.

The water quality parameter analyzed in (Table 2) indicated that the levels of water temperature, salinity, water pH, transparency, conductivity, hardness, calcium, ammonia, and nitrite were suitable (Boyd, 1998; Boyd, 2014; Shelley & Lovatelli, 2011). However, the alkalinity in this study exceeded the optimal level for mud crab culture. The alkalinity in the mud crab pond ranged from 165.83 ± 8.77 to 262.63 ± 6.53 mg/L as $CaCO_3$. Boyd (1998) reported the optimal range for alkalinity in brackish water ponds is 75-125. The high alkalinity observed in this study was attributed to the addition of limestone before pond preparation. The excessive alkalinity negatively affected the growth of crabs. In another study, Aji et al. (2019) reported that lobsters exhibited increased glucose levels as a response to environmental conditions with an alkalinity of 240 mg/L $CaCO_3$. This increase in glucose levels indicated that the lobsters were under stress. Environmental stress can lead to increased metabolic activity in animals, which can affect hormone mechanisms such as pH and salinity. Consequently, high alkalinity may disrupt the biochemical processes in crabs, leading to stress, reduced survival rates, and hindered growth (Aji et al., 2019; Wang et al., 2020). During the late culture period, there was insufficient dissolved oxygen in the pond, with an average value of 2.76 mg/L. The low oxygen levels during this period resulted in the accumulation of uneaten feed and microorganisms, which consume more oxygen during the decomposition of organic matter, leading to oxygen depletion. The dissolved oxygen in the "Monosex Culture of the Mud Crab (*Scylla serrata*) at Three Stocking Densities with Gracilaria as Crab Shelter" ranged from 3.5 to 8.0 mg/L (Trino et al., 1999). Overall, the water quality in the mud crab pond was deemed acceptable, except for the alkalinity and dissolved oxygen levels, which were outside the acceptable range. As Boyd (1998) reported, slow growth can be expected when the dissolved oxygen levels continuously remain low, ranging from 2 to 5 mg/L. Therefore, it is evident that the alkalinity and dissolved oxygen levels in this study were not within the

acceptable range. It was found that the total suspended solids were elevated during the rainy season, attributed to the presence of phytoplankton in the green mud crab culture pond. Salinity levels, however, remained within the recommended range at 23-28 parts per thousand.

Prior to the commencement of crab cultivation, lime material in the form of $CaCO_3$ was used. Boyd (1998) reported that the optimal sediment pH range for aquaculture ponds is between 7.5 and 8.5. A sediment pH of 7-8 is the most conducive for the activity of soil microorganisms and soil bacteria. The sediment pH among four stocking densities was determined to be at an appropriate level for a mud crab pond. Despite the fact that the feeding volume in high stocking density culture was higher compared to a low stocking density culture, the ammonia levels in the sediment among all stocking densities did not show significant differences. The presence of ammonia nitrogen in the sediment was found to be influenced by uneaten feed in this study. It is possible that there was a similar amount of uneaten feed in each of the stocking densities, which could explain why the ammonia levels in the sediment did not vary significantly among all stocking densities.

The mineralized nitrogen in soil, with a pH range of 5-8, is primarily present in the form of ammonium ($NH4^+$). Within the sediment, microorganisms, and plants uptake a significant amount of ammonia N, which subsequently converts into organic nitrogen. However, it is important to note that ammonia can be easily lost from both sediment and water through the nitrification process, ultimately escaping into the air (Boyd, 1995). The growth of mud crabs remains unaffected in this study, as the sediment pH levels for all stocking densities were within the optimal range. However, it is important to note that an increase in pH above 8.5 can lead to the toxicity of ammonia, which can hinder the growth of mud crab (Boyd, 1998).

Nitrite (NO_2^-) is a crucial intermediate in the both nitrification process, where ammonia is converted to nitrate, and the denitrification process, where nitrate is reduced to nitrogen gas. In this study, higher stocking densities, such as 3 crabs/m² compared to 1 crab/m², result in increased organic waste from feed and metabolic activities in 3 crabs/m². This organic matter accumulates in the sediment, driving microbial decomposition and raising oxygen demand, particularly near the sediment-water surface. When oxygen levels deplete the accumulation of nitrite in sediment can released into water bodies where both water and sediment quality deteriorate. High levels of nitrite can impact the oxygen-carrying capacity of crabs by reducing levels of oxyhemocyanin and increasing energy catabolism, which can result in hypoxia in tissues, reduced growth rates, and higher mortality rates (Hargreaves, 1998; Hong et al., 2009).

Throughout the entire culture period, the decrease in dissolved oxygen levels in the pond caused by the rainy season and the decomposition of organic matter can have an impact on the process of nitrification and denitrification. Boyd (1995) revealed that crustacean blood contains hemocyanin, a compound with copper instead of iron in fish blood; however, nitrite can be harmful to crustaceans. In aquaculture ponds, phosphorus plays a crucial role in the growth of phytoplankton as Funge-Smith and Briggs (1998) reported that 24% and 84% of nitrogen and phosphorus accumulation in pond soil were not recovered in the harvested period. Additionally, 63.5% of phosphorus accumulation in soils was observed in semi-intensive shrimp culture, with the main source being feed (Páez-Osuna et al., 1997). Phosphorus accumulates in sediment from feed mainly in this study. In ponds with clay soil, the absorption of phosphorus increased (Boyd et al., 2002). The accumulation of nitrogen and phosphorus ultimately leads to the production of nitrogenous waste, which contributes to the deterioration of the pond.

It was noted in this study that the concentration of organic matter slightly exceeded the optimal range among all stocking densities. The recommended organic matter concentration range of 1-3% is deemed suitable for aquaculture ponds (Boyd et al., 2002). Organic matter concentration originates from uneaten feed and dead plankton. Microorganisms require substantial amounts of oxygen to break down organic compounds, leading to oxygen depletion in the surface sediment. The consumption of soil oxygen is contingent upon the oxygen levels present in the water, serving as an indicator of the mineralization process and benthic community metabolism. Under anoxic conditions within the sediment layer, toxic substances resulting from the decomposition of organic compounds may diffuse from the sediment to the water, impacting both mud crab and water quality (Avnimelech & Ritvo, 2003). The excessive accumulation of organic matter can lead to the release of sediment, which can adversely affect the growth and survival of mud crabs.

All mud crab ponds underwent renovations. The new pond at the bottom contains a high concentration of clay particles. According to other research, the ideal clay particle for aquaculture ponds should be a minimum of 20% to 30% (Boyd & Thunjai, 2002). The suitable soil textures for mud crab culture include sandy clay, clay loam, and sandy clay loam (Trino et al., 1999; Antony et al., 2019). However, the soil texture of the four stocking densities consisted of clay, which is not conducive to optimal mud crab growth. The presence of clay texture may hinder the crabs' ability to hide in clay and dig burrows, as the particles are tightly compressed with no open spaces in between (Boyd, 1995).

Three ponds were discovered to contain Phylum Cyanobacteria, Actinobacteria, and Bacteroidota, with the latter being identified as the dominant phylum, as indicated by previous research. Zhang et al. (2019) reported that the bacterial structure in water from vannamei shrimp ponds was examined across different cultures. In traditional ponds, the dominant phylum consisted of Proteobacteria, Tenericus, Bacteroidetes, and Cyanobacteria, while in intensive ponds, the dominant phylum was reported to be Proteobacteria, Bacteroidetes, Tenericus, and Cyanobacteria. Mutoti et al. (2022) reported that Cyanobacteria, a group of blue-green algae or phytoplankton, was found to increase within the aquaculture pond, potentially due to factors such as nutrient flow, temperature, light, pH, and the presence of uneaten feed. The blooming of cyanobacteria can lead to hypoxic conditions at night and the production of cyanotoxins, which may negatively impact the growth of mud crab. Alfiansah et al. (2018) reported that the microbial community in water from semi-intensive and intensive shrimp ponds revealed the presence of the pathogen *Vibrio parahaemolyticus* in both systems. However, the results of this study indicated that no pathogenic microbes were found in the three ponds.

5. Conclusions

The primary objective of this study was to examine the water quality, sediment quality, microbial community in water, and growth performance at different stocking densities of mud crab culture in earthen ponds. Although there were no significant differences observed in water quality and sediment quality among the different stocking densities, certain parameters such as alkalinity and dissolved oxygen, soil texture, organic matter, and nitrite sediment had an impact on the growth of mud crab, along with the occurrence of cannibalism within each stocking density. Notably, mud crab cultured at stocking densities of 1 and 1.5 crab/m^2 exhibited a favorable growth performance. The microbial community analysis revealed that the dominant phyla present were Cyanobacteria, Actinobacteria, and Bacteroidota across the three ponds. To ensure the longevity of mud crab ponds, it is recommended to remove organic matter and dry the pond after harvesting.

Human/Animal ethics declaration

The letter of approval for animal care was approved by the Institutional Review Board of Kasetsart University (ACKU64-FIS-003)

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