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A Study of the composition of phytoplankton (*Scylla paramamosian*) in an earthen mud crab pond

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Abstract Phytoplankton in a mud crab pond conducted from June to August 2021 at Samutsongkram fisheries research station of Kasetsart university, Samutsongkram province that found 18 species and 5 phyla of phytoplankton which identified to be blue green algae (1 species), green algae (2 species), diatom (12 species), dinoflagellate (1 species) and euglena (2 species). The culture period was during the rainy season, which affected the growth of the phytoplankton. Throughout the culture period, the dominant group comprised diatoms (61.74% of total phytoplankton), followed by blue green algae (31.76 %) and euglena (4.24%), respectively. By the 8th week of the culture period, the phytoplankton density had decreased in all groups. Phytoplankton density of the diatom group had a negative relationship with dissolved oxygen; r= -0.814, (P<0.01). Phytoplankton density of the green algae, dinoflagellate, and euglena groups showed negative relationships with temperature: r=-0.970, (P<0.01), r= -0.879, (P<0.01), r=-0.818, (P<0.01), respectively, and the phytoplankton density of blue-green algae had relationships with salinity: r= 0.692, (p<0.05) and conductivity r= 0.708, (p<0.05). Monitoring of changing ecological factors is crucial for aquaculture ponds. This information can be used to develop useful guidelines for mud crab farming in earthen ponds.

Keywords: Phytoplankton, Water quality, Mud crab, Earthen pond

Introduction

Mud crabs are economically important aquatic animals in Asia (Samidjan *et al.*, 2022). They comprise high market value aquatic production in Thailand, especially large mature males, or females with ripe ovaries and large mature males (Nooseng, 2015; Fazhan *et al.,* 2017). In 2020, the value of mud crab farming increased, and total mud crab production in Thailand was 2,555.81 tons, valued at 876.65 million Baht (US$25.6 million) (Department of Fisheries Thailand, 2021). In aquatic systems, light energy and mineral nutrients play

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significant roles in determining the outcomes in primary production (Peeters *et al.,* 2013). Generally, trash fish are fed to mud crabs (Shelly and Lovatelli, 2011); in aquaculture ponds. Using fresh fish feed produces a higher level of nutrients and total organic carbon and phosphorus than does a pellet diet in a pond, but this higher nutrient level can cause deterioration of water quality (Meng *et al.,* 2018; Deng *et al.,* 2019). As phytoplankton uptake nutrients for their growth, excessive nutrients generate algae bloom, which leads to decreased oxygen, adversely affecting the growth of mud crabs. Ultimately, this causes farmers to lose income. Obviously, phytoplankton have a relationship with water quality and nutrient loading, and increased cyanobacteria and dinoflagellate are undesirable in an aquaculture pond. Measuring changes in the structure of plankton populations can be an effective method for assessing water quality in aquaculture ponds (Meng *et al.,* 2018; Rahman *et al.,* 2018).

Environmental factors and pond management are vital to mud crab production, and aquaculture ponds should be evaluated using a variety of criteria, such as those for social, economic, and ecological or environmental sustainability (Bosma and Verdegem, 2011). Understanding the relationship between water quality and phytoplankton biomass is an important environmental factor for maintaining a good yield of mud crabs. The purpose of this study was to understand the composition of phytoplankton and its relationship to water quality.

Materials and methods

Site selection and pond management

The research was carried out at three mud crab earthen ponds located at Samutsongkram Fisheries Research Station in Samutsongkram Province. Water quality and phytoplankton samples were collected from June to August, 2021. The three earthen ponds used for mud crab culture had a total area of 800 m^2. Each pond contained 1,500 crabs, and, in each, an aerator was in operation. Water was obtained from nearby canals; the depth for mud crab rearing was maintained at approximately 1 meter, and water was exchanged twice a week. The mud crabs were fed chopped trash fish in an amount equivalent to approximately 10 % of body weight twice a day.

Water quality sampling and assessment

Water samples were taken from four distinct sites within the three mud crab ponds at a depth of 30 cm below the surface. Water quality parameters were monitored daily (between 7.30 and 8.30 a.m.). Daily tests were carried out for factors such as dissolved oxygen; water temperature, using DO meter model YSI, Pro20; pH, using pH meter model YSI, Pro20; conductivity, using conductivity meter model Ez 9909 SP; salinity recorded by Portable Refractometer, model RHS10ATC; and transparency, using a Secchi disc. Water samples were also measured twice a week for such factors as total alkalinity, total hardness, total ammonia, and nitrite. Water samples were measured monthly for factors such as total suspended solid and biochemical oxygen demand (BOD). The APHA, AWWA, and WEF (2012) standards for examining water and wastewater were followed in all water quality measurements.

Total phytoplankton estimation

A total volume of 50 liters of water was subjected to filtration through a 20-millimeter plankton net monthly. The sampling collected 4 replications per pond, which were taken from the same locations as the water sampling. The phytoplankton samples were fixed with a 4% concentration of formalin, and were taxonomically categorized using relevant phytoplankton taxonomic documents, after which the phytoplankton density was investigated.

Data analysis

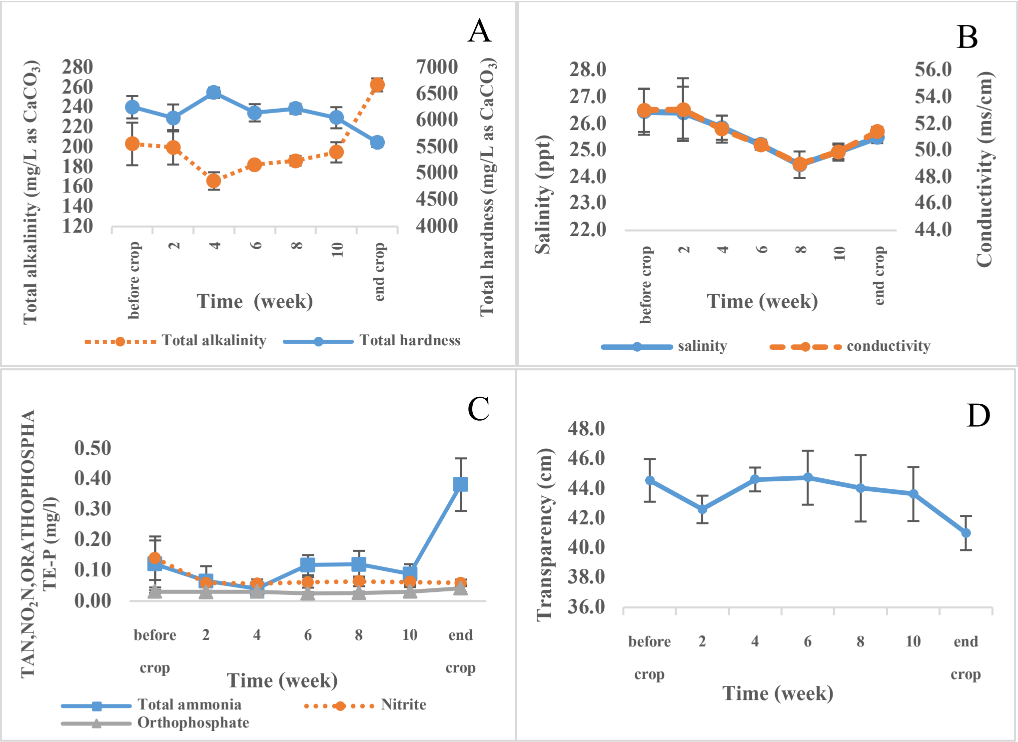
Data were collected in relation to the monthly water quality indices and phytoplankton samples and were then analyzed by correlation. Before testing the correlation between phytoplankton and water quality indices, the normality was examined. If the data met the parametric statistic condition, Pearson's correlation was applied; otherwise, a Spearman correlation was conducted by the SPSS version 27 (IBM Corp., 2020). Analysis of the phytoplankton community by measures such as density, diversity index, evenness index and dominant index followed Krebs (2001).

Results

Water quality variables in mud crab pond

Measured results of water quality factors such as dissolved oxygen, pH, water temperature, salinity, and transparency in the mud crab ponds tended to be lower due to the rainy season, while total alkalinity demonstrated the tendency to rise because of liming application. In terms of nutrients, nitrite and orthophosphate were low and quite stable during the culture period, while the total ammonia concentration increased through to the end of crop, resulting in high proportions of total ammonia and orthophosphate. Because uneaten feed can cause ammonia in water, the ratio of total ammonia increased through to the end of crop as feed was continually added to the mud crab ponds.

The water quality analysis results were compared with the water quality recommended for mud carb cultivation by the Thai Agricultural standard, ACFS (2016). Physical indices such as total suspended solids and transparency did not meet the ACFS (2016) recommendations. The total suspended solids in all periods were higher than 150 mg/L and the transparency was less than 40 cm. throughout the study. Both indictors were high in the rainy season, which was the period of study. Total alkalinity was higher than recommended from the beginning of the period until the end of crop. Although total ammonia tended to increase, in terms of mud crab culture, total ammonia concentration was still within the defined safety limit.



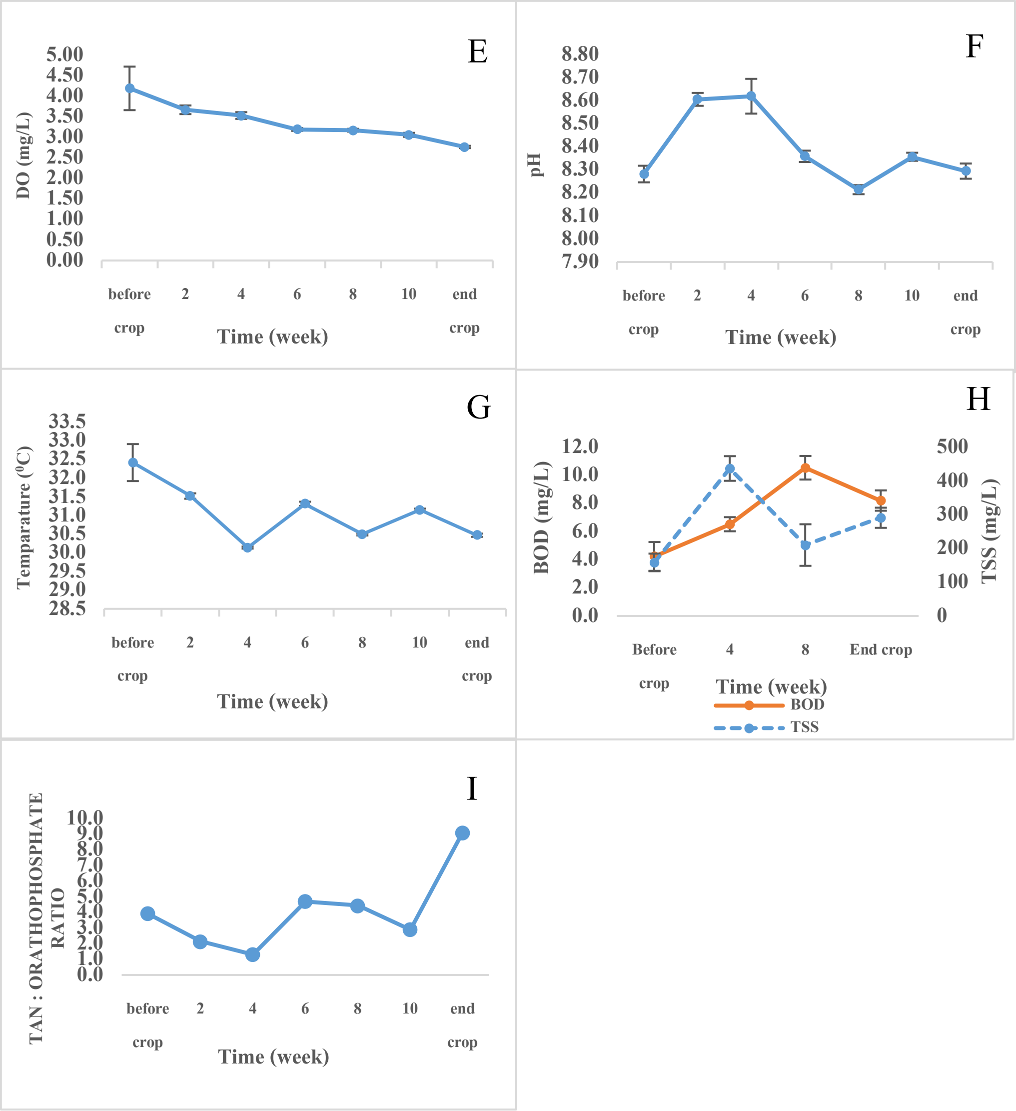


Figure 1. Biweekly and monthly variation of physical and chemical water quality indices in mud crab culture (Fig A-H). The ratio of total ammonia nitrogen and orthophosphate displayed in fig. I. The error bar showed the standard deviation

Phytoplankton community structure and ecological index in mud crab ponds

The analytical results of the phytoplankton community structure from the three mud crab ponds were reported. In this study, the phytoplankton species were identified within 5 groups, with 18 species comprising blue green algae (1 species), green algae (2 species), diatom (12 species), dinoflagellate (1 species) and euglena (2 species) (Table1). Prior to the culture period, it was found that the phytoplankton were dominated, in terms of quantity, by the diatom group (52.26 %) and blue-green algae (47.74 %). With respect to the key indices, the average phytoplankton diversity index, evenness index, dominance index, and species richness index were 1.08±0.04, 0.21±0.04, 0.76±0.07 and 0.63±0.05, respectively. The dominance index value was greater than 0.5, while the species richness index rose throughout the first month of culture since the phytoplankton were increasing., although the greatest number of plankton species were found during the second month of rearing. Increases were seen in the diversity index and the dominance index during the culture period, and the dominance index value was highest during the first month of the culture period. On the other hand, the evenness index decreased throughout the first month of the culture period (Table 3).

Table 1. Average density of phytoplankton community structure in mud crab pond

Specie composition	**Average density of phytoplankton community structure (cells/liter)**			
	Before culture	**1 month**	**2 months**	**The end of crop**
Division Cyanophyta				
Class Cyanophyceae				
Oscillatoria sp.	261.0±239.5	108.3±71.7	10.1±1.4	74.7±38.4
Division Chromophyta				
Class Bacillariophyceae				
Cocconeis sp.	1.3±0.5	20.9±11.1	3.30±1.4	
Gyrosigma sp.	1.3±1.0	209.4 ±157.1	18.0±14.2	38.5±12.9
Navicula spp.		8.60±5.2	1.33±0.49	
Trachyneis sp.		-	1.33±0.49	
Nitzschia spp.	121.33±108.8	1.80 ±0.9		
Entomoneis robusta (Mc Call) Reimer	161.7±121.7	6.60±5.0		6.33±2.80
Campyrodiscus sp			1.67±0.49	
Surirella sp.				1.53±0.79
Synedra sp.		1.40±0.5		12.0±4.07
Cyclotella sp.		128.80±82.6	25.7±6.5	71.7±38.5
Coscinodiscus sp.		1.3±1.0	4.1±1.7	4.1±1.7
Aulacoseira granulata (Ehrenberg) Simonsen				10.93±4.4

Remark: an average of phytoplankton in three mud crab pond

Table 1. (Continue)

Specie composition	**Average density of phytoplankton community structure (cells/liter)**			
	Before culture	**1 month**	**2 months**	**The end of crop**
Class Dinophyceae				
Protoperidinium spp.		60.7±51.8	3.87±0.91	2.33±0.49
Division Chlorophyta				
Class Chlorophyceae				
Oocystis parva W.West		62.1±40.5		
Spirogyra sp.			2.33±0.49	3.00±0.89
Division Chlorophyta				
Class Euglenophyceae				
Euglena sp.		26.8±13.4	9.9±1.53	10.00±8.1
Lepocinclis salina Fritsch		8.13±4.30		

Remark: an average of phytoplankton in three mud crab pond

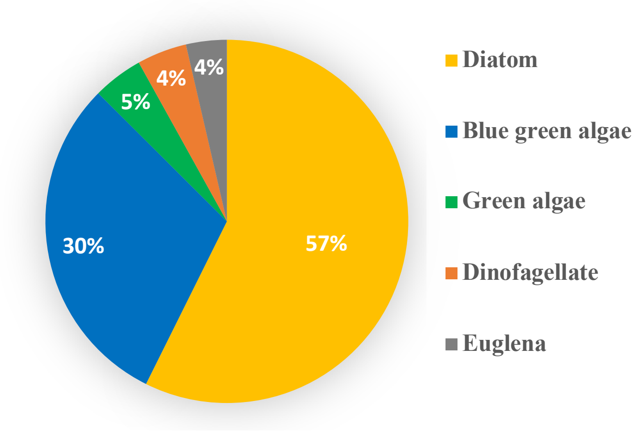


Figure 2. Total composition of phytoplankton in mud crab pond

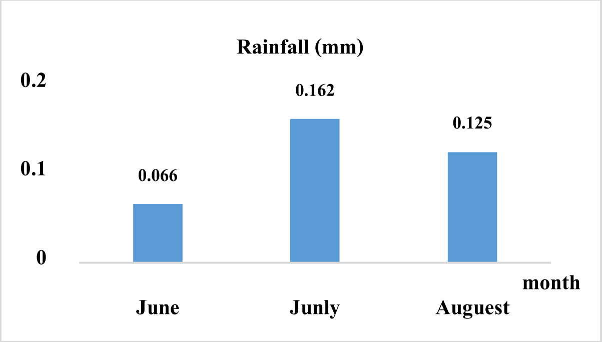


Figure 3. Average of rainfall from June to August at Samutsongkram fisheries research station kasetsart university, Samutsongkram province in 2021. (Recorded by rainfall meter model HOBO U30_Station)

Table 2. changes of phytoplankton community structure in mud crab pond

Phytoplankton group	**Total phytoplankton density (cells/L)**			
	Before culture	**1 month**	**2 months**	**The end of crop**
Blue-green algae	783 (47.74%)	325 (16.80%)	30.4 (12.42%)	224.2 (31.76%)
Green algae		186.4 (9.63%)	7.0 (2.86%)	9.0 (1.27%)
Diatom	857 (52.26%)	1,136.4 (58.73%)	166.2 (67.89%)	435.8 (61.74%)
Dinoflagellate		182.2 (9.42%)	11.6 (4.74%)	7 (0.99%)
Euglena		104.8 (5.42%)	29.6 (12.09%)	29.9 (4.24%)
Total	1,640	1,934.8	244.8	705.9

Remark: The percentage of phytoplankton during culture period

The three most abundant phytoplankton groups were blue green algae; the most dominant species was *Oscillatoria* sp. 74.7±38.4 (cells/L), while, in the diatom group, *Cyclotella* sp. 71.77±38.5 and *Gyrosigma* sp. 38.5±12.9 (cells/L) were the most abundant species at the end of crop.

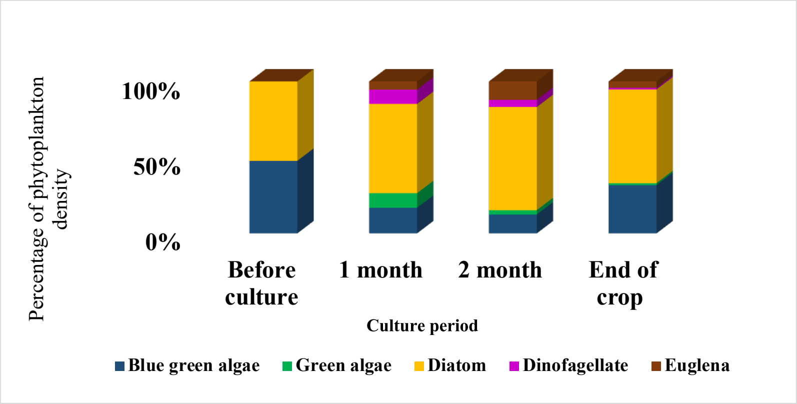


Figure 4. Composition of phytoplankton density group each culture period

Table 3. Ecological index of phytoplankton in mud crab pond

Culture period	**Ecological index**			
	Diversity index	**Evenness index**	**Dominant index**	**Species richness index**
Before culture	1.08±0.04	0.21±0.04	0.76±0.07	0.63 ±0.05
1 month	1.82±0.22	0.14±0.02	0.86±0.02	1.89±0.15
2 months	1.92±0.15	0.17±0.01	0.83±0.01	2.29 ±0.14
The end of crop	1.65±0.13	0.15±0.01	0.85±0.01	1.83±0.004

Correlation coefficient between phytoplankton and water qualities factors

Correlation coefficient analysis was used for comparison between water quality factors and the average density of five phytoplankton groups, comprising the blue-green algae, green algae, diatom, dinoflagellate, and euglena groups (Table 4). The correlation coefficient revealed that the phytoplankton density of the green algae, dinoflagellate, and euglena groups had negative relationships with temperature (r= -0.970, P<0.01), (r= -0.879, P<0.01), (r=-0.818 P<0.01), respectively, but had positive relationships with total suspended solids (r=0.828, P<0.01), (r=0.714, P<0.01), (r=0.775, P<0.01), respectively. In addition, the density of dinoflagellate had a negative relationship with nitrite (r=-0.714, P<0.01), while blue green algae had a positive relationship and diatom a negative relationship with salinity (r= 0.692, P<0.05) and (r= -0.684, P<0.05), respectively. Moreover, blue green algae had a relationship with conductivity (r= 0.708, P<0.05) and diatoms had a negative relationship with dissolved oxygen (r=-0.814, P<0.01).

Table 4. Correlation efficiency (r) between water quality variables and phytoplankton community structure

Variables	**Correlation coefficient (r)**					
	Blue-green algae	**Green algae**	**Diatom**	**Dino flagellate**	**Euglena**	**Total phyto plankton**
Dissolved oxygen	0.323	-0.287	-.814**	-0.211	-0.325	-0.196
	(p=0.306)	(p=0.366)	(p=0.001)	(p=0.510)	(p=0.303)	(p=0.540)
pH	0.487	0.545	-0.549	0.285	0.501	0.035
	(p=0.108)	(p=0.067)	(p=0.064)	(p=0.369)	(p=0.097)	(p=0.914)
Temperature	0.102	-.970**	-0.145	-0.879**	-0.818**	0.236
	(p=0.753)	(p=0.000)	(p=0.652)	(p=0.000)	(p=0.001)	(p=0.461)
Salinity	0.692*	-0.316	-.684*	-0.357	-0.183	0.280
	(p=0.013)	(p=0.317)	(p=0.014)	(p=0.255)	(p=0.569)	(p=0.379)
Transparency	-0.136	-0.310	-0.303	-0.043	-0.180	0.014
	(p=0.674)	(p=0.326)	(p=0.339)	(p=0.894)	(p=0.576)	(p=0.965)
Conductivity	0.708*	-0.354	-0.537	-0.421	-0.257	0.364
	(p=0.010)	(p=0.259)	(p=0.072)	(p=0.173)	(p=0.419)	(p=0.244)
Total ammonia	0.313	-0.352	0.060	-0.499	-0.435	0.063
	(p=0.322)	(p=0.262)	(p=0.852)	(p=0.099)	(p=0.157)	(p=0.845)
Nitrite	0.224	-0.501	0.092	-0.714**	-0.310	0.469
	(p=0.484)	(p=0.097)	(p=0.777)	(p=0.009)	(p=0.327)	(p=0.124)
Orthophosphate	0.204	0.012	-0.188	-0.082	0.272	0.358
	(p=0.526)	(p=0.969)	(p=0.559)	(p=0.801)	(p=0.392)	(p=0.253)
Hardness	0.343	0.185	-0.529	0.329	0.120	-0.322
	(p=0.276)	(p=0.565)	(p=0.077)	(p=0.297)	(p=0.711)	(p=0.308)
Alkalinity	0.077	-0.309	0.476	-0.583*	-0.338	0.545
	(p=0.812)	(p=0.328)	(p=0.118)	(p=0.047)	(p=0.283)	(p=0.067)

Remark: * * Correlation is significant at (P<0.01), * Correlation is significant at (P<0.05)

Discussion

The phytoplankton community structure in mud crab ponds was reported in five composition groups: blue-green algae, green algae, diatoms, dinoflagellate, and euglena. In this study, diatoms (52.26%) and blue green algae (47.74%) were the most abundant before culture, while diatoms (61.74%) and blue green algae (31.76%) had the most abundance of phytoplankton density at the end of crop. Boyd (1989) reported that in brackish water ponds of moderate or high salinity, diatoms generally comprise a major phytoplankton, but, when salinity is low, blue green algae will be the dominant species. Case *et al* (2008) reported on phytoplankton density in shrimp culture ponds, identifying 51 species comprising diatoms (69%), Pyrrophyta (8%), Cyanophyta (12%), Euglenophyta (4%), and Chlorophyta (6%).

During the first month (June) of the culture period, it was found that some of the phytoplankton populations like green algae (9.63 %), dinoflagellate (9.42 %), and euglena (5.42 %) increased. By contrast, the abundance of phytoplankton from all groups fell in the second month (July) of the culture period because the rainfall in July (0.162 mm) was higher than in June (0.066 mm). Despite this increased rainfall in July, total suspended solids decreased slightly; a decline in phytoplankton biomass might be the reason for the total measured suspended solid reduction in the pond water. Water color in ponds is also affected by clay particles during rainfall. Sources of suspended solids in aquaculture ponds include clay particles, detritus, plankton, and bacteria (Boyd, 1998). Suspended particles are displayed in various colors by phytoplankton blooms including green, blue-green, yellow, brown, red, and even black. In terms of color, suspended mineral particles can be as diverse as soils (Boyd and Tucker 2014). In this study, the dominant phytoplankton density comprised mainly diatoms (61.74%) and blue green algae (31.76%). During the entire culture period, the phytoplankton groups with the greatest density were diatoms (57.0 %), blue green algae (30.0%), and green algae (5.0 %). The high proportion of diatoms in this research corresponds to the study of Abide by Mondal *et al.* (2020), who found diatoms (71%) and green algae (79%) to be the two most dominant types of phytoplankton in a mud crab (*Scylla olivacea*) co-culture with different fish species in confined brackish water ponds.

The experiment took place during the rainy season, which reduced the amount of sunlight and its related effects on the density of phytoplankton. Nutrient concentration, light, pH and temperature can each affect the phytoplankton biomass, with differences during or outside of the rainy season (Sun and Boyd, 2013). However, the findings of this study report higher phytoplankton density compared to Samidjan *et al.* (2022), who reported that the average phytoplankton density in a polyculture pond of milk fish and mud crabs was 118.75 cell/L.This research, by contrast, found more than 1,131.37 cell/L during the culture period.

It was discovered that water quality indices including salinity, conductivity, total suspended solid while dissolved oxygen, temperature, nitrite, and alkalinity had a positive relationship with phytoplankton density, but a negative relationship with phytoplankton biomass. This finding agrees with the research of Zhang *et al.* (2021) who reported that animal species in aquaculture, conductivity and high nutrient loading in pond affect phytoplankton diversity.

The water quality indices mentioned above can be used as indicators of changes in specific phytoplankton density in mud crab ponds.

In this study, diatoms and blue green algae were the most abundant phytoplankton, and they were associated with salinity, conductivity, total alkalinity, and total suspended solids. The concentration of all ions in a body of water is referred to salinity (Boyd and Tucker, 2014). Salinity fluctuation affects phytoplankton diversity, and nutrient enrichment may encourage the competitive dominance of a small number of species (Paeal and Tucker, 1995; Larson and Belovsky, 2013). Moreover, diatoms need silica to grow; increasing silica and nitrogen may enhance the growth of diatoms while decreasing the growth of blue green algae (Yusoff *et al.*, 2002). In this study, *Oscillatoria* sp. (453.5 cells/L) was the most abundant phytoplankton; however, in the late culture period, *Oscillatoria* sp. (74.7 cells/L) was the more abundant species due to the level of nutrients increasing throughout the culture period because blue green algae biomass has a relationship with high nutrient supply. Blooming of blue green algae (cyanobacteria) in aquaculture pond results in oxygen depletion in the water, and some species can produce toxin (Paeal and Tucker,1995). It was also found that green algae, dinoflagellate, and euglena are associated with temperature, total suspended solids, and nitrite.

In conclusion, the majority of water quality indicators fell into suitable ranges for mud crab culture, although dissolved oxygen was slightly lower than 4 mg/l. Samples taken prior to culture showed the lowest phytoplankton diversity index. By the end of the first month of the culture period, the species richness index had increased and the evenness index had decreased. During the rainy season, dissolved oxygen should be monitored carefully due to detritus from dead plankton, and because increasing the levels of nutrients can lead to increased phytoplankton populations with a resulting reduction in available dissolved oxygen. Excessive of growth phytoplankton, including algae growth, leads to reduced oxygen in the pond bottom, die offs, and accumulation of toxic compounds such as ammonia, nitrite, and hydrogen sulfide (Case *et al.*, 2008); however, suitable and adequate management can positively affect the healthy growth of mud crab cultures in earthen ponds.

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