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Productivity of Phytoplankton by Using Different Organic Fertilizers in the Glass Aquarium

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Abstract
The experiment intended to estimate the productivity using different organic fertilizer and to identify the species of phytoplankton in the aquarium tanks. The study was conducted in the laboratory of Department of Fisheries and Marine Bioscience (FMB), Jashore University of Science and Technology, Jashore, Bangladesh during November to December, 2013. Phytoplankton samples for culture were collected from fisheries experimental pond by using conical-shaped monofilament nylon net (phytoplankton net). Three treatment such as cow dung (T_1), chicken manure (T_2) and control (T_3) were designed. Each treatment had two replications. In case of T_1 and T_2, 10 g of fertilizer was used in each aquarium tank. Sampling of phytoplankton for counting and identification was done every week and 1 litre of water sample was taken from each tank. Phytoplankton sample for identification were preserved by Lugol's solution. Three physicochemical parameters namely water temperature, dissolved oxygen (DO) and pH were measured in every sampling week during the study period. Water quality parameters of the aquarium tank varied with the variation of sampling week. There was no significant difference between water temperature, pH and DO in case of three treatment. The mean water temperature, pH and DO were 21.85±1.95 °C, 8.38±0.23 and 5.55±0.58 mg/l, respectively. The abundance of phytoplankton in T_1, T_2 and T_3 were 25–65, 25–105 and 6–20 individual/l, respectively. The phytoplankton abundance were influenced by different organic fertilizer and noticed higher in number by using chicken manure. Irregular relationship between phytoplankton abundance and water temperature was found. Phytoplankton showed positive relationship with DO. In this study 15 species of phytoplankton were identified under four groups namely Chlorophyta, Cyanophyta, Bacillariophyta and Euglenophyta. Chlorophyta (52%) was the dominant group in the aquarium. The findings of the present study will help to improve the management strategies of water quality, for estimating the productivity of phytoplankton and for the best use of organic fertilizer especially chicken manure.

Keywords: *Phytoplankton, organic fertilizers, glass aquarium*

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INTRODUCTION
Plankton is the main natural food particles in pond ecosystem [1]. Plankton is a microscopic organism that originates the base of food chain and food web in all aquatic ecosystems. It is an enormous group of aquatic organism drifting about in water under the action of water movement. These creatures are mostly small, many of them are minute, and their structure can only be seen clearly with the aid of a compound microscope, with the exception of some large animals, such as some medusa (*Chynea*, *Physalia* etc), heteropods (pterotrachea) and tunicates (Pyrosoma). Although they belong to different taxa, they have one thing in common, i.e. owing to the lack of locomotion organ (like fish fins); they are weak in locomotion and can only drift about on water at mercy of waves and currents, being incapable of moving anywhere

as fish do [2]. The first links in food chains in inland waters are phytoplankton and are an indicator of production level [3]. Aquatic environments are subject to high temporal variation, with frequent reorganization of relative abundance and species composition of phytoplankton, as a result of interaction between physical, chemical and biological variables [4]. Scientific management for good production of fish in water bodies such as ponds, lakes, sometime requires practical knowledge of the environmental factors of water which affect the aquatic community, as well as the fish production [5]. The zooplankton forms the principle source of food for fish within the water body [6] and zooplankton feeds on phytoplankton. Phytoplankton is the only source of food for the tiny zooplankton. Water quality, i.e. the physicochemical and biological characteristics of water, plays a big role on plankton productivity as well as the biology of the cultured organism and final yields. Water quality determines the species optimal for culture under different environments [7]. The overall productivity of a water body can easily be deducted from its primary productivity, which forms the backbone of the aquatic food chains [8]. Both the qualitative and quantitative abundance of plankton in a fishpond are of great importance in managing the successful aquaculture operations, as they vary from location to location and pond to pond within the same location even within similar ecological conditions [9]. Plankton inhabits oceans, seas, lakes, and ponds [10, 11]. Local abundance varies horizontally, vertically and seasonally. The primary cause of this variability is the availability of light. All plankton ecosystems are driven by the input of solar energy, confining primary production to surface waters, and to geographical regions and seasons having abundant light. A secondary variable is nutrient availability. Although large areas of the tropical and subtropical oceans have abundant light, they experience relatively low primary production because they offer limited nutrients such as nitrate, phosphate and silicate. This results from large-scale ocean circulation and water column stratification. The main production systems for freshwater aquaculture in Bangladesh are extensive and semi-intensive pond poly culture of Indian and Chinese major carps [12]. The growth of fish is strongly correlated with increase in phytoplankton and zooplankton productivity as a result of fertilization. Under poly culture system, the fertilizers increase the level of primary productivity, dissolved oxygen (DO), pH and total phosphorus [13]. The growth of fish is strongly correlated with increase in phytoplankton and zooplankton productivity as a result of fertilization [12]. The relationship between the physical and chemical environment and phytoplankton species composition has been the subject of much discussion [14]. The use of organic and inorganic fertilizers provides basic nutrients and elements required for the production of phytoplankton and zooplankton which serve as a major source of food for fish [15]. Supplementary feeding plays a vital role in semi-intensive system, offering the best means to enhance fish production within shortest possible time. Supplementary feed exerted a significant effect on the body weight, fork length and total length of fish species [16]. In Bangladesh there are millions of ponds and lakes where extensive fish culture is mainly practiced depending on natural food (phytoplankton) which is produced through fertilization [17]. Moreover, systematic studies for selecting suitable organic fertilization between cow dung and chicken manure to produce more plankton for the culture of fish and to increase the fisheries production. Considering the above facts, the present study was therefore, conducted to know the phytoplankton productivity by using cow dung and chicken manure; to identify the species of phytoplankton; and to know the relationship between physicochemical parameters and phytoplankton productivity in treatments.

MATERIALS AND METHODS

Study Area

The experiment was conducted in the laboratory of Department of Fisheries and Marine Bioscience (FMB), Jashore University of Science and Technology (JUST), Jashore, Banagladesh (23°14'01.8"N 89°07'31.3"E). This study was conducted from November to December 2013.

Experimental Design

The experiment was designed in three treatments (T_1, T_2 and T_3) to know the production of plankton in different organic fertilizer and without fertilization. The treatment with cow dung was treated as T_1, treatment with chicken manure was T_2 and treatment with only supply water was treated as T_3 means control (Table 1). Every treatment had one more replication. All the aquariums were same in size and same amount of water were used in every tank.

Aquarium Preparation

The experiment was conducted in rectangular glass aquarium (36-inch x 14 inch x 15 inch), each containing 10 l of tap water. The aquariums were placed near the window for proper lighting. The aquariums were washed properly with running water. These aquarium tanks were placed in the fisheries laboratory.

Sample Collection

Plankton samples were collected from the Fisheries experimental pond in JUST campus. The sample was collected by passing water through conical-shaped monofilament plankton net. It was collected from different parts of the pond. Sample collection was varied with water depth. Collected plankton samples were then taken into big glass jar with water and carried to the Fisheries laboratory for culture and kept into the aquarium slowly with care. About 25 l of pond water was filtered to collect the plankton samples for culture in the aquarium tank.

Use of Organic Fertilizer

Cow dung and chicken manure were used for this experiment. For culture, 10 g of cow dung was used in each tank of T_1 and 10 g of chicken manure in each tank of T_2. At first the collected fertilizers were weighted in electric balance and mixed with water and then sieved with net. Then the solution was mixed with tank water. The solution was sieved to remove other materials in the fertilizer and to get a pure solution. The solution was mixed with water properly.

Plankton Collection and Preservation

Plankton samples were collected in 7 days interval on each sampling date from November to December 2013 by conical-shaped monofilament nylon net (plankton net). About 1000 ml of water was passed through the net from each tank at a time. The mesh size of the plankton net was 90 μm and the diameter of the net at mouth was 30 cm. The plankton condensed at the end of the plankton net. Then it was collected in a beaker in 25 ml of water and fixed firmly. After collection the plankton materials were preserved with Lugol's solution (20 g potassium iodide and 10 g iodine crystals dissolved in 200 ml distilled water) [18]. Lugol's solution was added in an amount of 0.3 ml per 50 ml of sample. After preservation the plankton samples were observed through photographic microscope and photos of the plankton were taken.

Sampling Periods

The experiment was done from November to December 2013. Sampling for identification of phytoplankton was conducted at seven days interval.

Counting

Phytoplankton sample was counted under photographic microscope (Axio cam ERc 5s with axiovixim driver, Carl Zeiss Germany). Glass slide was used for plankton counting. The glass slide was set under the microscope before putting water. Then some drops of water was kept over the glass slide using dropper. Before putting water, the glass slide was cleaned properly. Then the water was observed, the plankton were counted and photos of the plankton were taken. By moving the mechanical stage, the entire bottom of the slide area was examined carefully. About 10–15 ml of water was examined. After this the plankton in 25 ml of water were counted. So 1 l water contains the plankton species in 25 ml

***Table 1:** Experimental Design for Different Treatment for the Production of Phytoplankton.*

Treatment	Tank name	Fertilizer
T_1	A_1	Cow dung
	A_2	
T_2	B_1	Chicken manure
	B_2	
T_3	C_1	No fertilizer
	C_2	

of water. After that this plankton population was multiplied with 10 l of water and the whole plankton population was calculated.

Measurement of Physicochemical Parameter

Temperature

Water temperature was measured at each tank using a mercury thermometer of (0–50) ºC range. The thermometer was kept into tank water for about 1 min. Thereafter the thermometer was kept up and the temperature was measured by observing the centigrade scale of thermometer and the temperature was recorded. The unit of this instrument was ºC.

pH

Water pH was measured by an electrometric pH meter model no: EZDO, 7200. Before using the instrument, it was calibrated with pH 7 and pH 10 buffer solutions. Before taking each reading, the electrode was washed well by distilled water.

Dissolved Oxygen (DO)

Dissolved oxygen (DO) was measured by DO meter model no: YK- 22DO, made in Taiwan.

Classification and Identification of Observed Phytoplankton

Phytoplankton cells were enumerated under a photographic microscope by using glass slide. Recognition of species is a matter of experience. Phytoplankton genera and species were identified using variety of bibliographic references. Identification was done by using different books and checklist of scientists especially followed by Arnold and Vuuren *et al.* [19, 20].

Statistical Analysis

Standard deviations of water quality parameters were done using Microsoft Excel Program 2007 and Statistical Program for Social Science (SPSS).

RESULTS

Water Quality Parameters During Culture Period

Water Temperature

Temperature showed considerable variations during the study period. The maximum water temperature which was 24.9°C was recorded in 1st week and minimum was 19.1 ºC which was record in 2nd week (Figure 1). The highest and lowest water temperature was found in T_1.

Water pH

Highest pH was 8.7 and lowest was 8.00 recorded in 2nd week and 1st week, respectively (Figure 2). The lowest and highest pH was found in T_1.

Dissolved Oxygen (DO)

The range of DO was 4.8–6.7 mg/l. The highest DO was 6.7 mg/l which was recorded in 4th week and the lowest DO was 4.8 mg/l which was recorded in 3rd week (Figure 3). The highest and lowest DO was found in T_3 and T_1, respectively.

Productivity of Phytoplankton in Different Week with Different Organic Fertilizer

The productivity of plankton was measured from each aquarium tanks. The number of plankton cells was counted after processing in the laboratory. The productivity was calculated as the number of unit per litre. The productivity of different groups of phytoplankton was studied weekly, which showed fluctuation both quantitatively and qualitatively and are shown in Figure 4. The productivity in case of T_1 (cow dung) for 1st, 2nd, 3rd, and 4th week was 46, 65, 49 and 15 unit/l, respectively; in case of T_2 (chicken manure) for 1st, 2nd, 3rd, and 4th week was 105, 82, 66 and 25 unit/l, respectively; in case of T_3 (control) for 1st, 2nd, 3rd, and 4th week was 15, 20, 11 and 6 unit/l, respectively.

Diversity of Phytoplankton

All divisions of phytoplankton were identified [19, 20] and four divisions of plankton were found; namely, Chlorophytes, Cyanobacterias, Bacillariophyta and Euglenophyta are the most common freshwater algae.

Abundance and Diversity of Phytoplankton

The abundance of different groups of phytoplankton was studied during the culture period. The abundance of phytoplankton varied from 105 unit/l to 6 unit/l (Table 2). The phytoplankton showed a weekly variation both qualitatively and quantitatively. The highest abundance was 105 unit/l, found in T_2 and it was found with seven different varieties of phytoplankton. The highest phytoplankton variation was found in T_1 and eight varieties were found. At the first of the culture period the production of phytoplankton per litre was high but at the end of the culture period the production per litre was decreased as well as the number of species per litre was also decreased.

Division	Genus	Species	Availability
Chlorophytes	*Schroederia*	*Schroederia sp.*	+++
	Doctylococcus	*Doctylococcus sp.*	+++
	Hormidium	*Hormidium sp.*	++++
	Ankistrodesmus	*Ankistrodesmus falcatus*	++++
	Zygnema	*Zygnema sp.*	++++
	Gonatozygon	*Gonatozygon sp.*	++++
	Chlorella	*Chlorella vulgaris*	+++++
Cyanobacterias	*Oscillatoria*	*Oscillatoria sp.*	++++
	Nodularia	*Nodularia sp.*	++++
	Chroococcus	*Chroococcus dispersus*	++++
	Anabaena	*Anabaena circinalis*	++++
Bacillariophyta	*Thalassiosira*	*Thalassiosira decipens*	++++
	Nitzschia	*Nitzschia paradoxa*	++++
	Asterionella	*Asterionella sp.*	++++
Euglenophyta	*Euglena*	*Euglena gracilis*	++++

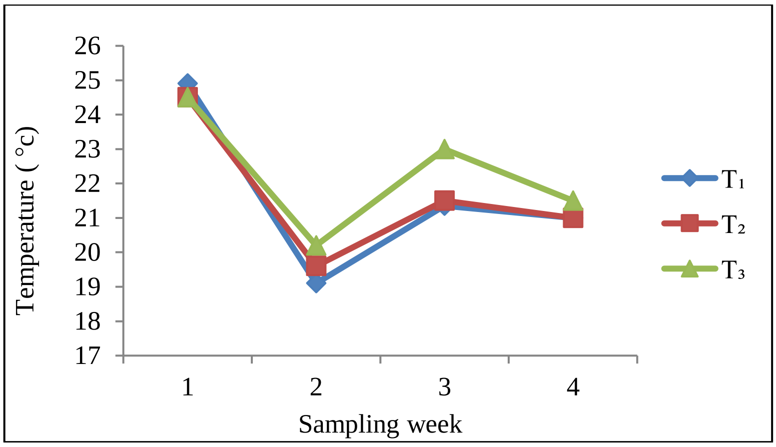

Fig. 1: *Average Water Temperature.*

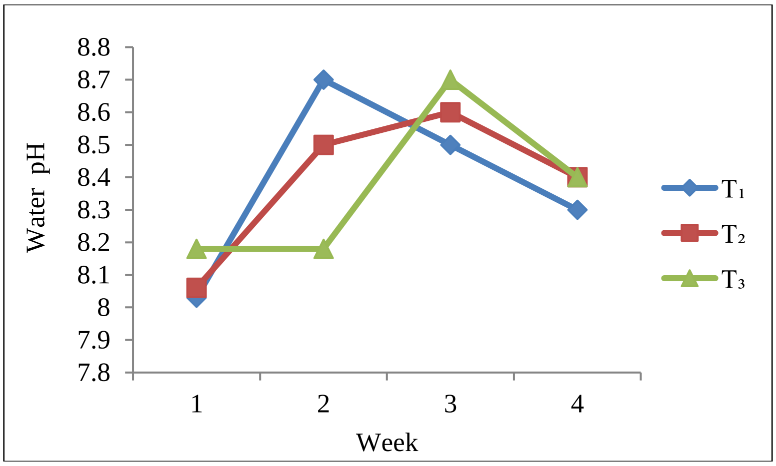

Fig. 2: *Average Water pH in Different Treatment.*

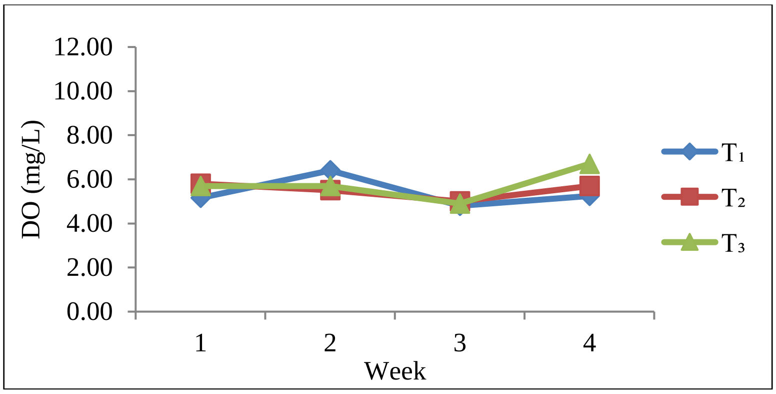

Fig. 3: *Average Dissolved Oxygen in Different Treatment.*

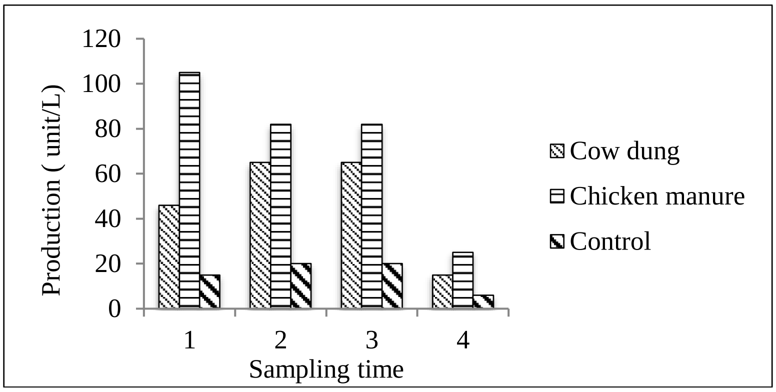

Fig. 4: *Productivity of Phytoplankton in Different Weeks with Different Organic Fertilizer.*

Table 2: *Phytoplankton Abundance and Diversity in Culture Period.*

Treatment	Abundance (unit/l)				Diversity of species			
	12.11.13	19.11.13	26.11.13	03.12.13	12.11.13	19.11.13	26.11.13	03.12.13
T_1	46	65	49	15	8	8	4	1
T_2	105	82	66	25	7	6	3	5
T_3	15	20	11	6	4	3	2	2

Abundance of Different Groups of Phytoplankton (Individual/litre)

Treatment 1

Table 3: *Abundance of Different Groups of Phytoplankton (Individual/l).*

Group	12.11.13	19.11.13	26.11.13	03.12.13
Cyanophyta	10	2	0	9
Chlorophyta	15	32	26	0
Bacillariophyta	8	2	3	0
Euglenophyta	6	3	0	0
Unidentified	0	0	0	0

Treatment 2

Table 4: *Abundance of Different Groups of Phytoplankton (Individual/l).*

Group	12.11.13	19.11.13	26.11.13	03.12.13
Cyanophyta	24	13	21	2
Chlorophyta	27	26	19	8
Bacillariophyta	9	4	0	0
Euglenophyta	0	0	0	3
Unidentified	3	0	0	0

Treatment 3

Table 5: *Abundance of Different Groups of Phytoplankton (Individual/l).*

Group	12.11.13	19.11.13	26.11.13	03.12.13
Cyanophyta	9	6	7	3
Chlorophyta	3	6	0	1
Bacillariophyta	0	0	0	0
Euglenophyta	3	0	0	0
Unidentified	0	0	0	0

Abundance of Different Groups

Treatment 1

At the first week the variation was not significantly different. But in the second week, the Chlorophyta was very high than in other the third week; small amount of Bacillariophyta were also seen. In the last week of culture period, only Cyanophyta were seen and other four groups were not present (Figure 5).

Treatment 2
During the culture period Chlorophyta was dominant and Euglenophyta was in lowest number (Figure 6).

Treatment 3
In treatment 3, Cyanophyta was dominant in all the culture period and Bacillariophyta was not present (Figure 7). In third week only Cyanophyta was recorded and other groups were not present. Chlorophyta was more or less similar in all the culture period.

Total Abundance
Total abundance of different group of phytoplankton during the culture period was dominated by Chlorophyta (52%) and then Cyanophyta (33%) which is shown in Figure 8. It was also shown that the abundance of Bacillariophyta and Euglenophyta was 8% and 7%, respectively.

Relationship Between Phytoplankton Abundance and Water Quality Parameters
Treatment 1
The abundance was high in the second week but the temperature was low in that week. After second week the production decreased gradually but the temperature was more or less same. Other parameters such as DO and pH showed no variable relationship with the production (Figure 9).

Treatment 2
Relationship between phytoplankton abundance and different water quality parameters in case of treatment 2 (Figure 10) showed no variation with the value of DO and pH. At the last of culture period, the production decreased but DO and pH remain more or less same.

Treatment 3
In case of treatment 3, the relationship between plankton production and different water quality are given in Figure 11. The production was too low in treatment 3.

DISCUSSION
In nature, most of the organisms subsist on live food consisting of plants and animals obtained from the environment. The initial source of food for many larval organisms is phytoplankton. Phytoplankton forms the basis of food chain [21]. The natural bloom of phytoplankton is not enough to obtain adequate phytoplankton levels for the production of fish. Fertilizers increase the natural fertility of culture ponds, so fertilizer is used [1]. In the present study, phytoplankton was cultured in aquarium with two organic fertilizers (cow dung and chicken manure). The present work gives some information about productivity of phytoplankton by

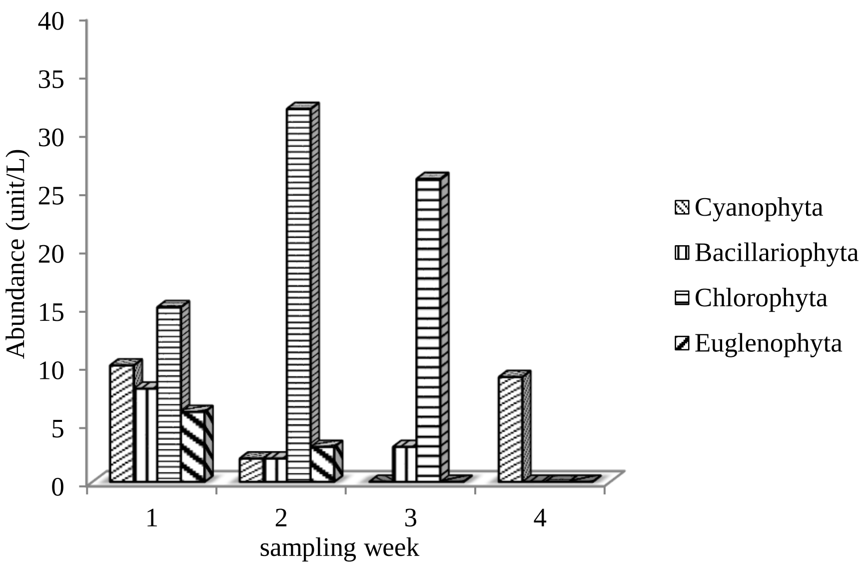

Fig. 5: *Phytoplankton Abundance in Culture Media Prepared with Cow Dung.*

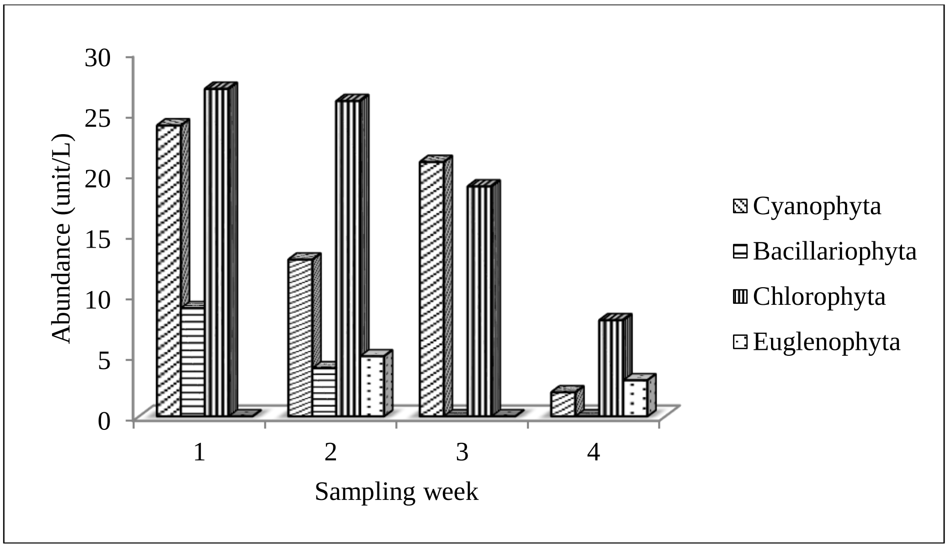

Fig. 6: *Phytoplankton Abundance Found in Culture Media Prepared with Chicken Manure.*

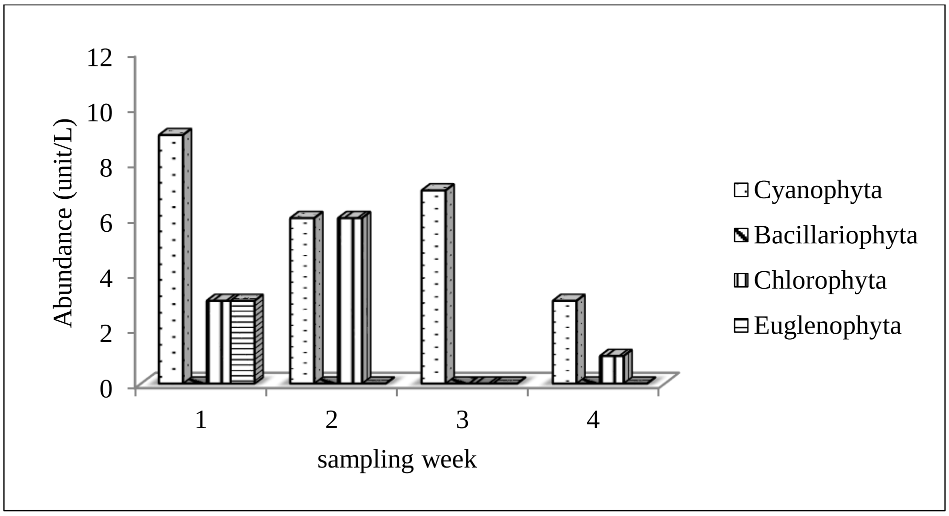

Fig. 7: *Phytoplankton Abundance in Culture Media Prepared with Only Supply Water.*

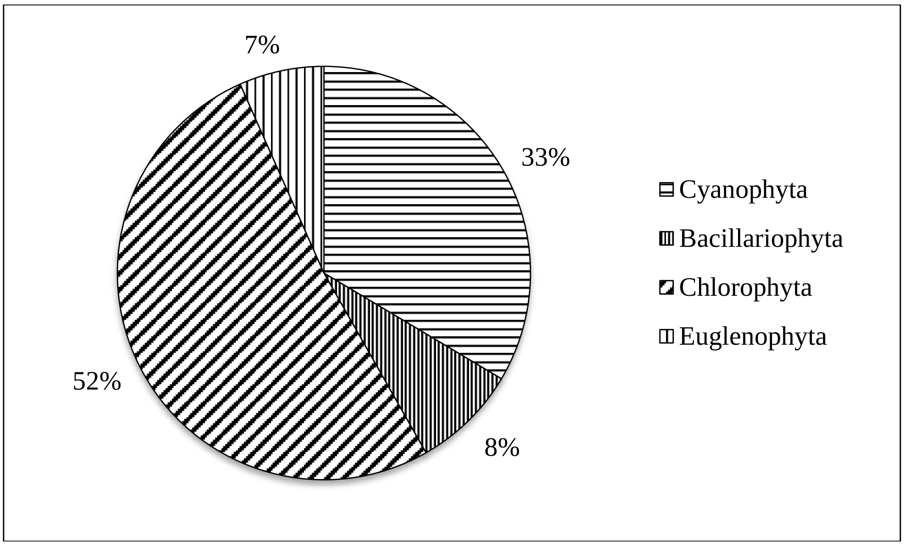

Fig. 8: *Total Abundance of Different Group of Phytoplankton During Culture Period.*

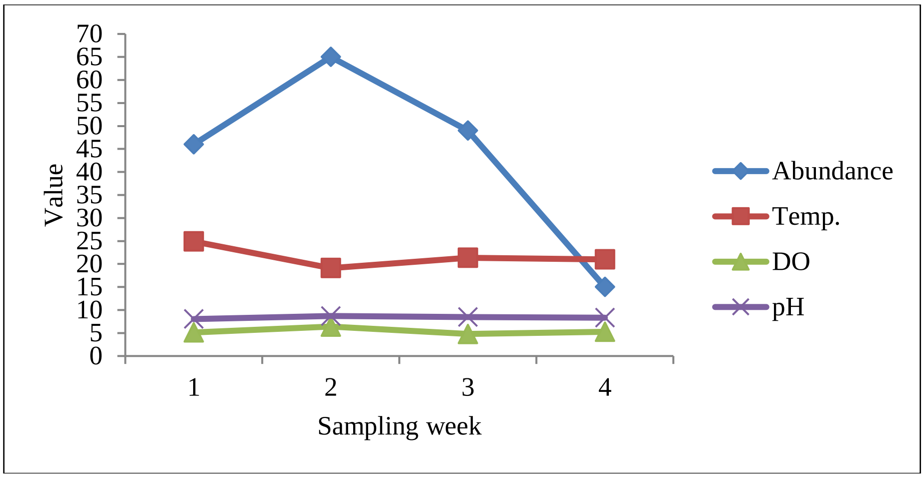

Fig. 9: *Relationship Between Phytoplankton Abundance and Water Quality Parameters for Treatment with Cow Dung.*

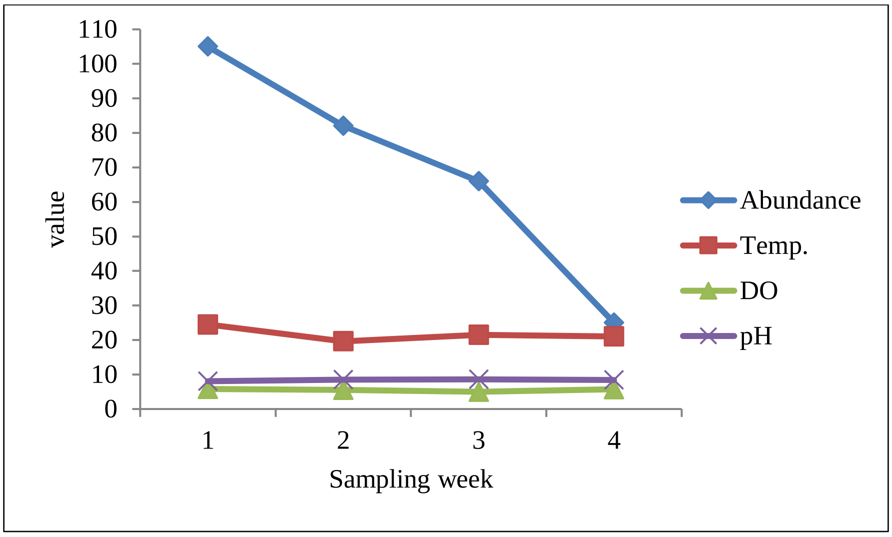

Fig. 10: *Relationship Between Phytoplankton Abundance and Water Quality Parameters for Treatment with Chicken Manure.*

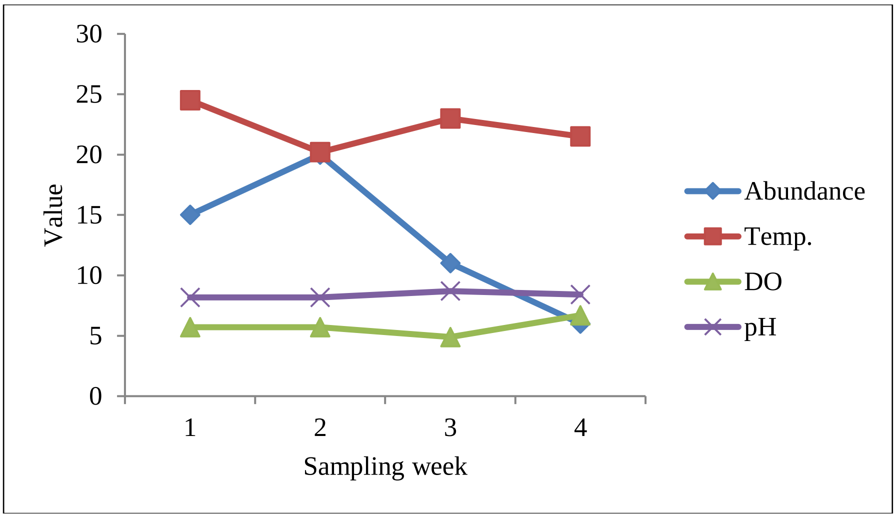

Fig. 11: *Relationship Between Phytoplankton Abundance and Water Quality Parameters for Treatment Only with Supply Water.*

organic fertilizer, to understand the best use of organic fertilizer (which fertilizer is more suitable), and the proper management of physicochemical parameter for phytoplankton production. During the study period the abundance of phytoplankton found in T_1, T_2 and in T_3 was 25–65, 25–105 and 6–20 individual/l, respectively. We found that the abundance of phytoplankton is high in case of T_2, moderately high in case of T_1, and lowest abundance was found in T_3. Organic fertilizer affects the production of phytoplankton and provides some necessary nutrients needed for phytoplankton growth. According to Ponce-Palafox *et al.* [3], phytoplankton concentration was also higher in ponds fertilized with sheep and pig manure than chemical fertilizer. Among the organic fertilizer chicken manure would be very effective for plankton production than cow dung. That is why the production was higher in that case. The productivity in case of T_1 for 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} week was 46, 65, 49 and 15 individual/l, respectively; in case of T_2 for 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} week was 105, 82, 66 and 25 individual/l, respectively; in case of T_3 for 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} week was 15, 20, 11 and 6 individual/l, respectively. For T_2 the productivity of phytoplankton was high in the first week. Two reasons behind this. The effect of fertilizer and physicochemical parameters were good in the first week. The productivity reduced gradually after the first week. But in T_1 the production was higher in 2^{nd} week; reason may be the concentration of DO. In that week DO was higher in T_1 so production was also higher. The plankton sample was to be put on the aquarium in 5 November, 2013 and the first counting was done on 12 November, 2013. But after that week most probably the effect of fertilization was reduced. So the plankton production was also reduced. The result of present study is more or less similar with the study of Mia *et al.* [22, 23]. During the study period 15 species of phytoplankton under four groups was identified. These were Cyanophyta (4), Chlorophyta (7), Bacillariophyta (3) and Euglenophyta (1). The observed species were *Hormidium sp., Doctylococcus sp., Schroederia sp., Ankistrodesmus falcatus, Zygnema sp., Gonatozygon sp., Chlorella vulgaris, Oscillatoria sp., Chroococcus disperse, Oscillatoria sp., Chroococcus disperses, Nodularia sp., Anabaena circinalis, Thalassiosira decipens, Nitzschia paradoxa, Asterionella sp., and Euglena gracilis.* As this experiment was conducted during November to December the plankton sample for culture from pond was collected at this time. The phytoplankton abundance in pond varied with different months and in different season, place and water body [24, 25]. So distinct types of plankton were cultured that were present in the pond water. This is similar to species composition of Khulna University pond given by Saha [26]. Study of Noakhali district, Bangladesh [24], Jashore district, Bangladesh [25], lower Meghna River [27], tropical mangrove estuary [11], Malaysian estuary [28], Merlimau, Malacca [29], Sarawak, Malaysia [10], showed some relevant result regarding species distribution and abundance; some species are relevant with this present study and several species are comparatively alien for the present study. During that time the fluctuation of phytoplankton population in different treatment varied with time. The total number of phytoplankton was dominated by Chlorophyta about 52%. Similar result was also given by Saha [26]. In treatment 1, 2 and 3 the abundance of Chlorophyta was high in 2^{nd} and 3^{rd} week. The reason may be the reproduction cycle of different group of phytoplankton. All the groups do not multiply at the same rate. Some multiply rapidly and some multiply slowly. The life of different phytoplankton may be the reason because phytoplankton survives few hours to several week. The fluctuation was the impact of different physicochemical parameters on plankton population. Different water quality parameters affected the abundance of phytoplankton in various ways. There was a relationship between phytoplankton abundance with different water quality parameters. Temperature affected the abundance of phytoplankton. The abundance varied with the variation of temperature. During our study period temperature varied from 19.1 ºC to 24.9 ºC. The temperature was within optimal ranges (18.3 ºC–37.8 ºC) for the production of plankton in tropical ponds [30, 31]. In treatment 1, at the first week of culture period, phytoplankton shows positive relationship with the temperature. In the 2nd week the temperature decreased but the production increased. At the last of the culture period temperature was more or less same but the production decreased. It was same for treatment 2 and treatment 3 which is shown in Figures 24, 25 and 26. Phytoplankton shows irregular relationship with temperature. The reason could be the effect of organic fertilizer. At the last the organic fertilization effect could be reduced and the production also reduced. So, phytoplankton showed negative relationship in the present study. The pH in the aquarium water varied from 8.03 to 8.7. The water remains slightly alkaline. The present study showed no relationship between pH and phytoplankton production. But Hossain and Chowdhury [32], and Islam and Saha [33] found positive relationship between pH and primary production. The recorded DO varied from 4.8 mg/l to 6.7 mg/l. The productivity increased with increasing DO in case of all treatments. Miah *et al*. [34] showed positive correlation of DO with phytoplankton abundance in ponds of Agricultural University campus, Mymenshing. Several water quality studies [35–38] were performed by researches and their outcomes were relevant with this present study. In last of the work, DO increased but productivity of plankton decreased gradually. The reason could be the organic fertilization effect. At the last organic fertilizer may not work properly so the production decreased.

CONCLUSION

The result of the study revealed that chicken manure is more effective than cow dung as fertilizer in water for primary productivity. The present study would be helpful as baseline information for developing monitoring, management and successful use of fertilizer in the pond for fish culture. There was lacking of technical support such as limitations in counting and identification of plankton so further study is needed for further better result.

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