Study of Physico-chemical Parameters, Planktonic Diversity and Bacterial Load of *Clarias batrachus* Cultivation Pond at Bankura, WB, India

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Abstract

The exploitation of the natural habitats, uncontrolled introduction of allied exotic fishes and pathogenic infections has brought down the condition of Asian catfish, Clarias batrachus to quite a deplorable state. Currently, a worldwide effort is on the run to cultivate the endangered species in both natural and semi-intensive manner. An extensive study was performed to evaluate the synergistic effect of physico-chemical parameters, planktonic diversity and bacterial flora of C. batrachus cultivation on its productivity. The values of the physicochemical parameters were between 18 to 25°C for temperature, 6.5 to 7.7 for pH, 3.1 to 4.8 mg/L for dissolved oxygen, 21 to 27 mg/L alkalinity, 70 to 128 mg/L for salinity, 78 to 128 mg/L for total hardness and 176 to 260 mg/L for total dissolved solids. The amount of total ammonia, nitrate and nitrite of the treated pond were 0.02 to 0.06 ppm, 0.1 to 0.4 ppm and 0.1 to 0.5 ppm respectively. Five predominant groups of zooplanktons and five classes of phytoplanktons were identified throughout the study. The viable bacterial counts of pond water and sediment mud were found in low range during winter in comparison to summer season. The bacterial flora consisted mainly of gram negative rods.

Keywords: Aquaculture, *Clarias batrachus*, Physico-chemical parameter, Phytoplanktons, Zooplanktons, Bacterial load.

Introduction

Asia has witnessed an impressive annual growth rate in aquaculture sector over last three decades and presently is the largest contributor of aquatic production worldwide¹. *Clarias batrachus* (Linn.) is considered as one of the most demanding aquatic products in Southern Asia for its adaptability, excellent nutritional profile and high economic importance. However, the species is presently on the verge of extinction and wrestling to cope up with several challenges. The screening and use of probiotic strains and proper farm set up for *C. batrachus* cultivation are in major focus to retrieve the situation.

Water is the most prime abiotic factor that supports life in this world. Particularly the physico-chemical properties of water monitor the life of aquatic organisms living in it. Any change in the water quality has direct influence on biotic communities where different species of flora and fauna exhibit great variations in their responses to the altered environment^{2,3}. Rapid industrialization, indiscriminate use of chemical fertilizers and pesticides in agriculture are causing drastic pollution in aquatic environment leading to deterioration of water quality and depletion of aquatic biota. Maintenance of water quality for the cultivation of aquatic species in this context is very important.

Planktons are tiny microscopic organisms that play an important role in indicating the water quality, eutrophication status and productivity of a freshwater body^{4,5}. The density and distribution of planktons are mainly depended physicochemical factors of water and available nutrients⁶. They help to convert static food energy from primary to secondary trophic level⁷. As they contain minute concentration of bioaccumulated toxic substances they are considered to be much suitable food item for aquatic fishes. The planktons not only increase fish production but also help in bioremediation of heavy metals and other toxic material⁸. The limnological properties also have considerable influence on the bacterial load of fish ponds⁹. Bacteria comprise of a significant portion of planktonic biomass and are responsible for contributing productivity in aquatic systems¹⁰. They play a fundamental role in nutrient recycling by decomposition and mineralization of organic matter¹¹. Bacteria are responsible for many of the dominant chemical processes that occur in fish ponds. They also help to maintain the delicate balance between the host and environment. However, they can also be the primary or secondary pathogens and cause many infectious aquatic diseases¹²

There is dearth of information about the appropriate hydrobiological parameters of *C. batrachus* cultivation in

Bankura district as no such report had been provided by any researcher till date. Generally, the ponds of this locality have high potentialities for cultivation of *C. batrachus* but the hydrobiological features may not always be satisfactorily good enough to support catfish production mainly due to anthropogenic pressure and lack of awareness among the fish farmers. Considering these facts, the present investigation was planned to observe the limnological parameters, planktonic diversity and bacterial biomass of *C. batrachus* culture pond throughout the year. It may also help in screening of probiotics from autochthonous sources that can be more effective in treating aquatic diseases.

Materials and methods

Culture Pond: A semi-intensive earthen pond at Ramsagar (latitude 23°06' and longitude 87°15') of Bankura District, West Bengal, India was selected as the study area. The area of the pond was 0.06 ha with an average depth of 1.2 \pm 0.8 m. The pond was surrounded with agriculture field. Limited number of plants exists on the earthen bank and has 15 - 20 cm bottom mud. The pond was free from industrial effluents and had no history of use of probiotics or any other bacterial additives. The pond was mainly dependent upon the supply of groundwater along with intermittent rain water throughout the year. C. batrachus fingerlings were introduced into the pond in the month of July' 2015 at a density of 10-15 fry per m². This stocking density however can be multiplied to many more extent. It has been found that they attain maturity at the end of the year (June' 2016) maintaining almost 75% harvesting rate. Fish yield data shows that the average C. batrachus production in this study site is 12 ± 2 t ha⁻¹ year⁻¹.

Limnological Assessment of Pond Water: Water samples were collected at 50 ± 10 cm depth of the surface whereas sediment mud was collected from bottom of the pond. It was done at the last week of each month in clean plastic air-tight bottles in between 8 A.M - 9.30 A.M. Water temperature was measured by hydro-thermometer; pH by digital pH meter (Systronics Model – 335); Total dissolved solid (TDS) was measured using a Universal Pocket Digital TDS Meter (CPEX10001). The alkalinity, salinity, dissolve oxygen (DO), calcium, magnesium, ammonia, nitrate, nitrite, free CO₂ was measured following standard methods of APHA¹³.

Collection of Planktons: Zooplanktons and phytoplanktons were collected by plankton net no. 25 of mesh size of approximately 0.064 mm. 50 L water was filtered through hand plankton net wherein plankton concentration used to accumulate in the specimen tube of 100 mL at the tail end of the net. The samples were immediately preserved in 4% formalin after collection. It was allowed to settle down for a day. The detailed study of the plankton was performed by using Olympus inverted stereoscopic microscope (Model- MLX-B) fitted with a Nikon camera. Identification of Zooplankton was done according to Battish¹⁴ where as phytoplanktons were classified as per modern taxonomic system.

Bacteriological analysis: Water and mud samples were serially diluted and petriplated on different agar media. The plates were then incubated at 37°C temperature for 24-48h. The enumeration of bacteria was determined and expressed in cfu/mL on agar plates with the help of Quebec colony counter. The plate counts were carried out in triplicates and the average of the three respective readings were taken. Colony morphology namely configuration, elevation, margin were recorded. Gram character of each bacterial isolate was thoroughly studied and their shape and size were observed.

Statistical analysis: The Pearson linear correlation matrix among variables at 5% probability was drawn according to the Microsoft Excel and Statplus software. The knowledge about the relationship is helpful for maintaining the water quality of the pond. A contour plot was drawn to represent different limnological parameters. pH, temperature, ammonia, nitrate were taken as X axis, nitrite, dissolve oxygen, alkalinity, CO₂ as Y axes and other parameters namely salinity, total hardness, Ca², Mg²⁺, TDS were taken as Z axis. The contour diagram was then plotted with respect to those axes with the values of bacterial load of pond water and sediment using MATLAB R2009b software.

Results and discussion

Physico-chemical Parameters of the Culture Pond: The physico-chemical parameters of *C. batrachus* culture pond were studied (Table-1) throughout the year. They were further analyzed to obtain linear correlation (Table-2).

Temperature plays a significant role on the haematology and metabolic activities of the aquatic organisms¹⁵. Ahmad et al. 16 reported that temperature has negative and significant correlation with planktonic organisms. Water temperature also plays a central role in regulating the quantity and quality of bacterial flora 17. In the present study, highest water temperature (23°C-25°C) was observed in summer season and lowest (18°C-19°C) in winter. The viable bacterial count was found in low range during winter season comparing to the summer season. The temperature has shown a significant and positive relation with bacterial flora.

The maintenance of pH is one of the most important attributes of aquaculture farming. The pH values of culture pond water ranged from 6.5 to 7.7. The pH increased during summer season and decreased during monsoon and winter. The high pH in summer season may be due to lower level of water and increased photosynthesis of some microorganism resulting high production of free CO₂ which makes the water little alkaline ^{18,19}. A shift to an alkaline environment or to an acidic environment is stressful for bacteria ²⁰⁻²². The capacity of bacteria to survive and grow at alkaline pH has widespread importance in the epidemiology of pathogenic bacteria as well as cultivation of aquatic organisms ²³.

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Table-1a: Physico-chemical parameters and bacterial load of *C. batrachus* culture pond.

Month	pН	Temperature (°C)	Ammonia (ppm)	Nitrate (ppm)	Nitrite (ppm)	Dissolve Oxygen (mg/L)	Alkalinity (mg/L)	CO_2
Jul	6.6	23	0.04	0.1	0.3	3.4	24	25
Aug	6.7	23	0.03	0.3	0.2	3.8	26	26
Sep	7.5	22	0.02	0.2	0.1	3.9	24	24
Oct	7.2	21	0.04	0.3	0.5	4.1	24	23
Nov	7	21	0.04	0.4	0.4	4.2	23	22
Dec	6.8	20	0.03	0.3	0.3	4.3	22	21
Jan	6.7	19	0.04	0.2	0.3	4.6	22	19
Feb	6.5	18	0.02	0.1	0.3	4.8	21	18
Mar	6.9	24	0.04	0.4	0.5	4	24	26
Apr	7.3	23	0.04	0.3	0.3	3.6	25	29
May	7.7	25	0.05	0.3	0.3	3.1	27	28
Jun	6.5	24	0.06	0.2	0.4	3.2	26	30

Table-1b: Physico-chemical parameters and bacterial load of *C. batrachus* culture pond.

Month	Salinity (mg/L)	Total Hardness (mg/L)	Ca ²⁺ (mg/L)	Mg^{2+} (mg/L)	TDS	Bacterial Load of pond water (cfu/ mL)	Bacterial Load of mud (cfu/g)
Jul	98	128	52	57	246	8.2×10^{3}	8.7×10^4
Aug	100	120	50	55	260	2.9×10^4	7.8×10^4
Sep	90	118	48	53	235	6.1×10^{3}	5.6×10^4
Oct	85	90	49	53	202	5.2×10^3	5.1×10^4
Nov	85	90	47	52	186	8.6×10^2	1.4×10^4
Dec	80	85	46	51	180	6.7×10^2	2.8×10^4
Jan	75	80	45	50	176	6.6×10^2	4.5×10^3
Feb	70	78	43	47	184	5.8×10^2	1.6×10^3
Mar	120	80	45	52	200	8.6×10^{3}	3.4×10^4
Apr	126	82	47	54	201	8.1×10^4	4.4×10^5
May	128	110	49	53	206	8.5×10^4	4.7×10^5
Jun	116	115	50	57	208	8.9×10^4	5.1×10^5

Average growth of *C. batrachus* $150 \pm 10 \text{ g}$)

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Table-2a: Correlation of Physico-chemical parameters and bacterial load of *C. batrachus* cultivation.

	pН	Temp.	NH ₃	Nitrate	Nitrite	DO	Alkalinity	CO ₂	Salinity	ТН	Ca ²⁺	Mg ²⁺	TDS	BLW	BLM
рН	1														
Temp	0.3782	1													
Ammonia	0.0101	0.5847*	1												
Nitrate	0.4027	0.3217	0.2205	1											
Nitrite	-0.1712	0.0835	0.5439	0.4209	1										
DO	-0.3338	-0.9175**	-0.6419*	-0.0546	0.0378	1									
Alkalinity	0.4182	0.9109**	0.5740	0.2522	-0.0442	-0.9027**	1								
CO ₂	0.3093	0.9327**	0.6226*	0.2450	0.0680	-0.9165**	0.9084**	1							
Salinity	0.3991	0.9215**	0.6029*	0.3450	0.1485	-0.8293**	0.8341**	0.9300**	1						
ТН	0.0578	0.5579	0.1586	-0.3184	-0.4502	-0.6966*	0.6150*	0.4871	0.2697	1					
Ca ²⁺	0.1113	0.6332*	0.4266	-0.1093	-0.1163	-0.7889**	0.7026*	0.6129*	0.3822	0.8718**	1				
Mg ²⁺	0.0325	0.7609**	0.5517	0.0054	-0.0142	-0.8504**	0.7476**	0.7973**	0.5829*	0.7640**	0.9151**	1			
TDS	0.0462	0.5303	-0.1044	-0.1977	-0.4564	-0.5399	0.5724	0.4661	0.2891	0.8565**	0.7490**	0.6891*	1		
BLW	0.2884	0.6786*	0.6572*	0.0687	-0.0054	-0.7740**	0.7846**	0.8320**	0.8110**	0.2699	0.3661	0.5004	0.1300	1	
BLM	0.3042	0.6617*	0.6887*	0.0182	0.0243	-0.7896**	0.7445**	0.8170**	0.7892**	0.2729	0.3824	0.5112	0.0816	0.9879**	1

BLW=Bacterial load of water, BLM=Bacterial load of mud. * = P(< 0.05). ** = P(< 0.01).

The concentration of DO reflects the physical and biological processes prevailing in water²⁴. It is an important aquatic parameter in the context of farming of any aquatic species. Oxygen depletion in water leads to poor feeding of fish, starvation and subsequent mortality²⁵. The concentration of DO was found in low range.

The least dissolved oxygen (3.1 mg/L) was recorded in May and the highest (4.8 mg/L) in February. DO was inversely

proportional (r = -0.917) to the temperature at a given time. As water temperature rises, the solubility of oxygen decreases.

The alkalinity of pond water is an important parameter in determining the biotic composition of the system^{26,27}. In this culture pond, high alkalinity (26-27 mg/L) was recorded during summer season and low (21-22 mg/L) in winter.

The high concentration of nitrogenous compounds in culture pond may cause a barrier to transport oxygenated-blood in aquatic organisms that results fish mortality and decreased production²⁸. The content of ammonia (0.02 to 0.06 ppm), nitrate (0.1 to 0.4 ppm) and nitrite (0.1 to 0.5 ppm) in culture pond didn't accumulated to lethal concentrations. The presence of such nominal levels of dissolved nitrogenous compounds usually doesn't have direct effect on aquatic species.

Free CO_2 is highly soluble in water and thus contribute as the main source of carbon pathway in the nature. Plants absorb the free carbon dioxide present in both atmosphere and water. Carbon dioxide in water bodies is deposited by the respiratory activity of aquatic animals. It has often been found to be negatively correlated with zooplankton abundance²⁹. Carbon dioxide content of the studied pond water ranged from 18 mg/L to 30 mg/L.

Higher salinity (128 mg/L) was observed in summer and lower during post-monsoon and winter months. The salinity content was well within the acceptable range of aquaculture farming and found to be positively correlated with bacterial load. However, further increase of salinity may result a sharp decrease in bacterial flora.

The hardness of water indicates water quality. In the present investigation, water was moderately hard (78 mg/L to 128 mg/L) throughout the year. Total hardness of water usually has been found to be positively correlated with zooplankton abundance³⁰.

Calcium is an essential prerequisite for physiological function of all life forms. The Ca^{2+} ion contributed (r=0.871) to the hardness of water (Table-2). The concentration of Ca^{2+} was higher (52 mg/L) in the month of July and lower (43 mg/L) in February. The magnesium values generally follow the same trend with calcium in aquaculture ponds. They both are essential plant nutrients and are taken up by the plants through the hydroponic components. The Mg^{2+} values of the studied pond ranged from 47 to 57 mg/L.

Total Dissolved Solid (TDS) is a combination of inorganic salts, organic matter and other suspended dissolved particles in water³¹. The TDS content was well within the permissible range of aquaculture. It was higher (246-260 ppm) in monsoon probably due to run-off water from agriculture fields.

In the present study, the limnological properties of pond water were within the permissible range for fresh-water aquaculture^{32,33}. The contour plot (Figure-1) has shown a positive and consistent impact of the physicochemical parameters on the bacterial load which in turn may contributed higher growth, development and survival rate of *C. batrachus*. The figure supports that our data are consistent since the contour plot is confined and divergent. Hence, in numerical point of view, it may be suggested that, these limnological parameters are most suitable regarding the cultivation of *C. batrachus*. Thus, the synergistic effect of physico-chemical parameters of

pond water created favourable and stable environment for the growth of *C. batrachus* throughout the farming phase.

Planktonic Diversity: The physico-chemical parameters of the pond water may affect the growth of phyto- and zooplanktons which belong to the dietary range of *C. batrachus*³⁴. Five major groups of zooplanktons (Figure-2) were observed and identified through this study. Overall, three species of Cladocera, two of Copepoda, four of Rotifera, two of Ostracoda, four of larva and protozoa were observed (Table-3). Cladocera was the most important group. They feed on smaller zooplanktons and are most sensitive to pollutants. The newly hatched fry generally feeds on small zooplankton like *Artemia* nauplii where as medium and large fingerlings mainly feed on Copepoda and Cladocera³⁵.

A phytoplankton community enriches the systems with dissolved oxygen, natural feed and minimizes pathogenic and unwanted microbial population. Conversely, phytoplanktons often produce high levels of ammonia which discourages fish feeding and growth of the Clarias Sp. 36. In the present study, five classes of phytoplanktons were observed and identified (Figure-3). Two of them belong to Cyanophyceae, five of Chlorophyceae, one of Bacillariophyceae, one of Charophyceae and three of Zygnematophyceae (Table-4). Dawah and Gomaah³⁷ reported that cyanobacteria were present in nearly all catfish culture ponds during summer and accounted for more than 75% of the total phytoplankton biomass in most ponds. These benthic organisms play significant role in fish feeding and in the transfer of matter and energy in fresh water ecosystem. Thus they become important component of food webs, primary and secondary production, energy flow and nutrient recycling in freshwater aquaculture¹¹.

Bacterial Flora: The viable bacterial count in pond water was found in low range $(5.8\times10^2 \text{ to } 6.6\times10^2 \text{ cfu ml}^{-1})$ during winter in comparison to summer $(8.5\times10^4 \text{ to } 8.9\times10^4 \text{ cfu ml}^{-1})$. Similar kind of observations was reported by Chowdhury et al.³⁸. In their findings, the bacterial count was 1.3×10^4 to 5.6×10^5 cfu/mL in catfish pond water. In the pond sediment, the bacterial counts were in the range of 1.6×10^3 to 4.5×10^3 cfu g⁻¹ during the winter and 4.7×10^5 - 5.1×10^5 cfu g⁻¹ during the summer season (Table-1).

The average bacterial density was almost ten-times higher in the pond sediment than pond water. The temperature may play a vital role in development of bacterial biomass. Higher counts were recorded during summer season in contrast to winter in both cases. The bacterial flora obtained from pond water (Table-5) and sediment mud (Table-6) consisted mainly of Gram negative rods.

The quality of aquatic species can be regulated by monitoring the bacteria of growing ponds, since this affect the storage life and the quality of fish products. The growth rate of bacteria is mostly controlled by their rapid response to physical, chemical or environmental changes. Table-3: Predominant zooplankton species of C. batrachus culture pond of Ramsagar, Bankura.

Groups	Cladocera	Copepoda	Rotifera	Ostracoda	Larva and Protozoa	
G	Daphnia sp.	Cyclops sp.	Brachionus bidentata	Cypris sp.	Artemia larva	
	Bosmina sp.		Brachionus quadridentatus		Zoea larva	
Genera	M. '	Diaptomus sp.	Keratella tropica	Stenocypris sp.	Paramecium sp.	
	Moina sp.		Asplanchna sp.		Euglena sp.	

Table-4: Predominant phytoplankton species of C. batrachus culture pond of Ramsagar, Bankura.

Groups	Cyanophyceae	Chlorophyceae	Bacillariophyceae	Charophyceae	Zygnematophyceae	
	Oscillatoria Sp.	Hydrodictyon Sp.			Spirogyra Sp.	
		Closterium Sp. (Desmids)		Cosmarium Sp	Cosmarium Sp. (Desmids)	
Class	Anghama Co	Scenedesmus Sp. Pennate Diatom	Coleochaete Sp.			
	Anabaena Sp. Stigeoclonium Sp. Volvox Sp.	Stigeoclonium Sp.		•	Zygnema Sp.	
		Volvox Sp.				

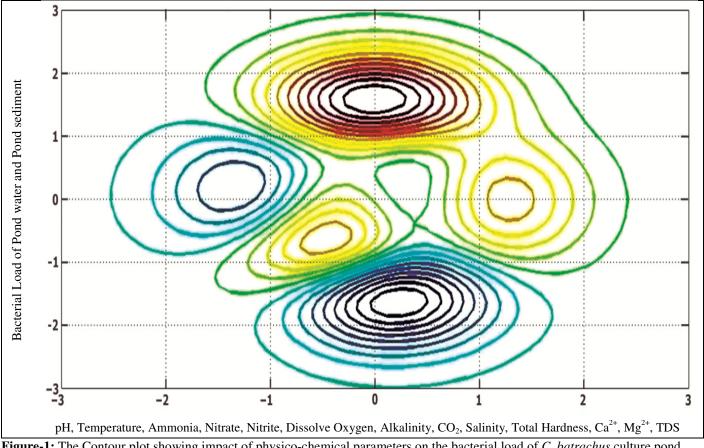


Figure-1: The Contour plot showing impact of physico-chemical parameters on the bacterial load of *C. batrachus* culture pond.

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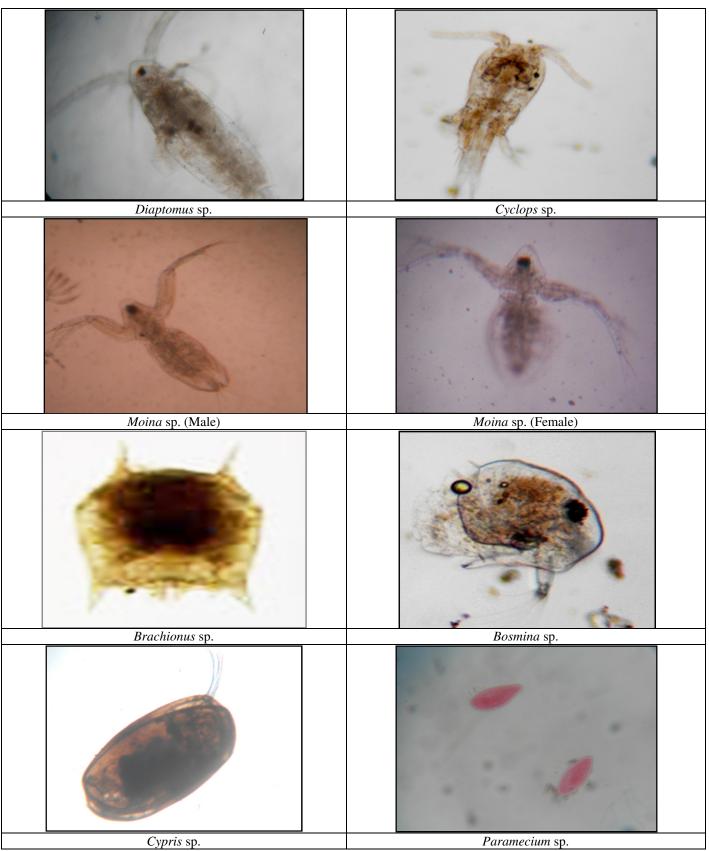
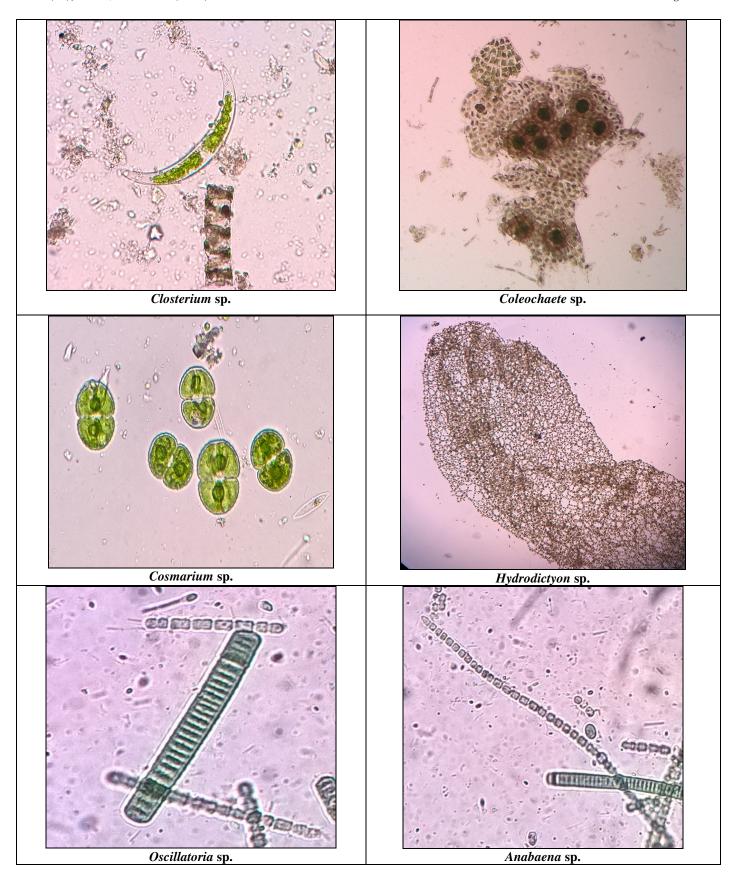


Figure-2: Diagrammatic representation of predominant zooplanktons from *C. batrachus* culture pond water.



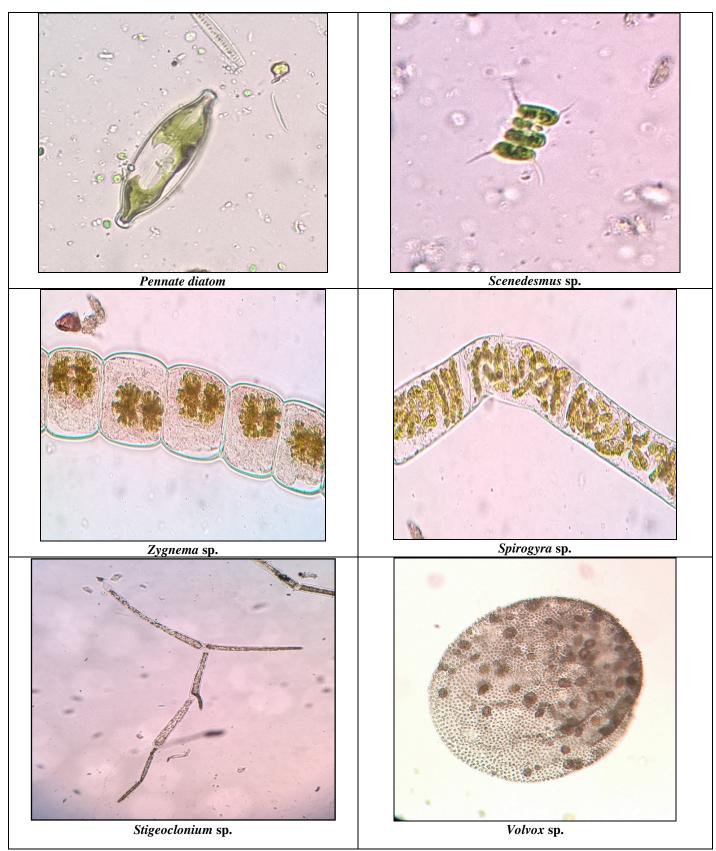


Figure-3: Diagrammatic representation of predominant phytoplanktons obtained from *C. batrachus* culture pond water.

Table-5: Morphological characteristics and gram nature of predominant bacterial isolates isolated from *C. batrachus* culture pond.

Strains	Configuration and Elevation	Margins	Surface	Colony Size (mm)	Cultivation Time	Media	Growth Temperature	Gram Character and Shape
PKA33	Circular, Umbonate	Erose	Rough	4	24 h	NA	30 °C	Gram (-) cocci
PKA34	Irregular,	Undulated	Rough	2	24 h	NA	30 °C	Gram (-) rod
PKA35	Circular, Flat	Entire	Smooth	3	24 h	NA	30 °C	Gram (-) rod
PKA36	Circular, Flat	Undulated	Smooth	4	24 h	NA	30 °C	Gram (+) spore- forming rod
PKA37	Circular, Flat	Erose	Smooth	5	24 h	NA	30 °C	Gram (+) rod
PKA38	Irregular, Flat	Undulated	Smooth	2	24 h	NA	30 °C	Gram (-) rod
PKA39	Circular, Convex	Entire	Rough	3	24 h	NA	30 °C	Gram (-) rod
PKA40	Irregular, Flat	Entire	Smooth	2	24 h	NA	30 °C	Gram (-) short rod
PKA41	Irregular, Flat	Lobate	Smooth	2	24 h	NA	30 °C	Gram (-) spore- forming rod
PKA42	Circular, Raised	Erose	Smooth	3	24 h	NA	30 °C	Gram (+) spore- forming rod

NA- Nutrient Agar.

Table-6: Morphological characteristics and gram nature of predominant mud isolates of C. batrachus culture pond.

Strains	Configuration and Elevation	Margins	Surface	Colony Size (mm)	Cultivation Time	Media	Growth Temperature	Gram Character and Shape
PKA43	Circular, Umbonate	Entire	Rough	2	24 h	NA	30 °C	Gram (-) rod
PKA44	Irregular, Flat	Entire	Rough	2	24 h	NA	30 °C	Gram (-) cocco- bacilli
PKA45	Circular, Raised	Erose	Smooth	3	24 h	NA	30 °C	Gram (+) spore- forming rod
PKA46	Irregular, Flat	Undulated	Smooth	3	24 h	NA	30 °C	Gram (-) rod
PKA47	Circular, Flat	Entire	Smooth	4	24 h	NA	30 °C	Gram (-) long rod
PKA48	Irregular, Flat	Undulated	Smooth	2	24 h	NA	30 °C	Gram (-) rod
PKA49	Irregular, Flat	Erose	Rough	3	24 h	NA	30 °C	Gram (-) rod
PKA50	Circular, Raised	Entire	Smooth	4	24 h	NA	30 °C	Gram (-) cocci
PKA51	Irregular, Flat	Erose	Smooth	5	24 h	NA	30 °C	Gram (-) cocci
PKA52	Circular, Flat	Entire	Smooth	2	24 h	NA	30 °C	Gram (-) short rod
PKA53	Circular, Flat	Undulated	Rough	3	24 h	NA	30 °C	Gram (+) spore- forming rod
PKA54	Irregular, Flat	Erose	Smooth	4	24 h	NA	30 °C	Gram (+) rod
PKA55	Irregular, Convex	Undulated	Smooth	4	24 h	NA	30 °C	Gram (-) cocci

NA- Nutrient Agar.

Conclusion

The study provides an updated concept on the effect of physicochemical parameters, planktonic diversity and bacterial flora with predominance of Gram negative species on successful cultivation of *C. batrachus*. Although each factor plays its individual role in *C. batrachus* cultivation, the synergistic effect of various parameters also have direct influence on the productivity. This study has an immense potential as a source of information about suitable hydrobiological features to the catfish farmers. It can generate high yield and restore the endangered species from further deterioration in its experimental site as particular and the regions of Asia-Pacific in general.

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