Chapter 1

Study of the physico-chemical characters of water of a normal habitat of Clarias batrachus at monthly interval

1.1 Introduction

Aquaculture pond is a dynamic equilibrium which exhibits constant fluctuation due to prevailing physical and chemical processes. Water is a matrix of dissolved gases, inorganic substances, and organic matters. The physico-chemical properties of water generally govern the life of aquatic organisms living in it. Aquatic animals need to adapt to variable environment due to sudden fluctuation in water quality. The leftover feed, fecal matter of fishes and decomposition of organic material accumulate nitrogenous substances that are detrimental to aquaculture practices. Industrial effluents, chemical fertilizers, pesticides and other anthropogenic approaches in adjacent areas are also concomitantly polluting the aquatic environment. In addition, pathogenic infections and algal bloom cause deterioration of water quality and depletion of aquatic diversity. Aquaculture productions are thus depended upon maintenance of a steady state of those limnological parameters. Proper exchange of gaseous substances, reduction of nitrogenous wastes, balancing of planktonic diversity and microbial population are crucial for fish growth and metabolism.

Plankton are the diverse floating microscopic organisms present in a freshwater body. Their density and distribution range indicate the water quality, availability of nutrients, eutrophication status and productivity of the system (Pradhan al. 2008). Phytoplankton are capable of photosynthesis to trap solar energy; the static food energy is subsequently carried from primary to secondary trophic level by other plankton (Shashikanth and Kumar 2009). Plankton are often been considered under dietary range of *C. batrachus*. They also monitor the culture water through bioremediation of toxic molecules (Apiratikul and Pavasant 2008). There is a direct relationship between the limnological properties and microbial load of culture water of a fish pond (Roy and Barat

2011). Bacteria can considerably contribute to the productivity of aquatic systems (Muylaert et al. 2002) through decomposition and mineralization of organic matter (Azam et al. 1983). They are also helpful in the maintenance of aquatic eco-systems. However, pathogenic bacteria may decrease the aquatic production through generation of aquatic diseases (Lightner 1996). The study of planktonic diversity thus is important for proper validation of a cultivation pond.

At present, a worldwide effort is conducted for the sustainable cultivation of *C. batrachus* in both natural and semi-intensive manner. The cultivation of indigenous *C. batrachus* will not only restore the species but also provide community nutrition in a district like Bankura (22°38′ to 23°38′ N and 86°36′ to 87°46′ E), WB, India, where a large sector of people are thriving under poverty. The ponds of this geographical region have considerable potentiality though the production fails to attend the expected level. Considering this lacuna, an extensive study was performed throughout the year to evaluate the hydrobiological parameters, planktonic diversity and bacterial flora of a natural *C. batrachus* culture pond.

1.2 Materials and Methods

1.2.1 Study area

A semi-intensive earthen pond (area 0.6 ha, depth 1.2±0.8 m) at Ramsagar (latitude 23°06' and longitude 87°15') of Bankura District, West Bengal, India was selected as the study area (Fig. 1.1). It is a rectangular perennial lentic pond that depends upon occasional rain water and underground water resources. The pond is situated far away from the industrial sector and is devoid of any chemical disposal. Probiotics or any other bacterial adjuvant has never been applied to the culture pond. *C. batrachus* fingerlings (stocking density: 10-

15 fry per m^2) are generally introduced to the culture pond during monsoon and harvested in a year to obtain high yield (12±2 t ha^{-1} $year^{-1}$).

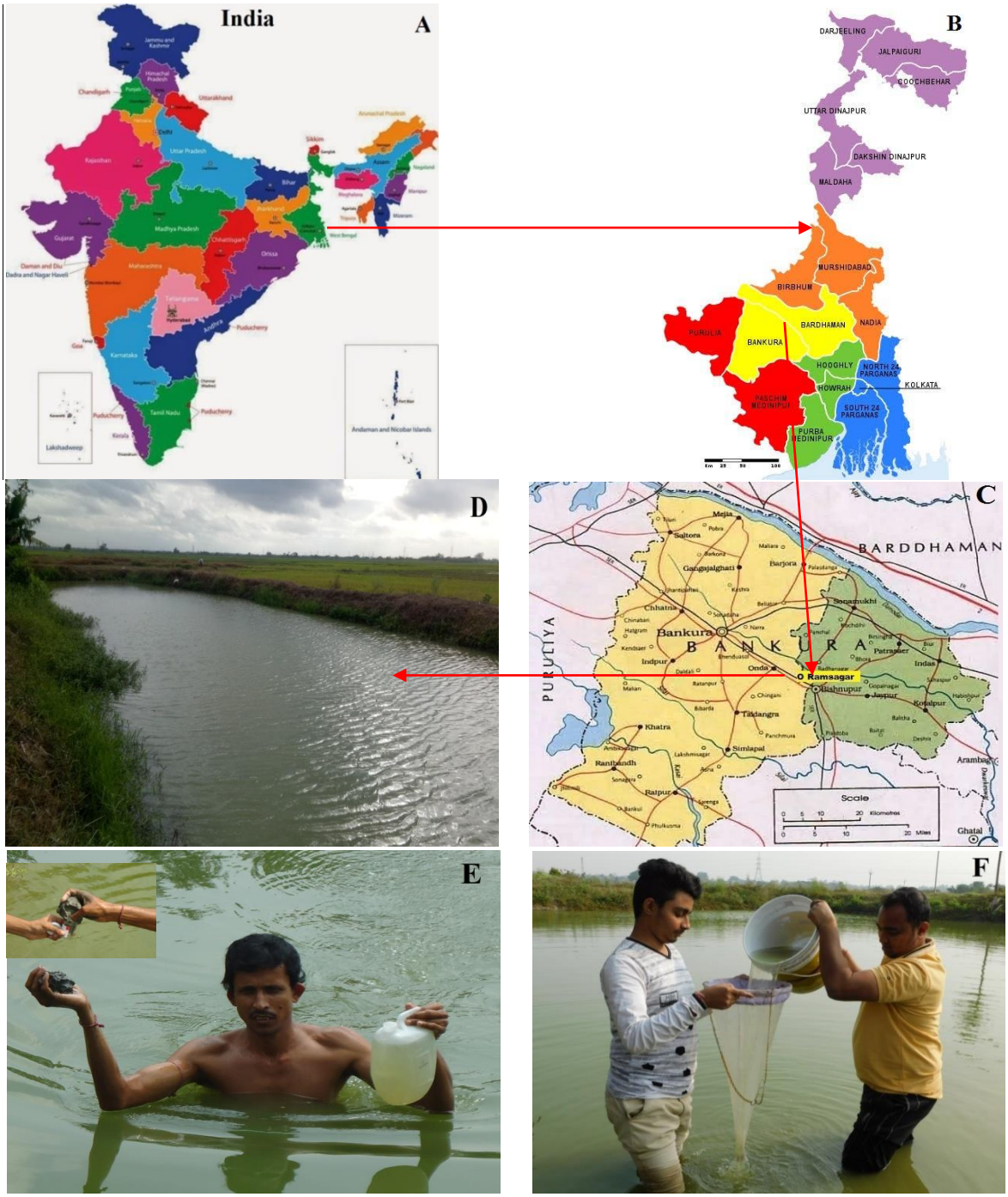

Fig. 1.1: Geographical location of the study area and sample collection: (A) Map of India; (B) Map of West Bengal; (C) Map showing the position of Ramsagar in Bankura district; (D) Study pond; (E) Collection of water and mud samples; (F) Collection of plankton through hand plankton net.

1.2.2 Physico-chemical assessment of culture pond water

Water samples were collected (50±10 cm depth of the water surface) randomly in the morning at the last week of every month in sterile container. Standard BOD bottles were employed for the sampling of water for the estimation of dissolved oxygen. The physicochemical parameters were measured following the standard protocol (APHA 2008).

1.2.2.1 Water temperature

A hydro-thermometer (marked with 0.01-graduated centigrade) was used to measure the surface water temperature at the study site.

1.2.2.2 Hydrogen ion concentration (pH)

The pH of water samples were spot evaluated with a pencil pH meter and further verified through digital pH meter (Systronics Model - 335).

1.2.2.3 Total dissolved solid (TDS)

The TDS level of the culture pond water was assessed using a digital TDS Meter (CPEX10001).

1.2.2.4 Dissolved oxygen (DO)

The concentration of Dissolved oxygen was measured by Winkler's (Montegomery et al. 1964) method. 1 ml of manganous sulphate solution and 1 ml of alkaline iodide solution was added immediately to the water sample (in BOD bottle) at the collection spot. It was kept in dark for 10 min. Concentrated H_2SO_4 (1 ml) was then added and thoroughly shaken to dissolve the precipitate. 50 ml of the sample was taken in a conical flask; 1-2 drops of starch solution was added and titrated the solution with N/40 thiosulphate to attain a colourless end point very carefully. The calculation of DO was performed through the following formula:

Concentration of DO (ppm) = Amount (ml) of thiosulphate required × 4 (Montegomery et al. 1964).

1.2.2.5 Free carbon di-oxide (CO_2)

Free CO_2 shows interdependence with pH and bicarbonate equilibrium. 50 ml of water sample was taken in a white porcelain basin. 3-4 drops of phenolphthalein indicator was added to it and titrated against N/44 NaOH with constant stirring till pink colour appears as the end point. Free CO_2 was calculated through the formula:

Concentration of free CO_2 (ppm) = ml of N/44 NaOH required × 20.

1.2.2.6 Total alkalinity

The total alkalinity was determined by titrimetric method. 1-2 drops of methyl orange indicator was added to the 50 ml of water sample in a conical flask and titrated with 0.02(N) H_2SO_4, until the end point was indicated a color change from yellowish to pinkish. It was calculated through the formula:

Total alkalinity (ppm) = ml of 0.02(N) H_2SO_4 consumed × 20.

1.2.2.7 Salinity

Salinity is the measures of salt (such as sodium chloride, magnesium and calcium sulphates and bicarbonates) dissolved in a water body. It is an important factor in determining many aspects of the chemistry of natural waters and of biological processes. 2-3 drops of K_2CrO_4 indicator was first added to the water sample (25 ml) and titrated against N/100 AgNO3, to measure the amount of chlorinity. The salinity was then calculated through the formula:

Salinity (ppm) = Chlorinity (ppm) × 1.805 + 0.03.

1.2.2.8 Dissolved inorganic nitrogen

Inorganic forms of nitrogen often dissolved in cultivation ponds and constitute ammonium (NH_4^+), nitrate (NO_3^-) or nitrite (NO_2^-) ions. The determination of nitrogenous contents was done following Kjeldahl method.

1.2.2.8.1 Ammonium and nitrate

250 ml of filtered water sample was taken in Kjeldahl flask and fitted with the distillation set. 20 ml of H_3BO_3 solution followed by 10 ml of 40% NaOH solution was then added to the flask. The condenser was fitted immediately. The distillation process was continued until 40 ml of distillate get accumulated in the receiving flask. The distillate was then titrated against 0.02 (N) H_2SO_4 till a pinkish colour appears.

For the determination of nitrate, 20 ml of 4% H_3BO_3 solution was taken in another conical flask and placed under the condenser tip. 0.5 g Davarda's alloy was added to the water sample and distillation process is continued until the water evaporates. The distillate was then titrated until a pinkish colour develops. The ammonium and nitrate was determined through the formula:

N (ppm) in the form of ammonium (NH_4^+), nitrate (NO_3^-) = $\frac{A}{V} \times 280.$

Where, A = ml of 0.02 (N) H_2SO_4 required for titration; V= Volume of water sample used.

1.2.2.8.2 Nitrite

The collected water samples (90 ml) were immediately treated with 2 ml 6 N HCl and 5 ml sulphanilamide solution due to unstable nature of nitrite. It was then preserved for further analysis. The treated water sample was mixed gently and allowed to settle for three min. 1 ml ammonium sulphamate was added to it, waited for another three min followed by addition of 1 ml napthylethylenediamine solution. The total volume was adjusted to 100 ml

with distilled water. The absorbance of the solution was subsequently measured at 530 nm through UV-VIS Double Beam Spectrophotometer (LI-2802; Lasany®, India). A standard curve was simultaneously plotted by using various concentration of the standard solution. The nitrite was calculated using the formula:

N (ppm) nitrite (NO_2^-) = A × 1.11.

Where, A = Observed concentration of nitrite of the water sample compared to the standard curve.

1.2.2.9 Calcium and Magnesium

The EDTA was carefully standardized. 5 ml of 0.01 N Ca solution was taken in a volumetric flask and the volume was made 25 ml by addition of distilled water 5 ml of 4 N NaOH was added to it and 25 mg of Murexide indicator was added to give a orange red colour. It was then titrated against 0.01 (N) EDTA till a purple colour appears.

1.2.2.9.1 Calcium

5 ml of water sample was taken in a volumetric flask and the volume was made 25 ml by addition of distilled water. 5 ml of 4 (N) NaOH was added to it and 25 mg of Murexide indicator was added to give orange red colour. It was then titrated against 0.01 (N) EDTA till a purple colour appears.

1.2.2.9.2 Magnesium

5 ml of water sample was taken in a volumetric flask and the volume was made 25 ml by addition of distilled water. 1 ml of Ammonium chloride – Ammonium hydroxide buffer and 3-4 drops of EBT was added to give a wine red colour. It was then titrated against 0.01 (N) EDTA till a blue colour appears. The concentrations of calcium and magnesium contents were calculated through the formula:

Ca (ppm) = used EDTA (ml) × F × 40.

Mg (ppm) = used EDTA (ml) for Ca + Mg - used EDTA (ml) for Ca) × F × 24.

(F = 5/V, V = volume of EDTA used).

1.2.2.10 Total hardness (TH)

The total hardness was determined by titration with standard ethylene diamine tetraaceatic acid (EDTA). The total calcium (as $CaCO_3$ equivalent) was obtained by multiplying the calcium (ppm) in water with 50.04/20.04. Similarly, total magnesium (as $CaCO_3$ equivalent) was obtained by multiplying the magnesium (ppm) in water with 50.04/12.16. The total hardness of water was determined by addition of these two results.

1.2.3. Collection and analysis of plankton

Sampling method

Plankton were collected by flushing 50 L of culture water through hand plankton net (No. 25 standard grade; aperture size: 0.064 mm) made up of bolting silk. The plankton were eventually accumulated in the specimen tube (100 ml conical flask) fitted at the tail end of the net.

Fixation and preservation

The collected samples were preserved immediately in 4-5% formalin and allowed to settle down for a day in a dark place.

Identification

The specimens were observed in a watch glass, mounted on a cover slip and subsequently identified under phase contrast microscope (RXLr-4; RADICAL, India) and binocular microscope (CH20i; Olympus, India). The photographic images were precisely captured (Coolpix S3100; Nikon, India) for further analysis. The identification of plankton was

carried out based on morphological features and special structures (Needham and Needham 1978; Battish 1992).

1.2.4. Bacteriological analysis

Water and mud samples were collected precisely in the sterile stopper container from different location of the culture pond. The samples were serially diluted (10^{-1} to 10^{-10}) with double-distilled water. The diluted samples (10^{-5} to10^{-9}) were aseptically inoculated on nutrient agar plates by spread-plate technique and incubated at 37 °C temperature for 24-48 h. The work was done in triplicates. The colony morphology was observed under Olympus stereo microscope. The bacterial load of pond water and sediment were enumerated with the help of digital colony counter (Model- 362; Environmental & Scientific Instruments Co., India). The isolates were studied with Gram characterization.

1.2.5. Assessment of limnological conditions

The physico-chemical factors must be thoroughly assessed to figure out the ecophysiology of a natural waterbody. Each factor substantially contributes the ecosystem and establishes the dynamics of aquatic body. Therefore, alteration of a single factor may have potential impact on others. Hence, the study of inter-relationship among variables through statistical approach is pertinent to evaluate limnological phenomena (Hutchinson 1957). The Pearson linear correlation matrix among variables at 5% probability was calculated through Microsoft Excel software. A contour surface plot was drawn by 2-D interpolation method using MATLAB R2009b software to analyze the effect of X-axis (physico-chemical factors) on Y-axis (bacterial load of pond water and sediment).

1.3 Results and Discussion

1.3.1. Physico-chemical parameters of the culture pond

The physico-chemical parameters of *C. batrachus* culture pond were evaluated thoroughly (Table 1.1) and the relationship among major physicochemical parameters was represented by the multiple scattered diagrams following the Pearson Correlation Matrix (Table 1.2) The highest water temperature (24±0.58 °C) was observed in summer season. It may be due to bright atmosphere, clear sunshine and decreased water level. The water temperature of the study pond was lowest (18.5±0.50 °C) in winter. A positive correlation of water temperature was observed with pH, free CO_2, alkalinity, salinity, total hardness, calcium, magnesium and TDS. However, the dissolved oxygen showed negative correlation ($P<0.01$) with the temperature. Dutta and Patra (2013) have also reported similar kind of observation. The depth and volume influences the thermal and light penetration capacity of the culture pond. It also affects on dissolved oxygen, biomass production and yield. The metabolic activities of aquatic organisms are regulated by temperature (Al-Deghayem et al. 2017). It has negative and significant correlation with planktonic organisms (Ahmad et al. 2012). Water temperature also monitors the quantity and quality of bacterial flora (Bisht et al. 2014). The average viable bacterial count was found in low range in both pond water ($6.37\pm0.29\times10^2$) and sediment ($11.37\pm8.35\times10^3$) during winter season as compared to the summer season. The physico-chemical parameters are related to fluctuation of planktonic algae and benthic algae.

The pH regulates the metabolic and other physiological processes of aquatic organisms. The pH of the study pond (6.95±0.11) is most favourable for aquaculture farming. The average value increased during summer season (7.3±0.23) because of intense

photosynthesis of aquatic organisms and decreased water level. A somewhat acidic pH was observed in monsoon (6.65±0.05) possibly due to run-off water from surrounding agricultural areas. The pH value has shown positive correlation with alkalinity, salinity, free CO_2 and bacterial load whereas the negative correlation was observed with DO and nitrite content. Jhingran (1982) prescribed a pH range of 6.5 to 9.0 for maximum aquaculture productivity. The effect free CO_2 on pH value in summer season was reported by Shiddamallayya and Pratima (2008). Maurer et al. (2005) has studied the effect of acidic or alkaline shift of pH on bacterial population.

Alkalinity is the ability of water to resist changes in pH. The total alkalinity is a measure of the quantity of bases (carbonates, bicarbonates, borate, hydroxides and phosphate) present in pond water. The alkalinity of the study pond was high (25.33±0.88 mg/l) during summer and low (21.5±0.5 mg/l) in winter. It has shown positive correlation with calcium, magnesium, free CO_2, total hardness, TDS and bacterial load. A total alkalinity of at least 20 mg/l is ideal for pond productivity (Dutta and Patra 2013). High alkalinity often cause turbidity and retards the growth of aquatic species. The alkalinity of the study pond was found to be suitable for aquaculture farming.

In the experimental pond, the concentration of ammonia was recorded as 0.04±0 ppm, nitrate as 0.26±0.03 ppm and nitrite as 0.33±0.03 ppm, which indicates the hospitable environment for catfish cultivation. Bhatnagar and Devi (2013) stated that a nitrate-nitrogen level of 0.1 to 4.5 ppm in aquatic pond is an indicator of good productivity. Hargreaves (1998) reported that a minimum amount of nitrification takes place in earthen catfish ponds. The increased concentration of nitrogenous compounds often results fish mortality and subsequent production loss (Crab et al. 2007). However, very negligible

amount of dissolved nitrogenous substances were observed in the study pond that doesn't generate any adverse impact on aquatic species.

Total dissolved solid (TDS) content (inorganic salts, organic matter and other suspended dissolved particles in water) was higher (253±7 ppm) in monsoon season in the cultivation pond might be due to run-off water from agricultural field. The TDS (207±7.73 ppm) of the study pond showed negative correlation with NH_3, Nitrate, Nitrite and DO. James (2000) stated that a TDS value of ≤400 mg/l is permissible for diverse fish cultivation. A comparative lower amount of TDS is considered more desirable for breeding and farming of aquatic organisms (Utang and Akpan 2012).

Dissolved oxygen of the study pond was higher (4.57±0.15 mg/l) in winter and lower (3.57±0.26 mg/l) in summer season. The decreased concentration of DO in summer season is mainly attributed to the high surface water temperature which subsequently reduces the oxygen holding capacity of the pond water. A similar kind of observation on DO in a perennial pond of Jhabua in Madhya Pradesh was reported by Vyamzal (1995). In the present study, the DO content (3.92±0.15 mg/l) was found suitable for aquaculture farming. It shared negative correlation with temperature, free CO_2, alkalinity and salinity. Boyd (1982) also observed an inversely proportional relationship between DO and free CO_2. The depletion of oxygen in waterbody leads to poor feeding, starvation and subsequent mortality of aquatic animals (Bhatnagar and Garg 2000). Ellis (1937) stated that a DO concentration of 3.0 mg/l or less can raise the level of toxic metabolites which affect adversely on fish growth and metabolism. Diffusion of atmospheric oxygen through air-water interface, photosynthesis of aquatic plants, physical and biological processes prevailing in water, tidal flow, algal growth, together influence the DO content of culture

water (Pedersen et al. 2013).

The average free carbon dioxide of the study pond was 24.25±1.10 mg/l which may be considered a little higher in the context of fish farming. Free CO_2 showed positive correlation with pH, temperature, NH_3, nitrate and alkalinity. The higher intent of free CO_2 in the present study may be due to the collection of water samples in early morning. Hargreaves and Brunson (1996) stated that the free CO_2 increases in the morning and decreases afterwards due to interactive fluctuating pattern of DO and free CO_2. The primary sources of free CO_2 in water bodies include aquatic respiration and decomposition of organic matter. Phytoplankton communities are often adversely affected by the increased CO_2 (Coello-Camba et al. 2014).

Salinity refers to the amount of salts (sodium chloride, calcium, magnesium, bicarbonate salts and potassium sulphate) dissolved in a water body. Higher salinity (124.67±2.40 mg/l) was observed in the experimental pond in summer and lower in winter (75±2.89 mg/l). The salinity was found to be positively correlated to temperature, NH_3, alkalinity and free CO_2. Fish possess osmoregulatory mechanisms to sustain and adapt to variable environmental salinities (Lisboa et al. 2015).

The culture water of the perennial study pond was moderately hard (98±5.38 mg/l) throughout the year. It showed positive correlation with the temperature, alkalinity, free CO_2 and negative correlation with the nitrate, nitrite and DO. Hard water has the capacity of buffering the effects of toxic heavy metals. It also promotes zooplankton abundance (Chiba et al. 2018). Harmon et al. (2003) observed a significant impact of water hardness on aquaculture productivity.

Table 1.1: Measurement of physico-chemical parameters and bacterial load of *C. batrachus* culture pond as studied at monthly interval.

Month	pH	Temp. (°C)	Ammonia (ppm)	Nitrate (ppm)	Nitrite (ppm)	DO (mg/l)	Alkalinity (mg/l)	CO_2	Salinity (mg/l)	TH (mg/l)	Ca^{2+} (mg/l)	Mg^{2+} (mg/l)	TDS (mg/l)	BLW (cfu/ml)	BLM (cfu/g)
Jul	6.6±0.06	23±0.12	0.04±0	0.1±0	0.3±0.01	3.4±0.06	24±0.15	25±0.33	98±0.44	128±0.88	52±0.58	57±0.17	246±0.5	8.2×10^3	8.7×10^4
Aug	6.7±0.12	23±0.06	0.03±0	0.3±0.01	0.2±0	3.8±0.03	26±0.09	26±0.29	100±0.88	120±0.88	50±0.44	55±0.29	260±1.15	2.9×10^4	7.8×10^4
Sep	7.5±0.03	22±0.03	0.02±0	0.2±0	0.1±0	3.9±0.03	24±0.17	24±0.17	90±0.33	118±0.58	48±0.17	53±0.29	235±0.58	6.1×10^3	5.6×10^4
Oct	7.2±0.06	21±0.15	0.04±0.01	0.3±0	0.5±0.02	4.1±0.03	24±0.15	23±0.58	85±0.17	90±0.33	49±0.44	53±0.33	202±1.20	5.2×10^3	5.1×10^4
Nov	7±0	21±0	0.04±0	0.4±0.01	0.4±0.01	4.2±0.09	23±0.03	22±0.58	85±0.58	90±0.88	47±0.29	52±1.15	186±0.58	8.6×10^2	1.4×10^4
Dec	6.8±0.09	20±0.07	0.03±0	0.3±0	0.3±0	4.3±0.03	22±0	21±0	80±0.88	85±0.58	46±0.17	51±0.44	180±1	6.7×10^2	2.8×10^4
Jan	6.7±0.12	19±0.07	0.04±0.01	0.2±0	0.3±0.01	4.6±0.15	22±0.07	19±0.17	75±0.29	80±1	45±0.17	50±0.17	176±0.58	6.6×10^2	4.5×10^3
Feb	6.5±0.03	18±0.09	0.02±0	0.1±0	0.3±0	4.8±0.03	21±0.13	18±0	70±0.17	78±0.33	43±0	47±0.17	184±0.88	5.8×10^2	1.6×10^3
Mar	6.9±0.03	24±0.03	0.04±0	0.4±0.01	0.5±0.01	4±0.03	24±0.06	26±0.1	120±0.88	80±0.58	45±0.17	52±0.58	200±0.58	8.6×10^3	3.4×10^4
Apr	7.3±0.12	23±0.06	0.04±0	0.3±0	0.3±0.01	3.6±0.09	25±0.15	29±0.33	126±0.88	82±0.58	47±0.29	54±0.58	201±0.33	8.1×10^4	4.4×10^5
May	7.7±0.09	25±0.07	0.05±0.01	0.3±0	0.3±0	3.1±0	27±0.21	28±0.33	128±0.73	110±0.33	49±0.17	53±0.29	206±0.58	8.5×10^4	4.7×10^5
Jun	6.5±0	24±0.03	0.06±0.01	0.2±0	0.4±0.02	3.2±0.09	26±0.06	30±0.33	116±1.30	115±0.58	50±1.15	57±0.29	208±1	8.9×10^4	5.1×10^5

The results were given in (Mean±S.E.)

BLW= Bacterial load of pond water, BLM=Bacterial load of mud

Table 1.2: Pearson correlation matrix of physico-chemical parameters and bacterial load of *C. batrachus* cultivation pond.

	pH	Temp.	Ammonia	Nitrate	Nitrite	DO	Alkalinity	CO_2	Salinity	TH	Ca^{2+}	Mg^{2+}	TDS	BLW	BLM
pH	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						
TH	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

* = P (< 0.05).

** = P (< 0.01).

TH= Total hardness; BLW= Bacterial load of pond water, BLM=Bacterial load of mud

The concentration of Ca^{2+} was found to be higher (51±1 mg/l) in monsoon and lower (44.67±0.88 mg/l) in winter. The Ca^{2+} ion contributed significantly ($P< 0.01$) to the hardness of water. It has also shown positive correlation with temperature, NH_3, free CO_2, alkalinity and negative correlation with nitrogenous substances. The result of the concentration of magnesium in the experimental pond followed the same trend with calcium in aquaculture pond (highest: 56±1 mg/l in summer, lowest: 49.33±1.20 mg/l in winter). It showed positive correlation with temperature, NH_3, alkalinity, salinity and total hardness. Calcium and magnesium are essential micronutrients of aquatic environment. Certain concentrations of Ca^{2+} and Mg^{2+} are required for the growth of planktonic algae in an aquaculture pond (Boyd and Scarsbrook 1974). The effect of Ca^{2+}:Mg^{2+} ratio on the growth of endemic cyprinid fish *Gobiocypris rarus* was evaluated by Luo et al. (2016).

The contour plot of the combined effects of individual parameters (pH, Temperature, Ammonia, Nitrate, Nitrite, Dissolve Oxygen, Alkalinity, CO_2, Salinity, Total Hardness, Ca^{2+}, Mg^{2+} and TDS) with bacterial load of pond water and sediment is presented in Fig. 1.2. The representation clearly denotes that the variations of physico-chemical parameters are associated with the bacterial load of pond water and sediment. The plot provided a topographical view of physico-chemical parameters against bacterial load of pond water and sediment. The figure predicted the value of the process output (Y-axis) for a particular combination of physico-chemical parameters inputs. It helped to visualize the effects two process output on the variation of shaking of the process inputs. It also assesses the contour region around the optical solution. The solution is relatively flat somewhere, that means the optimum is robust to variation of the said two factors. The contour region is not relatively flat in some places which indicated the deviation of both factors (Y-axis) could have serious

consequences. The figure supported that our data are consistent since the contour plot is confined and convergent. 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.

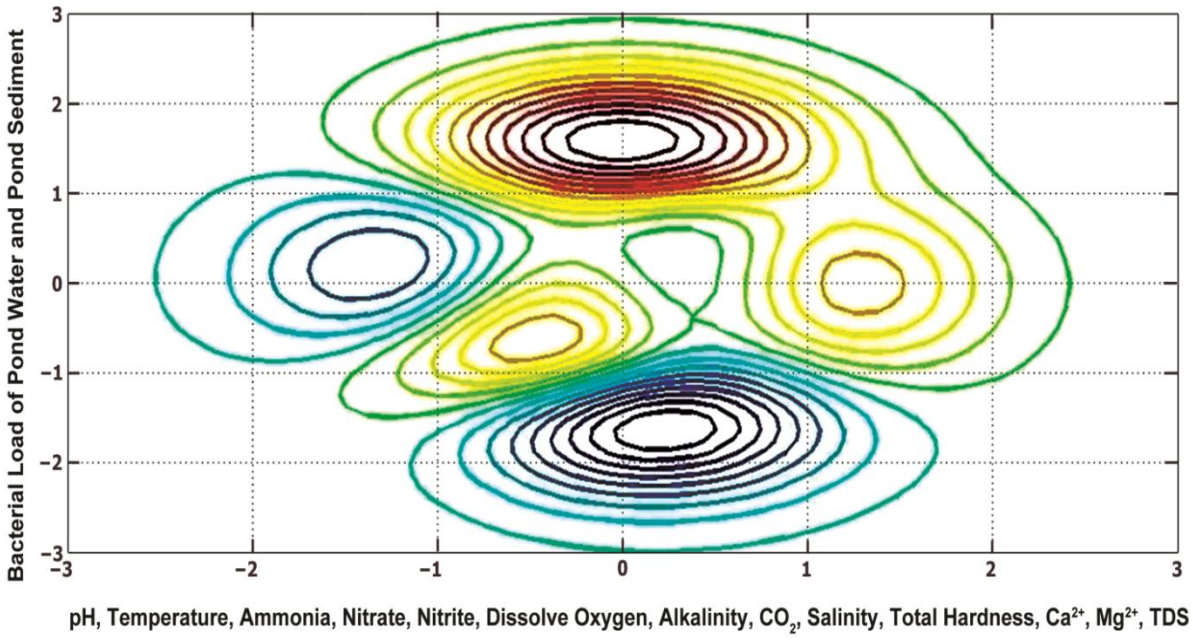

Fig. 1.2: The contour plot showing impact of physico-chemical parameters on the bacterial load of *C. batrachus* culture pond.

1.3.2. Plankton diversity

1.3.2.1 Zooplankton

Fifteen genera under five major groups of zooplankton were observed and identified through this study (Fig. 1.3). The distribution pattern includes four genera of Rotifera, three of Cladocera, two of Copepoda, two of Ostracoda and four of larva and protozoa (Table 1.3). The study revealed the dominance of Rotifera over other zooplankton. El-Feky (2017) has studied the zooplankton diversity in *C. gariepinus* culture pond at Al-Mahmoudia canal and Nubaria canal, Egypt and obtained similar kind of observation. Cladocera generally feed on smaller zooplankton and are regarded as most sensitive bio-indicator.

C. batrachus generally consume benthic algae, chironomid larvae, debris, finfish, insect egg, molluscs, zooplankton, weeds and worms. The newly hatched fry feeds on small zooplankton like *Artemia nauplii* where as medium and large fingerlings mainly feed on Copepoda and Cladocera (Knud-Hansen et al. 1990). Oladele and Omitogun (2016) successfully used zooplankton (Cladocera and Copepoda) as a low-cost feed for larval and fry stages of *C. gariepinus*. The density and diversity of zooplankton is often influenced by the physico-chemical parameters of pond water (Courtenay et al. 1974).

Table 1.3: Predominant zooplankton observed in *C. batrachus* culture pond.

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

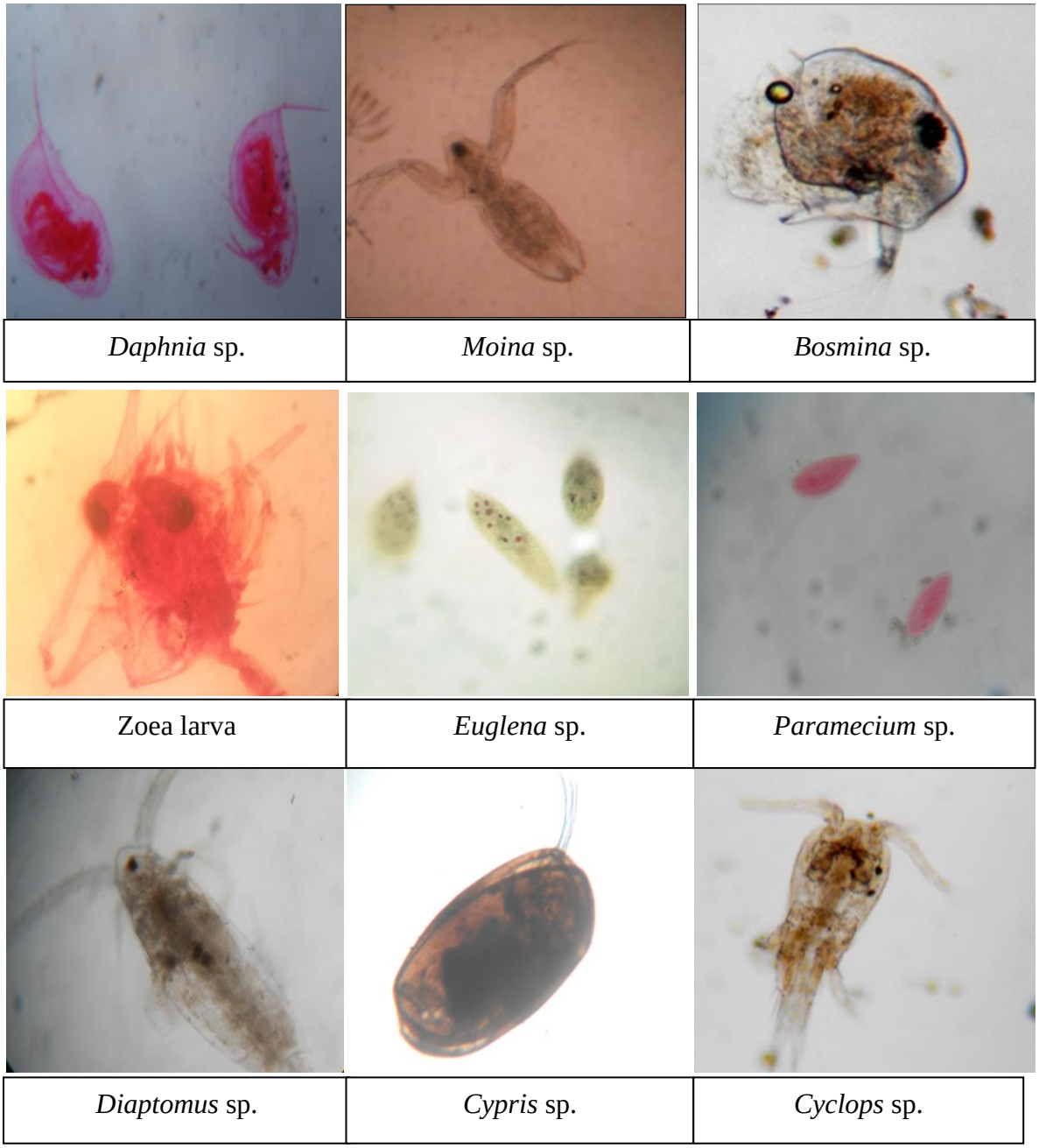

Fig. 1.3: Photograph of zooplankton observed in *C. batrachus* culture pond.

1.3.2.2 Phytoplankton

Five major groups of phytoplankton were observed in the present investigation (Fig. 1.4). The *Oscillatoria* and *Anabaena* were belongs to Cyanophyceae; *Hydrodictyon*, *Closterium*, *Scenedesmus*, *Stigeoclonium* and *Volvox* represented Chlorophyceae. The Bacillariophyceae and Charophyceae included Pennate Diatom and *Coleochaete* respectively. The Zygnematophyceae was represented by three members (Table 1.4). Dutta and Patra (2013) studied the phytoplankton diversity of Jamunabundh in Bishnupur, WB and reported similar kind of observation. Dawah and Gomaah (2005) observed that predominance of cyanobacteria in catfish culture ponds during summer season. It plays a vital role in fish feeding in freshwater ecosystem. The application of fertilizer in culture pond subsequently enhances the phytoplankton population (Chakrabarti and Jana 1998). Phytoplankton population provides stability to the pond ecosystem. Phytoplankton not only reduce toxic substances but also enrich the culture pond with dissolved oxygen and natural feed. A healthy phytoplankton bloom also minimizes microbial contamination. Conversely, die-offs phytoplankton often discourage the feeding and growth of the *Clarias* Sp. (Tucker et al. 1984).

Table 1.4: Predominant phytoplankton observed in *C. batrachus* culture pond.

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

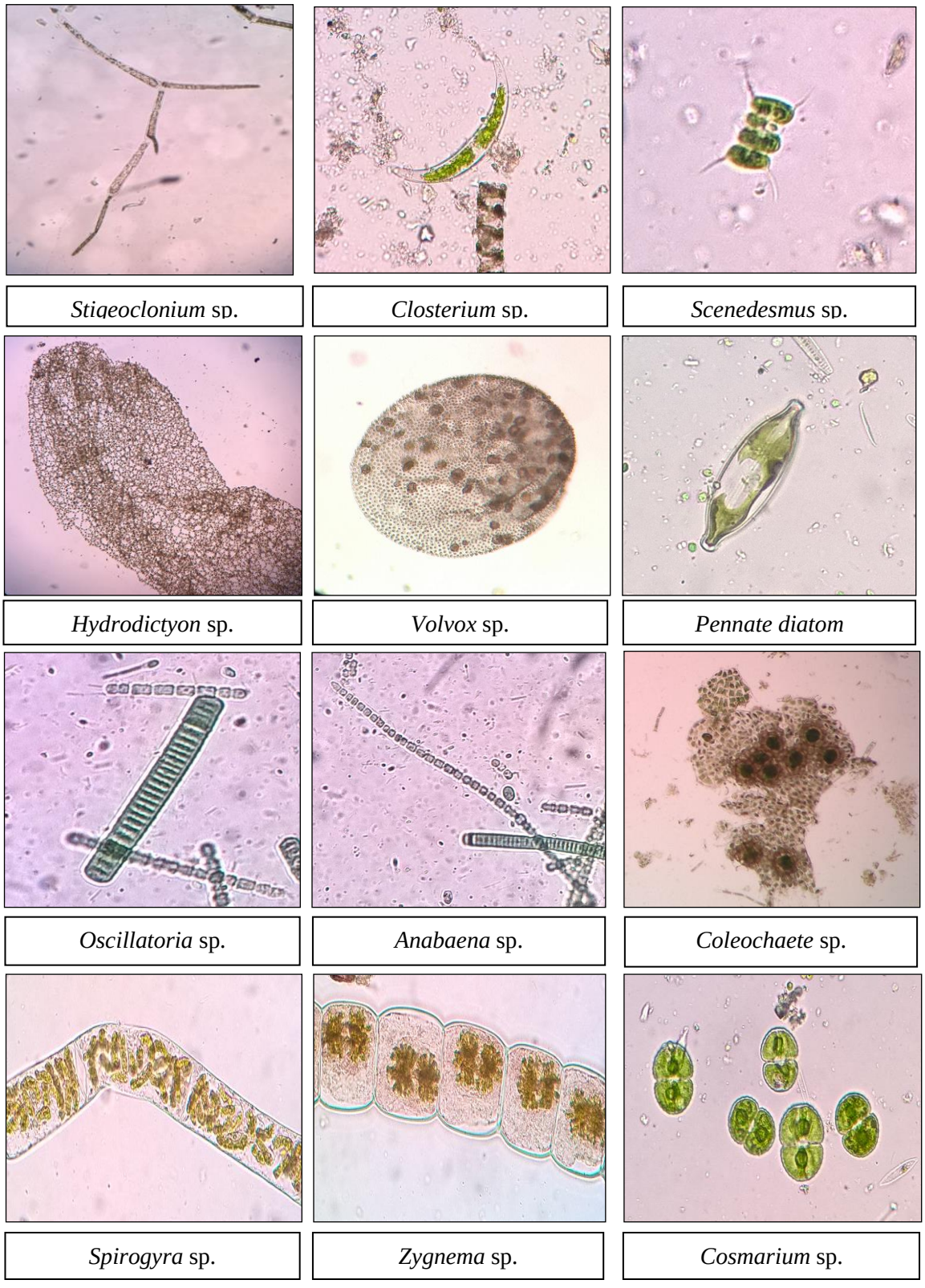

Fig. 1.4: Photograph of phytoplankton observed in *C. batrachus* culture pond.

1.3.3. Bacterial flora

The quantitative assessment of bacterial load of study pond was carried out. The bacterial load of culture water was higher in summer season and lower in winter. The bacterial load of pond sediment has followed the same trend. The results specified the seasonal influence on the bacterial load. This may be due to the variation of water temperature and other physico-chemical factors in different season. The bacterial count showed positive correlation with temperature, NH_3, Mg^{2+}, free CO_2, alkalinity and salinity in the present study. However, the bacterial load of pond sediment was considerably higher than the culture water. Bisht et al. (2014) studied the seasonal variation of bacterial flora in a *Cyprinus carpio* aquaculture pond at Uttarakhand and reported similar kind of observation. Chowdhury et al. (1994) also observed higher bacterial load in the pond sediment than pond water. The growth rate of bacteria is regulated by the physico-chemical or environmental stimuli (Rodriguez et al. 2018).

The qualitative analysis of bacterial flora of pond water (Table 1.5) and sediment (Table 1.6) was thoroughly performed. The study revealed the predominance of Gram negative rod-shaped bacteria in both pond water (70%) and sediment (76.92%). Al-Harbi and Uddin (2003) obtained similar kind of observation in a tilapia culture pond in Saudi Arabia. The bacterial flora often reflects the quality of aquatic environment Shewan and Hobbs (1967). *Edwardsiella tarda*, *Vibrio harveyi*, *Vibrio vulnificus*, *Aeromonas hydrophila*, *Flavobacterium columnare*, *Yersinia ruckeri*, *Moritella viscosa* are aquaculture pathogens that often contaminate culture water and cause subsequently fish mortality (Sudheesh et al. 2012).

Table 1.5: Colony characteristics and Gram nature of predominant bacterial isolates of *C. batrachus* culture pond water.

Strains	Configuration and Elevation	Margins	Surface	Colony Size (mm)	Gram Character and Shape
PKA33	Circular, Umbonate	Erose	Rough	4	Gram negative cocci
PKA34	Irregular,	Undulated	Rough	2	Gram negative rod
PKA35	Circular, Flat	Entire	Smooth	3	Gram negative rod
PKA36	Circular, Flat	Undulated	Smooth	4	Gram positive spore-forming rod
PKA37	Circular, Flat	Erose	Smooth	5	Gram positive rod
PKA38	Irregular, Flat	Undulated	Smooth	2	Gram negative rod
PKA39	Circular, Convex	Entire	Rough	3	Gram negative rod
PKA40	Irregular, Flat	Entire	Smooth	2	Gram negative rod
PKA41	Irregular, Flat	Lobate	Smooth	2	Gram negative spore-forming rod
PKA42	Circular, Raised	Erose	Smooth	3	Gram positive spore-forming rod

The isolates were cultivated on nutrient agar (NA) media at 30 ºC for 24 h

Table 1.6: Colony characteristics and gram nature of predominant mud isolates of *C. batrachus* culture pond.

Strains	Configuration and Elevation	Margins	Surface	Colony Size (mm)	Gram Character and Shape
PKA43	Circular, Umbonate	Entire	Rough	2	Gram negative rod
PKA44	Irregular, Flat	Entire	Rough	2	Gram negative cocco-bacillus
PKA45	Circular, Raised	Erose	Smooth	3	Gram positive spore-forming rod
PKA46	Irregular, Flat	Undulated	Smooth	3	Gram negative rod
PKA47	Circular, Flat	Entire	Smooth	4	Gram negative long rod
PKA48	Irregular, Flat	Undulated	Smooth	2	Gram negative rod
PKA49	Irregular, Flat	Erose	Rough	3	Gram negative rod
PKA50	Circular, Raised	Entire	Smooth	4	Gram negative cocci
PKA51	Irregular, Flat	Erose	Smooth	5	Gram negative cocci
PKA52	Circular, Flat	Entire	Smooth	2	Gram negative short rod
PKA53	Circular, Flat	Undulated	Rough	3	Gram positive spore-forming rod
PKA54	Irregular, Flat	Erose	Smooth	4	Gram positive rod
PKA55	Irregular, Convex	Undulated	Smooth	4	Gram negative cocci

The isolates were cultivated on nutrient agar (NA) media at 30 ºC for 24 h

1.4 Conclusion

The study focuses on the natural habitat of *Clarias batrachus* (Linn), the Asian catfish. The culture conditions of the cultivation pond were evaluated by measuring the physico-chemical properties and studying the microbial load of pond water and sediment. The planktonic diversity in this natural habitat was the indication of the synergistic effect of these conditions. Five groups of zooplankton and five groups of phytoplankton were observed predominantly throughout the year. The bacterial count in the culture water and sediment was low in winter in respect to the summer. The bacterial flora mainly contained Gram negative rods. The temperature ranged from 18±0.09 to 25±0.07 ºC, pH from 6.5±0 to 7.7±0.09, dissolved oxygen from 3.1±0 to 4.8±0.03 mg/l, alkalinity from 21±0.13 to 27±0.21 mg/l, salinity from 70±0.17 to 128±0.73 mg/l, total hardness from 78±0.33 to 128±0.88 mg/l, total dissolved solids from 176±0.58 to 260±1.15 mg/l. Ammonia, nitrate and nitrite contents were recorded as 0.02±0 to 0.06±0.01 ppm, 0.1±0 to 0.4±0.01 ppm and 0.1±0 to 0.5±0.02 ppm respectively. The ecology of a waterbody affects the habitability and abundance of flora and fauna in perennial ponds. Each factor had substantial contribution on fish production, but the interrelationship between the characters was considered to be more pertinent in aquatic ecosystem. This new and updated concept of study of the natural habitat of *C. batrachus* would be a reference in maintaining the conditions more natural to generate higher production of the fish. It would also ensure the species to remain sustained in its native area.