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Comparative Study on Physico-chemical Parameters of water, Zooplankton and Macrophytes Diversity under the Exposure of Farm-made Prebiotics in a Semi-intensive Aqua-Farming System

Subhas Das, Sukhendu Dey, Dhriti Dipon Ghosh, Ashis Kumar Panigrahi

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ABSTRACT

The present study intended to investigate the variation of the physico-chemical status, zooplankton and macrophytes diversity of three semi-intensive ponds (each having 0.40 acre of water area) under Khano village of Galsi-II Block of Purba Bardhaman district, West Bengal, India. One was marked with control (C: Fed only with locally available fish feed) and two others ponds (P1 and P2: Fed with farm-made prebiotics plus locally available fish feed) were considered as treatment. The 'farm-made prebiotics' (made of fish-meal, ground-nut oil cake, bar-yeast, jaggery: Fermented) was applied directly into the pond water fortnightly at the rate of 15 kg per acre fortnightly up to three months. The ponds were reared with *Catla* (*Catla catla*): Rahu (*Labeo rohita*): Mrigal (*Cirrhinus mrigala*) at the ratio of 3:4:3. The water samples were drawn on day15, day30, day45, day60, day75 and day 90 at six corners of each pond. The average value was considered while computing the results of physico-chemical parameters, viz., transparency, temperature, pH, electrical conductivity (EC), carbon dioxide (CO_2), dissolved oxygen (DO), total hardness (TH), total alkalinity (TA), phosphate (PO_4^{3-}), chloride (Cl^-), ammoniacal-nitrogen (NH_4^+-N), nitrate-nitrogen (NO_{3-}-N), sodium (Na^+) and potassium (K^+). The variations in the diversity of zooplankton and macrophytes in the study area were also measured. A marked difference in the case of physico-chemical parameters as well as the zooplankton and macrophytes diversity were observed under prebiotic exposure which may finally help in better growth of the fish species under prebiotic exposure. Finally, the fish farmers will be highly benefited from the application of such farm-made prebiotics.

Keywords Pond, Physico-chemical parameters, Zooplankton, Macrophytes, Farm-made prebiotics.

Subhas Das[1], Sukhendu Dey[2], Dhriti Dipon Ghosh[3], Ashis Kumar Panigrahi[4]*

[4]Professor

[1,2,3,4]Aquaculture, Fisheries and Eco-toxicology Laboratory, Pro-Vice-Chancellor's Research Group, The University of Burdwan, West Bengal 713104, India

Email : panigrahi.ashis@gmail.com
*Corresponding author

INTRODUCTION

It is quite praise worthy to know that about one billion population of our country is earning based on aquaculture practices which seems to be a fast-growing industry in present scenario in Indian context (Obi *et al.* 2016), where its production was noted about

46% of total world fish production as on 2018 (FAO 2020) and now Asia topped on aquaculture production (Rohani *et al.* 2021).

To meet up the gap of demand-supply chain in the aqua-industry due to recent gearing towards intensification of higher production, the disease has become a serious threat where antibiotics and other chemotherapeutics have become an obligatory item to combat the situation, but rampant use of these items has led to resistance towards microbes resulting into seeping through food chain (Kari *et al.* 2022. Tanwar *et al.* 2014). While searching on alternatives, prebiotics play an important role to avoid microbial resistance, especially against the use of antibiotics in maintaining the health management of aquaculture species (Kari *et al.* 2021, Song *et al.* 2014, Zulhisyam *et al.* 2020). Prebiotics are broadly used in enhancing growth performances by development and growth of the gut microbiota (Ramos *et al.* 2017), disease control (Li *et al.* 2019), better immune response (Selim and Reda 2015), and also decreasing the abiotic stressor while culturing the target aqua-species (Hoseinifar *et al*. 2014). On the other hand, improved yield, better water quality, quality food-fish with less fat in muscle and liver, and reduced muscular cholesterol are also been reported while treating the cultured fish-species with choline chloride directly into the pond water and thus offering a better aquatic waterbody for sustainable pisciculture (Das *et al.* 2020a, 2021b, 2022).

While concentrating on prebiotic, it is nothing but a long chain sugar containing fiber like compound or substrate derived mostly from plant-based items acts as food for the beneficial microbes in the host's digestive tract (Mohammadi *et al.* 2020, Mountzouris 2022), and it is quite resistant in nature under acidic condition in stomach, fermented under the aerobic condition to promote the development as well as the growth of the gut microbiota or the probiotic within host to uplift its health status (Davani-Davari *et al.* 2019). Commonly used sources of prebiotics include edible mushrooms (Balakrishnan *et al.* 2021), fruits, beans, vegetables, microalgae, seaweeds, milk of the animals (Ahmadifar *et al.* 2019, Van Doan *et al.* 2020, Rashidian *et al.* 2020, Elumalai *et al.* 2021). Moreover, its mode of action is largely based on preventing the adhesion of pathogens to the epithelial cells by functioning as a receptor as well as a modulator of the host immune system and thus finally controlling the inflammation within the host cell (Al-Sheraji *et al*. 2013, Mohammadi *et al.* 2021).

Though it has been observed that so many commercial grade prebiotics, e.g., containing β-glucan, insulin, mannan-oligosaccharide (MOS) fructooligosaccharides (FOS), galactooligosaccharide (GOS), arabinoxylan oligosaccharide (AXOS), oligosaccharides are being used now-a-days in enhancement of growth performances (Ebrahimi *et al.* 2012, Selim and Reda 2015, Ismail *et al.* 2019, Li *et al.* 2019), as immunostimulants (Ebrahimi *et al.* 2012, Abu-Elala *et al.* 2018, Mohammadian *et al.* 2021), and to combat disease invasion (Ebrahimi *et al.* 2012, Abu-Elala *et al.* 2018, Ismail *et al.* 2019, El-Nobi *et al.* 2021) to aquatic animals under commercial aqua-farming conditions and additional supplementation of prebiotics in aqua-feed is also been observed to indulge promotion of more proliferation of beneficial gut microbiota within the aquatic animals resulting into health betterment of the host fish-species (Wee *et al.* 2022), but prevalence of prebiotics on physico-chemical properties and subsequent effect on macrophyte vegetation in the aquatic body are not been elaborated judiciously where the entire productivity of the aquatic body (pond) is solely depending on the water-quality parameters of the pond.

On the other hand, the planktonic diversity in case fresh water pisciculture system is also an important concern for production purposes (Dhore and Lachure 2014, Bhute and Harney 2017), while the macrophytes play as a good indicator representing the pond water quality and subsequent contamination into the culturable aquatic body from different sources of anthropogenic activities (Kumar *et al.* 2022). Moreover, Hassan *et al.* (2013) also reported strongly in their study about the intra-relationship between water-quality parameters and planktonic diversity of freshwater bodies.

So, the present study is designed to highlight the comparative alternation in the physico-chemical parameters with the abundance of zooplankton and macrophyte vegetation within the waterbody under semi-intensive aqua-farming condition due to exposure of 'farm-made prebiotics' while culturing the target fish species.

MATERIALS AND METHODS

Culture pond preparation and procurement of fish species for culture

Three experimental ponds comprising each having 0.40 acre of water area were taken into consideration for the purpose of experimentation during the study period (October to December) and were prepared as per pisciculture culture protocol (Jhingran 1982, Ayyappan and Ahmad Ali 2007), finally, the acclimatization, liberation of advanced fingerlings (1800 fish species per pond) into the experimental ponds (one control pond (C): Fed only with local feed having 30 % protein at the rate of four percent per day of total biomass upto three months; two treatments (T: P1 and P2: Fed with local feed as per the control pond plus farm-made prebiotics)), i.e., the fish-species for culture (*Catla catla, Labeo rohita,* and *Cirrhinus mrigala* at a ratio of 3:4:3), manuring, fertilization, and subsequent feeding management were maintained as per standard aqua-farming procedure under a semi-intensive aqua-farming system (Das *et al.* 2021b, 2022).

The 'farm-made- prebiotics' (comprising fish-meal, ground-nut oil cake, bar-yeast, jaggery and water; fermented in aerobic condition for about 18 to 22 hours and applied directly into the pond water) was applied into the treatment ponds (P1 and P2) fortnightly at the rate of 15 kg per acre throughout the experimental tenure of culture.

Collection of water sample for analysis of limnological parameters

All the water samples were collected in sterilized water containers from a depth of 10-15 cm of water surface of each pond. The physico-chemical status of the water, viz., transparency, temperature, pH, electrical conductivity (EC), carbon-dioxide (CO_2), dissolved oxygen (DO), total hardness (TH), total alkalinity (TA), phosphate (PO_4^{3-}), chloride (Cl-), ammoniacal-nitrogen (NH_4^+-N), nitrate-nitrogen (NO_3^--N), sodium (Na^+), and potassium (K^+) were analyzed by the using the standard methods of APHA (2012). The transparency, temperature, pH, electrical conductivity (EC) and dissolved oxygen (DO), were analyzed at the time of sample collection. For analyzing the other physico-chemical parameters, water samples were preserved in the plastic water sampling one liter bottle and then shifted into the laboratory. All the physico-chemical parameters were randomly analyzed after collecting the samples six times in 15 days interval during the entire three months tenure of the experiment and finally all the data were expressed as mean ± standard deviation (n =6).

Collection and analysis of zooplankton species

Zooplankton specimens were collected from the same experimental ponds with plankton net having a mesh size of 50 to 55 µm and then those were preserved in 4% formaldehyde solution. The zooplanktons were identified under the light microscope (DM 2000) and finally those zooplanktons were taxonomically characterized (Sarkar *et al.* 2020).

Collection and analysis of macrophytes

The different aquatic macrophyte samples were collected from three different experimental ponds (control: lat: N 23^{0}33'555", long: E 87^{0}73'525"; treatment (P1): lat: N 23^{0}33'65", long: E 87^{0}73'545"; treatment (P2): lat: N 23^{0}33'756", long: E 87^{0}73'515") during the study period (October to December). Different macrophytes were collected directly by using hand and long-handled hook. After the collection of the macrophytes, specimens were handled with care and were washed thoroughly then shocked with filter paper. Finally, those were kept in polythene zipper bags and then shifted into the laboratory for further analysis. Identification was done by following the methodology described by Kodarkar (1996), Cook (1996), Sarkar *et al.*(2020).

RESULTS AND DISCUSSION

Analysis of physico-chemical parameters of experimental ponds

Different physico-chemical parameters of water samples at an interval of 15 days, i.e., D15, D30, D45, D60, D75, and D90 (D = day) were collected

Table 1. Analysis of physico-chemical parameters of pond water under prebiotic exposure in experimental ponds in comparison to control in field condition.

Parameter	Unit	Control	Treatment (P1)	Treatment (P2)	p value	
Trans	cm	28.25±0.20	22.25±0.50	21.20±0.60	<0.01	**
Temp	^{0}C	22.25±0.40	21.20±0.50	22.30±0.10	<0.01	**
EC	µS/cm	540.00±2.50	575.00±3.00	595.00±3.20	<0.01	**
pH		7.20±0.18	7.80±0.12	8.25±0.25	<0.05	*
CO_2	mg/l	5.25±0.20	4.60±0.15	4.80±0.25	<0.01	**
DO	mg/l	5.40±0.10	5.90±0.30	6.80±0.25	<0.01	**
TA	mg/l	281.00±3.00	290.00±1.20	310.00±1.50	<0.01	**
PO_4^{3-}	mg/l	0.40±0.02	0.58±0.02	0.90±0.06	<0.01	**
TH	mg/l	140.20±1.70	162.25±0.80	188.20±1.20	<0.01	**
Cl-	mg/l	85.20±0.50	92.50±0.60	85.00±0.10	<0.01	**
NH_4^+-N	mg/l	0.80±0.02	1.10±0.08	0.90±0.01	<0.01	**
NO_3^--N	mg/l	0.31±0.02	0.60±0.04	0.40±0.04	<0.01	**
Na^+	mg/l	62.50±0.80	68.50±1.00	72.50±1.50	<0.01	**
K^+	mg/l	15.50±0.30	16.50±0.40	18.50±0.30	<0.01	**

Data are represented as mean ± SD (n=6). One-way ANOVA (single factor) conducted, significance: **p<0.01 and *p<0.05. D-day. Avg. value taken from D15, D30, D45, D60, D75 and D90.
P1 and P2= Treatment ponds fed with farm-made prebiotics with locally available fish feed; C= control pond: fed only with locally available fish feed [study period (October to December)].

from three respective ponds, viz., C (control), P1and P2 (as replica of treatment ponds) throughout the experimental tenure. The results of P1 and P2 were represented as an average value (n=6) and compared with the control and finally synchronized in Table 1.

Transparency under the present study ranged from 21.20±0.60 to 28.25±0.20 cm where the control pond depicted the highest value; whereas, the significant (p<0.01) highest value of temperature, electrical conductivity, and pH was showed in P2 pond (22.30±0.10 ^{0}C, 595.00±3.20 µS/cm and 8.25±0.25 respectively). On the other hand, CO_2 ranged significantly (p<0.01) from 4.60±0.15 to 5.25±0.20 mg/l; where the maximum value was shown in control pond (5.25±0.20 mg/l). Interestingly, the values of dissolved oxygen, total alkalinity, phosphate (PO_4^{3-}) and total hardness were noticed significantly (p<0.01) maximum in P2 pond (6.80±0.25, 310.00±1.50, 0.90±0.06 and 188.20±1.20 mg/l respectively). Furthermore, P1 pond depicted the highest values of chloride (Cl^-), ammoniacal -nitrogen (NH_4^+-N), and nitrate-nitrogen (NO_3^--N), e.g., 92.50±0.60, 1.10±0.08 and 0.60±0.04 mg/l respectively. Sodium and potassium were noticed significantly (p<0.01) highest in P2 pond (72.50±1.50 and 18.50±0.30 mg/l respectively).

The health of the aquatic ecosystem is directly related with the physico-chemical parameters of the pond water and which is finally linked with the production of the pond ecosystem. Transparency, ranges from 30 to 60 cm in aquaculture pond is adequate for fish culture (Boyd and Lichtkoppler, 1982), but according to Bhatnagar and Devi (2019), 15 to 40 cm of transparency is good for intensive and semi-intensive culture systems and below 12 cm may be detrimental to cause stress to culture species. In the present study, both in treatment as well as control ponds, it was very much within the optimum range between 21.20±0.60 and 28.25±0.20 cm, which seems to be congenial for the pond culture.

Temperature above or below the optimum range may decrease fish growth and cause mortality due to extreme conditions (Volkoff and Rønnestad 2020). Usually, it ranges from 26.06 to 31.97 ^{0}C and is suitable for warm water fish culture (Boyd 1982); again, the temperature between 25 and 32 ^{0}C is ideal for tropical fish culture (Bhatnagar and Devi 2019), and less than 12 ^{0}C are assumed to be stressful (Bhatnagar and Devi 2019). Our study revealed the optimum temperature range of 21.20±0.50 to 22.30±0.10 ^{0}C, which seems to moderate to lowered valued temperature but the it would not hamper the fish productivity as well as zooplankton development into the experimental ponds.

Electrical conductivity (EC) is largely depending upon the degree of increase or decrease of soluble ions in water, and directly influences the productivity of the culture pond (Kumari *et al.* 2019). In the present study, EC in the treatment conditions at the end of the experiment depicted the maximum activity, while in the control pond at the end of the experiment was lowest. It might be assumed that the ion exchange was increased gradually in the treatment conditions and became maximum in P2 pond contributing to high yield of fish and abundance of zooplankton. The pond under control condition was less reactive due to non-abundance of sufficient total soluble solids (Robotham *et al.* 2021).

In aquaculture system, pH range of 6.4 to 8.3 is favorable for fish growth (Swain *et al.* 2020), fishes may undergo stress between 4.0 and 6.5, and 9.0 and 11.0; death of fish species may occur at less than 4.0 pH and greater than 11.0 (Bhatnagar and Devi 2019). In tropical areas, the ideal range lies between 6.7 and 9.5 and optimum is 7.5 to 8.5, while less than 4.0 and greater than 10.5 pH is dangerous to fish health (Bhatnagar and Devi 2019). In our findings, it was laid in the range from 7.20 ± 0.18 to 8.25 ± 0.25 which seemed to be the normal and congenial of fish culture in the tropical plain.

In the aquaculture system, free carbon dioxide (CO_2) at less than 10 mg/l is assumed to be ideal, while 5.0 to 8.0 mg/l is also required for photosynthetic activity; 12.0 to 15.0 is sub-lethal and 50.0 to 60.0 mg/l is lethal to the culture fishes in tropical areas (Bhatnagar and Devi 2019), whereas, according to Fatima *et al.* (2021), CO_2 less than 5.0 mg/l is supposed to be beneficial for better productivity of the fishes under culture system. Here, the free CO_2 level varied within 4.60±0.15 to 5.25±0.20, which indicated the congenial range for fish culture for treatment ponds but control pond depicted the highest CO_2 concentration which may be detrimental to the culture fish species.

Dissolved oxygen (DO) attributes the physical and biological occurrences prevailing in the water (Devi *et al.* 2017), it is also an indicator of pollution in water bodies (Amankwaah *et al.* 2014). Several authors established the different ranges of DO for pisciculture, usually, 5.0 mg/l is desirable for aquaculture system and greater than 14.0 mg/l is lethal to fish fry, producing gas bubble disease (Bhatnagar and Devi 2019), while, Nsonga (2014) observed that DO of 6.5 mg/l or above 5.0 mg/l is the ideal for warm water fish culture; and for air-breathing fishes, 4.0 mg/l is sufficient to survive (Fatima *et al.* 2021). The DO level in the present experiment revealed a minimum value of 5.40±0.10 mg/l in the control condition and a maximum of 6.80±0.25 mg/l in the treatment condition under P2 pond. On the other hand, DO concentration is mainly dependent on temperature fluctuations, dissolved salts, wind velocity, pollution load, photosynthetic activity and respiration rate in any aquatic body, while, the low level of DO in summer due to higher decomposition rate, elevated temperature and limited flow of water into the aquatic system (Fatima *et al.* 2021).

The total alkalinity (TA) is a factor for the measurement of productivity and condition of the water body (Cuevas-Madrid *et al.* 2020). Several researchers stated the range of TA aquaculture practice like less than 20 mg/l of TA indicates the poor status, while 80-200 mg/l is desirable for pisciculture in warm areas and more than 300 ppm is undesirable due to the non-availability of CO_2 (Bhatnagar and Devi 2019), whereas, Fatima *et al*. (2021) mentioned that 50-300 mg/l is the ideal for fish culture. Although the present study revealed the minimum and maximum values of 281.00±3.00 and 310.00±1.50 mg/l of alkalinity in the control and treatment pond (P2) respectively, but during treatment condition under prebiotic exposure, the ponds showed a slightly elevated range than the ideal range making an effective buffering mechanism in the aquatic ecosystem.

The total hardness (TH) mainly represents the content of calcium and magnesium salts (Cuevas-Madrid *et al*. 2020); calcium and magnesium are mandatory items to the fish for metabolic reactions, as well as bone and scale formation (Bhatnagar and Devi 2013). In our experiment, it ranged from 140.20±1.70 to 188.20±1.20 mg/l. Under control condition it was slightly lower may be due to less ionic movements in ponds and less abundance of other salts, resulted in adverse effects in terms of productivity, but in the treatment conditions, the values remained quite higher (162.25±0.80 and 188.20±1.20 mg/l) which finally depicted higher productivity with the help of higher content of zooplankton and controlled macrophyte vegetation. Thirupathaiah *et al.* (2012) reported the higher TH in summer than to winter due to decrease in water volume and increase in the rate of evaporation at high temperature. According to Bhatnagar and Devi (2019), the TH level below 20.0 mg/l causes stress, 75-150 mg/l is ideal for fish, and higher than 300.0 mg/l is lethal to fish under the pond culture system.

Phosphates are known to be one of the most important growth-limiting factors in eutrophication and responsible for producing some desirable and undesirable ecological effects in the aquatic system (CCME 2006). The aquatic system usually gets the phosphate in terms of orthophosphate from the domestic waste, mainly phosphate-based detergents and also from agro-based fertilizers, used in pisciculture (Kurzbaum *et al.* 2017). Stone and Thomforde (2004) pointed out that the phosphate level of 0.06 mg/l is suitable for fish culture, while Bhatnagar and Devi (2019) suggested that 0.05-0.07 mg/l is optimum and productive and 1.0 mg/l is better for plankton production in fish culture pond. In our study the phosphate ranged from 0.40±0.02 to 0.90±0.06, which indicated a slightly elevated phosphate level in treatment conditions but control depicted a less concentration. The treatment ponds might lead to excess production of plankton but satisfactory production has been depicted under the experimental tenure.

Chloride maintains the osmotic balance and mineral contents in the aquaculture ponds (Bhatnagar and Devi 2013). Our analysis revealed that the chloride content ranged from 85.00±0.10 to 92.50±0.60 mg/l, which indicated the abundance of chloride ions in the prebiotic-fed ponds under the experiment. Whereas, a slightly elevated range of chloride content showed the suitability for the aquatic vegetation and fish productivity under the freshwater aquaculture system (Devi *et al.* 2017).

Ammoniacal-nitrogen (NH_3^+-N) is the main nitrogenous waste generated by aquatic animals, via metabolism and is excreted across the gills (Cao *et al.* 2007); a higher concentration of ammonia in aquaculture is caused due to fish excreta (Nyanti *et al.* 2012). It is also established that ammonia levels between 3.0 and 4.0 mg/l may be toxic for tropical fishes under culture; the desired concentration for freshwater fish is well below the level of 0.05 mg/l (Ip and Chew 2010, Devi *et al.* 2017). According to EPA (1973) and Jhingran (1988), 0.02 mg/l is sufficient to maintain the optimum health condition of warm water fish culture. Moreover, Karnatak and Kumar (2014) explained the presence of a high concentration of ammonia in aquaculture due to the high stocking density of fish, high feeding rates, reduced dissolved oxygen level. The present study disclosed the range from 0.80±0.02 to 1.10±0.08 mg/l with an indication that the level was slightly elevated in treatment conditions in both the seasons because of the generation of various nitrogenous by-products due to the application of prebiotics, but posed no such harmful effects or mortality because of better aquaculture practices.

Torno *et al*. (2018) reported the suitable range of nitrate concentration for the aquaculture is 0.2 to 10.0 mg/l, while Fatima *et al.* (2021) noticed the favorable range of 0.1 mg/l to 4.0 mg/l. In our experiment, it was ranged from 0.31±0.02 to 0.60±0.04 mg/l, which represented the satisfactory level of nitrate concentration throughout the experimental period. It is also mentioned that addition of prebiotics might also contribute to the high levels of nitrate into the culture ponds (Rao 2011), which was in consonance of our experiment.

The present study estimated the sodium ranged from 62.50±0.80 to 72.50±1.50 mg/l which seemed to be well within the permissible limit (200 mg/l) as prescribed by Indian standards. Mishra *et al.* (2009) reported to some extent similar results in the pond water at Varanasi varied between 40.80 and 93.50 mg/l. Usually, enhanced sodium concentration was evidenced in ponds due to runoff after precipitation, the addition of detergents, and soaps (Sajitha and Vijayamma 2016).

Potassium may generally become a limiting nutrient during the eventual addition of N and P fertilizers in the aqua-pond. So, the estimation of potassium ions in pond water is necessary (Devi *et*

Table 2. Zooplankton species diversity under prebiotic exposure under experimental ponds in comparison to control in field condition.

Parameters	Phylum	Family	Control	Treatment (P1)	Treatment (P2)
Moina sp.	Arthopoda	Moinidae	+	+	+
Daphnia sp.	Arthopoda	Daphniidae	+	++	++
Diaptomus sp.	Arthopoda	Diaptomidae	-	++	++
Cyclops sp.	Arthopoda	Cyclopidae	+	++	++
Cypris sp.	Arthopoda	Cyprididae	-	+	+
Bosmina sp.	Arthopoda	Bosminidae	-	+	+
Keratella sp.	Rotifera	Brachionidae	+	-	-
Brachionus sp.	Rotifera	Brachionidae	++	-	-

(-) = 0 number of species per 10 ml of sample; (+) = 1 to 5 number of species per 10 ml of sample; (++) = 6 to 10 number of species per 10 ml of sample.
P1 and P2= Treatment ponds fed with farm-made prebiotics with locally available fish feed; C= Control pond: Fed only with locally available fish feed [study period (October to December).

al. 2017). Generally, the major sources of potassium from nature are feldspar, some micas, and clay minerals (Sajitha and Vijayamma 2016). The presence of moderate to a good amount of potassium (K) in the water of an aquaculture pond endorses the higher concentration of clay and organic matter content in the pond bottom with alkaline pH conditions (Singh *et al.* 2008). The present study depicted the amount of potassium ranging from 15.50±0.30 to 18.50±0.30 mg/l which seemed to be congenial for pisciculture and planktonic development as also reported by Mishra *et al.* (2009).

Zooplankton distribution study

A total of eight numbers of species were identified in the entire study period, out of those, six species were arthropods, viz., *Moina* sp., *Daphnia* sp., *Diaptomus* sp., *Cyclops* sp., *Cypris* sp., *Bosmina* sp. and two of were rotifers, namely, *Keratella* sp. and *Brachionus* sp. normally, *Brachionus* sp., *Cyclops* sp., *Keratella* sp., *Moina* sp. and *Daphnia* sp. were commonly distributed (also called dominant species) in control pond, while *Daphnia* sp., *Diaptomus* sp., *Cyclops* sp., were very common (also called dominant species) in treatment ponds during the entire period of experimentation (Table 2).

Worldwide, experimentation of pond health status and ecosystem's integration was studied through zooplankton diversity pattern, macrophyte distribution as well as water physiological parameters (Liu *et al.* 2013, Srichandan *et al.* 2015). In the present investigation, six arthropods and two rotifer species were recorded. Similar to our findings Haque (2015) reported nine zooplankton species from Krishna Sayer lake with maximum abundance in summer. Zooplankton species were comparatively higher in treatment ponds compared to control. This phenomenon indicated the hyper nutria condition of the ponds at prebiotic exposure and low water level (Haque 2015). It is noteworthy that under the prebiotic exposure, the abundance of *Cyclops* sp., *Daphnia* sp., *Diaptomus* sp., and *Cypris* sp. superseded the abundance of other zooplankton species. Our study displayed similarity with previous reports conducted on some specific habitats such as Guaraíras Lagoon (Almeida *et al.* 2012) and Krishna Sayer lake (Haque 2015) showing maximum zooplankton abundance under higher primary productivity.

Macrophyte distribution study

Different macrophytes were observed during the entire experimental period, e.g., *Lemna* sp. (Major) Lemna sp. (Minor), *Azolla* sp., *Pistia* sp., *Hydrilla* sp., *Nymphaes* sp., *Ipomoea* sp., *Eichhornia* sp. and *Ceratophyllum* sp., belonging to three separate groups, viz., free floating, rooted submerged and rooted emerged (Table 3). Maximum species diversity in macrophyte community was observed in control pond compared to treatment.

Table 3. Macrophytes species distribution under prebiotic exposure under experimental ponds in comparison to control in field condition.

Macrophytes	Types	Family	Control	Treatment (P1)	Treatment (P2)
Lemna sp. (major)	Free-floating	Lemnaceae	√	√	√
Lemna sp. (minor)	Free-floating	Lemnaceae	√	√	√
Azolla sp.	Free-floating	Salviniaceae	×	√	√
Pistia sp.	Free-floating	Araceae	√	√	√
Hydrilla sp.	Rooted submerged	Hydrochorideae	√	√	√
Nymphaes sp.	Rooted free floating leaves	Nymphaeaceae	×	√	√
Ipomoea sp.	Emergent	Convolvulaceae	×	√	√
Eichhornia sp.	Free-floating	Pontederiaceae	×	√	√
Ceratophyllum sp.	Free submerged	Ceratophyllaceae	√	√	√

(√) = Present, (×) = Absent.

Pond macrophytes are important plant communities, which are regulating the nutrient cycling and productivity of pond (Pal *et al.* 2014). They provide food, habitat for other aquatic species, remove excessive carbon dioxide, suspended materials and produce good oxygen flux in the aquatic ecosystem. During the study period, nine different types of perennial macrophytes, viz., *Lemna* sp. (Major), *Lemna* sp. (Minor), *Azolla* sp., *Pistia* sp. (free floating), *Hydrilla* sp. (rooted submerged), *Nymphaes* sp. (rooted free-floating leaves), *Ipomoea* sp. (emergent), *Eichhornia* sp.(free floating), *Ceratophyllum* sp. (free submerged) had been noticed and identified in those three ponds. Submerged macrophytes played vital role for mineral recycling in aquatic ecosystem (De *et al.* 2019). Several studies demonstrated that proliferation of free-floating species namely *Lemna* sp. (Major), *Hydrilla* sp. *Nymphaes* sp., and *Eichhornia* sp. caused very good impacts not only to multiple uses of water but also to other communities (Pereira *et al.* 2012). On the other hand, comparatively lower abundance of free-floating and submerged macrophytes in treatment ponds might be marked for growth of phytoplankton. Further, the growth of macrophytes is dependent on water depth (Cazzanelli *et al.* 2008). Accordingly, macrophytes are considered as vital bioindicators of water quality measurement of aquatic ecosystem and incidence of macrophytes diversity primarily depend on nutrient load of the aquatic ecosystem (Poikane *et al.* 2015).

CONCLUSION

From the present study it can be inferred that a marked as well as notable difference has been observed in zooplankton diversity, macrophyte vegetation and the various physico-chemical parameters of the pond water under the exposure of farm-made prebiotics. While, others are concentrating basically on the increment of aquaculture production, immune system enhancement, promoting disease resistance, but the present study is specifically concentrated on the water quality of the pond in a semi-intensive pisciculture system under home-made, cost-effective, easy-to-handle prebiotic, i.e., prebiotic mixture. Though ammoniacal-nitrogen (NH_3^+-N), chloride (Cl^-) content in the pond water have been elevated under prebiotic exposure, but it revealed a balanced aqua-farming which finally led to a marked higher Na^+, K^+, EC concentration and phosphate values; finally resulted into a healthy waterbody congenial for fish culture. Moreover, an elevated abundance of certain zooplankton, especially, *Daphnia* sp., *Diaptomus* sp., *Cyclops* sp. have made the waterbody available for natural-foods which may finally resulting into higher productivity. Nonetheless, dosing of the prebiotic mixture into the waterbody must be taken care of to avoid adverse effect on the culturable aquatic animal. Thus, there is a still gap between scientific know-how and practical application of prebiotics during aquaculture practices. So, a controlled and measured application of proper prebiotic alike the present experiment may enhance the fish productivity, betterment of pond water as well as controlled abundance of zooplankton and macrophyte vegetation. Finally, the fish farmers may be highly benefited from the application of such home-made as well as farm-made prebiotics as suggested.

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