EFFECT OF DIFFERENT SUBSTRATES ON FISH PRODUCTION AND WATER QUALITY IN PERIPHYTON BASED POLYCULTURE OF MAJOR CARPS

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ABSTRACT

The study was designed to assess the effects of periphyton grown on different substrates on water quality and production of major carps cultured in zero water exchange system. Three experimental treatments included; T1 (Control): without substrate, T2: using PVC pipes substrates, T3, using Bamboo poles substrates. Fish seed of *Labeo rohita: Cirrhinus mrigala and Gibelion catla* were stocked in cemented tanks in 1:1:1 ratio at stocking density of 3 fishm^{-2} for 60 days period. Survival rate of *L. rohita*, *C. mrigala* and *G. catla* was significantly higher (p<0.05) in substrates based treatments. In T1, T2 & T3, it was found to be 34.09%, 81.82% & 90.91% for *L. rohita*; 22.73%, 100.0% & 100.0% for *C. mrigala* and 36.36%, 90.91% & 100.0% for *G. catla* respectively. Total fish biomass of *L. rohita*, *C. mrigala* and *G. catla* harvested at the end of trial was 2.24, 4.32 and 1.42 times higher in treatment with bamboo poles as substrates and 1.18, 3.64 and 0.75 time higher in PVC pipes based treatment than that of control. Concentration of ammonia and nitrite was found to be significantly low in treatments with periphyton than control. High fish survival in periphyton based systems can be attributed to bioremediation through bioprocesses of their ubiquitous microbial biofilms.

Keywords: Periphyton biomass, Biofilms, water quality, major carps, fish production.

INTRODUCTION

Aquaculture, a rapidly developing food production system, ensures increasing demand of the protein supply for growing world population. However, sustainable development of the sector necessitates to cope with the challenges of negative environmental impacts due to production of nutrient rich water and high feed cost. Several novel techniques have been practiced on this basis that improve the nutrient conversion efficiency in the aquaculture water. One of these strategies is based on the development of microbial biofilms that are attached to a substratum within the culture unit and referred as periphyton (Azim and Little, 2006). Periphyton can be considered as a microecosystem composed of a complex matrix with embedded microorganisms attached to the substratum that has the natural ability to respond and recuperate from stress (Sabater *et al.*, 2007). This composite of sessile aquatic biota comprises of protozoans, fungi, bacteria, planktons, benthic organisms and invertebrates along with calcium carbonate and detritus attached to a submerged substratum (Azim and Asaeda, 2005).

Due to their higher nutrient conversion efficiency, periphyton matrix not only reduces the negative effects of pond water effluents but also improves the water quality within the aquaculture system, thereby, leading to higher fish production (Wu, 2017). Materials that provide large surface area like tree branches, aquatic vascular plants, bamboos, and synthetic polymers (e.g. PVC pipes) can be used for periphyton production (Ekram-Ul-Azim, 2001). Periphyton based systems, therefore, can serve as feasible option to be employed for sustainable aquaculture practices.

In Pakistan, various anthropological activities and overfishing have led to the loss of biodiversity in our natural water bodies and reduction in wild fish catches (Shafi *et al.*, 2018). Sustainable development of aquaculture sector is, therefore, a crucial prerequisite to ensure the food security for the increasing population of the country and to fill the gap between the demand and supply of fish and fish products. Although aquaculture is progressing at fast rate in the country and enormous research is being carried out to get increased fish production through natural and supplementary feeding, none of the earlier investigations have evaluated the effect of using periphyton in fish culture systems. The present study was, therefore, carried out to evaluate the effect of periphyton based systems on fish culture and water quality to aid in the use of low cost technologies for development of aquaculture sector. The study assessed the effect of using natural and synthetic substrates for periphyton growth on water quality and fish survival & production in polyculture system of major carps in zero water exchange (ZWE) system. Bamboo poles and PVC pipes were used in this study to compare the efficacy of natural and artificial substrates for periphyton growth.

MATERIALS AND METHODS

Tanks preparation and fertilization: Fish culture experiment was conducted for 60 days (October-November, 2018) to evaluate the effect of periphyton on fish growth. The experiment was conducted in cemented tanks at Fisheries Research and Training Institute, Lahore, Pakistan. Average surface area of each tank was 20.7 m^2 with average water depth of 0.90 m. Bottom of tanks was covered with 0.30 m thick soil layer. There were three experimental treatments; T1: without substrate, T2: with PVC pipes as substrate and T3: with bamboo poles as substrate. Each experiment treatment was tested in duplicate. Submerged bamboo pole and PVC stick with uniform surface area (0.06 m^2) were installed at a rate of 5 substrates/m^2 in T2 and T3. Additional surface area provided by substrates installation was 5.5 m^2 in each treatment. In order to use optimum pond fertilization, fertilizer programme that provided N: P_2O_5 of 1.1:1.0 was adopted following Azim *et al.* (2001a). Cow dung, urea and single super phosphate (SSP) were applied in tanks at the rate of 10 kg, 0.050 kg and 0.100 kg per tank, respectively and tanks were left for 15 days to allow sufficient production of periphyton on the provided substrates.

Fish Stocking and Tank Management: Fish seed of *L. rohita: C. mrigala: G. catla* (stocking ratio; 1:1:1) with initial weight of 0.81 g ± 0.08 g, 3.16 g ± 0.16 g and 3.9 g ± 0.55 g respectively were stocked in six experimental tanks at the stocking density of 3 fish/m^2. Total initial fish biomass stocked in each tank was recorded. Fish was supplied with soybean meal based supplementary feed (31% crude protein) at the rate of 2% fish body weight in each tank daily. Consumption of supplementary feed by fish was ensured by regularly monitoring the deposition of any uneaten feed on bottom mud of tanks. Urea and SSP were applied at the rate of 0.025g and 0.050g/tank respectively on weekly basis throughout the experimental period (Azim *et al.*, 2001a). Due to low water level and bottom soil content in tanks, organic manure was used only at initiation of experiment. There was no exchange of water (ZWE system) during the entire experimental period and fresh water was added only to compensate for water lost by evaporation and maintain it at constant level in tanks.

Water Quality Monitoring: Water abiotic quality parameters were measured on weekly basis and included temperature, dissolved oxygen (mgL^{-1}), pH, electrical conductivity (EC, μScm^{-1}), total alkalinity (mgL^{-1}), total hardness (mgL^{-1}), chloride content (mgL^{-1}), total ammonia-N (TAN, mgL^{-1})), nitrite-N (mgL^{-1}), nitrate-N (mgL^{-1}), soluble reactive phosphorous (mgL^{-1}). Unionized ammonia-N content (mgL^{-1}) was calculated according to Boyd and Tucker (2012) using water TAN, pH and temperature. Water samples were collected between 10:00-11:00 on each sampling day, 30 cm below the water level. Temperature was measured by direct insertion of a laboratory thermometer in the tank water. Dissolved oxygen, pH and EC were measured with the aid of electronic meters. Total alkalinity, total hardness, chloride content, nitrite-N, nitrate-N and soluble reactive phosphorous were measured using standard methods of APHA, 2012. Total ammonia-N was measured using Nessler method (ASTM, 2008).

Monitoring of Periphyton and Primary Productivity: For determination of moisture and ash content, periphyton mass were taken by scraping the respective substrate with the help of scalpel from an area of 94.20 cm^2. Moisture and ash content of periphyton was determined following Azim *et al.* (2001a). For qualitative and quantitative analysis of phytoplankton and periphyton, water and periphyton samples were collected on weekly basis from the second month of experiment. For determination of plankton density, method of Azim *et al.* (2001b) with slight modification was adopted. Water sample (1 L) of water was collected 30 cm below the water surface and filtered through a phytoplankton net with 37 μm mesh size. Residual biomass (RBM) retained on the top of the net was transferred quantitatively to a glass cylinder and made up to 100 ml with distilled water. The RBM samples were preserved with concentrated formalin and stored in sealed plastic vials till further analysis. Sedgewick-Rafter counting chamber (SRC) was used for quantification of phytoplankton and zooplankton. RBM sample was shaken gently and 1 ml was poured on Sedgewick-Rafter cell (1000 grids). Number of plankton in three randomly selected grids was counted and used to determine planktonic density. For periphyton analysis, an area of 12.56 cm^2 from one substrate in each tank (bamboo pole/ PVC pipe) was scrapped with the help of a scalpel and periphyton biomass was transferred to a plastic bottle with 50 ml distilled water. Samples were preserved with concentrated formalin for future examination. The compact mass of periphyton was gently grinded in the glass tissue grinder, transferred to glass cylinder, made up to 500 ml with distilled water and quantified using SRC (Azim *et al.*, 2001b). Qualitative analysis of phytoplankton and periphyton was carried out according to Ward and Whipple (1959) and Prescott (1984).

Fish Harvest: Tanks water was drained and fish were harvested at the end of 60 days culture period. Total fish biomass (gross production; gtank^{-1}) and survival rate in each tank was recorded and fish growth and production parameters were calculated.

Statistical Analysis: Collected data on fish production, survival and water quality parameters was statistically analyzed through one-way ANOVA followed by post hoc analysis (Least Significant Difference Test) at

significance level of .05. Independent t- test was used to compare the proximate quality of periphyton mass in two treatments ($p<.05$). All statistical analysis was performed using SPSS version 22 following Field (2013).

RESULTS AND DISCUSSION

Fish culture has been associated with the discharge of nutrient rich aquaculture water containing compounds of nitrogen and phosphorous as well as organic matter in the receiving water bodies causing degradation of their water quality (Crab *et al.*, 2007; Mugg *et al.*, 2007). Moreover, drained water may introduce pathogenic microorganisms leading to poor microbial quality of receiving water reservoir (Thompson *et al.*, 2002). Management of ammonia and other nitrogenous substances in pond involves optimum operation of biological processes in culture water that can maintain content of toxic compounds within safe limits. Bioprocesses involved in development and growth of periphyton within the culture system amplify nutrient conversion efficiency in water leading to increased transformation of toxic ammonia and nitrite to innocuous compounds (Sabater *et al.*, 2007).

Although periphyton based aquaculture systems have been focused in earlier studies, most of the work in this field was done at the start of 21st Century in Bangladesh (Wahab *et al.*, 1999; Azim *et al.*, 2001a; Ekram-Ul-Azim, 2001; Azim, *et al.*, 2002; Azim *et al.*, 2004; Rai *et al.*, 2008). In some recent investigation, researchers have investigated the potential of periphyton based fish culture in Nepal and Brazil (Tortolero *et al.*, 2016; Jha *et al.*, 2018). The present study was a first pilot investigation to assess the potential of using periphyton in fresh water aquaculture in Pakistan.

Fish Survival and Production: Fish survival rate and production in substrates based treatments was significantly higher than that of control (Table 1). Survival rate of fish showed marked differences among treatments with and without substrate. For substrate based treatments, survival rate of all three fish species ranged from 80% to 100% while it was found to vary from 22% to 38% in the case of control (Table 1). Total fish biomass (gross production; $gtank^{-1}$) of *L. rohita, C. mrigala* and *G. catla* harvested at the end of trial was 2.24, 4.32 and 1.42 times higher in treatments with bamboo poles as substrates than that of control (without substrate). For PVC pipes based treatments, total fish biomass was 1.18, 3.64 and 0.75 times higher than control treatment for *L. rohita, C. mrigala* and *G. catla* respectively. The results are in agreement with those of Azim *et al.* (2004), Cavalcante *et al.* (2011) and Reboucas *et al.* (2012) who have also reported higher fish production in periphyton based culture systems due to improved water quality. A comparison of the two

substrates for periphyton growth showed that use of bamboo poles resulted in higher survival rate and fish production of *L. rohita* in comparison to PVC pipes. However, survival rate and production of *L. rohita* was significantly higher in PVC pipes based treatment than that of control.

Average weight gain and specific growth rate (SGR) of all three fish species didn't differ significantly between the substrate based treatments and control ($p<.05$). Results of comparable fish growth in control treatment are in contradiction with those of Azim *et al.* (2002), Azim *et al.* (2004) and Rai *et al.* (2008) who reported improved growth performance of carps in the presence of periphytic communities. According to these studies, periphyton served as natural feed for fish resulting in higher fish growth. However, findings of present study are in agreement with those of Tortolero *et al.* (2016) and Jha *et al.* (2018) who showed that weight gain of carps in control treatment (without substrate and periphyton) was comparable to that of substrate based treatments. Tortolero *et al.* (2016) has attributed higher fish growth in control treatment to the periphytic communities grown on walls of tanks. In present study, the walls of tanks were periodically scrapped to remove any periphyton growth. It was also observed during the experiment that fish in substrate based treatments was consuming periphyton. However, higher fish growth in control treatment can be attributed to lower fish survival that led to reduced fish stocking density in this treatment. Due to low fish density, higher amount of supplemented feed was available to the fish that led to higher growth.

A comparison of two types of substrates showed that average weight gain of all fish species were higher in bamboo poles based treatment (16.82 g ± 2.01 g, 14.37 g ± 1.12 g and 13.27 g ± 1.56 g for *L. rohita, C. mrigala* and *G. catla* respectively) as compared to those achieved in PVC pipes based treatment (12.86 g ± 0.50 g, 12.42 g ± 0.30 g and 9.52 g ± 1.73 g for *L. rohita, C. mrigala* and *G. catla* respectively). Similar results have been reported by Jha *et al.* (2018) who achieved higher fish growth with the use of 20% density of natural substrates as compared to three evaluated densities of artificial substrates.

Monitoring of Water Quality: Results of water quality analysis are presented in Table 2. Presence or absence of substrate didn't affect water dissolved oxygen, pH, electrical conductivity, total alkalinity, total hardness, chloride content which were found to be within suitable range for fish culture. These results are in accordance with those of Azim *et al.* (2001a), Ahsan *et al.* (2014) and Tortolero *et al.* (2016) who also reported that substrates availability didn't affect general water quality parameters of aquaculture water.

Graphical presentation of average content of nitrogenous compounds and variation of nitrite-N, nitrate-N and phosphorous content in water has been

shown in Figure 1 (a-d). Content of TAN, unionized ammonia-N and nitrite-N was significantly higher ($p<.05$) in control treatment than in substrate based treatments. Average unionized ammonia-N in control treatment (0.012 mgL^{-1} ± 0.0002 mgL^{-1}) was found to be 3.8 times higher than its content in bamboo based treatment (0.0036 mgL^{-1} ± 0.002 mgL^{-1}) and 2.3 times higher than detected in PVC pipes based treatment (0.0043 mgL^{-1} ± 0.0012 mgL^{-1}). The results of higher unionized ammonia-N observed in control treatment are in agreement with earlier investigations carried out on periphyton based fish culture studied in tanks (Langis *et al.*, 1988; Thompson *et al.*, 2002) and in ponds (Azim *et al.*, 2002; Azim *et al.*, 2004) who have reported significantly higher ammonia concentration in control than that of periphyton based treatment due to higher rate of nitrification in the latter.

Level of toxic nitrogen compounds in water must be maintained within suitable limits for health and optimum growth of cultured fish that consequently leads to higher fish production. Problems of higher ammonia concentration in fish ponds are often dealt with occasional or regular water exchange that not only involves the pollution of receiving water reservoirs but is also uneconomical practice for large ponds (Tucker and Hargreaves, 2012). In general, maximum unionized ammonia-N concentration is recommended to be less than 0.02 mgL^{-1} for freshwater aquatic life (United States Army Corps of Engineers, 1982). Lethal concentration (LC50, 24 hours) of unionized ammonia-N has been reported to be 0.055 mgL^{-1} and 0.045 mgL^{-1} for *C. mrigala* and *G. catla* respectively (Tilak *et al.*, 2002; Iqbal *et al.*, 2013). In present study, highest mean unionized ammonia-N level (0.012 mgL^{-1}, detected in control treatment) was less than maximum acceptable level of 0.02 mgL^{-1}. However, it has been reported that unionized ammonia-N concentration follow a diurnal cycle in ponds and may vary from trace level in the morning to more than 1.0 mgL^{-1} ammonia-N in mid-afternoon (Boyd, 2012). In current investigation, water quality assessment was carried out in the morning, therefore, there is possibility that in control treatment, unionized ammonia-N may rise to critical levels in the late hours of the day leading to fish mortality. However, this aspect of water quality requires further investigation.

Nitrite-N ranged from 0.028 mgL^{-1} ± 0.00 mgL^{-1} in PVC pipes based treatment to 0.056 mgL^{-1} ± 0.065 mgL^{-1} in control. Maximum acceptable levels for nitrite-N are species specific and depend on pond conditions, however, a value of less than 0.60 mgL^{-1} for freshwater aquatic life is recommended (with water chloride content higher than 10 mgL^{-1}) (Nordin *et al.*, 2009). Nitrite-N content was found to be significantly higher in control treatment in present study, however, its average content (0.056 mgL^{-1}) was less than maximum acceptable value (0.60 mgL^{-1}). An examination of variation in nitrite-N

during experimental period reveals that it remained consistently higher in control ponds after 3 weeks of initiation of experiment till 8th week (Figure 1-b). Content of nitrate-N remained below the maximum recommended limit of 32.8 mgL^{-1}. Moreover, there was no significant difference in nitrate-N content found in three treatments (Figure 1-c).

Water phosphorous content was found to be significantly lower in T1 (control) than T2 and T3 (Figure 1-d). Its averaged content ranged from 0.240 mgL^{-1} ± 0.077 mgL^{-1} in control treatment to 0.613 mgL^{-1} ± 0.057 mgL^{-1} in PVC based treatment. According to Milstein *et al.* (2003), major flow of phosphorous in ponds is linked with phytoplankton development and process of decomposition occurring at the pond bottom whereas nitrogen cycling is mainly connected to periphyton based heterytrophic and autotrophic processes. Differences in TAN, unionized-ammonia-N, nitrite-N and water phosphorous content were not significant among two substrate based treatments.

Periphyton Biomass: Periphyton biomass was found to be higher in the case of bamboo poles (9.22 mgcm^{-2} ± 0.45 mgcm^{-2}) than that of PVC pipes (7.98 mgcm^{-2} ± 1.41 mgcm^{-2}), however, the difference was not significant statistically (Table 3). Periphyton biomass depends on multiple factors including nature of substrate, fertilization rate, taxonomic composition of communities colonizing on substrate, fish stocking combination and stocking ratio. Azim *et al.* (2001a) have reported periphyton biomass of 2.4 mgcm^{-2} with 100% *Gibelion catla* and 0.68 mgcm^{-2} with 100% *L. rohita*. Tortolero *et al.* (2016) also reported higher periphyton on natural substrate (3.55 mgcm^{-2} ± 2.57 mgcm^{-2}) than artificial one (0.69 mgcm^{-2} ± 0.27 mgcm^{-2}) at 10% substrate density.

Moisture and ash content of periphyton biomass in two treatments has been shown in Table 3. There was no significant difference in proximate quality of periphyton grown on two different substrates. Moisture and ash content of periphyton ranged from 79% - 80% and 40% - 46% respectively in two treatments. Ash free dry weight (AFDW) is an indicator of total organic mass in periphyton comprising of autotrophic and heterotrophic communities, alochthonous material and dead periphyton communities. According to the classification of Lakatos (1989), less than 25% AFDW is considered as indicator of organic periphyton. In present study, there was no difference in composition of periphyton grown on natural or synthetic substrate on the basis of AFDW and periphyton biomass in both treatments was identified as inorganic-organic periphyton (Lakatos, 1989).

Taxonomic composition of periphyton: Quantitative analysis of water biotic parameters has been presented in Table 4. Classes of phytoplankton and zooplankton found in three treatments and periphyton found in two

substrates based treatments have been shown in Table 5 and Table 6 respectively. For phytoplankton and zooplankton, there was no significant difference in their densities within the treatments (Table 4). Number of phytoplankton genera identified in three treatments was 8, 7 and 6 for PVC pipes based treatment, control and bamboo poles based treatment respectively. For zooplankton, 5 genera were identified in control and PVC pipes based treatment and 4 in bamboo poles based treatment. For periphyton, members of Bacillophyceae

were found to be significantly higher (p<.05) in bamboo poles based treatment than PVC pipes based treatments. However, there was no significant difference between the two substrate based treatments for members of Cyanophyceae. *Gonium sp*.; member of Chlorophyceae was only found in bamboo poles based treatment. Nine genera of periphyton were identified on bamboo poles while five were detected on PVC pipes. The results are in agreement with those of Tortolero *et al.* (2016) who also reported higher periphyton genera on natural substrate.

Table 1. Production and Survival of major carps (Mean ± SE)

Fish species	Treatment	T1 (Control)	T2 (With PVC pipes)	T3 (With bamboo poles)
L. rohita	Initial biomass (g)	18.65 ± 1.25[a]	21.90 ± 3.5[a]	19.10 ± 1.6[a]
	Final biomass (production, gtank[-1])	112.90 ± 18.20[a]	246.50 ± 10.90[b]	365.60± 26.20[c]
	Survival rate (%)	34.09 ± 2.27[a]	81.82 ± 0[b]	90.91 ± 0[c]
	Average weight gain (g)	14.17 ± 1.37[a]	12.86 ± 0.50[a]	16.68 ± 2.01[a]
	Average initial weight (g)	0.79 ± 0.07[a]	0.81 ± 0.11[a]	0.82 ± 0.065[a]
	Average final weight (g)	14.96 ± 1.44[a]	13.67 ± 0.60[a]	17.51 ± 2.08[a]
	SGR	4.90 ± 0.013[a]	4.72 ± 0.15[a]	5.08 ± 0.07[a]
C. mrigala	Initial biomass (g)	78.80 ± 2.40[a]	73.30 ± 0.90[a]	73.05 ± 0.45[a]
	Final biomass (production, gtank[-1])	73.15 ± 14.63[a]	339.30 ± 7.90[b]	389.2 ± 25.20[b]
	Survival rate (%)	22.73 ± 4.54[a]	100.0 ± 0.0[b]	100.0 ± 0.0[b]
	Average weight gain (g)	11.31 ± 0.10[a]	12.42 ± 0.30[a]	14.37 ± 1.12[a]
	Average initial weight (g)	3.32 ± 0.065[a]	3.0 0 ± 0.060[a]	3.32 ± 0.020[a]
	Average final weight (g)	14.63± 0.071[a]	15.42 ± 0.36[a]	17.69 ± 1.14[a]
	SGR	2.53 ± 0.07[a]	2.73± 0.005[a]	2.78 ± 0.01[a]
G. catla	Initial biomass (g)	86.65 ± 3.65[a]	81.3 ± 4.90[a]	86.55 ± 4.35[a]
	Final biomass (production, gtank[-1])	165.00 ± 25.00[a]	288.97 ± 49.83[b]	394.25 ± 26.66[b]
	Survival rate (%)	36.36 ± 4.54[a]	90.91 ± 0.0[b]	100.0 ± 0.0[b]
	Average weight gain (g)	14.74 ± 0.18[a]	9.52 ± 1.73[a]	13.27 ± 1.56[a]
	Average initial weight (g)	4.74 ± 0.52[a]	3.62 ± 0.53[a]	3.88 ± 0.40[a]
	Average final weight (g)	18.96 ± 1.00[a]	13.14 ± 2.26[a]	17.14 ± 1.16[a]
	SGR	2.52 ± 0.15[a]	1.16 ± 0.24[a]	2.4 8± 0.29[a]

Mean values with different superscript letters in the same column (for each fish species) are statistically significant (p<.05)

Table 2. Water abiotic parameters (Mean ± SE)

Water abiotic parameters	Optimum/ recommended value	T1 (Control)	Treatments T2 (With PVC pipes)	T3 (With Bamboo poles)
Temperature (°C)	20-32	23.74 ± 0.02[a]	23.59 ± 0.65[a]	23.76 ± 0.025[a]
DO (mgL[-1])	>5	5.71 ± 0.004[a]	5.27 ± 0.11[a]	5.08 ± 0.04[a]
pH	6.5-9.0	8.66 ± 0.078[a]	8.49± 0.076[a]	8.41 ± 0.028[a]
EC (µScm[-1])	1176	588.72 ± 3.722[a]	604.67 ± 12.44[a]	605.72 ± 0.28[a]
Total alkalinity (mgL[-1])	>50	338.59 ± 5.12[a]	355.66 ± 10.90[a]	365.96 ± 3.51[a]
Total hardness (mgL[-1])	> 50	291.41 ± 4.40[a]	296.56 ± 8.72[a]	316.97± 5.82[a]
Chloride content (mgL[-1])	>20	25.75 ± 0.57[a]	22.73 ± 0.15[a]	25.17 ± 0.05[a]
TAN (mgL[-1])	---	0.081 ± 0.007[a]	0.036 ± 0.007[b]	0.038 ± 0.008[b]
Unionized NH_3-N (mgL[-1])	<0.02	0.012 ± 0.0002[a]	0.0036 ± 0.002[b]	0.0043 ± 0.0012[b]
Nitrite-N (mgL[-1])	<0.60*	0.056 ± 0.006[a]	0.031 ± 0.003[b]	0.028 ± 0.0004[b]
Nitrate-N (mgL[-1])	<32.8	0.249 ± 0.023[a]	0.173 ± 0.065[a]	0.088 ± 0.017[a]
Phosphorous (mgL[-1])	Variable	0.240 ± 0.077[b]	0.589 ± 0.075[a]	0.613 ± 0.057[a]

Mean values with different superscript letters in the same row are statistically significant (p<.05),
*: for chloride content >10 mgL[-1]

Table 3. Characteristics of Periphyton biomass in substrate based treatments

Parameter	Treatment	
	T2 (With PVC pipes)	T3 (With bamboo poles)
Periphyton biomass (mgcm^{-2})	7.98 ± 1.41	9.22 ± 0.45
Moisture content (%)	80.86 ± 1.13	79.51 ± 1.81
Ash content (%)	45.23 ± 5.58	40.96 ± 4.76
Ash free dry matter (%)	54.77 ± 5.58	59.04 ± 4.80
Periphyton class	Inorganic-Organic Periphyton	Inorganic-Organic Periphyton

Mean values with different superscript letters in the same row are statistically significant ($p<.05$)

Table 4. Water biotic parameters in three treatments (unitscm^{-2} unless stated otherwise, Mean ± SE)

| Water biotic parameters	T1 (Control)	T2 (With PVC pipes)	T3 (With Bamboo poles)
Phytoplankton	$1.04 \times 10^5 \pm 1.024\ 10^{3a}$	$2.6 \times 10^3 \pm 2.0 \times 10^{3a}$	$1.1 \times 10^3 \pm 5.30 \times 10^{2a}$
Zooplankton	16.3 ± 8.0^a	9.5 ± 5.2^a	5.7 ± 2.0^a
Periphyton			
i. Cyanophyceae*	---	$2.8 \times 10^6 \pm 2.9 \times 10^{5a}$	$11.1 \times 10^6 \pm 7.10^{6a}$
ii. Bacillariophyceae	---	$3.4 \times 10^5 \pm 4.4 \times 10^{3a}$	$8.06 \times 10^4 \pm 6.3 \times 10^{5b}$
iii. Unicellular algae	---	$2.6 \times 10^5 \pm 1.3 \times 10^{5a}$	$1.4 \times 10^6 \pm 2.6 \times 10^{4a}$
iv. Chlorophyceae	---	ND	1.3×10^4

*: Fragments cm $^{-2}$; ND: Not detected; Mean values with different superscript letters in the same row are statistically significant ($p<.05$)

Table 5. List of planktons identified in three treatments.

T1 (Control)		T2 (With PVC pipes)		T3 (With Bamboo poles)	
Phytoplankton	Zooplankton	Phytoplankton	Zooplankton	Phytoplankton	Zooplankton
i. Chlorophyceae	i. Eurotatoria	i. Chlorophyceae	i. Eurotatoria	i. Chlorophycea	i. Eurotatoria
a. *Gonium sp.*	a. *Polyarthra sp.*	a. *Gonium sp.*	a. *Polyarthra sp.*	a. *Trochiscia sp.*	a. *Polyarthra sp.*
b. *Pediastrum sp.*	b. *Filinia sp.*	b. *Pediastrum sp.*	b. *Filinia sp.*	b. *Gonium sp.*	ii. Monogononta
c. *Scenedesmus sp.*	ii. Monogononta	c. *Haematococcus sp.*	ii. Monogononta	c. *Pediastrum sp.*	a. *Keratella sp.*
ii.Bacillariophyceae	a. *Keratella sp.*	ii. Bacillariophyceae	a. *Keratella sp.*	d. *Treubaria sp*	b. *Brachionus sp.*
a. *Amphipleura sp.*	b. *Brachionus sp.*	a. *Bacillaria sp.*	b. *Brachionus sp.*	ii. Cyanophyceae	iii. Maxillopoda
iii. Cyanophyceae	iii. Maxillopoda	iii. Cyanophyceae	iii. Maxillopoda	a. *Aphanocapsa sp.*	a. *Diaptomus sp.*
a. *Aphanocapsa sp.*	a. *Diaptomus sp.*	a. *Aphanocapsa sp.*	a. *Diaptomus sp.*	iii. Euglenoidea	
iv. Euglenoidea		iv. Euglenoidea		a. *Euglena sp*	
a. *Euglena sp.*		a.*Euglena sp*			
v.Trebouxiophyceae		v. Trebouxiophyceae			
a. *Actinastrum sp.*		a. *Crucigenia sp*			
		vi.Zygnematophyceae			
		a. *Closterium sp.*			

Table 6. List of periphyton identified in substrate based treatments

T2 (With PVC pipes)	T3 (With Bamboo poles)
i. Cyanophyceae	**i.Cyanophyceae**
a. *Gloeotrichia sp.*	a. *Gloeotrichia sp.*
ii. Bacillariophyceae	b. *Oscillatoria sp.*
a. *Neidium sp.*	c. *Hyella fontana*
b. *Diatomella sp.*	**ii. Bacillariophyceae**
c. *Bacillaria sp.*	a. *Navicula sp.*
d. *Gomphoneis sp.*	b. *Mastogloia sp*
	c. *Anomoeoneis sp.*
	d. *Bacillaria sp.*
	e. *Amphipleura sp.*
	iii. Chlorophyceae
	a. *Gonium sp.*
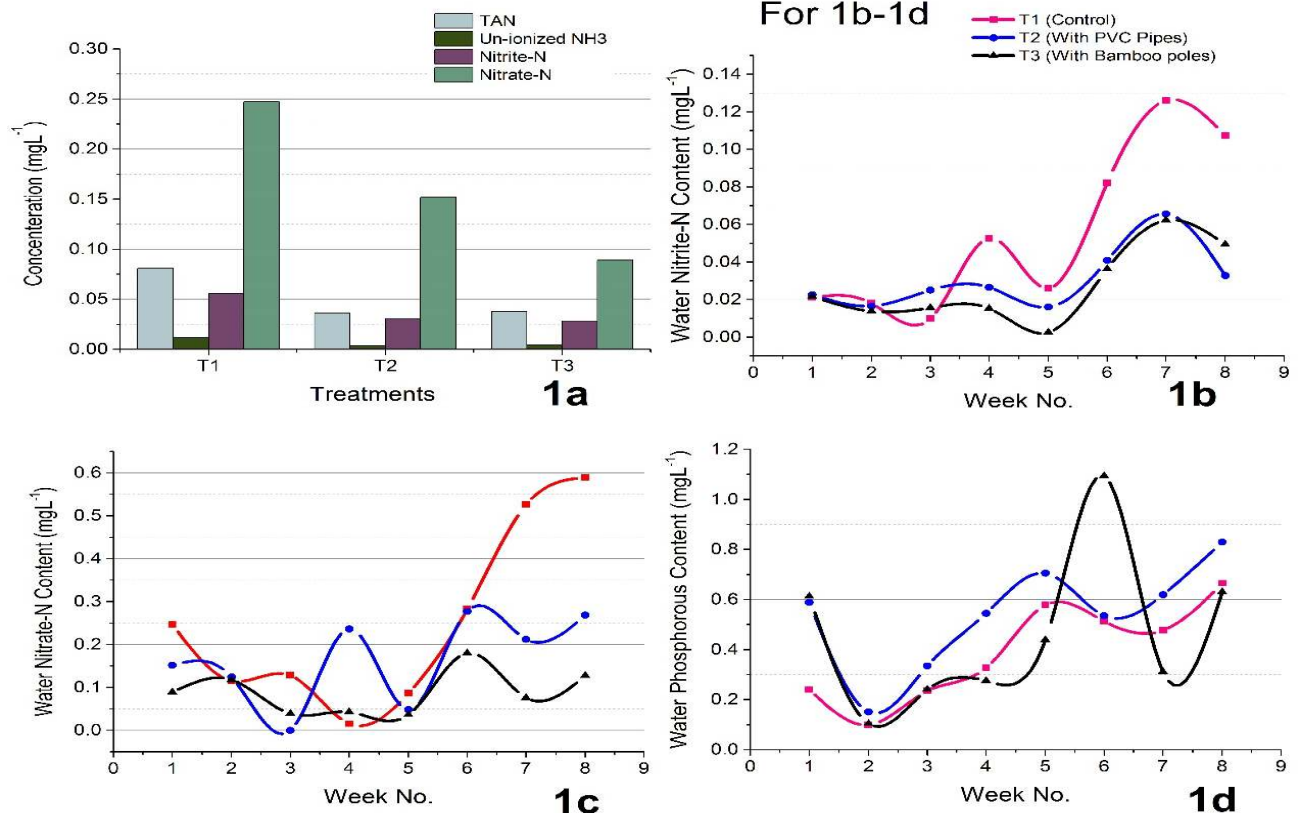
Figure 1. a: Average content of water nitrogenous compounds in three treatments, variation in b; water nitrite-N content, c; water nitrate-N content, d; water phosphorous content during experimental period.

Conclusion: The present investigation has shown the potential of periphyton based culture in ZWE systems for polyculture of major carps through higher fish production and survival. In future, there is the need for extensive investigation of water quality in periphyton based ZWE systems for sound apprehension of the processes governing the development of ubiquitous microbial films and their role in nutrient conversion efficiency of the water column. Moreover, there is the need to investigate the potential of other low cost substrates and optimization of their density in culture system.

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