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Quantitative and qualitative analysis of periphyton composition in carp polyculture system

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Abstract
A study was conducted for quantitative and qualitative analysis of periphyton composition and the production potential of carps with periphyton based aquaculture system. During the study a total number of 39 genera of periphytic community were identified. Among them 28 genera of algae periphyton comprised of four different groups, Bacillariophyceae (10 genera), Chlorophyceae (12), Cyanophyceae (4) and Euglenophyceae (2) as well as 11 genera of periphytic animal community comprised of Protozoa (2 genera), Rotifera (4), Copepoda (2), Cladocera (3) along with macrobenthic invertebrate (Chironomids larvae) were recorded from bamboo substrate used for periphytongrowth.A declining trend was detected in periphytondry matter (DM), ash free dry matter (AFDM) and ash content value in both the treatment as the period of the experiment augmented, which indicates effective grazing on periphyton by cultured fish species.

Keywords: Periphyton, quantitative, qualitative, carp polyculture

1. Introduction
Fish occupies an important place in the lives of the people of the State and fish farming has been one of the most common practice in the rural areas of the region. Thus the fishery sector is considered as an important economic means for upliftment of the socio-economic status of the rural in the region. Supplemental feeding is an important management measure in semi-intensive and intensive aquaculture for enhancing fish production. However, the cost of feed constitutes one of the most expensive cost in terms of fish production. In order to reduce the cost of production an alternative approach to this conventional system is to provide pond with substrates for the growth of periphyton that can be eaten by herbivorous or planktivorous fish. The provision of substrate in conventional culture system can provide extra source of food and thus can reduce the need of supplementary feed and lower the cost of production. The qualitative and quantitative assessment of periphyton and its communities will depict its potential in carp polyculture system.

Periphyton based aquaculture (PBA) is a new innovative pond management strategy and was proposed as a suitable technique to increase fish production in rural ponds in South Asia, particular in Bangladesh and India. Studies have demonstrated significantly higher fish production over controls with the addition of various substrates [1].

Rai *et al*., [2] conducted a comparative study on rice straw mat and kanchi (bamboo sticks) as substrates for production of major carps in periphyton-based polyculture systems. No significant differences were reported in the densities of phytoplankton and zooplankton among the treatments. The abundance of macrozoobenthos in pond sediment were 361-101, 320-104 and 275-90 individual m^2 in the control, rice straw and kanchi treatments, respectively. Potential of some locally found biodegradable and non-degradable substrate to harbor periphyton in cement tanks fertilized with poultry manure were evaluated [3]. Among the substrates, earthen tiles harbored negligible amount of periphyton. The phytoperiphyton genera encountered on the substrate belonged mainly to *Chlorophyceae* (14 genera), followed by *Cyanophyceae* (2 genera), *Chrysophyceae* (1 genus), *Bacillariophyceae* (1 genus), and *Dinophyceae* (1 genus). *Nauplius*, *Keratella*, *Diaptomus*, *Cyclops*, *Moina*, *Chironomus* and insect eggs were the zooplankton encountered on substrates. All five families of phytoplankton present on the substrates were also found in tank water. They have found 26 genera of plankton in periphyton while tank water had only 24genrea.

Tippayadara *et al*. [4] studied on the periphyton community in wastewater from swine farm along with bamboo substrate. The planktonic communities were mainly consisted of 8 of genera phytoplankton and 5 of genera zooplankton. The dominant has focus of phytoplankton was *Cyanophyta* (*Microcystis*) and zooplankton, such as *Protozoa* (*Amoeba*). Phytoplankton communities were comprised three groups *Bacillariophyta* (3), Chlorophyta (3) and *Cyanophyta* (2) genera. Zooplankton community comprised three groups *Discoba* (1), *Rotifera* (1) and *Protozoa* (3) genera. The dominant phytoplankton was *Cyanophyta* (*Microcystis*), while the dominant zooplankton was *protozoa* (*Amoeba*). Among the all groups *Cyanophyta* was found dominant followed by *Chlorophyta*, whereas *Bacillariophyta* was poorly represented. Among the zooplankton group, *Protozoa* was the dominant followed by *Discoba* and *Rotifera*.

Pandey *et al*. [5] evaluated on plankton communities in several natural and synthetic substrate for biofilm formation in in-vitro condition. The phytoplankton communities consisted principally of three groups belonging to *Bacillariophyceae* (4 genera), *Chlorophyceae* (6 genera) and *Cyanophyceae* (2 genera). *Chlorophyceae* was found the most dominant group among phytoplankton in all substrates.

Bharti *et al*. [6] reported on planktonic communities from four different substrates namely, paddy straw, sugarcane bagasse, plastic sheet and tile in fibre reinforced plastic (FRP) tanks of 500 Liter capacity. The phytoplankton communities were consisted of four groups viz. *Bacillariophyceae* (7 genera), *Chlorophyceae* (10 genera), *Cyanophyceae* (2 genera) and *Euglenophyceae* (1 genus). Among them *Chlorophyceae* was the most dominant group of phytoplankton.

Mohapatra *et al* [7] conducted an experiment in laboratory to observe periphytic growth and their planktonic communities in four types of plastic sheets, such as polyethylene (PE), polypropylene (PP), fiber reinforced plastic (FRP) and acrylic placed inside the glass aquaria filled with fertilized freshwater for 45 days. Significant difference in periphyton quantity per unit area of the plastic sheets was found among the treatments and the volume from FRP sheet was higher (7.10±0.26 ml/0.1 m^2) than the polyethylene (4.43±0.35 ml/0.1 m^2), polypropylene (3.35±0.20 ml/0.1 m^2) and acrylic sheet (2.32±0.31 ml/0.1 m^2). Tortolero *et al*., [8] studied on the periphyton development in two types of substrates, natural (*macrophyte*, *Pistia stratiotes*) and artificial (plastic screen) in various densities. Natural substrate harboured higher periphyton biomass (1.48±0.09 mg/cm^2) as well as species diversity (28 genera) than the artificial substrate (0.84±0.12 mg/cm^2, 20 genera). In the natural substrate treatment, 28 genera of microalgae were identified among the periphyton community of which 16 (57.1%) belonged to *Chlorophyta*, 5 (17.9%) to *Heterokontophyta*, 4 (14.3%) to *Cyanophyta* and 3 (10.7%) to *Euglenophyta*. Periphyton from artificial substrate consisted of 20 genera of which 13 (65%) belonged to *Chlorophyta*, 3 (15%) to *Cyanophyta*, 3 (15%) to *Euglenophyta* and 1 (5%) to *Heterokontophyta*

The aim of the present investigations was to evaluate periphyton biomass as in the carp polyculture system

2. Materials and Methods

The experiment was carried out for a period of 90 days (w.e.f. February to May, 2017) at College of Fisheries, Assam Agricultural University.

The experiment was carried out using Completely Randomized Design (CRD) in 12 nos. of outdoor rectangular cement concrete tanks. All the tanks were of same size (5.5m x 4m x 1m) having a surface area of 22m^2 each and provided with 5 cm thick soil bottom.

Prior to start of the experiment all the tanks were cleaned properly and exposed to sunlight for few days until the bottom mud gets cracked. Further, after estimation of pH, quicklime (CaO) was applied @ 250 kg ha^{-1}. After filling the tanks with water fertilization was done with raw cow dung, urea and single super phosphate @ 10,000, 100 and 50 kg ha yr^{-1} respectively.

During the study, bamboo (*Bambusatulda*) locally known as *Jati bah* was used as substrate to facilitate periphyton growth. The bamboo poles were then placed vertically with the help of plastic rope in six tanks and maintained a density of 45 pieces in each tank. The total surface area for colonization of periphyton on additional bamboo substrate was 7.48 m^2, which was about 34% of total surface area of the tanks. After the installation of substrates nearly a month was waited for periphyton development as 15 to 30 days were required for maximum periphyton biomass colonization on substrate [9]. After a month there was a good growth of periphyton which were seen on the outer surface of the substrates.

2.1 Study of taxonomic composition of periphyton

Periphyton samples were collected at an interval of 15 days. Three bamboo poles were selected randomly from each tank and periphyton samples taken carefully. A 2 x 2 cm^2 surface area of each substrate 30 to 50 cm below the water surface was removed carefully by scalpel blade. Pooled samples were re-suspended in 50 ml of distilled water and preserved in 5% formalin in sealed plastic tubes. Periphytonwas enumerated using Sedge Wick Rafter (SR) counting cell. All plankton cells or units occurring in 10 randomly selected fields of the chamber were counted using a binocular microscope. Periphytonnumbers were estimated using the formula given by Azim *et al*. [1].

$$N = \frac{P \times C}{S} \times 100$$

Where, N = number of periphyton cells or units cm^2 surface area

P = total number of periphyton units counted in 10 fields

C = volume of final concentrate of the sample (ml)

S = area of scraped surface (cm^2)

Identification of periphyton to genus level was performed using keys from [10, 11, 12, 13].

2.2 Determination of periphyton biomass

The periphyton biomass growing on the substrates, viz. dry matter (DM), ash free dry matter (AFDM), ash percentage were determined at 15 days interval following standard methods [14] and pigment concentrations were chlorophyll – a and pheophytin – a were measured following APHA, method [15].

Chlorophyll a (µg cm^2) = $[26.7 (664_b – 665_a) V_1]/ (V_2L)$

Pheophytin a (µg cm^2) = $[26.7 \{1.7(664_b – 665_a)\} V_1]/ (V_2L)$

Where,

V_1 = volume of extract (ml)

V_2 = volume of sample (cm^2)

L = light path length of cuvette (cm)

664_b, 664_a = optical density of 90% acetone extract before and after acidification respectively

2.3 Water quality monitoring

Water samples were collected from all tanks between 09:00 –

10:00 hours at 15 days interval. Few parameters like temperature, transparency were recorded at the spot, while for other parameters viz. Hydrogen ion concentration (pH), Dissolved oxygen, total alkalinity, total hardness, Nitrate nitrogen, ammonia nitrogen, phosphate – phosphorous and chlorophyll samples were brought to the laboratory for analysis following APHA method [15].

3. Results and Discussion

3.1 Qualitative and quantitative analysis of periphyton composition

3.1.1 Taxonomic composition of periphyton

The periphyton community on bamboo substrate comprised members of four groups of algae and four groups of animal communities in both treatments are depicted in (Table1 and Figure1).

Table 1: List of periphyton community recorded from the bamboo substrate in experimental tanks during the experiment.

Taxonomic group	Genera
Bacillariophyceae	*Navicula, Tribonema, Fragillara, Cyclotella, Synedra, Diploneis, Caloneis, Cymbella, Nitzschia, Melosira*
Chlorophyceae	*Scenedesmus, Cosmarium, Microspora, Ulothrix, Tetraedron, Chlorella, Spirogyra, Pediastrum, Volvox, Uronema, Closterium, Cladophora*
Cyanophyceae	*Microcystis, Oscillatoria, Chroococcus, Spirulina*
Euglenophyceae	*Euglena, Phacus*
Protozoa	*Epistylis, Vorticella*
Rotifera	*Keratella, Brachionus, Platyias, Lecane*
Copepoda	*Cyclops, Diaptomus*
Cladocera	*Daphnia, Ceriodaphnia, Moina*
Macrobenthic invertebrate	Chironomid larvae

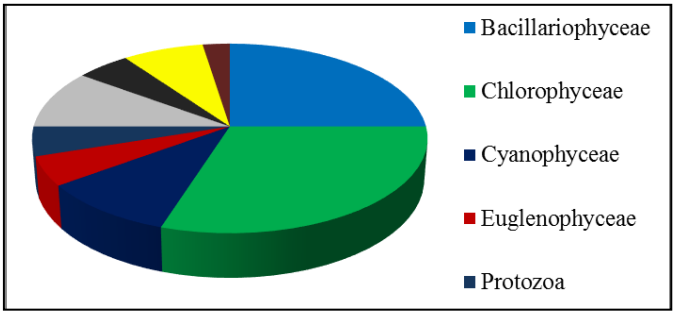

Fig 1: Graphical representation of periphytic organism composition recorded during the experiment

3.2 Periphyton density (nos./cm^2)

Periphyton density (nos./cm^2) were fluctuated in both treatments and varied between 2483 ± 124 to 8153 ± 456 nos./cm^2 during the experiment (Table2). In both the treatments periphyton density gradually decreased and did not able to regain the initial density due to continuous grazing pressure of fishes.

3.3 Periphyton biomass

3.3.1 Dry matter (mg/cm^2)

Dry matter (DM) content of periphyton in treatment with only substrate and treatment with substrate plus feeding were measured and ranged from 0.45 ± 0.008 mg/cm^2 to 2.12 ± 0.01 mg/cm^2 during the experiment (Table 3). Dry matter content of periphyton was gradually decreased in both the treatments as the experiment proceeded.

3.3.2 Ash free dry matter (mg/cm^2)

Ash free dry matter (AFDM) of periphyton was exhibited a decrease trend as experiment proceeded. In T1, the highest value of AFDM was 1.49 ± 0.008 mg/cm^2 recorded on first day of sampling and lowest value was 0.33 ± 0.005 mg/cm^2 measured on 60th day of sampling (Table 3).

3.3.3 Ash content (mg/cm^2)

Highest ash content value of periphyton was 0.62 ± 0.01 mg/cm^2 and 0.64 ± 0.02 mg/cm^2 recorded on first day of sampling in T1 and T2 respectively. In T1 lowest value was 0.12 ± 0.003 mg/cm^2 measured on 60th day of sampling. In T2 lowest value was 0.38 ± 0.01 mg/cm^2 recorded on 90th day of sampling. Mean value data and graphical presentation of ash content are tabled (Table 3).

3.3.4 Ash percentage (%)

As percentage of periphyton in both treatments was fluctuated and varied between 27.20 ± 0.28 to 30.51 ± 0.34% (Table 3). In T1 the highest value was 30.33 ± 0.76% measured on 30th day of sampling and the lowest value was 27.20 ± 0.28% measured on 60th day of sampling.

3.3.5 Chlorophyll-*a* (µg/cm^2)

Chlorophyll *a* content of periphyton was measured and expressed in µg/cm^2 and mean data is tabled (Table 3). In T1, the highest value was 11.02 ± 0.10 µg/cm^2 and the lowest value was 7.26 ± 0.22 µg/cm^2 measured on first and 75th day of sampling respectively.

3.3.6 Phaeophytin-*a* (µg/cm^2)

Phaeophytin-*a* content of periphyton was measured and values were expressed in µg/cm^2 and tabled in (Table 3). In T1 highest value was 1.34 ± 0.01 µg/cm^{2}and lowest value was 0.78 ± 0.03 µg/cm^2 recorded on first and 75th day of sampling respectively.

Table 2: Periphyton biomass and pigment content recorded in T1 and T2 sampling at 15 days interval (February – May).

Parameter	Treatment	Sampling Days						
		0	15	30	45	60	75	90
Periphyton density (nos./cm^2)	T1	7983 ± 516	6378 ± 339	4476 ± 218	3172 ± 131	2483 ± 124	3252 ± 173	2636 ± 128
	T2	8153 ± 456	6949 ± 364	5968 ± 351	5430 ± 216	6125 ± 237	5752 ± 177	4706 ± 164
Dry matter (mg/cm^2)	T1	2.12 ± 0.01	1.69 ± 0.05	1.13 ± 0.03	0.68 ± 0.02	0.45 ± 0.008	0.83 ± 0.02	0.68 ± 0.01
	T2	2.12 ± 0.01	1.87 ± 0.02	1.69 ± 0.01	1.46 ± 0.01	1.68 ± 0.01	1.50 ± 0.05	1.27 ± 0.02
Ash free dry matter (mg/cm^2)	T1	1.49 ± 0.008	1.19 ± 0.03	0.79 ± 0.03	0.47 ± 0.02	0.33 ± 0.005	0.59 ± 0.01	0.49 ± 0.01
	T2	1.48 ± 0.01	1.31 ± 0.01	1.21 ± 0.01	1.05 ± 0.008	1.20 ± 0.01	1.06 ± 0.05	0.89 ± 0.01
Ash content (mg/cm^2)	T1	0.62 ± 0.01	0.49 ± 0.02	0.34 ± 0.006	0.20 ± 0.008	0.12 ± 0.003	0.24 ± 0.01	0.19 ± 0.003
	T2	0.64 ± 0.02	0.57 ± 0.003	0.48 ± 0.01	0.40 ± 0.003	0.48 ± 0.006	0.44 ± 0.01	0.38 ± 0.01
Ash %	T1	29.83 ± 0.60	29.29 ± 0.42	30.33 ± 0.76	30.24 ± 0.09	27.20 ± 0.28	28.66 ± 0.41	29.37 ± 0.29
	T2	30.23 ± 0.92	30.51 ± 0.34	28.68 ± 0.57	27.62 ± 0.11	28.70 ± 0.14	29.64 ± 1.00	30.47 ± 0.50
Chlorophyll- *a* (µg/cm^2)	T1	11.02 ± 0.10	10.37 ± 0.16	9.58 ± 0.18	8.22 ± 0.27	7.50 ± 0.20	7.26 ± 0.22	8.17 ± 0.09
	T2	12.11 ± 0.07	11.46 ± 0.20	10.18 ± 0.14	8.28 ± 0.11	7.66 ± 0.02	8.38 ± 0.10	9.39 ± 0.10
Phaeophytin- *a* (µg/cm^2)	T1	1.34 ± 0.01	1.28 ± 0.006	1.15 ± 0.02	0.88 ± 0.05	0.79 ± 0.03	0.78 ± 0.03	0.90 ± 0.01
	T2	1.44 ± 0.01	1.37 ± 0.02	1.19 ± 0.03	1.03 ± 0.02	0.90 ± 0.01	0.99 ± 0.02	1.09 ± 0.01

*Values are given as mean of 7 sampling days ± S.E. (n = 3)

Table 3: Average periphytic biomass, density and pigment content in treatment T1 and T2 during the three months of experiment.

Treatment	Periphyton density (nos./cm^2) Mean ± S.E.	DM (mg/cm^2) Mean ± S.E.	Ash (mg/cm^2) Mean ± S.E.	Ash %	AFDM (mg/cm^2) Mean ± S.E.	Chlorophyll-*a* (µg/cm^2) Mean ± S.E.	Phaeophytin-*a* (µg/cm^2) Mean ± S.E.
T1	4338 ± 792^a	1.08 ± 0.12^a	0.31 ± 0.03^a	29.27 ± 0.26	0.76 ± 0.08^a	8.87 ± 0.30	1.02 ± 0.04
T2	6154 ± 421^b	1.66 ± 0.05^b	0.48 ± 0.01^b	29.41 ± 0.29	1.17 ± 0.04^b	9.64 ± 0.35	1.14 ± 0.04

Values are given as mean of 7 sampling days and 3 replications ± S.E. (n = 63)
The means in a column with different superscripts are significantly different (P<0.05, pair t test).

3.4 Water quality parameters

Water quality parameters of an environment can greatly influence the productivity and ecology of the environment. The physico-chemical parameters of all the experimental tanks were analyzed for a period of three months (February to May 2017). Samples were collected from all the experimental tanks at 15 days interval and results obtained are presented in Table – 4 and 5.

Table 4: Water quality parameters (mean ± SE) of the experimental tanks recorded at 15 days interval during the three months of culture period (Feb-May).

Parameters	Tanks	Sampling Days						
		0	15	30	45	60	75	90
Temperature (°C)	T0	24.16 ± 0.03	26.13 ± 0.08	28.26 ± 0.03	30.36 ± 0.17	32.05 ± 0.17	32.90 ± 0.40	34.33 ± 0.08
	T1	23.43 ± 0.06	25.30 ± 0.20	27.23 ± 0.20	28.77 ± 0.43	30.69 ± 0.27	32.91 ± 0.40	32.81 ± 0.25
	T2	23.56 ± 0.06	25.26 ± 0.08	27.4 ± 0.11	29.43 ± 0.13	31.13 ± 0.12	32.21 ± 0.49	33.14 ± 0.04
	T3	23.26 ± 0.03	25.43 ± 0.14	27.60 ± 0.26	29.40 ± 0.26	31.20 ± 0.36	32.56 ± 0.24	33.86 ± 0.08
Transparency (cm)	T0	35.19 ± 0.90	31.68 ± 0.44	29.23 ± 1.04	27.16 ± 0.56	26.53 ± 1.08	31.13 ± 1.90	33.90 ± 1.04
	T1	27.32 ± 0.88	31.24 ± 1.68	33.85 ± 2.08	36.46 ± 1.51	41.36 ± 1.74	43.15 ± 1.48	45.92 ± 0.69
	T2	29.87 ± 0.23	31.91 ± 0.37	34.02 ± 0.29	36.31 ± 0.26	38.59 ± 0.14	40.19 ± 0.20	41.91 ± 0.10
	T3	33.32± 0.87	30.58 ± 0.58	27.25 ± 0.35	24.75 ± 0.61	19.54 ± 0.51	18.27 ± 0.88	18.00 ± 0.88
pH	T0	7.08 ± 0.08	7.32 ± 0.04	6.83 ± 0.03	7.08 ± 0.05	7.28 ± 0.04	7.78 ± 0.04	8.06 ± 0.18
	T1	7.24 ± 0.07	7.69 ± 0.03	7.34 ± 0.11	7.72 ± 0.09	8.65 ± 0.01	8.92 ± 0.10	8.61 ± 0.10
	T2	7.11 ± 0.06	7.37 ± 0.04	7.08 ± 0.05	7.66 ± 0.10	7.81 ± 0.03	8.29 ± 0.14	8.08 ± 0.04
	T3	7.17 ± 0.13	7.31 ± 0.08	7.06 ± 0.05	7.41 ± 0.12	7.70 ± 0.09	8.75 ± 0.09	8.34 ± 0.09
DO (mgl^{-1})	T0	5.70 ± 0.06	5.35 ± 0.08	5.65 ± 0.07	4.79 ± 0.11	4.71 ± 0.10	4.54 ± 0.13	4.79 ± 0.15
	T1	6.83 ± 0.02	6.74 ± 0.03	6.80 ± 0.03	6.99 ± 0.06	7.25 ± 0.08	7.12 ± 0.07	6.97 ± 0.09
	T2	6.23 ± 0.10	5.98 ± 0.11	6.08 ± 0.12	6.23 ± 0.07	6.01 ± 0.06	6.10 ± 0.07	6.39 ± 0.09
	T3	6.22 ± 0.19	5.89 ± 0.33	5.14 ± 0.10	5.60 ± 0.02	5.63 ± 0.12	4.93 ± 0.08	4.60 ± 0.20
Total alkalinity (mgl^{-1} as $CaCO_3$)	T0	111.07 ± 4.69	129.83 ± 4.28	135.43 ± 1.18	147.91 ± 6.24	150.09 ± 0.77	153.53 ± 1.48	150.70 ± 1.35
	T1	125.43 ± 2.43	138.45 ± 1.56	148.61 ± 1.66	157.23 ± 1.82	148.41 ± 2.22	167.07 ± 3.33	180.32 ± 2.23
	T2	123.14 ± 1.51	138.88 ± 1.33	144.57 ± 2.08	162.91 ± 3.54	163.60 ± 1.64	180.59 ± 4.04	184.78 ± 3.19
	T3	112.96 ± 1.48	119.90 ± 2.98	137.68 ± 1.15	147.93 ± 0.89	134.74 ± 0.99	155.08 ± 1.85	161.23 ± 3.49
Total hardness (mgl^{-1} as $CaCO_3$)	T0	121.52 ± 2.99	115.69 ± 3.21	119.73 ± 5.53	133.96 ± 5.01	138.69 ± 2.04	131.93 ± 3.12	123.64 ± 1.18
	T1	112.64 ± 4.32	99.10 ± 1.17	115.66 ± 1.68	127.01 ± 0.85	137.33 ± 1.84	138.05 ± 1.53	129.70 ± 2.66
	T2	121.60 ± 1.82	109.36 ± 3.44	124.63 ± 2.66	135.98 ± 2.45	140.63 ± 0.55	130.71 ± 1.18	129.28 ± 1.84
	T3	125.05 ± 1.82	108.97 ± 1.70	123.88 ± 2.81	131.02 ± 3.96	140.26 ± 2.17	141.65 ± 0.62	133.94 ± 1.23
Nitrate-nitrogen (µgl^{-1})	T0	5.30 ± 0.08	4.87 ± 0.05	5.02 ± 0.05	4.22 ± 0.04	4.52 ± 0.08	4.29 ± 0.07	4.25 ± 0.05
	T1	7.47 ± 0.17	8.23 ± 0.23	9.03 ± 0.14	8.85 ± 0.13	9.13 ± 0.00	9.66 ± 0.04	10.34 ± 0.39
	T2	8.15 ± 0.15	8.92 ± 0.17	8.36 ± 0.20	7.96 ± 0.20	9.34 ± 0.12	9.71 ± 0.15	9.92 ± 0.10
	T3	6.99 ± 0.05	6.55 ± 0.03	6.67 ± 0.03	5.93 ± 0.09	5.59 ± 0.05	5.28 ± 0.04	5.17 ± 0.02
Ammonia-nitrogen (µgl^{-1})	T0	16.26 ± 0.53	14.6 ± 0.86	12.82 ± 0.71	12.49 ± 0.74	13.52 ± 0.78	15.34 ± 1.15	16.07 ± 0.55
	T1	7.12 ± 0.09	7.92 ± 0.13	8.59 ± 0.26	9.30 ± 0.15	10.13 ± 0.20	10.41 ± 0.30	9.24 ± 0.55
	T2	11.80± 0.43	12.29 ± 0.42	12.65 ± 0.33	12.18 ± 0.30	11.70 ± 0.22	11.40 ± 0.17	10.82 ± 0.16

	T3	15.36 ± 0.36	14.38 ± 0.49	14.80 ± 0.57	15.60 ± 0.77	16.57 ± 0.54	16.93 ± 0.61	17.41 ± 0.63
Phosphate-phosphorus (μgl^{-1})	T0	0.10 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
	T1	0.26 ± 0.01	0.24 ± 0.02	0.23 ± 0.01	0.21 ± 0.02	0.19 ± 0.01	0.21 ± 0.02	0.22 ± 0.03
	T2	0.26 ± 0.01	0.25 ± 0.02	0.24 ± 0.01	0.24 ± 0.03	0.22 ± 0.01	0.21 ± 0.01	0.21 ± 0.02
	T3	0.15 ± 0.01	0.16 ± 0.02	0.14 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.12 ± 0.01
Water chlorophyll-*a* (μgl^{-1})	T0	89.36 ± 1.70	94.29 ± 1.13	97.26 ± 2.05	100.53 ± 0.80	103.43 ± 1.46	101.27 ± 4.90	87.98 ± 0.59
	T1	87.73 ± 0.75	95.79 ± 2.96	94.52 ± 0.58	92.52 ± 0.77	87.14 ± 1.04	82.59 ± 3.08	78.61 ± 1.36
	T2	81.97 ± 0.65	86.06 ± 2.00	92.58 ± 1.79	98.87 ± 0.67	101.20 ± 1.46	101.87 ± 2.84	109.66 ± 2.00
	T3	89.12 ± 1.30	93.83 ± 1.83	102.01 ± 1.16	101.61 ± 2.10	110.72 ± 2.17	108.33 ± 1.32	105.10 ± 3.92

*Values are given as mean ± SE. (n = 3)

Table 5: Average water quality parameters (mean ± SE) of different treatments during the 3 months of experiment (Feb-May).

Treatment	Parameters									
	Temperature (ºC)	Transparency (cm)	pH	DO (mgl^{-1})	Total Alkalinity (mgl^{-1})	Total Hardness (mgl^{-1})	Nitrate-Nitrogen (μgl^{-1})	Ammonia-Nitrogen (μgl^{-1})	Phosphate (μgl^{-1})	Chlorophyll-*a* (μgl^{-1})
T0	29.74 ± 0.77	30.69 ± 0.75	7.34 ± 0.09	5.07 ± 0.10^{a}	139.80 ± 3.37	126.45 ± 2.08	4.64 ± 0.09^{a}	14.44 ± 0.40^{a}	0.08 ± 0.004^{b}	96.30 ± 1.43
T1	28.59 ± 0.72	37.04 ± 1.47^{b}	8.02 ± 0.14	6.95 ± 0.04^{b}	152.22 ± 3.81	122.78 ± 3.04	8.96 ± 0.06^{c}	8.96 ± 0.25^{b}	0.22 ± 0.01^{a}	88.41 ± 1.42
T2	28.86 ± 0.74	36.11 ± 0.91^{b}	7.63 ± 0.10	6.14 ± 0.04^{c}	156.92 ± 4.73	127.45 ± 2.23	8.91 ± 0.16^{c}	11.84 ± 0.16^{c}	0.23 ± 0.009^{a}	96.06 ± 2.08
T3	29.04 ± 0.80	24.53 ± 1.29^{a}	7.67 ± 0.13	5.43 ± 0.13^{a}	138.50 ± 3.73	129.25 ± 2.43	6.02 ± 0.15^{b}	15.86 ± 0.29^{a}	0.14 ± 0.005^{c}	101.53 ± 1.73

Values are given as mean ± SE. (n = 63)

The means in a column with different superscripts are significantly different (P<0.05, Tukey-HSD test).

In the present investigation a total number of 28 genera of algal periphyton were identified which comprised of Bacillariophyceae (10 genera), Chlorophyceae (12), Cyanophyceae (4) and Euglenophyceae (2). Among the algal group Chlorophyceae was found to be most dominant followed by Bacillariophyceae, Cyanophyceae and Euglenophyceae in both treatments (T1 and T2). These large numbers of algae periphyton had colonized on the bamboo substrates. In the present study a total of eleven genera of animal community belonging to protozoa (2), rotifera (4), copepoda (2) and cladocera (3) were recorded. A number of macrobenthic organisms, especially chironomid larvae, were often observed moving around the surface of the bamboo during periphyton sampling.Azim *et al*., [16] also recorded 13 genera of animal community belonging to crustacea and rotifera and some other macrobenthic invertebrates, especially chironomid larvae and oligochaetes from periphyton on bamboo substrates in aquaculture ponds. However a lesser nos. of animal genus were observed in T1 might be due to higher grazing pressure by fishes, mostly by catla which is reported to primarily feed upon zooplankton in natural condition [17]. Present findings are similar with Azim *et al*.,[1], Azim *et al*.,[18], Azim *et al*.,[19], Wahab *et al*., [20], Wahab *et al*., [21] where they had also recorded Chlorophyceae as the most dominant among algal group.

Periphyton density (nos./cm^2) was significantly (p<0.05) higher in treatment T2 than T1. This is may be due to lesser grazing by the fishes on periphyton due to availability of another source of food (i.e. supplementary feed) as well as the extra nutrients received through the leftover supplementary feed [22].

3.5 Periphyton Biomass

A decreasing trend was observed in Periphyton dry matter (DM), ash free dry matter (AFDM) and ash content values in both the treatments as the experiment days increased, which indicated effective grazing on periphyton by cultured fishes. Similar observation was also made byAzim *et al*., [23] they reported that after 14 days of fish stocking periphyton biomass in tilapia ponds decreased sharply and remained at a significantly lower level throughout the period of study. [24] also observed decreased pigment concentration of periphyton with time in tanks stocked with mahseer and fringe-lipped carp. Grazing could substantially reduce the periphyton biomass [25, 26, 27]. Fish can graze on these concentrated food items (periphyton) more efficiently than filter feeding on planktonic foods only [28, 29]. In this present study fishes were observed grazing on the periphyton grown on the surface of the bamboo substrates. It was hypothesized that the periphyton grown on the bamboo surface was a readily available feed which might have enhanced the growth rate and production of IMC fingerlings in the treatment tanks with substrate compared to that of the control ponds. This has been reflected in the higher gain in weight of fish in the tanks having bamboo substrates than control.

Feeding had a significant effect on periphyton DM, AFDM, and ash content but not on the other parameters. Periphyton DM, AFDM and ash content were significantly (p<0.05) higher in treatment T2 than only substrate treatment T1 [30]. Keshavnath *et al*., [31] also recorded significantly higher periphyton DM and AFDM in substrate tanks with feeding than only substrate tanks.

A negligible difference in pigment concentration between feeding and without feeding tanks was also observed byKeshavnath *et al*. [24]. Keshavnath *et al*., [32] had not found any significant (p<0.05) difference in pigment concentration among several biodegradable substrates including bamboo.

In the present study, values of periphyton DM ranged from 0.45 to 2.16 mg/cm^2 [1]. reported higher values of DM ranged from 3.12 ± 0.20 to 4.89 ± 0.26 mg/cm^2.Rahman, S.M.S. [33]found DM concentration of periphyton ranged from 0.43 to 4.20 mg/cm^2 which had been found to be similar to the present study. Jiywam, W. [34] also recorded DM (mean ± S.E.) value of 1.14 ± 0.57 mg/cm^2 and ranged between 0.41 and 3.17 mg/cm^2.

The ash free dry matter (AFDM) and ash content of periphyton grown on bamboo substrates found to range from 0.32 mg/cm^2 to 1.51 mg/cm^2 and 0.12 mg/cm^2 to 0.69 mg/cm^2. Keshavnath *et al*., [31] also observed higher mean AFDM value (0.53 - 0.54 mg/cm^2) in the tanks with feeding. Jiywam, W [34] also recorded similar value of AFDM (mean ± S.E.) 0.53 ± 0.38 mg/cm^2 and ranged between 0.15 and 2.01 mg/cm^2.

The chlorophyll-*a* values of periphyton varied during the study period which ranged from 7.26 to 12.11 µg/cm^2.Jana *et al*., [35] also observed similar value (13.5±1.1 µg/cm^2) of chlorophyll a from their investigation. Azim *et al*., [16] reported no significant different of periphyton chlorophyll *a*

concentration per unit surface area among three different substrates.

3.6 Water quality parameters

During the study period, water quality parameters of the experimental tanks were found to be within permissible limits recommended for warm water fish culture [36].

Water temperature in the experimental tanks ranged from 23.2 °C to 34.5 °C in all treatments. Suitable range of water temperature for fish culture was 25 ºC to 35 ºC recommended by Aminul, I.M. [37]. Rahman *et al*., [38] found water temperature 26.06 °C to 31.97 °C which was within the suitable range for pond fish culture. Though slightly lower temperature was observed in tanks with substrate but did not showed significant (p<0.05) difference from other treatments. Lower temperature in substrate tanks could be attributed to the shading effect of substrates [31].

The transparency of a water body normally indicates its productivity. It is usually affected by several factors such as silting, microscopic organisms, suspended organic matter, latitude, the season, and the intensity of sunlight [39]. In the present study, average transparencies values were 30.69 cm, 37.04 cm, 36.11 cm and 24.53 cm in T0, T1, T2 and T3 respectively. Addition of substrate showed significance (p<0.05) difference in transparency value with only feeding treatment but did not vary significantly (p<0.05) from control. It might be due to entrapping of organic detritus and dissolved suspended solids, remove nutrients from water column, organic matter breakdown by periphyton assemblage as stated byAzim *et al*., [16]. Periphyton substrates tend to entrap suspended organic material and it is likely to be more during supplementary feeding due to uneaten feed and fish feces [30]. In treatment T3, transparency was found to be lowest among the all treatments which may be due to accumulation of leftover feed, organic particles and fish feces.

The pH values fluctuated and ranged from 6.7 to 9.3 in all experimental tanks. The average pH values were 7.34, 8.02, 7.6 and 7.6 in T0, T1, T2 and T3 respectively. The pH values were slightly in alkaline range in all the tanks which indicated good pH conditions for biological production. According to Boyd, C.E. [36], suitable pH range for fish pond should be 6.5 to 7.5. The permissible range of pH is 6.5–8.5 for fisheries (EMECS, 2001); 6-9 for aquaculture pond (EEC, 1976).

Average Dissolved oxygen concentrations were found to be in desirable (5.07 to 6.95 mgl^{-1}) range for carp culture in the all treatment groups. Dissolved oxygen concentration of 6 mgl^{-1} is required for better growth of fish [40]. Fish cannot survive when DO content is less than 3mg/L [41]. The mean values were 5.07, 6.95, 6.14 and 5.43 in treatment T0, T1, T2 and T3 respectively. However treatment with substrate was found to be significantly (p<0.05) higher DO concentration than the control tanks. It might be due to the effect of photosynthetic activity of periphyton in tanks with substrates led to higher DO concentration. Water turbulence induced by the grazing activity of the fishes might have also increase dissolved oxygen diffusion, particularly at the uppermost area of the water column [42]. Significant effect of substrate density on dissolved oxygen concentration was also observed byKeshavnath *et al*., [31]. Azim *et al*., [19] also recorded higher DO concentration in treatment with substrate surface area of 75% of tanks water surface area than control tanks. However in treatment T0 and T3 did not showed significant (p>0.05) difference in DO concentration.

Total alkalinity values were found to be higher during sampling period and showed gradual increasing trend and ranged from 102 to 189 mgl^{-1} in all treatments. Among treatments the alkalinity values did not varied significantly (p>0.05). The mean values were 139.80, 152.22, 156.92 and 138.50 mgl^{-1} in T0, T1, T2 and T3 respectively. Similar value was also reported by Tippayadara *et al*., [4] in substrate based culture system. The higher alkalinity value indicated higher nutrient turn over and productivity in tanks with substrates. [42] gave the range of total alkalinity as 0.0 - 20.0 ppm for low production, 20.0 - 40.0 ppm- low to medium, 40.0 - 90.0 ppm- medium to high production and above 90.0 ppm productive.

The total hardness values were ranged from 92 to 144 mgl^{-1} in all treatments. The average values were 126.45, 122.78, 127.45 and 129.25 mgl^{-1} in treatment T0, T1, T2 and T3 respectively. Similar values were also recorded by [4] from their experimental tanks with and without bamboo substrates. There was no significance (p<0.05) difference in hardness value among the treatments groups.

The Nitrate-nitrogen values were found to be in the ranged of 4.15 to 11.12 μgl^{-1} in all treatments. The mean nitrate values were 4.64, 8.96, 11.84 and 15.86 µgl-1 in T0, T1, T2 and T3 respectively, which showed significant difference (p<0.05) among treatments. In T1 nitrate values were significantly (p<0.05) higher than T3 and control. There was an increasing trend of nitrate values in T1 and T2 and showed significant (p<0.05) difference from T3 and control throughout the study period. The treatment T1 showed the highest nitrate value at the end of the study period. There was no significant (p> 0.05) different between T1 and T2. Nitrate-nitrogen in the treatments with substrate showed an increasing trend indicating enough nitrifying activities and oxidation process of nitrite to nitrate. In tanks with substrate nitrifying bacteria could colonize on provided substrate that were located in water column, which resulted in enhanced nitrification [43, 44, 45]. However T3 showed significantly (p<0.05) higher nitrate value compared to control which could be attributed to nitrogen input through supplementary feed [46]. In control, no increment in nitrate value and significantly (p<0.05) lower than other treatments throughout the study period was observed. This might be due to the absence of substratum for colonization of nitrifying bacteria.

In the present investigation mean values ammonia nitrogen were found to be 14.44, 8.96, 11.84 and 15.86 μgl^{-1} (ranged 6.94 – 18.25 μgl^{-1}) in T0, T1, T2 and T3 respectively. In treatment T1 and T2 ammonia values were significantly (p<0.05) lower than T3 and control. However-ammonia nitrogen values did not show any significant (p>0.05) different between T3 and control. In T1 ammonia-nitrogen concentration was found to be relatively lower and showed significantly (p<0.05) lower values than all other treatments. The provision of substrate had significant effect on lowering ammonia concentration in T1 and T2. This might be due to higher nitrification rates by periphyton assemblage. Langis *et al*.,[43] and Ramesh *et al*., [44]reported that the bacterial biofilms (periphyton) on the substrates reduced ammonia levels through promotion of nitrification. Nitrifying bacteria are known to improve water quality by converting highly nitrogenous toxins such as ammonia and nitrite into nitrate [47]. Lower ammonia nitrogen value in substrate tanks might be attributed to the establishment of nitrifying bacteria in the systems [45]. Several studies showed a positive effect of periphyton on nitrification, leading to lower ammonia concentrations. Comparatively higher ammonia concentration was observed in T3 which could be due to addition of supplementary feed and nitrogenous excretory products

leading to accumulation of ammonia [48]. Significant effect of feeding on increase in ammonia concentration was observed in only feeding treatment byKeshavnath *et al*., [31]. LikewiseAzim *et al*., [18] also recorded significantly higher total ammonia concentration in control and feed treatment compared to substrate treatments.

A higher phosphate value was recorded in treatment with substrate which was significantly (p<0.05) higher from T3 and control. Similar observation was also noticed by [32]. Higher phosphate indicated higher nutrient turn over and productivity in tanks with substrates.

The chlorophyll-*a* content of water did not show any significant (p<0.05) difference among the treatments. The mean values were 96.30, 88.41, 96.06 and 101.53 μgl^{-1} in treatments T0, T1, T2 and T3 respectively. In treatment with only substrate found to be lower chlorophyll a value, which might be due to effect of periphyton acting on accumulation of dissolved organic particle making water more transparent. Mean chlorophyll- *a* concentration in T3 was higher but did not significantly differ from other treatments. This might be due to supplementary feed which fertilized the tanks and enhanced plankton production [16].

4. Conclusion

A total of 39 genera of periphytic organisms were identified from the bamboo substrates. However a lesser number of animal genus were observed in T1 which may be due to higher grazing pressure by fishes, mostly Catla which is reported to primarily feed upon zooplankton in natural condition. In the present study, feeding had a significant effect on periphyton DM, AFDM and ash content but not on the other periphyton parameters. A decreasing trend was observed in periphyton DM, AFDM and ash content value in both the treatment as the duration of the experiment increased, which indicates effective grazing on periphyton by cultured fish species.

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