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Influence of periphyton substrate density on hydrobiological characteristics and growth performance of Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758) stocked in inland saline groundwater ponds

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Abstract
To investigate the optimum density of additional substrate required for obtaining higher growth of *Oreochromis niloticus* in brackish water ponds (salnity: 14~16 ppt, temperature: 27.4 ~ 32.3 ºC), three different densities of bamboo poles (at 375, 560 and 750 per pond of 375^{-2} meters) each in replicate of two were maintained. Fish fry (mean body weight 0.94-0.96g) were stocked in ponds at 10000 fish ha^{-1}. During the experimental period, all the water quality parameters remained within the optimum range. After a grow-out period of 100 days, a significant (P<0.05) increase in fish growth indicating parameters *i.e*., mean weight (g), length (cm), growth day^{-1} and specific growth rate (SGR) and higher values of productivity indicating parameters were observed in ponds provided with substrate density i.e.,560 bamboo poles. Higher values in dry matter contents and periphyton biomass were observed at a depth of 50 cm which coincided with the photosynthetic compensation depth. Periphytic dry matter, periphyton number, total pigment concentration, algal constitute of periphytic biomass and periphyton productivity were also significantly (P<0.05) higher in ponds provided with substrate density at 560 bamboo poles. In other words provision of substrate at 54 per cent of the total submerged surface area appeared to be sufficient for periphyton production and obtaining optimum growth of Nile tilapia. Findings suggest that with the provision of optimum density of the substrate, periphyton supported production technology can offer considerable potential for enhancing aquaculture production without any deleterious impact on pond ecosystem.

Keywords: Biofilms, Nile tilapia, periphyton, submerged surface, productivity

1. Introduction
In order to develop organic aquaculture, Periphyton-based system is considered to be a modern and eco-friendly approach in the global pond aquaculture. Periphyton is very preferable natural food for herbivorous and omnivorous fish species like tilapia [1] and many other fresh and brackish water fish species [2].

Periphyton-based aquaculture systems offer the possibility of increasing both primary production and food availability for fish which is an important consideration in resource-constrained countries. The feasibility of using periphyton based systems have been explored and found to enhance surface area for the development of attached algae to enhance productivity and thus, may provide sufficient food for the growing fish leading to higher fish production as compared to the traditional system of fish culture [3]. Thus, periphyton-based aquaculture could be an important step if aquaculture production by resource-poor farmers is to grow further. In many recent trials artificial hard substrates are being used in the aquatic ecosystem for the development of periphyton communities for enhancing fish growth/production [1, 2, 4].

Nile tilapia, *Oreochromis niloticus* (Cichlidae) is an important brackish water edible fish species which thrives well both in fresh and brackish waters and feeding mainly on zooplankton and zoobenthos but also ingest detritus and feed on lablab and phytoplankton [1]. Since, periphyton-based technology results in increasing the natural productivity of the water body and thus leads to *in vitro* food production [4], the technology appears to be economically viable as it can save the poor fish farmers on spending extra money on supplementary feeds for the fish [5, 6, 7].

Many trials have already been conducted world over including India and these studies have demonstrated that ponds provided with additional substrates for the development of periphyton had higher fish growth/production than those from substrate-free controls or from the ponds where the fish were fed on supplementary diet [8, 9, 10]. India has extensive salinisation having about 7.0 million hectares of saline affected soil which is spread in the Indus-Ganga plains of North-Western India and in the states of Rajasthan, Gujarat, Haryana and Punjab [11]. The surface as well as groundwater are saline which cannot be used for agriculture. The potential area, where aquaculture can be taken up, can be profitably utilized with the culture of saline water fish and shellfish species.

Our recent studies have revealed that inland saline groundwater with high salinities (10 ppt and above) can be profitably utilized for the culture of certain euryhaline fish species such as mullets, milkfish, Pearlspot and Nile tilapia [6, 7, 9]. Most of the studies were based on the viability of periphyton-based aquaculture technology rather than on optimizing the substrate surface area in relation to the total pond surface area [5, 12, 13, 14, 15] required for obtaining higher or optimum fish yield. Therefore, the present studies were planned to investigate to what extent the submerged surface area in brackish water ponds could be enhanced so as to obtain maximum fish yield without deteriorating the pond environment.

The major objectives of the studies were to evaluate the i) optimum density of the additional substrate required for obtaining higher fish growth/production, ii) impact of three different densities of additional substrate on physico-chemical characteristics of pond water, their nutrient status and also (iii) to examine the impact on pond productivity, plankton production, periphytic biomass and fish growth in stagnant brackish water fish ponds with salinity ranging from 14 ~ 16 ppt.

Materials and Methods

Experiments were conducted at the brackish water fish farm facility of the Department of Zoology and Aquaculture, CCS Haryana Agricultural University, Hisar (Lat. 29º, 10'N; Long 75º, 46'E), India in earthen ponds each measuring 15m×25m (area 375m^2, depth 1.5 m). Ponds were cleaned and quick-lime (CaO at 200 kg ha^{-1}) was applied and the ponds were filled with inland saline groundwater pumped from deep aquifers, allowed to stabilize for about two weeks. To maintain the desired level (1m) water was replenished as often as required. After the addition of first dose of fertilizer, ponds were filled with inland saline groundwater by pumping from bore wells and allowed to stabilize for about two weeks. To maintain the desired level (1m) water was replenished as often as required. Semi-dry cow-dung was thoroughly mixed in the pond water (in the ratio 1:3 w/v) before spreading the same on water surface. Irrespective of the treatments (I-III), semi-dry cow-dung (10000 kg ha^{-1} y^{-1}) was applied at biweekly interval to fertilize the ponds. Prior to the initiation of experiments, bamboo poles (1 meter long and 3.1 cm diameter) were fixed vertically at the bottom of ponds by digging 20 cm deep holes at an equal distance. Water salinity during the experimental period fluctuated between 14–16 ppt and temperature ranged between 27.4 ~ 32.3^{0}C. A total of three treatments each in replicate of two were maintained (I-III). Each treatment had different density of bamboo poles as additional substrate at 375, 560 and 750 per pond 375^{-2} meters, which resulted in an increase of submerged surface equal to 36, 54 and 72 m ^{2}.

Stocking

Two weeks after the application of the first dose of organic fertilizer, 30 days old fry of *O. niloticus* (mean body weight 4.02 ± 0.05g) were stocked at 10000 fish ha^{-1} i.e. 375 fish per pond of 375m^2. The duration of grow out period was 100 days.

Water quality monitoring

Water samples collection was initiated from day15 of the initiation of the experiment. Samples were obtained in replicate of four from each pond (i.e., 8 samples from each treatment) before sunrise. During the study period, a total of six sampling on six different dates were performed (15, 30, 45, 60, 75 and 100 days) for the study of physico-chemical characteristics, however only overall mean values of all the six observation dates are shown (see table 2). Temperature, salinity and water pH were recorded daily and the other physico-chemical parameters [(electrical conductivity, dissolved oxygen, BOD_5, carbonates, bicarbonates, alkalinity, chlorides, hardness, Ca++, Mg++, total kjeldahal nitrogen, NO_3-N, NO_2-N, NH_4-N, o-PO_4, SO_4, turbidity, and total dissolved solids (TDS)] were measured at six different time intervals (15,30,45,60,75 and 100 days) following APHA [16]. Net and gross primary productivity (NPP and GPP) were determined using the light and dark bottle technique following APHA [16].

Determination of periphyton biomass and pigment concentration

The periphyton biomass growing on the substrate was determined in terms of dry matter (DM) and pigment concentrations (chlorophyll *a* and pheophytin *a*) at bi-weekly intervals following the standard methods [16] beginning on Day-15 following the application of first dose of fertilizer. From each pond, three poles were selected and two, 3 cm × 3 cm samples of periphyton were taken at each of four depths (0, 25, 50, and 75 cm below the water surface) per pole. One sample out of two (i.e. 3 samples from each replicate pond/depth), was used to determine total dry matter and ash contents. Samples from all sampling dates, poles per replicate ponds and per depth were pooled and ashed in a muffle furnace at 550ºC for 6 hours. Dry matter (DM), ash free dry matter (AFDM), autotrophic index (AI), and ash content were calculated following APHA [16]. AI was calculated as follows:

AI = biomass (ash-free weight of organic matter, mg/ m^2)/ chlorophyll *a*, mg/m^2.

Ash values were also used to calculate periphyton productivity and expressed as follows:

Periphyton productivity (mg C/ m^2/day) = total ash weight (mg/cm^2) ×100 /t

Where, t = duration of experiment (100 days).

Out of the remaining 3 samples of each replicate per depth, two were used for determining periphyton abundance. Samples from each depth were suspended in 50 mL of distilled water and stored in plastic bottles. Periphytons were enumerated using a Sedgwick-Rafter cell according to the procedure described for planktons and calculated as follows:

N = P×C×100/S

Where, N = periphyton number/cm^2 (whether single-celled or multi-cellular, counted as one unit); P=total number of periphyton units counted in 10 fields of Sedgwick-Rafter cell; C = volume of final concentrate sample (mL); and S = area of scraped surface (cm^2).

The remaining sample from each replicate was used to determine chlorophyll *a* and pheophytin *a* contents following standard methods [16].

Plankton samples were also collected by passing 20L of water taken from five different locations (4 L from each location) of each pond through plankton net (mesh size 125 µm). The samples were then carefully transferred to a measuring cylinder and a volume of 50 ml with distilled water was made and preserved in small plastic bottles with 5 percent buffered formalin (concentrated sample). Plankton numbers were estimated using Sedgwick Rafter cell. One ml of the concentrated sample was placed on to the counting chamber and left to stand for 5 minutes to allow plankton to settle. Ten randomly selected fields of the chamber were counted under a binocular microscope and plankton density was calculated using the following formula:

Plankton (number/L) = 100[(number counted in ten fields) (conc. volume of sample in mL)]/volume of filtered pond water in L.

Identification of plankton to genus level was carried out using the keys of Ward and Whipple [17], Prescott [18] and Bellinger [19].

Fish harvesting

Post 100 days of stocking, all the substrates were removed, ponds were completely drained and all the fish were harvested. Total bulk weight and number of fish recovered from each treatment were recorded. Thereafter, weight (g) and length (cm) of the individual fish were also recorded. Specific growth rate (SGR), condition factor (k) and length-weight relationship (LWR) were calculated. Length-weight relationship (LWR) of fish was calculated according to the following equation:

W = c Ln (Logarithmic form of equation is log W = log c + n log L)

Where,

W = weight in kg, c = constant, n = exponential value of length and

L = length of fish in cm.

SGR and condition factor (k) were calculated. Fish carcass (initial and final), and periphyton were analysed following AOAC [20].

Statistical analysis

Data were subjected to multivariate analysis following Prein *et al.* [21]. Coefficient of correlation between different variables was determined by computer. ANOVA followed by Tukey test [22] was applied to find out significant differences between different treatments.

Results

Fish growth, survival and production (Table 1)

Survival appeared to be independent of the treatments and varied between 90.5-95.0%. ANOVA showed a significant (P<0.05) increase in mean fish weight, biomass and other growth parameters (length, biomass, growth per day and SGR) including condition factor in ponds provided with 560 number of bamboo poles. A decrease in growth parameters was observed in ponds provided with lower or higher density of the substrate. Condition factor (n =2.75±0.05) indicates the wellbeing of the fish, which also coincided Well with higher growth performance.

The individual mean weight of tilapia at harvest was higher i.e. 56..60 ± 1.71 g in ponds provided with substrate density i.e., 560 bamboo poles compared with 40.88 ± 1.22 g, and 30.48 ±1.11g in ponds provided with substrate density at 375 and 750 bamboo poles respectively. One way ANOVA showed a significant effect of substrate density on fish growth

Proximate carcass composition

Proximate carcass composition of *O. niloticus* indicated a significantly (P<0.05) higher accumulation of protein, fat, ash and phosphorus in fish grown in ponds provided with 560 number of bamboo poles as additional substrate in comparison with the other two treatments (Table 1), where the values remained low.

Table 1: Effect of three different substrate densities (375, 560 and 750) on growth performance and carcass field conditions – 100 days treatment composition (% wet weight) of *Oreochromis niloticus* under

Initial Fish Stock				Final Fish Stock (after 100 days)					
Substrate density/375m2	**Stocking density/**	**Mean fish weight (g)**	**Total biomass (g)**	**Survival (%)**	**Mean fish weight (g)**	**Total Biomass (kg)**	**SGR % g d-1 (SGRL cm d-1)**	***Growth d-1 (g)***	**Condition factor (k)**
	375 m2	**(length cm)**			**(Length cm)**				
375	375	0.95±0.03a	356.25±6.76a	90.5	40.88±1.22b	13.873±1.35b	3.76±0.04b	0.40±0.01b	2.09±0.06a
		(4.02±0.05)a			(12.53±0.15)b		(1.14±0.02)b		
560	375	0.94±0.03a	352.50±5.81a	95.0	56.60±1.71a	20.164±3.24a	4.08±0.04a	0.56±0.02a	2.75±0.05b
		(4.02±0.05)a			(13.59±0.15)a		(1.22±0.01)a		
750	375	0.96±0.03a	360.00±6.32a	94.0	30.48±1.11c	10.744±0.95c	3.46±0.05c	0.30±0.01c	2.25±0.10a
		(4.02±0.05)a			(10.39±0.19)c		(0.95±0.02)c		
Carcass Proximate Composition									
Substrate	**density/ 375m2**	**Moisture**		**Protein PProtein**		**Fat**		**Ash**	**Phosphorus**
Init	ial value	72.70±0.11			15.66±0.32	2.75±0.02		2.98±0.06	0.33±0.01
	375	68.44±0.11b			19.13±0.29ab	3.43±0.04b		3.96±0.03b	0.65±0.02b
	560	67.91±0.10c			19.67±0.33a	3.65±0.05a		4.28±0.06a	0.89±0.04a
	750	69.46±0.12a			18.36±0.35b	3.25±0.03c		3.71±0.07c	0.53±0.02c

All values are mean±SE of mean. Mean with the same letters in the same column are not significantly (P<0.05) different

SGR (% g d-1) = specific growth rate of weight = [(In Wtf–In Wti)×100]/t

SGRL (% cm d-1) = specific growth rate of length = [(In Lf–In Li)×100]/t

Growth per cent gain in body weight=[(Wtf-Wti)/Wti] × 100, where, Wti and Wtf denotes initial and final weight of fish respectively, Lf and Li denotes initial and final length (cm) of fish respectively and t represents time (days), duration of experiment (60 days), BW = Body weight, d=days.

Condition factor (k) = Wt×105/ L3, where Wt is weight of the fish in grams and L=Total length in millimetres.

Physico-chemical characteristics of water quality

Water conductivity fluctuated between 19.73± 0.51 to 19.97 ± 0.46 dSm-^{1}, pH remained alkaline in all the treatments. Dissolved oxygen (DO) concentrations remained at optimal levels in all the treatments with slightly higher values in treatment II (ponds with 560 bamboo poles as substrate). A review of the data further indicated that productivity indicating parameters (*viz*., turbidity, total alkalinity and TDS), nutrients (o-PO_4, SO_4) and carbonate. Bicarbonate, calcium and total hardness values remained significantly (P<0.05) higher in treatment II. On the other hand BOD_5, NO_2-N, NH_4-N remained significantly (P<0.05) lower and NO_3-N high in treatment II in comparison with the other two treatments (I, III). Not many variations in magnesium levels among the three treatments (Table 2) were observed.

Biological characteristics of pond water (Table 2)

Net primary productivity (NPP), gross primary productivity (GPP), and pheophtin *a* concentration were significantly (P<0.05) higher in treatment II in comparison with the other two treatments (I, III). The peak values of chlorophyll *a* were observed on 30th and 75th day (Fig 1), while, pheophytin *a* showed a peak value on 45th day of observation (Fig 2). Chlorophyll *a* and Epilethic chlorophyll *a* concentrations decreased with an increase in the density of the substrate, while no significant differences in epilithic pheophytin *a* concentrations were observed among the three (I.II.III) treatments. The water pheophytin and epilithic pheophytin *a*, epilithic phytoplankton and zooplankton were also significantly (P<0.05) higher in treatment II in comparison with treatment I and treatment III respectively.

Phytoplankton and zooplankton population and their species diversity (d) increased with increase in the substrate density up to the second treatment (II), however, thereafter at highest density of the substrate a decline in their values was observed.

Abundance and distribution of different taxa of phyto-and zooplankton (data not shown) indicated that Chlorophyceae (5 taxa) and Bacillariophyceae (2 taxa) were the dominant phytoplanktons. Zooplanktons were represented by Copepoda (3 taxa) and Rotifera (3 taxa). Further, none of these groups showed a stable community.

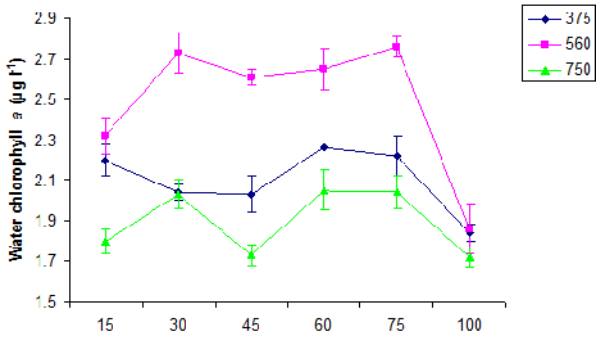

Fig 1: Bi-weekly variations in mean values of chlorophyll *a* concentrations in pond water stocked with *Oreochromis niloticus* provided with three different substrate densities (375, 560 and 750 per pond of 375^{-2} meters) of bamboo poles.

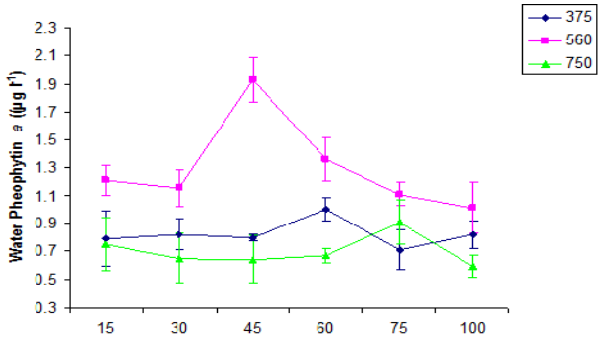

Fig 2: Bi-weekly variations in mean values of pheophytin *a* concentrations in pond waters provided with three different substrate densities (375, 560 and 750 per pond of 375^{-2} meters) of bamboo poles stocked with *Oreochromis niloticus.*

Periphyton and pigment concentration

Irrespective of the substrate density mean values of periphyton scraped from the bamboo substrate at 50 cm depth were 17947 numbers cm^{-2} (range 14,999 – 22,499 nos. cm-^{2}). Depth trend in periphyton growth indicated highest values (22499±1398 nos. cm-^{2}) at 50 cm depth in ponds provided with additional substrate at a density 560 in comparison with the other two treatments, i.e., at lower (20332±1038) and at the highest (19,687±1720 nos. cm-^{2}) density of the substrate. Fortnightly variations though revealed no definite trend in periphyton numbers, yet the, peak values at most of the depths were observed on sampling done on 45th day (Figs. 3, 4 and 5) and remained higher at a depth of 50 cm.

Though the mean values of DM, AFDM, ash and % of dry matter were higher at 50 cm of the depth in ponds provided with 560 number of bamboo poles, however, these values were not significantly different from the other two treatments. Irrespective of the substrate density, autotrophic index (AI), values for AFDM and DM decreased with an increase in substrate depth (Table 3), however, the values of these parameters were not significantly (P<0.05) higher in the treatment provided with 560 number of bamboo poles. Mean periphyton productivity, chlorophyll *a* (Figs. 6, 7 and 8)) and pheophytin *a* (Figs.9, 10 and 11) concentration also remained higher at 50 cm depth (Table 3). A similar trend was observed among all the treatments but highest values were observed in the ponds provided with 560 number of bamboo poles as an additional substrate.

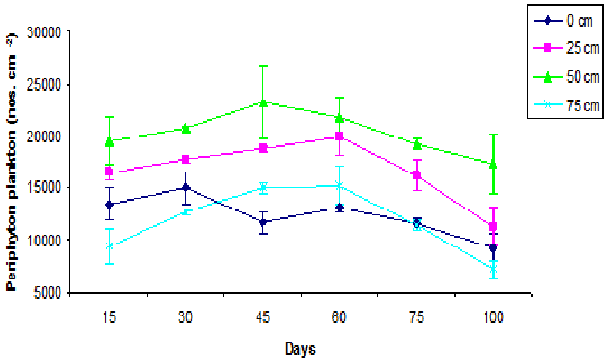

Fig 3: Bi-weekly variations in mean values of periphyton plankton density at different depths (0, 25, 50 and 75 cm) from ponds provided with 375 (per pond of 375^{-2} meters) numbers of bamboo poles as additional substrate.

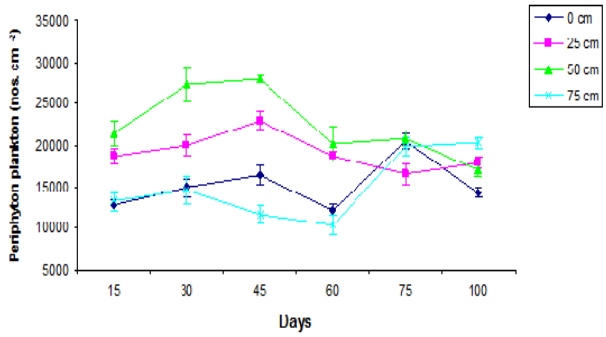

Fig 4: Bi-weekly variations in mean values of periphyton plankton density at different depths (0, 25, 50 and 75 cm) from ponds provided with 560 (per pond of 375^{-2} meters) numbers of bamboo poles as additional substrate.

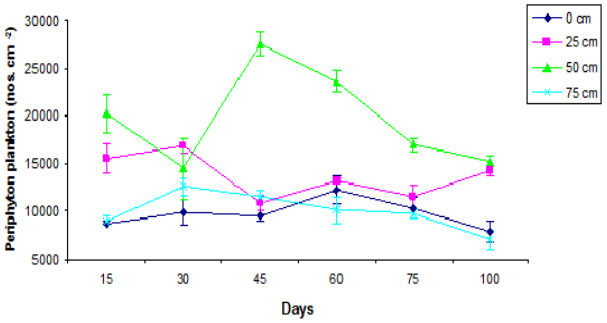

Fig 5: Bi-weekly variations in mean values of periphyton plankton density at different depths (0, 25, 50 and 75 cm) from ponds provided with 750 (per pond of 375^{-2} meters) numbers of bamboo poles as additional substrate.

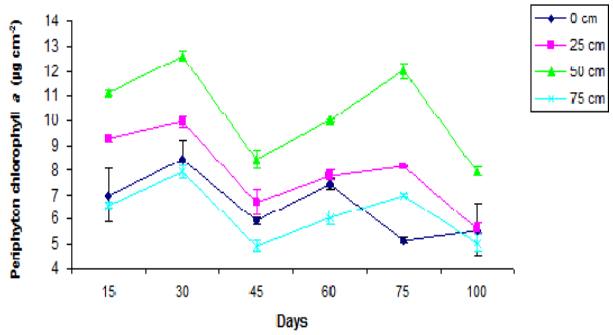

Fig 6: Bi-weekly variations in mean values of periphyton chlorophyll *a* concentrations at different depths (0, 25, 50 and 75 cm) from ponds provided with 375(per pond of 375^{-2} meters) numbers of bamboo poles as additional substrate.

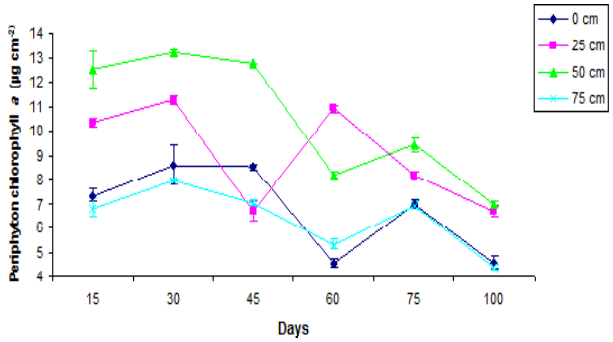

Fig 7: Bi-weekly variations in mean values of periphyton chlorophyll a concentrations at different depths (0, 25, 50 and 75 cm) from ponds provided with 560 (per pond of 375^{-2} meters) numbers of bamboo poles as additional substrate.

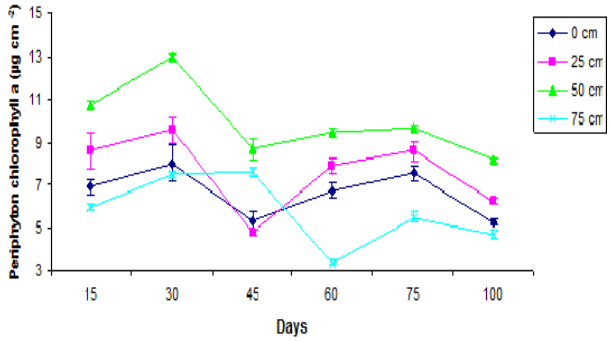

Fig 8: Bi-weekly variations in mean values of periphyton chlorophyll a concentrations at different depths (0, 25, 50 and 75 cm) from ponds provided with 750 (per pond of 375^{-2} meters) numbers of bamboo poles as additional substrate.

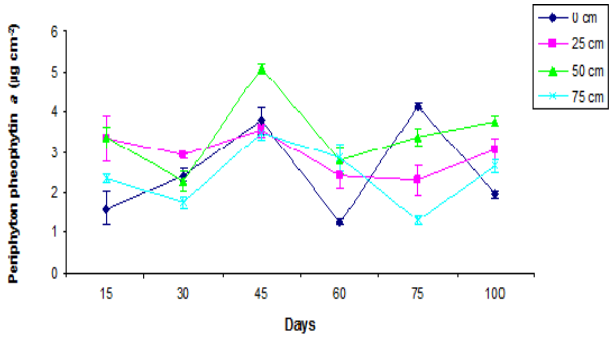

Fig 9: Bi-weekly variations in mean values of periphyton pheophytin a concentrations at different depths (0, 25, 50 and 75 cm) from ponds provided with 375 (per pond of 375^{-2} meters) numbers of bamboo poles as additional substrate.

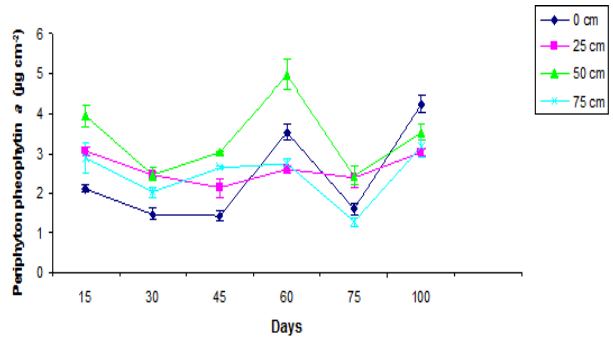

Fig 10: Bi-weekly variations in mean values of periphyton pheophytin a concentrations at different depths (0, 25, 50 and 75 cm) from ponds provided with 560 (per pond of 375^{-2} meters) numbers of bamboo poles as additional substrate.

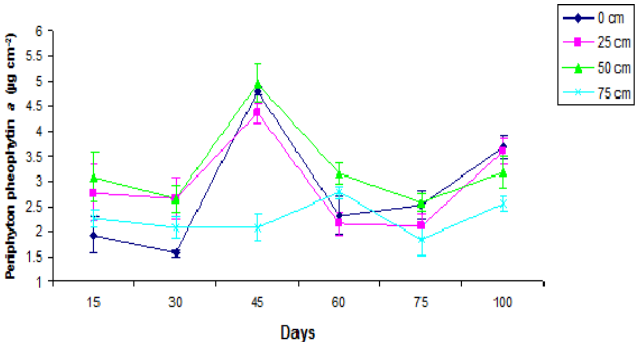

Fig 11: Bi-weekly variations in mean values of periphyton pheophytin a concentrations at different depths (0, 25, 50 and 75 cm) from ponds provided with 750 (per pond of 375^{-2} meters) numbers of bamboo poles as additional substrate.

Table 2: Physico-chemical and biological characteristics of pond water stocked with *Oreochromis niloticus* provided with three different substrate densities (375, 560 and 750) under field conditions – overall mean

Parameters		Substrate density/375 m2	
	350	560	750
Physico-chemical characteristics			
Electrical Conductivity (dSm-1)	19.73±0.51a	19.97±0.49a	19.73±0.46a
pH	8.32±0.04a	8.30±0.03a	8.27±0.03a
Dissolved oxygen (mg l-1)	4.23±0.23ab	4.72±0.22a	3.76±0.24b
BOD5 (mg l-1)	4.41±0.12b	4.29±0.14b	4.97±0.13a
Carbonates (mg l-1)	19.88±0.82b	24.83±1.00a	14.46±0.71c
Bio-carbonates (mg l-1)	274.63±10.28a	284.71±9.72a	244.54±8.56b
Total alkalinity (mg l-1)	294.33±10.34a	309.54±9.53a	259.00±8.91b
Chlorides (mg l-1)	4800.19±27.08a	4390.17±37.12c	4503.01±32.51b
Total hardness (mg l-1)	3139.58±50.77b	3316.67±50.99a	3020.83±51.65b
Calcium (mg l-1)	310.97±14.53b	367.91±17.33a	323.23±14.39b
Magnesium (mg l-1)	572.00±16.32a	585.09±15.68a	544.70±16.00a
Total Kjeldahl nitrogen (mg l- 1)	3.59±0.18b	4.42±0.18a	2.75±0.15c
NO3-N (mg l-1)	0.28±0.02b	0.40±0.02a	0.24±0.02b
NO2-N (mg l-1)	0.92±0.02b	0.82±0.01c	1.03±0.02a
NH4-N (mg l-1)	2.04±0.06a	1.83±0.06b	2.20±0.06a
o-PO4 (mg l-1)	0.06±0.00b	0.07±0.00a	0.04±0.00c
SO4 (mg l-1)	23.02±0.64b	31.05±0.48a	19.74±0.57c
Turbidity (NTU)	22.52±0.93b	28.17±1.20a	20.74±0.88b
TDS (mg l-1)	5035.10±92.04b	5480.21±94.91a	5259.90±102.43ab
Biological characteristics			
Net primary productivity (mg C l-1 d-1)	0.86±0.05b	1.30±0.05a	0.75±0.03b
Gross primary productivity (mg C l-1 d-1)	2.29±0.06b	2.98±0.05a	2.16±0.04c
Phytoplankton density (nos.l-1)	9695.83±857.27ab	11327.08±794.37a	7823.96±632.61b
Zooplankton density (nos. l-1)	4032.29±378.43b	6684.38±567.51a	3231.25±336.17b
Water chlorophyll *a* -1)	2.10±0.03b	2.49±0.06a	1.89±0.03c
Water pheophytin*a* -1)	0.82±0.06b	1.29±0.07a	0.70±0.06b
Epi-phytoplankton (nos. l-1)	7750.00±253.52b	8677.08±257.85a	7260.42±201.80b
Epi-zooplankton (nos. l-1)	3203.13±125.15b	3906.25±251.73a	3218.75±187.37b
Epi-chlorophyll *a* -1)	10.86±0.29ab	11.38±0.31a	10.26±0.26b
Epi-pheophytin*a* -1)	4.22±0.23b	3.84±0.18ab	3.41±0.19b

All values are mean±SE of mean. Water temperature during the experimental period ranged from 27.4 ~ 32.3 ^{0}C
All ponds had additional substrates for the development of periphyton

Discussion

Results of present studies have revealed that, all water quality parameters remained within the optimal range required for obtaining optimum growth of *O niloticus*. The pH of the pond water was alkaline and alkalinity remained higher in all the treatments, indicating that pond waters were well buffered. Mean weight gain, SGR and growth d $^{-1}$ of Nile tilapia were significantly (P<0.05) higher in the ponds where 560 number of bamboo poles were installed as substrate then in the other two substrate densities**.** Nile tilapia has attained a significantly (P<0.05) higher weight gain in treatment II, even though there were no significant differences in periphyton dry matter among the three treatments (I,II and III). This perhaps may be attributed to the availability of higher number of phytoplankton or other nutrients. High growth of *O niloticus* in brackish water ponds provided with additional substrate for periphyton production has already been shown by many workers [2, 8, 9, 23]. Further, many workers [2, 8, 9, 24, 26] have also demonstrated that algal ingestion rates in cichlids are much higher when food is presented as periphytic mat than when presented as plankton. Azim, *et al.* [5] evaluated the polyculture of Indian major carps (catla, rohu and kalbasu) in periphyton based ponds and had obtained similar results. Studies have also reported 35 per cent higher growth in grey mullet, *Mugil cephalus* and 73 per cent higher growth in milkfish, *Chanos chanos*, when grown in inland saline groundwater ponds with a provision of additional substrate for the development of periphyton [26, 27]. Kumar *et al*., [9] conducted a monoculture experiment on *O niloticus* and obtained higher growth in substrate ponds in comparison to feed (67%) and control (113%) ponds. Similarly, *Etroplus suratensis* has also been reported to grow 24 per cent and 99 per cent higher in treated ponds as compared to feed and control ponds, respectively [6]. *O niloticus* which thrives low in the food web i.e., it is a herbivore [1, 28] and feeds primarily on algae and algal based detritus, therefore, its high growth in the ponds provided with additional substrate can be attributed to an increase in primary production.

Substrate density had a significant (P<0.05) effect on the abundance of planktons, TDS, turbidity, kjeldahl nitrogen, ammonia (NH_4-N), and nitrate (NO_3-N) concentrations, net primary productivity (NPP), gross primary productivity (GPP), pigment concentrations (chlorophyll *a* and pheophytin *a*, epilithic plankton population and epilithic pigment concentration). Phytoplankton density (11327 + 794 nos. l^{-1}), zooplankton density (6684 + 567 nos. l^{-1}) and turbidity (28.17 ± 1.20 NTU) were higher in ponds provided with 560 numbers of bamboo poles as substrate, in comparison to 375 (9695 ± 857 nos. l^{-1}; 4032 ± 378 nos. l^{-1} and 22.52 ±0.93 NTU) or 750 (7823± 632 nos. l^{-1}; 3231 ± 336 nos. l^{-1}and 20.74 ± 0.88 NTU) bamboo poles as substrate density respectively.

Table 3: Effect of three different substrate densities (375, 560 and 750) on periphyton dry matter (DM), ash free dry matter (AFDM), ash content, ash (% dry matter), periphyton number, total pigment concentration, chlorophyll *a*, pheophytin*a* and autotrophic index (AI) at different depth

Parameters	375 Depth (cm)				560 Depth (cm)				750 Depth (cm)			
	0	25	50	75	0	25	50	75	0	25	50	75
Dry matter (DM) (mg cm^{-2})	1.27 ± 0.03^{bc}	1.37 ± 0.01^{b}	1.59 ± 0.03^{a}	1.09 ± 0.01^{c}	1.43 ± 0.01^{c}	1.50 ± 0.01^{b}	1.72 ± 0.03^{a}	1.14 ± 0.05^{c}	1.25 ± 0.02^{b}	1.31 ± 0.01^{ab}	1.46 ± 0.02^{a}	0.91 ± 0.01^{c}
AFDM (mg cm^{-2})	0.67 ± 0.03^{bc}	0.71 ± 0.01^{b}	0.77 ± 0.03^{a}	0.64 ± 0.02^{c}	0.89 ± 0.02^{b}	0.91 ± 0.03^{a}	0.94 ± 0.06^{a}	0.70 ± 0.01^{b}	0.61 ± 0.02^{b}	0.67 ± 0.02^{a}	0.72 ± 0.03^{a}	0.63 ± 0.01^{ab}
Ash (mg cm^{-2})	0.51 ± 0.01^{c}	0.56 ± 0.01^{b}	0.64 ± 0.01^{a}	0.52 ± 0.03^{c}	0.56 ± 0.01^{c}	0.64 ± 0.02^{b}	0.81 ± 0.03^{a}	0.45 ± 0.04^{d}	0.47 ± 0.01^{bc}	0.51 ± 0.01^{b}	0.60 ± 0.02^{a}	0.49 ± 0.02^{b}
Ash % of DM	36.0 ± 0.91^{bc}	39.5 ± 1.61^{b}	47.5 ± 1.37^{a}	34.5 ± 1.31^{c}	43.0 ± 0.81^{c}	48.0 ± 0.72^{b}	54.5 ± 1.05^{a}	42.0 ± 1.32^{c}	35.0 ± 0.87^{bc}	37.0 ± 1.12^{b}	45.0 ± 1.92^{a}	31.0 ± 1.43^{c}
Periphyton number (units cm^{-2})	12395 ± 1020^{c}	16753 ± 1044^{b}	20332 ± 1038^{a}	11874 ± 1145^{c}	15186 ± 1152^{c}	19104 ± 778^{b}	22499 ± 1398^{a}	14999 ± 1403^{c}	9770 ± 740^{c}	13707 ± 907^{b}	19687 ± 1720^{a}	10020 ± 639^{c}
Total pigment	9.12 ± 0.50^{c}	10.84 ± 0.37^{b}	13.81 ± 0.43^{a}	8.67 ± 0.31^{c}	9.17 ± 0.45^{c}	11.61 ± 0.38^{b}	13.91 ± 0.54^{a}	8.80 ± 0.30^{c}	9.44 ± 0.42^{c}	10.55 ± 0.48^{b}	13.22 ± 0.42^{a}	8.04 ± 0.33^{d}
concentration (μg cm^{-2})												
Chlorophyll *a* (μg cm^{-2})	6.59 ± 0.32^{c}	7.91 ± 0.23^{b}	10.36 ± 0.27^{a}	6.27 ± 0.18^{c}	6.77 ± 0.28^{c}	8.99 ± 0.30^{b}	10.50 ± 0.38^{a}	6.34 ± 0.18^{c}	6.64 ± 0.24^{c}	7.61 ± 0.30^{b}	9.95 ± 0.25^{a}	5.77 ± 0.23^{d}
Pheophytin*a* (μg cm^{-2})	2.53 ± 0.18^{bc}	2.93 ± 0.14^{b}	3.45 ± 0.16^{a}	2.40 ± 0.13^{c}	2.40 ± 0.17^{b}	2.62 ± 0.08^{b}	3.41 ± 0.16^{a}	2.46 ± 0.12^{b}	2.80 ± 0.18^{a}	2.94 ± 0.18^{a}	3.27 ± 0.17^{a}	2.27 ± 0.10^{b}
Autotrophic index (AI)	105.32^{a}	85.30^{b}	74.53^{c}	87.42^{b}	127.22^{a}	97.29^{bc}	85.81^{c}	106.41^{b}	102.78^{a}	81.28^{b}	72.96^{c}	97.54^{ab}
for AFDM												
AI values for DM	155.71^{a}	138.32^{c}	146.91^{bc}	149.62^{b}	201.61^{a}	161.25^{c}	157.27^{c}	173.78^{b}	153.24^{a}	132.51^{c}	140.47^{b}	142.37^{b}
Algal constitute of	36-42	41-45	45-57	40-51	42-54	48-65	49-63	44-58	34-38	37-45	48-55	39-54
periphyton biomass (%)												
Periphyton productivity (mg C cm^{-2} d^{-1})	0.55^{c}	0.65^{b}	0.84^{a}	0.43^{b}	0.62^{c}	0.76^{b}	0.97^{a}	0.54^{d}	0.51^{c}	0.64^{b}	0.81^{a}	0.41^{d}

All values are mean ± S.E. of mean. Means with the same letters in the same column are not significantly ($P<0.05$) different

Phytoplankton density

Irrespective of the density of the substrate, phytoplankton number and turbidity values showed a positive correlation, however, their values remained significantly (P<0.05) higher in ponds provided with 560 bamboo poles as substrate.

A significant positive (r=0.30) correlation was observed between chlorophyll *a* and pheophytin *a*. Data clearly indicated that low concentrations of pheophytin *a* were mostly preceded by high concentrations of chlorophyll *a* in all the treatments indicating a continuous breakdown of chlorophyll *a* as a result of grazing pressure exerted by the fish/zooplankton. Nayar and Gowda [29] have also observed an inverse relationship between chlorophyll *a* and pheophytin *a* concentration.

Nutrients viz. total kjeldahl nitrogen, nitrate and o-PO_4 were higher, while, total ammonia and nitrite were low in ponds provided with 560 bamboo poles as substrate indicating a higher ammonia uptake by periphyton. It has been reported that [30] the attached diatoms and filamentous Cyanobacteria (periphyton) were responsible for the largest uptake of ammonium ions from the water in intensive shrimp culture ponds. Other reasons might be higher abundance of phytoplankton (in treatment with 560 numbers of bamboo substrate), which assimilated ammonia from the water column [31]. Many other workers have also reported that the bacterial biofilm (periphyton) reduced ammonia through promotion of nitrification [32, 33]. Application of multiple regression models also showed a significant positive correlation of chlorophyll *a* with nutrients (NO_3-N r2=0.26, P<0.001; kjeldahl nitrogen r2=0.18, P<0.05 and o-PO_4 r2 = 0.04) and phyto plankton population with total kjeldahl nitrogen (r2=0.006) and NO_3-N (r2=0.66, P<0.001) clearly indicating that the pond productivity was positively correlated with the nutrient status. Periphyton biomass as measured by dry matter (DM) and pigment concentrations differed significantly (P<0.05) between different depths with higher values occurring at 50 cm depth. These results are in agreement with the findings of other workers [3, 8, 9, 13] who have reported maximum periphytic biomass levels coinciding with photosynthetic compensation depths.

Ash contents of the periphyton were high in ponds provided with 560 numbers of bamboo poles as the substrate and ranged from 42.0–54.5 of DM content; however, growth of the fish was not affected. High ash contents in our experiment might be attributed to the suspended particles entrapped in the periphyton community. The periphyton autotrophic index (AI) for dry matter values of three different treatments (375, 560 and 750) were 155.7, 201.6 and 153.2 respectively, which indicated higher amount of algae colonized on bamboo substrate [16] which directly affected fish growth.

Lower growth at lower density or at the highest density of the substrate may be attributed partly to the lower values in most of the productivity indicating parameters. Lower growth at highest density of the substrate can also be attributed to the crowding effect due to the insufficient space because of higher density of substrate. Thus this too might have acted as a limiting factor for the growth of the fish. Since there were no significant differences in the Dry matter (DM) of periphyton among the three treatments, therefore, this could be the only plausible explanation for lower fish growth under high density. The dose of fertilizer might also be a little lower for the higher density of the substrate to provide enough nutrients for microbial biofilm to grow/colonise on the substrate. An experiment conducted on the polyculture of carps using three different densities of the substrate (50,75 and 100 percent submerged area) had revealed highest fish production from ponds where the submerged surface was 100 percent indicating that the fish production increased linearly with increasing substrate surface [7]. Milstein *et al*. [34] used submerged plastic surfaces equivalent to 40% of the pond surface area in polyculture ponds containing 85% hybrid tilapia *(Oreochromis niloticus* x *O. aureus)*, together with a reduction of 40% of the amount of pelleted feed. The treatment improved nitrification and saved 40% of the feed costs, with only a 10% reduction in the tilapia growth rate and yield. In the present studies fish production though increased linearly with increasing substrate density, however it was limited only up to a maximum of 54 per cent of the submerged surface area. These results indicate that periphyton-based aquaculture is an appropriate technology for reducing production costs and allowing economically viable organic tilapia production.

From these studies, it can be concluded that in addition to increasing food supply, the presence of substrate in optimal density appears to reduce stress by acting as a shelter or hiding place. To conclude, these findings suggest that with the provision of optimum density of the substrate, periphyton supported production technology can offer considerable potential for enhancing aquaculture production without any deleterious impact on pond ecosystem. This technology can be considered as an alternative to organic farming. The nutrient re-cycling of the periphyton contributes to water purification as some algae are able to use ammonia and also help in removing phosphate from the water column,

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