



Impacts of Grazing by Milkfish (*Chanos chanos* Forsskal) on Periphyton Growth and its Nutritional Quality in Inland Saline Ground Water : Fish Growth and Pond Ecology

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Abstract: Two experiments (I and II) each under three different salinity levels (10, 15 and 20 ppt) were conducted to investigate the effects of addition of milkfish and substrate for periphyton development and its nutritional quality. Efforts were also made to investigate the hydrobiological parameters and growth of milkfish. Both experiments were run simultaneously with the difference that in experiment I, ponds were stocked with milkfish, while no fish were stocked in experiment II. No significant differences were observed in TDS levels among the three salinity treatments. Studies have revealed higher values of productivity indicating parameters (Alkalinity, NPP and GPP) under grazed conditions in comparison with the ungrazed conditions. A comparison of physico-chemical characteristics of pond water in between the two experiments (I and II) indicated not many variations. All parameters followed a trend similar to the ponds stocked with milkfish, except that BOD₅ values were slightly higher and DO levels were slightly lower under ungrazed conditions. SO₄ and o-PO₄ levels were similar in both the trials. Addition of fish only slightly affected inorganic N-species (NO₃N, NO₂N), however, NH₄N levels were significantly ($P < 0.05$) low, while Alkalinity and total Kjeldahl nitrogen were significantly ($P < 0.05$) high in treatment with fish at 15 ppt salinity. Irrespective of the water salinity, mean periphyton density scraped from the substrate increased with an increase in depth upto 50 cm in both the trials. A comparison of periphyton production/biomass and its pigment concentrations indicated significantly ($P < 0.05$) higher values for dry matter, ash free dry matter (AFDM), ash, ash % of dry matter, algal constitutes, autotrophic index in ponds with fish (grazed conditions). On the other hand, periphyton number (units cm⁻²), total pigment concentration, chlorophyll *a* and pheophytin *a* remained significantly ($P < 0.05$) higher in ponds without fish (ungrazed conditions). Results have also revealed a significant effect of salinity on fish growth with significantly ($P < 0.05$) higher growth occurring in ponds maintained at 15 ppt salinity. Fish carcass protein, fat and phosphorus, VSI and HSI values also coincided well with highest fish growth at 15 ppt salinity. Proximate composition of periphyton had revealed significantly ($P < 0.05$) higher nutritive value of samples obtained from ponds without fish.

Keywords: Milkfish, Inland Saline Water, Growth, Periphyton, Water Quality, Grazed and Ungrazed, Nutritional Quality of Periphyton

1. Introduction

Many of the herbivorous fish species specialize in feeding on larger benthic, epilithic or periphytic algae rather than on phytoplankton [21, 34]. Most such algae require hard substrates for attachment, which are usually absent in fish ponds. Algae growing on substrates and the associated bacterial and zooplanktonic biomass can be directly exploited by many a herbivorous fish species resulting in higher fish

yield [18, 20]. The introduction of hard surfaces in the water column induces the growth of biofilms and periphyton production, which enhances natural productivity of the water body and thus producing food for cultured aquatic organisms [3, 18, 29, 36]. Submerged substrates provide sites not only for the development of periphyton and microbial community, but also improve water quality by sequestering excess

nutrients, ammonia and phosphates etc. [26, 38]. Lots of research on the role of periphyton in fish growth under brackishwater conditions have already been carried out in our laboratory [12, 14, 16, 27, 28]. These studies have shown that herbivorous fish like milkfish [23], mullet [24], pearlspot [16, 27] and Nile tilapia [16, 28] grew faster and at higher rates in ponds provided with the additional substrate for the development of periphyton. Studies of Kumar *et al* [27, 28] on *Oreochromis niloticus* and *Etroplus suratensis* have also revealed that the fish growth was much higher in ponds provided with additional substrate in comparison to the fish grown in ponds provided with supplementary diet (with no additional substrate).

One of the most important inputs in aquaculture is the fish feed and it accounts for over 50-60 % of the total cost of fish production. Therefore, sustainability, viability and success of aquaculture mainly depend on the type of feed used and feed management. Since periphyton technology uses at best only organic manures without involving the use of any supplementary diets, therefore this technology appears to be economically viable and thus a way a step towards the development of organic farming [30, 31]. Our earlier studies have not taken into consideration the development/composition of periphytic biomass, their effect on hydrobiological parameters in ponds provided with additional substrate with and without fish. Milkfish are considered to be opportunistic and feed on anything from detritus to phytoplankton, zooplankton and filamentous algae, while their juveniles in their natural habitats commonly feed on bluegreen algae, diatoms, detritus, filamentous green algae, copepods and nematodes etc. Our studies on milkfish have revealed that it thrives very well in ponds provided with substrate for the development of periphyton [Jana *et al.*, 2006a]. Therefore, in the present studies, two experiments (I and II) under three different salinity levels (10, 15 and 20 ppt) were conducted. In experiment I ponds were stocked with milkfish (henceforth called grazed conditions), while, no fish were stocked in ponds in experiment II (henceforth called ungrazed conditions). Efforts were made to assess the (i) effect of variable salinity levels on periphyton production, and also (ii) to assess the effect of fish on periphytic proximate composition (nutritional quality), (iii) usual parameters like water quality, effect of water depth on periphyton productivity and effect of different salinity levels on fish growth were also monitored.

2. Materials and Methods

2.1. Pond Preparation and Experimental Design

Experiments were conducted in earthen ponds (15m×25m (area 375m², depth 1.5 m) at the brackishwater fish pond facility of the Department of Zoology and Aquaculture, CCS Haryana Agricultural University, Hisar (Lat. 29°, 10'N; Long 75°, 46'E), India, from April to August. Protocol for the maintenance of ponds and installation of bamboo substrates

were adopted as described in Kumar *et al* [27, 28].

Following two experiments (I & II) each in replicate of two under three different salinity levels (10, 15 and 20 ppt) were conducted with a difference that in trial I, ponds were stocked with milkfish, while no fish were stocked in ponds of trial II (Table 1).

Table 1. Protocol of experimental treatments.

Salinity (ppt)	Stocking density/375 m ²	Fertilization (kg ha ⁻¹ y ⁻¹)	Substrate density/375 m ²
Experiment I with fish (Grazed conditions)			
10	500	10,000	375
15	500	10,000	375
20	500	10,000	375
Experiment II without fish (Ungrazed conditions)			
10	-	10000	375
15	-	10000	375
20	-	10000	375

2.2. Stocking

In experiment I, Two weeks after the application of the first dose of organic fertilizer, 20 day-old milkfish fry (mean weight of 0.2 g) were stocked at 500 fish per pond. The duration of grow out period was 115 days.

2.3. Water Quality Monitoring

Water 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 seven (on 15, 30, 45, 60, 75, 90 and 115 days) samplings were done on seven different dates, however, only overall mean values of all the seven observation dates are shown. Temperature, salinity and pH were recorded daily, while the other physico-chemical parameters were measured on seven different dates following APHA [4]. Net and gross primary productivity (NPP and GPP) were determined using light and dark bottle technique [4].

2.4. Determination of Chlorophyll a, Pheophytin a and Plankton Biomass from Pond Water

Water samples were collected and analysed following the protocol described by Kumar *et al* [27, 28]

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.

Plankton species diversity (\bar{d}) was determined using the diversity index formula of Shannon and Weaver (40):

$d = - \sum (ni/N) \log_2 (ni/N)$; where, d = species diversity; ni = number of individuals of i th species, and N = total number of individuals.

Identification of plankton to genus level was carried out using the key [8, 33, 39].

2.5. 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. Dry matter (DM), ash free dry matter (AFDM), autotrophic index (AI), and ash content were calculated following AOAC (4).

AI was calculated as follows:

AI = biomass (ash-free weight of organic matter, mg m⁻²/chlorophyll *a*, mg/m²).

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

Periphyton productivity (mg C/ m²/day) = total ash weight (mg/cm²) × 100 / t

Where, t = duration of experiment (115 days).

Periphytons were enumerated using a Sedgwick-Rafter cell according to the procedure described for planktons and calculated as follows:

$N = P \times C \times 100 / S$

Where, N = periphyton number/cm² (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 (cm²).

The remaining sample from each replicate was used to determine chlorophyll *a* and pheophytin *a* contents following standard methods [4].

2.6. Proximate Composition of Periphyton

For determining proximate composition, periphyton samples (in replicate of six) from substrate implanted ponds with and without fish were scraped and analysed following AOAC (3). From each treatment, for comparison, periphyton samples (in replicate of four each 3×3 cm²) growing on the pond walls (epilithic) were also collected (at 15-day intervals) during the experimental period of 115 days for the study of periphyton population and pigment concentrations.

2.7. Fish Harvesting

Post stocking (115 days), substrates were removed, ponds were drained and all the fish were harvested, weighed and number of fish recovered from each treatment were recorded. Thereafter, weight (g) and length (cm) of the individual fish were taken. SGR, condition factor (k) and length-weight relationship (LWR) were calculated. Length-weight relationship (LWR) of fish was calculated according to the following equation:

$W = c \text{ Ln}$ (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.

2.8. Determination of VSI, HSI and Other Biochemical Parameters

From each salinity treatment in experiment I, eight fish were obtained and kept on an ice tray; viscera and liver of the fish were extirpated for the determination of viscero-somatic index (VSI) and hepato-somatic index (HSI). Fish carcass (initial and final), and periphyton samples were analysed following AOAC [3]. Energy contents of periphyton were calculated using the average caloric conversion factors of 0.3954, 0.1715, and 0.2364 kJ/g for lipid, carbohydrate and protein, respectively [19].

2.9. Statistical Analysis

The data were subjected to ANOVA to test the effect of replication and treatment using the following model:

$Y_{ijk} = \mu + R_i + T_j + e_{ijk}$; where, Y_{ijk} = kth observation of jth treatment of ith replications (Time); μ = overall mean; R_i = effect due to ith replications; T_j = effect due to jth treatment; and e_{ijk} = random error NID (0, σ^2). Arcsine transformation of the data presented in percentage was done before analysis of variance as described by Snedecor and Cochran [37] and means were compared using Tukey's test.

3. Results

3.1. Experiment I. Effect of Milkfish on Periphyton Growth and Hydrobiological Characteristics of Inland Saline Ground Water Ponds (Grazed Conditions)

In this experiment, fish growth, its carcass composition, in relation to periphyton growth, Physico-chemical and biological characteristics of water quality including periphyton pigment concentrations were investigated

3.2. Fish Growth and Carcass Composition

Survival of *Chanos chanos* at different salinities varied between 93 to 96%. The mean weight of milkfish increased from 0.2g to 29.55 g at 15 ppt salinity compared to 0.2 to 19.12g at 10 ppt and 0.2 to 13.33g at 20 ppt salinity. The mean length at 15 ppt was 15.82 cm compared to 13.87 cm at 10 and 13.33 at 20 ppt salinity. One way ANOVA revealed a significant ($P < 0.05$) increase in mean fish weight, length, specific growth rate and condition factor (k) in ponds provided with additional substrate at 15 ppt salinity in comparison with the other two salinity (10 and 20 ppt) treatments (Table 2). The exponential value of 'n' of LWR was also higher at 15 ppt salinity. Analysis of fish carcass revealed significantly ($P < 0.05$) higher values for protein, fat and phosphorus in fish grown in ponds provided with additional substrate at 15 ppt salinity. Similarly, VSI and HSI values were also significantly ($P < 0.05$) higher in fish grown at 15 ppt salinity in comparison with the other two salinity treatments (Table 2).

Table 2. Effect of different salinity levels (10, 15 and 20 ppt) on growth performance, viscero-somatic index (VSI), hepato-somatic index (HSI) and proximate composition (% wet weight) of milkfish, *Chanos chanos* in ponds provided with additional substrate-115 days treatment.

Salinity- (ppt)	INITIAL FISH STOCK			FINAL FISH STOCK (after 100 days)			SGR % g d ⁻¹ (SGRL)	Growth (g d ⁻¹)	Condition factor (cf/k)	Length weight relationship (LWR)
	Stocking density/ 375 m ²	Mean fish weight (g) (length cm)	Total biomass (g)	Survival (%)	Mean fish weight (g) (Length cm)	Total biomass (g)				
10	500	0.02±0.002a (1.32±0.04a)	9.06±1.14a	94	19.12±0.46b (13.87±0.12b)	8.98±0.21b	7.08±0.13b (2.36±0.03b)	0.191±0.004b	0.71±0.001b	W= -0.559 L ^{3.104}
15	500	0.02±0.002a (1.32±0.04a)	9.06±1.13a	96	29.55±1.04a (15.82±0.20a)	14.19±0.50a	7.51±0.12a (2.49±0.03a)	0.30±0.01a	0.74±0.01ab	W= -0.192 L ^{3.375}
20	500	0.02±0.002a (1.32±0.04a)	9.06±1.14a	93	13.33±0.52c (11.98±0.21c)	6.20±0.24c	6.71±0.11c (2.21±0.03c)	0.13±0.01c	0.77±0.02a	W= -0.571 L ^{3.097}

PROXIMATE COMPOSITION OF FISH-continuation of Tabl 2

Salinity (ppt)	Moisture	Protein	Fat	Phosphorus	Ash	Viscero-somatic index (VSI)	Hepato-somatic index (HSI)
Initial value	72.20±0.11	15.44±0.30	2.95±0.02	0.38±0.01	2.78±0.06	-	-
10	68.26±0.10b	20.13±0.28ab	3.73±0.04b	0.68±0.02b	3.76±0.03b	8.49±0.14b	1.64±0.04b
15	67.71±0.10b	20.67±0.33a	3.95±0.05a	0.92±0.04a	4.09±0.06a	10.18±0.15a	1.94±0.05a
20	69.26±0.12a	19.36±0.35b	3.55±0.03c	0.56±0.02c	3.51±0.07c	7.84±0.16c	1.45±0.05c

Temperature during the experimental period of 115 days fluctuated between 26.0 ~ 28.4°C

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 = [ln W_{tf} - ln W_{ti}] × 100 / t, SGRL (% cm d⁻¹) = specific growth rate of length = [ln L_f - ln L_i] × 100 / t

Growth per cent gain in body weight = [(W_{tf} - W_{ti}) / W_{ti}] × 100, where, W_{ti} and W_{tf} denotes initial and final weight of fish respectively, L_f and L_i denotes initial and final length (cm) of fish respectively and t represents time (days), duration of experiment (days), BW = Body weight, d=days.

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

Length-weight relationship (LWR): W = cLⁿ = log w = log l + n log l, where w = weight in kg, C = constant, n = exponential value of length and L = length of fish in cm.

3.3. Physico-Chemical and Biological Characteristics of Water (Expt. I)

Temperature during the experimental period (115 days) fluctuated between 26.0–28.4°C and pH remained alkaline in all the three treatments. Dissolved oxygen (DO) levels were significantly (P<0.05) higher in ponds maintained at 15 ppt salinity, while low values were observed in the other salinity treatments. Electrical conductivity (EC), total hardness, chlorides, calcium and magnesium levels increased with increase in salinity of the treatments. Nutrients (NO₂-N, o-PO₄, SO₄ and kjeldahl nitrogen) and productivity indicating parameters (Turbidity and total alkalinity) were significantly (P<0.05) higher, while BOD₅, and NH₄ levels remained

significantly (P<0.05) lower in ponds maintained at 15 ppt salinity. No significant differences were observed in TDS levels among the three salinity treatments (Table 3). Productivity indicating parameters (NPP and GPP) were significantly (P<0.05) higher in ponds maintained at 15 ppt salinity. Similarly, Phyto and zooplankton density, their species diversity, chlorophyll *a* and Pheophytin *a*, Epilithic phytoplankton and Epilithic zooplankton, zooplankton numbers, and Epilithic chlorophyll *a* and Epilithic pheophytin *a* concentrations were also significantly (P<0.05) higher in ponds maintained at 15 ppt in comparison with the other two (10 and 20 ppt) treatments (Table 3).

Table 3. Physico-chemical characteristics of pond water at three different salinity levels (10, 15 and 20 ppt) under grazed and ungrazed conditions- Overall mean of seven samplings done on seven different dates (during a period of 115 days).

Parameters	Grazed (Stocked with milkfish)			Ungrazed (No fish was stocked)		
	Salinity (ppt)			Salinity (ppt)		
	10	15	20	10	15	20
Electrical Conductivity dSm ⁻¹	14.56±0.39c	20.16±0.45b	26.38±0.56a	14.99±0.39c	20.30±0.43b	26.86±0.61a
pH	8.27±0.03a	8.28±0.03a	8.16±0.02ab	8.03±0.05ab	8.12±0.05a	7.91±0.06b
Dissolved oxygen mg l ⁻¹	4.73±0.12bc	5.22±0.13a	4.64±0.11b	4.61±0.10b	5.03±0.09a	4.79±0.10b
BOD mg l ⁻¹	2.05±0.05a	1.82±0.05b	2.17±0.06a	2.38±0.07a	2.12±0.07b	2.41±0.08a
Carbonates mg l ⁻¹	17.96±1.05a	16.98±0.77a	15.55±0.86ab	14.02±0.93a	12.14±0.70b	8.29±0.51c
Biocarbonates mg l ⁻¹	229.16±1.23a	222.45±2.07bc	210.18±1.10c	229.84±1.49a	226.25±1.40ab	218.68±2.00c
Total alkalinity mg l ⁻¹	247.29±1.12ab	269.43±1.39a	225.71±0.90b	243.79±1.80b	258.36±1.48a	227.00±2.09c
Chlorides mg l ⁻¹	3865.18±28.75c	4235.16±31.79b	4672.71±21.31a	3800.91±17.71c	4156.75±26.43b	4634.89±27.05a

Parameters	Grazed (Stocked with milkfish)			Ungrazed (No fish was stocked)		
	Salinity (ppt)			Salinity (ppt)		
	10	15	20	10	15	20
Total hardness mg l ⁻¹	3128.57±45.19c	3987.50±85.84b	4806.43±117.60a	3171.43±33.10c	4014.29±83.41b	4873.21±114.23a
Calcium mg l ⁻¹	288.54±12.00c	358.11±12.07b	474.23±18.65a	295.41±12.46c	376.86±13.01b	436.71±18.36a
Magnesium mg l ⁻¹	587.57±10.26c	758.07±16.55b	882.95±20.24a	603.93±7.37c	756.37±18.46b	917.21±28.67a
Total Kjeldahl nitrogen mg l ⁻¹	5.63±0.15b	6.67±0.16a	5.33±0.12bc	5.10±0.10b	6.13±0.17a	4.84±0.14c
NO ₃ -N mg l ⁻¹	0.60±0.12a	0.61±0.04a	0.50±0.09b	0.46±0.08a	0.56±0.10a	0.74±0.17a
NO ₂ -N mg l ⁻¹	0.75±0.04ab	0.69±0.03b	0.74±0.02ab	0.82±0.03a	0.79±0.04ab	0.75±0.04ab
NH ₄ -N mg l ⁻¹	2.60±0.04a	2.19±0.04b	2.62±0.06a	2.80±0.06a	2.59±0.08b	2.97±0.10a
o-PO ₄ mg l ⁻¹	0.04±0.002bc	0.05±0.002a	0.03±0.002bc	0.03±0.002a	0.04±0.002a	0.03±0.002a
SO ₄ mg l ⁻¹	33.81±0.69b	36.84±0.78a	37.21±0.57a	29.97±1.02c	36.24±0.81ab	33.77±1.36b
Turbidity NTU	25.70±1.37a	25.84±1.31a	21.32±1.19b	21.50±1.20ab	24.46±1.64ab	22.29±1.60ab
Total dissolved solids mg l ⁻¹	3110.45±270.81a	3434.36±283.41a	3513.43±270.91a	3116.68±265.92a	3451.25±293.26a	3308.21±271.61a

All values are mean±SE of mean. Water temperature during the experimental period ranged from 26.0–32.2°C

All ponds were provided with additional substrates in the form of bamboo poles for the development of periphyton

3.4. Biotic Community (Expt. I)

Phytoplankton were represented by chlorophyceae (6 taxa), Bacillariophyceae (7 taxa) and cyanophyceae (1 taxa). In case of Zooplankton, Rotifera (2 taxa) and Copepoda (2 taxa) represented the community.

3.5. Periphyton and Pigment Concentrations (Expt. I)

Irrespective of the salinity level, mean values of periphyton scraped from the bamboo substrate at 50 cm depth were 7465.0 number cm⁻² (range 5678.0 – 10230.0 numbers cm⁻²). Depth trend in periphyton growth indicated highest values (10230.0 numbers cm⁻²) at 50 cm in ponds maintained at 15 ppt salinity in comparison with 10 ppt (8993.0 numbers cm⁻²) and 20 ppt (9695 numbers cm⁻²). By-weekly variations had revealed no definite trend in periphyton numbers, however, peak values at most of the depths were observed in sampling done at 45 days interval, thereafter values levelled off at all the depths, though remained higher at 50 cm depth (Table 4). Significantly

(P<0.05) higher values for mean DM, AFDM and ash were observed at 50 cm substrate depth in 15 ppt ponds (Table 4). Irrespective of the salinity treatment, autotrophic index (AI) values for AFDM and DM decreased with an increase in substrate depth (Table 4). Bi-weekly variations in mean values of periphyton plankton density have revealed that irrespective of the salinity level, higher values were observed on 45th day, which were significantly higher at 15 ppt salinity (Fig 1). Mean periphyton productivity, chlorophyll *a* (Fig. 2) and pheophytin *a* concentrations (Fig. 3) remained significantly (P<0.05) higher at 50 cm depth (Table 4). No significant variations in periphyton chlorophyll *a* concentrations were observed with respect to time. Initially the values were high, which gradually declined with passage of time. Irrespective of the substrate depth or salinity level no definite trend in pheophytin *a* concentration at 15 ppt was observed. At other salinity levels lowest values were observed during the sampling done between 45-60 days (Fig 3). Irrespective of the salinity level, high concentration of pheophytin *a* was observed at 50 cm depth.

Table 4. Biological characteristics of pond water at three different salinity levels (10, 15 and 20 ppt) under grazed and ungrazed conditions- Overall mean of seven samplings done on seven different dates (during a period of 115 days).

Parameters	Grazed (Stocked with milkfish)			Ungrazed (No fish was stocked)		
	Salinity (ppt)			Salinity (ppt)		
	10	15	20	10	15	20
Net primary productivity mg C l ⁻¹ d ⁻¹	1.01±0.02c	1.89±0.04a	1.19±0.03b	0.96±0.02b	1.29±0.03a	1.04±0.02c
Gross primary productivity mg C l ⁻¹ d ⁻¹	2.07±0.05c	2.90±0.07a	2.19±0.05b	1.78±0.06b	2.30±0.07a	1.94±0.05c
Phytoplankton density nos. l ⁻¹	9763±219c	12040±234a	10960±332b	9531±286c	12549±256a	11576±438b
Zooplankton density nos. l ⁻¹	5710±135b	5076±136c	6330±203a	5750±226b	6723±227a	5375±251c
Phytoplankton (d)	1.40±0.15bc	1.99±0.20a	1.59±0.14b	1.44±0.17b	1.99±0.22a	1.31±0.14b

Parameters	Grazed (Stocked with milkfish)			Ungrazed (No fish was stocked)		
	Salinity (ppt)			Salinity (ppt)		
	10	15	20	10	15	20
Zooplankton (d)	1.14±0.21ab	1.36±0.21a	1.10±0.18b	1.07±0.12b	1.29±0.15a	1.02±0.11b
Chlorophyll <i>a</i> µg l ⁻¹	3.59±0.21c	4.65±0.21a	4.21±0.20b	3.73±0.12b	4.24±0.13a	3.52±0.11c
Pheophytin <i>a</i> µg l ⁻¹	1.46±0.10a	1.63±0.12a	1.76±0.12a	1.60±0.11a	1.57±0.11a	1.74±0.11a
Epilithic phytoplankton nos. l ⁻¹	5156±537b	7371±643a	5112±527b	5996±930b	7067±992a	5224±658c
Epilithic zooplankton nos. l ⁻¹	2460±194b	3295±214a	2223±182c	3232±450a	3510±277a	2642±242b
Epilithic chlorophyll <i>a</i> µg l ⁻¹	8.18±0.76c	8.94±0.76a	8.48±0.73c	7.38±1.02b	8.05±1.07a	7.36±1.00b
Epilithic pheophytin <i>a</i> µg l ⁻¹	2.87±0.22c	3.62±0.21a	3.32±0.16b	3.47±0.27a	3.75±0.26a	3.39±0.18ab

All values are mean±SE of mean. Water temperature during the experimental period ranged from 26.0~32.2°C

All ponds were provided with additional substrates in the form of bamboo poles for the development of periphyton

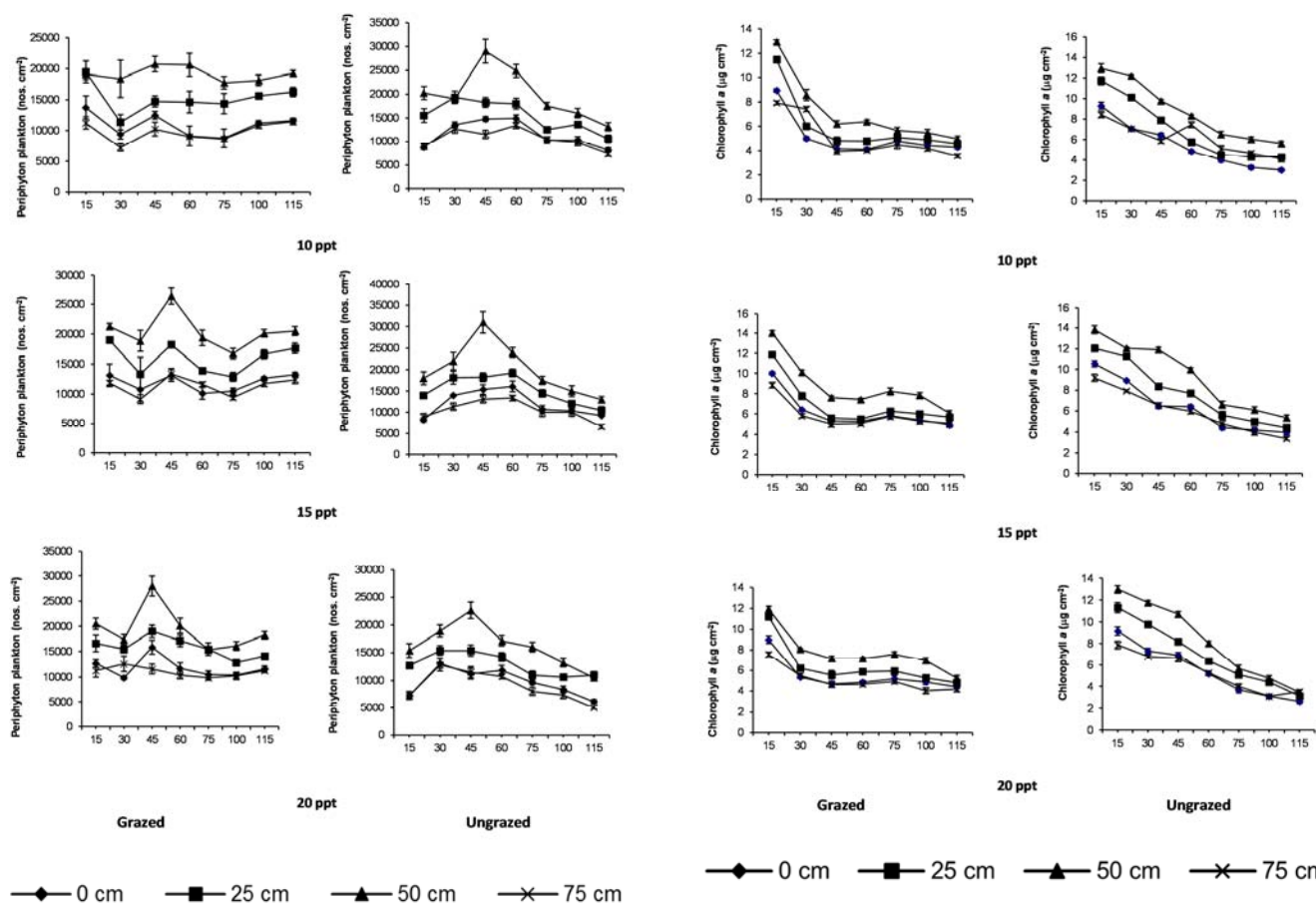


Fig. 1. Bi-weekly variations in mean values of periphyton plankton density at different depths (0, 25, 50 and 75 cm) from ponds having 10, 15 and 20 ppt saline water and provided with bamboo poles as additional substrate in grazed versus Ungrazed conditions.

Fig. 2. Bi-weekly variations in mean values of periphyton chlorophyll *a* concentrations at different depths (0, 25, 50 and 75 cm) from ponds having 10, 15 and 20 ppt saline water and provided with bamboo poles as additional substrate in grazed versus Ungrazed conditions.

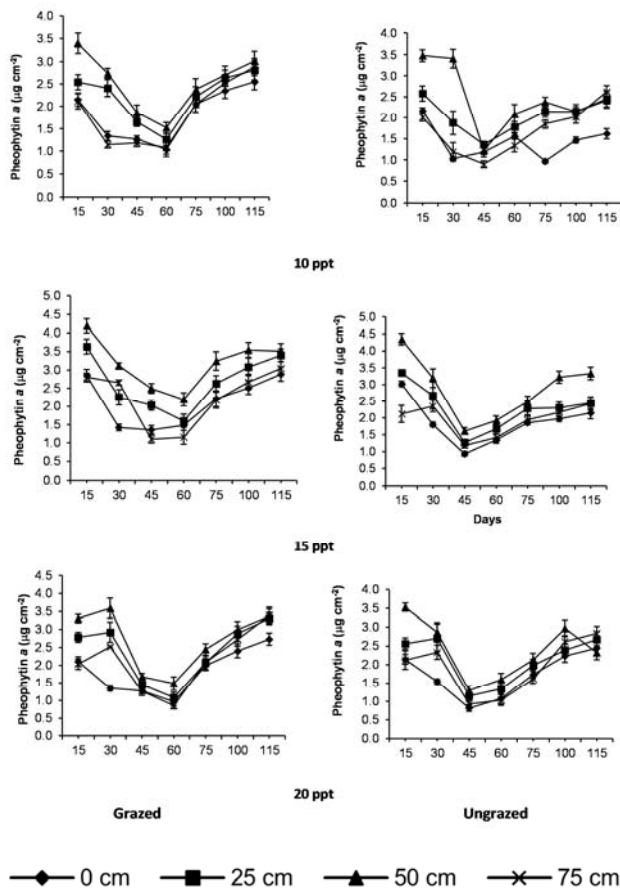


Fig. 3. Bi-weekly variations in mean values of periphyton pheophytin *a* concentrations at different depths (0, 25, 50 and 75 cm) from ponds having 10, 15 and 20 ppt saline water and provided with bamboo poles as additional substrate in grazed versus Ungrazed conditions.

3.6. Experiment II. Periphyton Growth and Hydrobiological Characteristics of Inland Saline Ground Water Ponds under Ungrazed Conditions)

In this experiment, along with periphyton growth Physico-chemical and biological characteristics of water quality including periphyton pigment concentrations were investigated. No fish were stocked in this experiment.

3.7. Physico-Chemical Characteristics of waTer (Expt. II)

Dissolved oxygen (DO) levels were significantly ($P < 0.05$) higher in ponds maintained at 15 ppt salinity, in comparison with the other two salinity treatments. Electrical conductivity (EC), total hardness, chlorides, calcium and magnesium levels increased with increase in salinity of the treatments. Nutrients (alkalinity, SO_4 and kjeldahl nitrogen) were significantly ($P < 0.05$) higher, while BOD_5 , and NH_4 levels remained significantly ($P < 0.05$) lower in ponds maintained at 15 ppt salinity. No significant variations were observed in the concentrations of $o-PO_4$, NO_3-N , NO_2-N among the three salinity levels. NPP and GPP values were significantly ($P < 0.05$) higher at 15 ppt salinity, while no significant differences were observed in TDS and turbidity levels among

the three salinity treatments (Table 3).

A comparison of physico-chemical characteristics of pond water in between the two experiments (I and II) indicated not many variations. All parameters followed a trend similar to the ponds stocked with milkfish, except that BOD_5 values were slightly higher, while DO levels were slightly lower under ungrazed conditions. SO_4 and $o-PO_4$ levels were similar in both the trials. Addition of fish only slightly affected inorganic N-species (NO_3-N , NO_2-N), however, NH_4-N levels were significantly ($P < 0.05$) low, while Alkalinity and total Kjeldahl nitrogen were significantly ($P < 0.05$) high in treatment with fish at 15 ppt salinity.

3.8. Biological Characteristics and Biotic Community of Pond Water (Expt. II)

Like under grazed conditions, epilethic phyto and zooplankton, epilethic chlorophyll *a* and pheophytin *a* values were significantly ($P < 0.05$) higher in ponds maintained at 15 ppt (Table 3). No definite trend in phytoplankton and zooplankton population was observed with respect to salinity. Plankton population for both phytoplankton and zooplankton was always higher in 15 ppt salinity ponds in comparison to the other two salinity treatments. Phytoplankton were represented by chlorophyceae (7 taxa), Bacillariophyceae (7 taxa) and cyanophyceae (2 taxa), while Rotifera (2 taxa) represented the zooplanktons community. A comparison of the biological characteristics of the two experiments (I and II) indicated that all parameters followed a trend similar to the ponds stocked with milkfish (grazed conditions), except that the values of most of the parameters were slightly higher under ungrazed conditions more so at 15ppt salinity.

3.9. Periphyton and Pigment Concentrations (Expt. II)

Irrespective of the salinity level and depth, mean values of periphyton scraped from the bamboo substrate were 9712 numbers cm^{-2} (range 4482-10035.0 number cm^{-2}). Depth trend in periphyton growth indicated highest values (10035.0 numbers cm^{-2}) at 50 cm in ponds maintained at 15 ppt in comparison with the other two treatments (10 ppt 9980 numbers cm^{-2} and 20 ppt 8123.0 numbers cm^{-2}) (Table 5). By-weekly variations revealed no definite trend in periphyton numbers, however, peak values at most of the depths were observed at 30 days sampling, which gradually decreased thereafter. Algal constitute of periphyton biomass (%) ranged from 31.27 to 62.12 at 50 cm substrate depth. Significantly ($P < 0.05$) higher values for mean DM, AFDM and ash were also observed at 50 cm substrate depth in 15 ppt ponds. Irrespective of the salinity treatments, autotrophic index (AI) values for AFDM and DM decreased with an increase in substrate depth upto 50 cm depth (Table 6). Mean periphyton productivity and chlorophyll *a* concentrations were significantly higher ($P < 0.05$) at 50 cm depth in 15 ppt ponds (Table 5). Bi-weekly variations in mean values of periphyton plankton density have revealed that irrspctive of the salinity level, higher values were observed on 45th day, which was significantly ($P < 0.05$) high at 15 ppt salinity (Fig. 1).

Periphyton chlorophyll *a* concentration was initially high, which gradually decreased with the passage of time and thus lowest values were observed at the end of observation period of 115 days (Fig. 2). Similarly, pheophytin *a* concentrations were initially high, which gradually declined and reached at the lowest level at the end of 60 days (Fig. 3), thereafter, again the values gradually rose and highest concentration at all depths and at all the three salinities were observed at the end of observation period of 115 days. Total pigment

concentration values were high at 50 cm depth in 15 ppt ponds.

A comparison of the periphyton and pigment concentration of the two experiments (I and II) indicated that periphyton number were higher under ungrazed conditions, while, values of DM, AFDM, AI, algal contents, periphyton productivity were higher under grazed conditions. Not many variations in algal contents and total pigment concentration were observed in between the two experiments.

Table 5. Effect of different salinity levels (10, 15 and 20 ppt) on periphyton dry matter (DM), ash free dry matter (AFDM), ash contents, ash (% of dry matter), periphyton number, total pigment concentrations, chlorophyll *a*, pheophytin *a* and autotrophic index (AI) at different depth – Grazed conditions.

Parameters	10 ppt				15 ppt				20 ppt			
	Depth (cm)				Depth (cm)				Depth (cm)			
	0	25	50	75	0	25	50	75	0	25	50	75
Dry matter (DM) mg cm ⁻²	1.92 ±0.01c	2.00 ±0.04b	2.43 ±0.01a	1.62 ±0.08d	1.82 ±0.02c	1.90 ±0.01b	2.85 ±0.05a	1.80 ±0.01c	1.79 ±0.01b	1.85 ±0.07ab	2.00 ±0.06a	1.71 ±0.06c
AFDM mg cm ⁻²	1.27 ±0.01ab	1.26 ±0.02b	1.32±0.05 a	1.11 ±0.11c	1.07 ±0.09bc	1.08 ±0.08b	1.51 ±0.14a	1.11 ±0.10c	1.15 ±0.02b	1.14 ±0.03b	1.51 ±0.14a	1.18 ±0.01b
Ash mg cm ⁻²	0.65 ±0.02c	0.74 ±0.03b	1.12 ±0.02a	0.52 ±0.01d	0.75 ±0.05c	0.82 ±0.05b	1.35 ±0.14a	0.68 ±0.06c	0.64 ±0.03c	0.71 ±0.07b	0.85 ±0.06a	0.54 ±0.05d
Ash % of DM	35.00 ±1.41b	37.00 ±1.63b	44.50 ±2.14a	32.00 ±1.34c	41.0 ±2.83b	43.0 ±2.81ab	47.0 ±2.24a	38.0 ±3.01c	35.7 ±1.27bc	38.0 ±2.47b	42.5 ±1.41a	31.4 ±2.05c
Peiphyton number units cm ⁻²	5455 ±597b	7605 ±802ab	8993 ±922a	4911 ±501b	5964 ±542b	7993 ±507ab	10230 ±1001a	5678 ±556b	5909 ±570c	8121 ±785ab	9695 ±1001a	5518 ±585c
Total pigment concentration µg cm ⁻²	6.54 ±0.32c	7.93 ±0.47b	9.62 ±0.31a	6.79 ±0.28c	8.03 ±0.27	9.23 ±0.42b	11.63 ±0.67a	7.78 ±0.29d	7.18 ±0.25c	8.51 ±0.29b	10.26 ±0.41a	6.99 ±0.18c
Chlorophyll <i>a</i> µg cm ⁻²	5.11 ±0.23c	5.89 ±0.30b	7.18 ±0.36a	5.08 ±0.24c	6.16 ±0.24c	6.95 ±0.30b	8.76 ±0.34a	5.84 ±0.19c	5.47 ±0.22bc	6.40 ±0.29b	7.73 ±0.27a	5.06 ±0.18c
Pheophytin <i>a</i> µg cm ⁻²	1.43 ±0.06c	2.04 ±0.08b	2.44 ±0.12a	1.71 ±0.09c	1.87 ±0.10cd	2.28 ±0.11b	2.87 ±0.13a	1.94 ±0.09c	1.71 ±0.09d	2.11 ±0.11b	2.53 ±0.13a	1.93 ±0.11c
Autotrophic index (AI)	247.80 ±1.20a	213.98 ±2.06bc	183.04 ±1.49d	217.05 ±1.20b	173.00 ±17.69ab	155.43 ±13.18c	171.88 ±16.20ab	191.00 ±21.28a	211.12 ±10.05b	178.47 ±7.81c	148.87 ±0.34d	232.71 ±2.24a
Algal constitute of periphyton biomass (%)	30.00– 31.80	27.80– 42.30	23.44– 48.21	30.40– 43.90	35.01– 39.47	33.32– 49.26	39.13– 56.90	35.92 – 41.28a	30.56 – 35.31	29.66 – 46.81	38.14 – 50.29	30.59 – 42.80
Periphyton productivity mg cm ⁻² d ⁻¹	1.24 ±0.03bc	1.26 ±0.02ab	1.32 ±0.05a	1.11 ±0.11c	1.07 ±0.09ab	1.08 ±0.08b	1.51 ±0.14a	1.12 ±0.10c	1.15 ±0.02ab	1.14 ±0.03ab	1.15 ±0.14ab	1.18 ±0.01a

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

Table 6. Effect of different salinity levels (10, 15 and 20 ppt) on periphyton dry matter (DM), ash free dry matter (AFDM), ash contents, ash (% of dry matter), periphyton number, total pigment concentration, chlorophyll *a*, pheophytin *a* and autotrophic index (AI) at different depth –Ungrazed conditions.

Parameters	10 ppt				15 ppt				20 ppt			
	Depth (cm)				Depth (cm)				Depth (cm)			
	0	25	50	75	0	25	50	75	0	25	50	75
Dry matter (DM) mg cm ⁻²	1.17 ±0.01c	1.24 ±0.03b	1.47 ±0.01a	1.04 ±0.04d	1.43 ±0.01c	1.52 ±0.01b	1.87 ±0.04a	1.44 ±0.01c	1.06 ±0.05b	1.05 ±0.02b	1.41 ±0.01a	0.96 ±0.04c
AFDM mg cm ⁻²	0.77 ±0.02b	0.78 ±0.01b	0.89 ±0.03a	0.71 ±0.08bc	0.97 ±0.06b	0.96 ±0.02b	1.06 ±0.06a	1.01 ±0.03a	0.70 ±0.60b	0.68 ±0.02b	0.78 ±0.04a	0.61 ±0.02c
Ash mg cm ⁻²	0.41 ±0.02b	0.46 ±0.03b	0.58± 0.02a	0.33 ±0.01c	0.46 ±0.02c	0.57 ±0.02b	0.81 ±0.01a	0.42 ±0.01c	0.36 ±0.01b	0.37 ±0.01b	0.63 ±0.02a	0.35 ±0.0b1
Ash % of DM	34.50 ±1.34bc	37.00 ±1.56ab	39.50 ±1.55a	31.7 ±2.69c	32.10 ±1.70b	37.20 ±1.41ab	43.20 ±1.06a	30.50 ±0.92c	33.7 ±1.41c	35.20 ±0.71b	44.5 ±1.98a	36.2 ±1.41bc
Peiphyton number units cm ⁻²	5766.00 ±602.00c	7746.00 ±884.00b	9980.00 ±1160.00a	5277.00 ±564.00c	6009.00 ±692.00bc	7580 ±846b	10035 ±1182a	5250 ±533c	4830 ±586c	6277 ±639b	8123 ±905a	4482 ±523c
Total pigment	7.24 ±0.27c	9.16 ±0.27b	11.30 ±0.41a	7.95 ±0.21c	8.53 ±0.41c	10.43 ±0.27b	12.59 ±0.62a	8.20 ±0.21c	7.24 ±0.31b	9.25 ±0.26a	10.88 ±0.41a	7.42 ±0.21b

Parameters	10 ppt				15 ppt				20 ppt			
	Depth (cm)				Depth (cm)				Depth (cm)			
	0	25	50	75	0	25	50	75	0	25	50	75
concentration												
$\mu\text{g cm}^{-2}$												
Chlorophyll <i>a</i>	5.42	6.94	8.78	6.10	6.43	7.77	9.41	5.97	5.41	6.90	8.19	5.30
$\mu\text{g cm}^{-2}$	$\pm 0.30\text{d}$	$\pm 0.39\text{b}$	$\pm 0.38\text{a}$	$\pm 0.22\text{c}$	$\pm 0.33\text{c}$	$\pm 0.39\text{b}$	$\pm 0.43\text{a}$	$\pm 0.28\text{d}$	$\pm 0.32\text{c}$	$\pm 0.39\text{b}$	$\pm 0.47\text{a}$	$\pm 0.25\text{c}$
Pheophytin <i>a</i>	1.82	2.22	2.52	1.85	2.10	2.66	3.18	2.23	1.83	2.35	2.69 \pm	2.12
	$\pm 0.09\text{c}$	$\pm 0.09\text{b}$	$\pm 0.11\text{bc}$	$\pm 0.11\text{c}$	$\pm 0.10\text{d}$	$\pm 0.12\text{b}$	$\pm 0.11\text{a}$	$\pm 0.12\text{c}$	$\pm 0.09\text{d}$	$\pm 0.12\text{b}$	0.13a	$\pm 0.12\text{c}$
Autotrophic index (AI)	141.26	112.70	101.46	113.09	150.18	123.06	113.35	167.11	129.82	97.96	95.18	115.09
	$\pm 1.34\text{a}$	$\pm 3.91\text{b}$	$\pm 1.92\text{c}$	$\pm 7.92\text{b}$	$\pm 8.21\text{ab}$	$\pm 5.42\text{c}$	$\pm 8.13\text{c}$	$\pm 7.76\text{a}$	$\pm 16.34\text{a}$	$\pm 3.81\text{c}$	$\pm 2.80\text{c}$	$\pm 3.56\text{b}$
Algal constitute of periphyton biomass (%)	31.60 – 33.60	34.73 – 37.00	39.94 – 42.20	31.20 – 35.80	37.20 – 40.28	36.57 – 56.00	31.27 – 62.12	36.75 – 50.65	32.60 – 33.06	32.83 – 49.12	27.12 – 54.16	31.73 – 45.49
Periphyton productivity	0.77	0.78	0.89	0.71	0.97	0.96	1.07	1.01	0.70	0.68	0.78	0.61
$\text{mg cm}^{-2} \text{ d}^{-1}$	$\pm 0.02\text{b}$	$\pm 0.01\text{b}$	$\pm 0.03\text{a}$	$\pm 0.08\text{b}$	$\pm 0.06\text{b}$	$\pm 0.02\text{b}$	$\pm 0.06\text{a}$	$\pm 0.03\text{a}$	$\pm 0.06\text{b}$	$\pm 0.02\text{c}$	$\pm 0.04\text{a}$	$\pm 0.02\text{d}$

All values are mean \pm SE of mean. Mean with the same letters in the same column are not significantly ($P>0.05$) different.

3.10. Proximate Composition of Periphyton Under Grazed and Ungrazed Conditions

Proximate analysis had revealed significantly ($P<0.05$) higher values of protein (37.9 ± 1.72), fat (4.2 ± 0.47) and energy (15.0 ± 0.39) in periphyton samples scraped from

ungrazed conditions at 50 cm. depth as compared with the periphyton samples obtained from grazed conditions (Table 7). These values were lower in samples of periphyton obtained from the other two depths (25 cm. and 75 cm.) and also from other two salinity levels (10 ppt and 20 ppt).

Table 7. Effect of different salinity levels (10, 15 and 20 ppt) on proximate composition of periphyton (% dry weight) under grazed and ungrazed conditions.

	Salinity (ppt)	Moisture	Protein	Fat	Ash	Energy (kJ g^{-1})
Grazed condition	10	28.72 \pm 1.12b	19.37 \pm 0.63c	1.86 \pm 0.13c	35.3 \pm 1.85b	11.59 \pm 0.18d
	15	30.01 \pm 1.02a	20.70 \pm 0.71c	1.91 \pm 0.15c	38.2 \pm 1.97a	12.1 \pm 0.20c
	20	27.98 \pm 0.94b	18.54 \pm 0.68cd	1.82 \pm 0.16c	34.1 \pm 1.54b	10.32 \pm 0.12e
Ungrazed condition	10	23.7 \pm 1.06cd	35.7 \pm 1.57a	3.8 \pm 0.53b	29.5 \pm 0.82c	13.39 \pm 0.41b
	15	24.3 \pm 1.11c	37.9 \pm 1.72a	4.2 \pm 0.47a	30.4 \pm 0.74c	15.0 \pm 0.39a
	20	22.6 \pm 0.95d	33.2 \pm 1.36ab	3.2 \pm 0.38b	28.6 \pm 0.91cd	12.51 \pm 0.32b

All values are mean \pm SE of mean. Mean with the same letters in the same column are not significantly ($P>0.05$) different

4. Discussion

4.1. Fish Growth and Carcass Composition

Survival, growth rate, mean weight gain, net biomass at harvest and exponential value of constant 'n' (LWR) of *C. chanos* were significantly ($P<0.05$) higher in ponds maintained at 15 ppt salinity compared with the other two salinities (10 and 20 ppt). The mean weight of *C. chanos* at 15 ppt was much higher than the fish grown at 10 or at 20 ppt. The exponential value of constant 'n' (LWR) for milkfish grown in ponds at 15 ppt salinity was higher than at other salinities. Carcass composition had revealed high accumulation of protein, fat and phosphorous. Higher viscero- somatic index and haepato somatic index also coincided well with the higher fish growth in ponds maintained at 15 ppt salinity perhaps because of higher production of periphyton. Similar results have also been obtained by many workers [16, 27, 28]. Since *C. chanos* is a herbivore and plankton feeder and thus high growth can be attributed to an increase in food availability through periphyton production in ponds maintained at 15 ppt. Dempster *et al* [9, 10] have 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.* [7] evaluated the polyculture of Indian major carps (*Catla catla*; *Labeo rohita* and *Labeo calbasu* in periphyton-based ponds and had obtained similar results. Studies of Jana *et al* [22, 23] have also reported 35% higher growth in grey mullet,

Mugil cephalus, and 73% higher growth in milkfish, *Chanos chanos*, when grown in inland saline groundwater ponds with a provision of additional substrate for the development of periphyton. Amisah *et al* [2] have reported higher growth in *Clarias gariepinus* in ponds provided with substrate for the growth of periphyton in comparison to control and feed ponds. Kumar *et al.* [27, 28] conducted monoculture experiments on *O. niloticus* and *E. suratensis* and reported higher growth of *O. niloticus* in treated ponds in comparison to feed (67%) and control (113%) ponds. Similarly, *E. suratensis* has been reported to grow 24% and 99% higher in treated ponds as compared to diet and control ponds, respectively. Studies of Jana *et al* [24] have revealed that growth of milkfish fry and fingerlings was significantly ($P<0.05$) higher in ponds maintained at 25 ppt salinity. Low growth at 20 ppt in the present studies may be attributed to the availability of low periphytic biomass as compared to ponds maintained at 15 ppt salinity.

4.2. Water Quality

In both the experiments (I and II) all water quality parameters remained within the optimal range required for the optimal growth of fish. The pH of the water was alkaline and alkalinity was higher in both the treatments, indicating that pond waters were well buffered. Due to non-stocking of fish in experiment II, productivity indicating parameters remained slightly higher under ungrazed conditions. Subsequent sampling had revealed higher values of productivity indicating parameters under grazed conditions. Many other workers [2, 5, 14, 16] have also reported similar results of low periphytic productivity as a result of grazing pressure exerted by the fish. SO_4 and o-PO_4 levels were similar in both the trials. Alkalinity and total Kjeldahl-nitrogen were significantly ($P < 0.05$) higher and NH_4 levels were significantly ($P < 0.05$) lower in treatments with fish. This may be attributed to the raking effect of fish which enhances the nutrient cycle in the system by converting periphyton into fish biomass and excreting inorganic nutrients. The excreted nutrients can be reutilized by the periphyton. The fish biomass represents a considerable amount of nitrogen that in the treatments without fish have remained in the system in another form. The low nutrient concentrations and the high transparency of the water (precluding light as a limiting factor), indicate that the nutrients were used mostly by the periphyton and in turn converted into fish biomass. Decrease in periphytic productivity under ungrazed conditions may be attributed to the accumulation of organic matter due to non-consumption/ degradation of the matter. Although no significant correlation between chlorophyll *a* and pheophytin *a* was observed, data clearly indicated that low concentrations of pheophytin *a* were mostly preceded by high concentrations of chlorophyll *a*, indicating a continuous breakdown of chlorophyll *a* as a result of grazing pressure exerted by the fish/zooplankton or due to auto shading under ungrazed conditions. Nayar and Gowda [32] have also observed an inverse relationship between chlorophyll *a* and phea pigments. Nutrients viz. total Kjeldahl nitrogen, nitrates and sulphates were high and ammonia ($\text{NH}_4\text{-N}$) was low in ponds stocked with fish (grazed conditions). In general, $\text{NH}_4\text{-N}$ remained higher than the usual limits during the entire study, which may be attributed to the high water salinity. Garg [11] and Garg and Bhatnagar [13] had also reported high levels of $\text{NH}_4\text{-N}$ with increase in salinity in carp culture ponds. Ramesh *et al.* (35) and many other studies [See 14 for references] had also reported that ponds with substrates had lower ammonia levels than control ponds and concluded that enhanced bacterial biofilms on substrates might have reduced ammonia levels through promotion of nitrification. In the present studies N-NH_4 levels remained low under grazed conditions, while high values were observed in ponds without fish (ungrazed). Low $\text{NH}_4\text{-N}$ levels under grazed conditions may be attributed to the availability of more nutrients resulting in the development of more bacterial biofilms-which might have reduced NH_4 levels through promotion of nitrification. Low DO, High BOD and $\text{NH}_4\text{-N}$ under ungrazed conditions may be attributed to the breakdown and accumulation of periphytic biomass under

ungrazed conditions. In general, the periphyton density remained higher at a depth of 50 cm. Density values at most of the depths showed a gradual increase upto the first 45 days, however, thereafter, at most of the depths a decline in their numbers was observed, which might have been due to the competition for substrate and nutrients and perhaps also due to the decrease in productivity of older periphyton.

4.3. Periphyton and Pigment Concentrations in Both the Experiments (I and II)

Periphyton biomass measured in terms of DM, AFDM and pigment concentrations (chlorophyll *a* and pheophytin

a), significantly ($P < 0.05$) increased with depths upto 50 cm; a decline thereafter in their values indicates that the euphotic zone lies upto 50 cm only. These findings are in accordance with those of many workers [29, 6, 22, 24, 27, 28, 25] who have also reported that the maximum periphyton biomass levels coincide with the euphotic zone. Azim *et al* [5] have reported no significant variations in periphyton production with respect to depth. As *C. chanos* feeds mostly on benthic and periphytic organisms as well as on lab-lab and detritus, this may also be the causal factor of decrease in periphyton density and pigment concentration at a depth of 75 cm. Ash contents of the periphyton were higher and ranged between 39.5–47.0 % of the DM content, however, growth of the fish was not affected. According to Horn [21], high ash contents are perhaps necessary for grinding algae with the pharyngeal jaws. High ash contents in periphyton samples might be attributed to the suspended particles entrapped in the periphyton community. This perhaps accounts for low turbidity in ponds provided with substrate at 15 ppt. Huchette *et al.* [20] and Azim *et al.* [6] have reported that AI fluctuated between 150 and 300, and 190 to 350 respectively, under ungrazed conditions. In the present studies, AI values were higher under grazed conditions (155.43 to 191.10) as compared to the ungrazed conditions (113.35 to 167.11). Low values of AI in the present studies may be attributed to the grazing pressure exerted by the fish, which primarily feed on attached planktonic flora and fauna. If 1 mg of chlorophyll *a* can be derived from 65–85 mg algal DM, then different depths (0, 25, 50 and 75 cm respectively). The bulk of the periphyton (24–80%) is thus not of an algal nature, confirming the importance of periphyton for attracting heterotrophs and trapping organic matter. Enhanced fish growth, weigh substrates at 15 ppt, which may be attributed to the high productivity and readily available food in the form of periphyton. A similar production enhancement has been reported in many other studies through the provision of additional substrates [see: 14, 17, 26 for references]

4.4. Nutrient Composition of Periphyton

Proximate composition of periphyton had revealed higher values of protein, fat and energy in samples obtained from ungrazed conditions as compared to samples analysed from grazed conditions where significantly low values of protein,

fat and energy were observed. These observations indicate that since periphyton possess high nutritive value, therefore, it can be used as an alternative to supplementary diets for many a fish species. Many other workers [1, 7, 16] have studied the nutritive composition of periphyton and had reported high nutritive contents of periphyton. These authors have also reported that the nutritive quality depends on several factors like grazing pressure, algal and bacterial taxonomic composition and most significantly to the substrate type used in the system. High digestive enzyme activity in the gut coupled with high growth of fish grown in ponds with substrate indicates the suitability of periphyton as a suitable protein source [14, 16, 27, 28].

5. Conclusions

These studies have revealed that the substrates for biofilm/periphyton development improved water quality through enhanced nitrification. Use of periphyton development technology precludes the possibility of using costly feeds and fertilizers, thus this technology in a way is a step towards developing organic farming. This technology appears to be cheap and appropriate for fish farmers especially in the resource poor countries

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References

- [1] Abwao J A, Boera PN, Munguti JM, Orina PS and Ogello EO (2014) The potential of periphyton based aquaculture for Nile tilapia (*Oreochromis niloticus* L.) production. a review. International Journal of Fisheries and Aquatic Studies 2(1) 147-152.
- [2] Amisah S, Adjei-boateng D, Afianu, DD (2008) Effects of bamboo substrate and supplementary feed on growth and production of the African catfish, *Clarias gariepinus*. J. Appl. Sci. Environ. Manage. 12(2) 25 - 28
- [3] AOAC (Association of Official Analytical Chemists) (2016) Official Methods of Analysis. Assoc. of Off. Anal. Chem. Inc., Arlington, USA, p.684.
- [4] APHA (2005) Standard method for the estimation of water and wastewater. American Public Health Association. American waste water association and water pollution control federation. 21st ed. Washington DC.
- [5] Azim ME, Wahab MA, van Dam AA, Beveridge MCM and Verdegem MCJ (2001a) The potential of periphyton-based culture of two Indian major carps, rohu *Labeo rohita* (Hamilton) and gonia *Labeo gonius* (Linnaeus). Aquacult. Res. 32: 209-216.
- [6] Azim ME, Wahab MA, van Dam AA, Beveridge MCM, Huisman EA and Verdegem, MCJ (2001b) Optimization of stocking ratio of two Indian major carps rohu (*Labeo rohita* Ham.) and catla (*Catla catla* Ham.) in a periphyton based aquaculture system. Aquaculture 203: 33-49.
- [7] Azim ME, Verdegem MCJ, Khatoon H, Wahab M.A, van Dam AA. and Beveridge, MCM (2002) A comparison of fertilization, feeding and three periphyton substrates for increasing fish production in freshwater pond aquaculture in Bangladesh. Aquaculture 212: 227-243.
- [8] Bellinger EG (1992) A key to common Algae. The institute of water and environment management, London.in Aquaculture. Chapman and Hall, London.P 319
- [9] Dempster PW, Beveridge MCM and Baird DJ (1993) Herbivory in tilapia *Oreochromis niloticus* (L.): a comparison of feeding rates on periphyton and phytoplankton. J. Fish Biol. 43: 385-392
- [10] Dempster PW, Baird DJ, Beveridge MCM (1995) Can fish survive by filter-feeding on microparticles? Energy balance in tilapia grazing on algal suspensions. J Fish Biol 47: 7-17.
- [11] Garg, S. K (1996) Brackish water carp culture in potentially waterlogged areas using animal wastes as pond fertilizers, Aquaculture International, 4: 143-155.
- [12] Garg S. K (2005) Role of periphyton in development of sustainable aquaculture technology for inland saline groundwater: A review, Indian J. Animal Sci. 75(11):1348-1353
- [13] Garg, S. K. and Bhatnagar, A. (1996) Effect of varying doses of organic and inorganic fertilizers on plankton production and fish biomass in brackish water fish ponds, Aquaculture Research, 27:157-166
- [14] Garg, S. K. and Bhatnagar, S (2016). Influence of periphyton substrate density on hydrobiological characteristics and growth performance of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) stocked in inland saline groundwater ponds. International Journal of Fisheries and aquatic studies. 4:444-452.
- [15] Garg S. K, Jana S. N. and Arasu, A. R. T., (2006) Determination of fertilization rate for optimum pond productivity and fish growth in inland saline groundwater ponds: Monoculture of grey mullet and milkfish. Asian Fish Sci., 19: 165-176.
- [16] Garg, S. K., Kumar, A., Arasu, A. R. T., Bhatnagar, A., Jana, S. N. and Barman, U. K., (2007) Effect of periphyton and supplementary feeding on growth performance and some aspects of nutritive physiology of Nile Tilapia, *Oreochromis niloticus* and Pearlscale, *Etroplus suratensis* under polyculture, J. Applied Aquaculture, 19(3): 19-45
- [17] Garg S. K, Barman UK and Bhatnagar A (2013) Optimization of fertilization rate for maximizing periphyton production on additional substrate and growth performance of milkfish, *Chanos chanos* (Forsskal) in stagnant inland saline ground water ponds. Journal of Nature Science and Sustainable Technology. 7(2):1-15
- [18] Hem S and Avit JLB. (1994) First results on "acadjas enclos" as an extensive aquaculture system (West Africa). Bulletin of Marine Sci. 55: 1038-1049.

- [19] Henken AM, Lucas H, Tijssen PAT, and. Machiels MAM (1986) A comparison between methods used to determine the energy content of feed, fish and faeces samples. *Aquaculture*, 58: 195-201.
- [20] Huchette SMH, Beveridge MCM, Barid DJ and Ireland M (2000) The impact of grazing by tilapias (*Oreochromis niloticus* L.) on periphyton communities growing on artificial substrate in cages. *Aquaculture*.186: 45-60.
- [21] Horn MH (1989) Biology of marine herbivorous fishes. *Oceanogr. Mar Biol. Ann. Rev.* 27: 167-272.
- [22] Jana S.N, Garg SK and Patra BC (2004) Effect of periphyton on growth performance of grey mullet, *Mugil cephalus* (Linn.) in inland saline groundwater ponds. *J. Appl. Ichthyol.* 20 (2): 10-17.
- [23] Jana S. N, Garg SK, Arasu ART, Bhatnagar A, Kalla A and Patra BC (2006a) Use of additional substrate to enhance growth performance of milkfish, *Chanos chanos* (forsskal) in inland saline groundwater ponds. *J. Applied Aquacult.*, 18(1): 1-20.
- [24] Jana SN, Garg SK and Patra BC (2006b) Effect of inland water salinity on growth performance and nutritional physiology in growing milkfish, *Chanos chanos* (Forsskal): field and laboratory studies. *J. appl. Ichthyol.* 22: 25-34.
- [25] Keshavanath P, Gangadhar B, Ramesh TJ, van Rooij JM, Beveridge MCM, Baird DJ, Verdegem, M. C. J. and van Dam, A. A. (2001) Use of artificial substrates to enhance production of freshwater herbivorous fish in pond culture. *Aquacult. Res.* 32: 189-197.
- [26] Keshavanath P, Gangadhar B, Ramesha TJ, Priyadarshini M, van Dam AA, Verdegem, MCJ and Beveridge MCM (2015) Impact of substrates and fish stocking density on growth and production of the Indian major carp, *labeo rohita* (ham.). *J Aqua Trop.* 30(1-2):1-14.
- [27] Kumar A, Bhatnagar A and Garg S. K (2009a) Growth performance carcass composition and digestive enzyme activity of pearlspot, *Etroplus suratensis* (Bloch) reared in inland saline groundwater ponds providing substrate or feed., *Live stock production for rural development*, 21(10), #180. pp 1-20.
- [28] Kumar A, Bhatnagar A, Garg S K and Jana SN (2009b) Growth performance of Nile Tilapia, *Oreochromis niloticus* (Linn) in relation to provision of substrate and supplementary feeding and grown in brackish water ponds. *Asian Fish Science*, 22 (4): 1071-1100.
- [29] Konan-Brou, A. A. and Guiral, D. (1994) Available algal biomass in tropical brackishwater artificial habitats. *Aquaculture* 119: 175-190
- [30] Milstein A, Joseph D, Peretz Y and Harpaz S (2005) Evaluation of organic tilapia culture in periphyton-based ponds. *The Israeli Journal of Aquaculture – Bamidgeh* 57(3): 143-155
- [31] Milstein A, Peretz Y, Harpaz S (2009) Culture of organic tilapia to market size in periphyton-based ponds with reduced feed inputs. *Aquac. Res.* 40, 55-59.
- [32] Nayar S and Gowda G. (1999) Phytobenthic production during bloom phase in a tropical coastal lagoon near Mangalore. *Indian J. Environ. Sci.* 3: 69-73.
- [33] Prescott GW (1962) *Algae of the Western Great Lake area*. Wm. C. Brown. Co., Dubuque, IA.
- [34] Prejs A (1984) Herbivory by temperate freshwater fishes and its consequences. *Environmental Biology of Fishes.* 10: 281-296.
- [35] Ramesh MR, Shankar KM, Mohan CV and Varghese TJ (1999) Comparison of three plant substrates for enhancing carp growth through bacterial biofilm. *Aquacult. Eng.* 19: 119-131.
- [36] Shilta M T, Chadha N K, Pandey P K, Sawant P B. (2015) Effect of biofilm on water quality and growth of *Etroplus suratensis* (Bloch, 1790). *Aquacult. Int.* (in press).
- [37] Snedecor G W and Cochran W C (1982) *Statistical methods*. Iowa State University Press, Ames. 1A, pp 507
- [38] van Dam AA, Beveridge MCM, Azim ME. (2002) The potential of fish production based on periphyto. *Rev. Fish Biol. Fish.* 12:1-31.
- [39] Ward HB and GC Whipple (1959) *Freshwater Biology*, John Wiley and Sons, pp. 1248.
- [40] Washington HG (1984) Diversity, biotic and similarity indices. *Water Res.* 18 (6): 653-694.