



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 ith 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 Table 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_t - ln W_i] × 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_t - W_i) / W_i] × 100, where, W_t and W_i 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

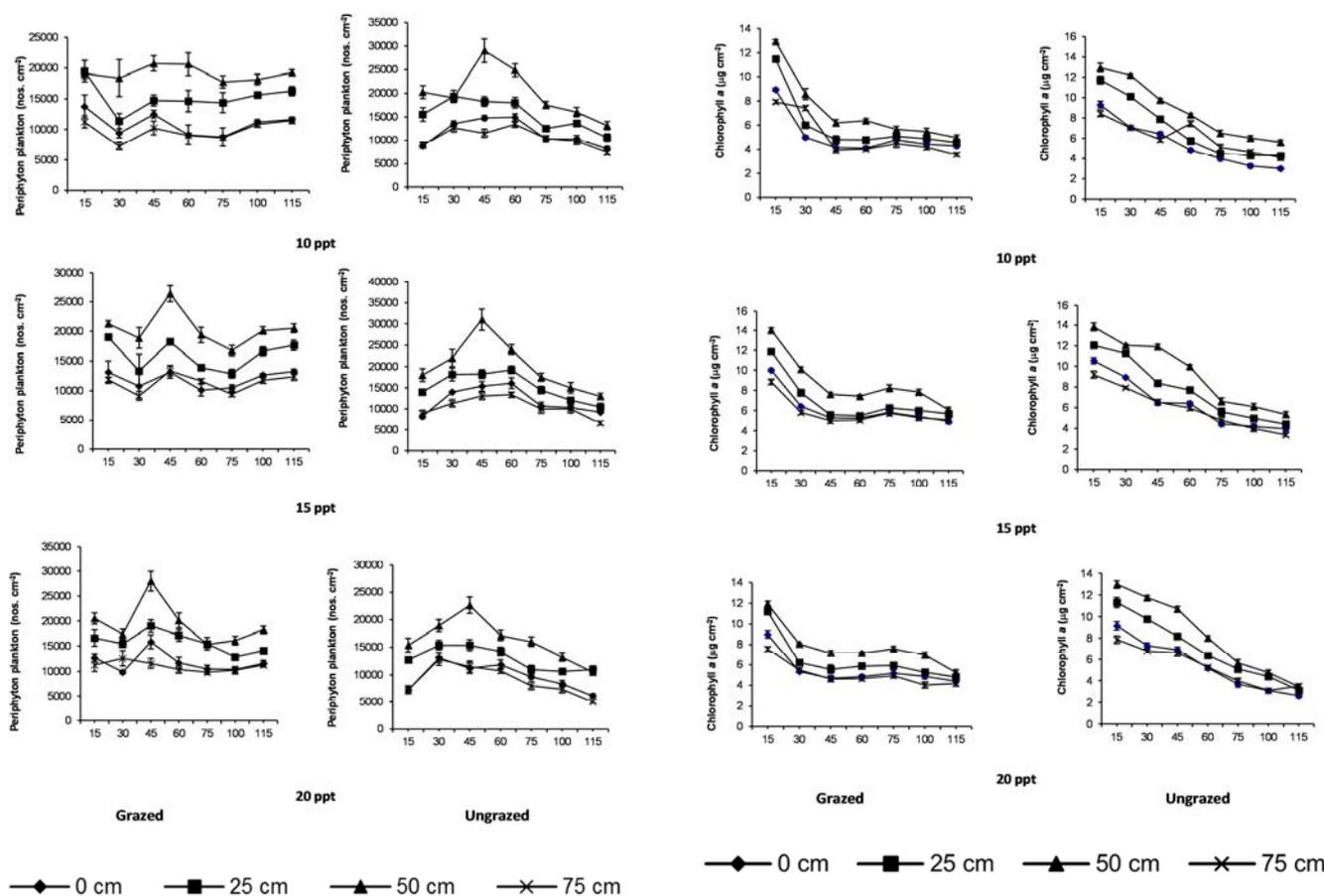


Fig. 1. Bi-weekly variations in mean values of periphyton plankton density at different depths (0, 25, 50 and 75 cm) from ponds having 10, 15 and 20 ppt saline water and provided with bamboo poles as additional substrate in grazed versus Ungrazed conditions.

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 ppt saline water and provided with bamboo poles as additional substrate in grazed versus Ungrazed conditions.

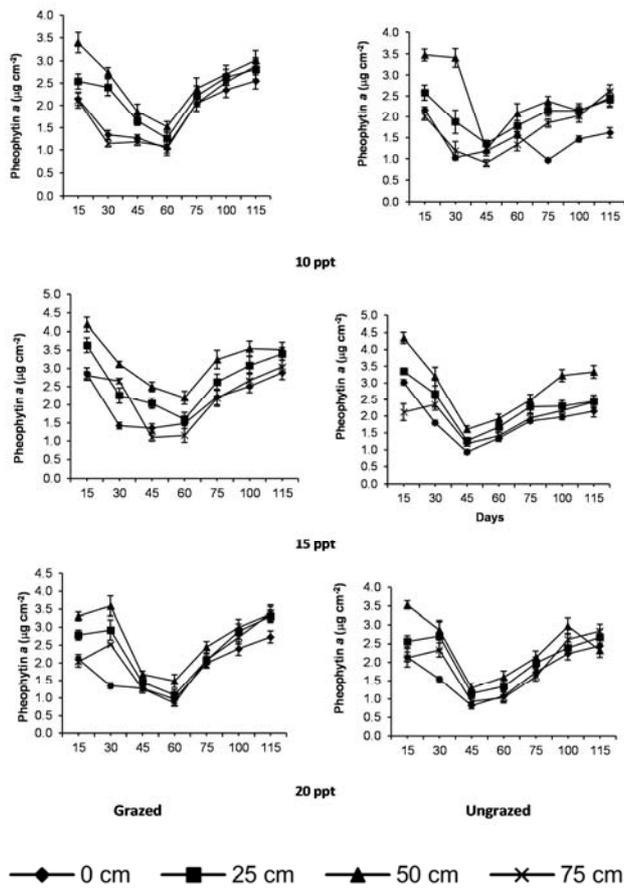


Fig. 3. Bi-weekly variations in mean values of periphyton pheophytin a concentrations at different depths (0, 25, 50 and 75 cm) from ponds having 10, 15 and 20 ppt saline water and provided with bamboo poles as additional substrate in grazed versus Ungrazed conditions.

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_3N , NO_2N), however, NH_4N 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 grazad 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 irrspetive 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 |
| | $\pm 0.30\text{d}$ | $\pm 0.39\text{b}$ | $\pm 0.38\text{a}$ | $\pm 0.22\text{c}$ | $\pm 0.33\text{c}$ | $\pm 0.39\text{b}$ | $\pm 0.43\text{a}$ | $\pm 0.28\text{d}$ | $\pm 0.32\text{c}$ | $\pm 0.39\text{b}$ | $\pm 0.47\text{a}$ | $\pm 0.25\text{c}$ |
| 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 | 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 (%) | | | | | | | | | | | | |
| Periphyton productivity | 31.60 | 34.73 | 39.94 | 31.20 | 37.20 | 36.57 | 31.27 | 36.75 | 32.60 | 32.83 | 27.12 | 31.73 |
| | ± 3.60 | ± 3.70 | ± 4.20 | ± 3.50 | ± 4.00 | ± 3.60 | ± 3.10 | ± 3.60 | ± 3.30 | ± 4.90 | ± 5.10 | ± 4.50 |
| $\text{mg cm}^{-2} \text{d}^{-1}$ | 0.77 | 0.78 | 0.89 | 0.71 | 0.97 | 0.96 | 1.07 | 1.01 | 0.70 | 0.68 | 0.78 | 0.61 |
| | $\pm 0.02\text{b}$ | $\pm 0.01\text{b}$ | $\pm 0.03\text{a}$ | $\pm 0.08\text{b}$ | $\pm 0.06\text{b}$ | $\pm 0.02\text{b}$ | $\pm 0.06\text{a}$ | $\pm 0.03\text{a}$ | $\pm 0.06\text{b}$ | $\pm 0.02\text{c}$ | $\pm 0.04\text{a}$ | $\pm 0.02\text{d}$ |

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 pheo 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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