RESEARCH ARTICLE

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Effect of Different Salinity and Ration Levels on Growth Performance and Nutritive Physiology of Milkfish, *Chanos chanos* (Forsskal) – Field and Laboratory Studies

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Abstract

To investigate the effect of inland groundwater salinity, and two ration levels on growth performance and nutrition physiology in milkfish, *Chanos chanos*, two experiments (Experiment 1 and 2) were conducted. In the first experiment (Expt. 1), a 100-day monoculture of *Chanos chanos* at two different salinities (10 and 25‰) was carried out in ponds and the fish were fed on two different (4% and 6% BW d^{-1}) ration levels. Irrespective of the salinity treatment, low ration favored high growth in fish grown at 25 ppt salinity. Carcass composition revealed high accumulation of protein, fat, energy and phosphorus in fish fed at low ration level and maintained at 25 ppt salinity. Irrespective of the salinity treatment, DO, BOD, pH and nutrients remained significantly ($P<0.05$) higher in ponds where the fish were fed at low ration level. Multivariate analysis revealed a significant positive correlation of nutrients and productivity indicating parameters with fish weight gain. The second experiment (Expt. 2) was conducted under laboratory conditions and the milkfish fry were exposed to five different salinity levels (10.0, 15.0, 20.0, 25.0 and 30.0 ‰) for 100 days. A control in fresh water (0.0 ppt) was also maintained. Irrespective of the salinity treatment, significantly ($P<0.05$) high growth, feed conversion efficiency and intestinal enzyme activity were observed in fish maintained at low (4%) ration level. Carcass composition, muscle and liver glycogen levels, muscle protein, viscero-somatic index (VSI) and hepato-somatic index (HSI) values were also significantly ($P<0.05$) affected not only by the salinity treatment but also by the ration level. Studies indicated that low ration level and high salinity favored high growth in milkfish.

Keywords: Different ration levels; growth; inland saline water; milkfish; nutrition.

1. Introduction

Inland saline groundwater has been successfully used in the United States and Middle East to culture a range of algae, crustaceans and finfish species such as tilapia, red drum, sea bream, eels and channel catfish [1-3]. In Australia also, inland saline groundwater from shallow and deep aquifers has also been found suitable for the growth and survival of a number of euryhaline finfish species [4]. Fish culture studies utilizing inland saline groundwaters were also carried out in India for the culture of common carp as well as the Indian major carps [5]. Since these fish species are stenohaline, therefore they do not perform well at higher salinities (>7.5 ppt). Our recent studies have revealed that inland saline groundwater with higher salinities (10 ppt and above) can be profitably utilized for the culture of certain euryhaline fish species such as mullets [6], milkfish [7], pearl spot and Nile Tilapia [8-10]. Water salinity has been shown to affect feed intake, protein requirements, feed conversion efficiency, digestibility and many other physiological functions in aquatic animals. The effect of salinity on feed utilization in fishes is not well understood. Lall and Bishop [11] and Macleod [12] observed that feed absorption efficiency in rainbow trout (*Salmo gairdneri*) decreased in high salinities, while DeSilva and Perera [13] found that salinity had no significant effect on digestibility in *Sarotherodon niloticus*. On the other hand, Ferraries *et al.* [14] and Conides *et al.* [15] in gilthead sea bream and Partridge and Jenkins [16] in black bream and Jana *et al.* [7] in milkfish observed a marked effect of salinity on digestibility. Ration size alters nutrient intake and feed efficiency as excess feeds may lead to nutrient leaching. Limited feeding, however, suppresses growth. Information on these lines in milkfish is scant with particular reference to inland saline groundwaters whose chemical composition is very different from sea water because of its high hardness and low contents of sodium, potassium and chlorides [17].

Many of the world's cultured species are euryhaline, additional information on the effects of salinity on nutritional physiology can therefore shed light on the possible interactions between osmoregulation and

feed utilization and thus increase our understanding on the nutritional and ecological physiology of euryhaline fish species such as milkfish. Among cultivable marine finfishes, milkfish *Chanos chanos* is known to grow quickly in coastal ponds and attain harvestable size in 6-12 months. Most of the studies on milkfish culture and its growth in India and elsewhere have been conducted in coastal areas using sea water/brackish water [18]. No attempts on milkfish culture have been made in semi-arid areas where plenty of high salinity (up to 30 ppt) inland saline groundwater is available. Since the effects of salinity on growth and feed conversion efficiency and digestibility are poorly known in milkfish, therefore present investigations are an attempt to, i) study the effect of inland saline ground water salinity and two (low and high) different ration levels on growth performance of milkfish under field conditions and, ii) study the effect of five different salinity levels on growth performance, feed utilization and digestive enzyme activity in fish maintained under laboratory conditions.

2. Methods

Experimental set up/design (Table 1)

Two experiments (1 and 2) were conducted to evaluate the effect of inland water salinity on growth performance and some aspects of nutritional physiology in *Chanos chanos*. In first experiment (Expt. 1), a 100 day's monoculture of milkfish (mean BW 0.02g) at two different salinities (10 and 25 ppt) was carried out in ponds fertilized with cow dung (10,000 kg ha^{-1} yr^{-1}). In each salinity treatment, the fish were fed on two (4% and 6% BW) different ration levels (on a compounded supplementary diet containing 40% protein.

Table 1: Protocol of experimental treatments.

Salinity (ppt)	Expt. 1, Under field conditions (Duration 100 days) Dietary regime (% BW)	Expt. 2, Under laboratory conditions (Duration 100 days)	
		Salinity (ppt)	Dietary regime (% BW)
10	4%	10, 10	4, 6
10	6%	15, 15	4, 6
25	4%	20, 20	4. 6
25	6%	25,25 30,30 0,0	4,6 4,6 4,6

2.1. Experiment 1: Effect of two different salinities (10 ppt and 25 ppt) and two different ration levels (4% and 6%) on growth performance and carcass composition of milkfish fry under field conditions

Studies were conducted at the brackish water fish pond facility of the Department of Zoology and Aquaculture, CCS Haryana Agricultural University, Hisar (latitude $29^0 10^/ N$; longitude $75^0 46^/ E$), India. The experiment was conducted in 15×25m (area 375 m^2) and 1.2m deep ponds from May to August 2005.

One month prior to the commencement of treatments, the ponds were cleaned; quick lime (CaO) at 200 kg ha^{-1} y^{-1} was applied and the ponds were then filled with inland saline groundwater. Two different salinities (10 and 25 ppt) in replicate of two were obtained from two aquifers discharging water of 10 and 25 ppt salinity, respectively. To maintain the desired level (90 cm), water was replenished as often as required. To fertilize the ponds, decomposed semi-dry cow dung @ 10,000 kg ha^{-1} yr^{-1} was applied at biweekly intervals, so that each treatment received about 580 kg of nitrogen ha^{-1} y^{-1}. Cow dung was dissolved in pond water before application.

2.1.1. Stocking

Four week-old fry (average weight and length 0.02 g and 1.36 cm respectively) pre-adapted to 15 ppt seawater (SW) were obtained from the southern coastal areas (wild catch) of India. Before initiating the experimental treatments, fry were adapted to the desired salinity of the inland saline groundwater by gradually lowering or raising the salinity level over a 7-day period. Stocking rate was kept at 10,000 ha^{-1}. Fish were fed twice daily (between 09.00-10.00 and 15.00-16.00 hours) on a diet containing approximately 40% protein [See 19 for details]. The feeding rate was adjusted every 15th day after weighing a representative sample of about 25-30 fish per treatment.

2.1.2. Water quality monitoring

Water samples were obtained in replicate of four from each treatment before sunrise for the study of physico-chemical characteristics and planktonic flora and fauna. Water temperature (^{o}C), salinity and pH were recorded daily; dissolved oxygen (DO) and conductivity were monitored at weekly intervals using multiline microprocessor (F/set-3, E-Merck). All other water quality parameters were monitored only thrice (30, 60 and 100 days) during the experimental period of 100 days [20].

2.1.3. Biotic communities and chlorophyll

For qualitative and quantitative estimations of planktonic flora and fauna, water samples were collected in replicate of four from each treatment at the end of the 45 and 100 days intervals. Plankton samples were obtained by passing 20 L water from five different locations through plankton net (mesh size 125 µm). Plankton densities were estimated using a Sedgwick Rafter cell under a binocular microscope. Plankton species diversity (d) was determined using Shannon and Weaver's diversity index formula [21]. Identification of planktons to genus level was carried out using suitable keys and monographs.

Net primary productivity (NPP) was determined by light and dark bottle method [20]. For chlorophyll *a*, a known amount of water was filtered through Whatman filter paper (No. 40) and determined following Boyd [22]. The filter papers along with the sample were put into a plastic tube. Thereafter, 5 ml 90% cold acetone was added in each tube and the filter paper was ground with a tissue grinder. After the filter paper was crushed, a further 5 ml of 90 per cent cold acetone was added, stirred and the tubes were transferred to a refrigerator for 24h. Thereafter, the tubes were centrifuged for ten minutes at 3000 rpm and the supernatant was decanted in glass cuvettes and absorption was read at 665 and 750 nm.

Chlorophyll *a* = 11.9 (E_{665}-E_{775}) V/L × 1000/S (µg L^{-1})

E_{665} = optical density of samples at 665 nm

E_{750} = optical density of sample at 750 nm

V = acetone volume used (ml)

L = volume of sample filtered (ml)

S = length of light path in the spectrophotometer (cm) =1cm

2.1.4. Fish growth and energy assimilation

Fish growth (length and weight) was monitored thrice at the end of 30 and 60 days taking a representative sample of 40-50 fish from each treatment; and at the end of 100 days post-stocking when the ponds were completely drained and all the fish harvested and counted. Individual lengths (cm) and weights (g) were recorded from each treatment. To evaluate the well being of the fish, condition factor (k) and LWR were also determined.

Energy assimilation was computed based on the caloric content of fish at initial sampling and at harvest as follows:

$$\text{Energy assimilated} = \frac{F \times 100}{\text{Average weight of fish}}$$

F = Energy in feed utilized

= Energy in feed (kCal g^{-1}) × Total feed consumed

2.2 Experiment 2: Effect of five different (10, 15, 20, 25 and 30 ppt) salinity treatments at low (4%) and high (6%) ration levels on growth performance and feed conversion efficiency in milkfish fry under laboratory conditions

The experiment was conducted under laboratory conditions (25.0±1^{0}C; LD 12:12) in transparent glass aquaria (60×30×30cm) with an aeration facility. Each aquarium was filled with 30 L water of the desired salinity, which was made, by mixing water from two different aquifers (discharging water of 10 and 30 ppt salinity, respectively). Each aquarium was then stocked with 15 fish (average BW and length 0.18 g and 2.89 cm, respectively). Four replicates of each treatment were maintained. All groups were fed daily at 4 or 6 per cent BW in two installments at 08.00 and 15.00 hours for 100 days on the formulated diet containing 40% protein [see 19 for details]. Fish were exposed to the diet for 4h during each ration; thereafter the uneaten food was siphoned out, stored and dried for calculating FCR. Fish were bulk weighed thrice during the experiment, with the feeding rate adjusted accordingly. Water in the aquaria was replenished daily with the desired salinity

level. Faecal matter voided by the fish was collected each morning by siphoning approximately 12h after removal of the uneaten food. Faeces were oven dried (at 60^0C) for subsequent analysis. Individual weights of the fingerlings were recorded at the beginning and at the end of the experiment with the help of a top pan electronic balance. Viscera of the fish were extirpated for the calculation of viscero-somatic index (VSI) and hepato-somatic index (HSI). Liver and muscle tissue were processed for the estimation of glycogen and muscle protein. The intestine was processed for the determination of protease enzyme activity, amylase, cellulase and lipase activity.

At the end of feeding trials, water samples from each aquarium were collected at 2-h interval for the estimation of excretory levels of total ammonia ($N\text{-}NH^+_4$) and reactive phosphate ($o\text{-}PO^-_4$) following APHA [20]. The concentrations of each metabolite obtained at the 2-h intervals were summed up and the quantity of nitrogen and phosphate excreted by the fish in aquaria water were calculated as follows:

$$\text{Total } N\text{-}NH^+_4/o\text{-}PO_4^- \text{ excretion (mg kg}^{-1}\text{ BW day}^{-1}) = \frac{N\text{-}NH^+_4/o\text{-}PO^-_4 \text{ (mg L}^{-1}) \text{ in water}}{\text{Fish biomass (kg) per L of water}}$$

Feed ingredients, experimental diets, faecal samples, fish carcass (initial and final) were analysed following AOAC [23]. Dry matter (after desiccation in an oven at 105^0C for 24 h), ash (incineration at 550^0C for 4 h in a muffle furnace) and nitrogen were determined using the micro-Kjeldahl method. Crude protein contents were estimated by multiplying nitrogen by a factor of 6.25. Crude fat contents were determined by petroleum ether extraction (Soxhlet's apparatus). Phosphorus from the feed/carcass was determined spectrophotometrically after acid digestion (nitric acid:perchloric acid, 10:1), NFE % was calculated by subtracting the total percentage of crude protein, crude fat, ash, moisture and crude fibre from 100. Chromic oxide levels in the diets as well as in the faecal samples were estimated spectrophotometrically. Apparent protein digestibility (APD) of the diets was calculated as follows:

$$APD = 100 - 100 \times \frac{\%Cr_2O_3 \text{ in diet}}{\%\ Cr_2O_3 \text{ in faeces}} \times \frac{\%\text{ nutrient in faeces}}{\%\text{ nutrient in diet}}$$

Energy contents of the diets and fish were calculated using the average caloric conversion factors of 0.3954, 0.1715 and 0.2364 kJ g^{-1} for lipid, carbohydrate and protein respectively [24].

2.3. Statistical analysis

Data were subjected to multivariate analysis. Coefficient of correlation between different parameters and multiple regressions between independent (hydrochemical parameter) and dependent variables (biological/productivity indicating parameters) were determined by computer. Length-weight relationship was calculated according to the equation:

$W = cL^n$ or $\log W = \log c + n \log L$

where, W = weight in kg, c = constant, n = exponential value of length and, L = length of fish in cm. Plankton species diversity (d) was determined using the diversity index formula of Shannon and Weaver [21].

$\overline{d} = -\sum (ni/N) \log_2 (ni/N)$

where, d = species diversity, ni = number of individuals of i^{th} species, N = total number of individuals.

ANOVA followed by Duncan's multiple range test (1955) was applied to determine significant differences between different salinity treatments maintained under laboratory conditions.

3. Results

3.1. Experiment 1: Effect of two different salinities (10 and 25 ppt) and two different ration levels (4% and 6%) on growth performance and carcass composition of milkfish fry under field conditions

3.1.1. Fish growth

Survival was independent of salinity level and varied between 80-86%. ANOVA revealed that irrespective of the salinity levels, a significant increase in weight gain and specific growth rate (SGR% g day^{-1}) were observed in groups fed at low ration level (4%) in comparison with the groups fed at high ration level (6%). Significantly ($P<0.05$) higher values in growth performance were observed in fish maintained at 25 ppt salinity where the

fish attained a mean BW of 56.5 g (mean length = 20.19 cm) in a culture period of 100 days. Growth rate per day at 4% ration level was 0.27g and 0.56g at 10 and 25 ppt respectively (Table 2).

Table 2. Effect of two different salinity levels (10 and 25 ppt) and two different ration levels (4% and 6% BW d^{-1}) on growth performance of *Chanos chanos* under field conditions-100 day treatment

Salinity-ppt (ration level -%)	INITIAL FISH STOCK			FINAL FISH STOCK (after 100 days)			SGR % $g\,d^{-1}$ (SGRL)	Growth d^{-1} (g)	Condition factor (cf/k)	Length weight relationship (LWR)
	Stocking density/ 375 m^2	Mean fish weight (g) (length cm)	Total biomass (kg)	Survival (%)	Mean fish weight (g) (Length cm)	Total biomass (kg)				
10 (4)	375	0.02±0.004a (1.36±0.08a)	7.97±1.49 a	80	27.25±1.20c (16.01±0.35c)	8.80±0.71c	7.27±0.19bc (2.47±0.07bc)	0.27±0.02c	0.66±0.03a	W=-0.21374855 $L^{3.21}$
10 (6)	375	0.02±0.003a (1.36±0.08a)	7.97±1.49 a	86	20.13±1.63d (14.26±0.20d)	6.94±0.56c	6.96±0.20c (2.36±0.06c)	0.20±0.02 d	0.68±0.03a	W=-0.2232434 $L^{3.12.}$
25 (4)	375	0.02±0.004a (1.36±0.08a)	7.97±1.45 a	84	56.50±3.21a (20.19±0.35a)	18.87±1.07a	8.01±0.20a (2.71±0.06a)	0.56±0.03a	0.68±0.01a	W=-0.1155966 $L^{3.32}$
25 (6)	375	0.02±0.004a (1.36±0.08a)	7.97±1.46 a	80	40.75±1.18b (18.05±0.26b)	13.00±0.38b	7.69±0.21ab (2.60±0.06ab)	0.41±0.01 b	0.69±0.02a	W=-0.1885318 $L^{3.23}$

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 Wtf–ln Wti)×100]/t
SGRL (% $cm\,d^{-1}$) = specific growth rate of length =[ln Lf–ln Li)×100]/t
Growth per cent gain in body weight=[(Wtf-Wti)/Wti] × 100, where, Wti and Wtf denotes initial and final weight of fish respectively, Lf and Li denotes initial and final length (cm) of fish respectively and t represents time (days), duration of experiment (60 days), BW = Body weight, d=days.
Condition factor (k) = $Wt\times10^5/L^3$, $Wt\times10^5/L^3$ where Wt is weight of the fish in grams and L=Total length in millimeters.
Length-weight relationship (LWR) : $W=cL^n$ = 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.

At the end of 100-day culture period, no significant variations in 'k' values were observed; however, highest values of 'k' were observed in fish grown at 25 ppt salinity and fed at low ration level. LWR indicate that 'n' values were found to obey cube law; however, highest values were observed in fish grown at 25 ppt salinity fed at low ration (Table 2). VSI and HSI values also followed growth patterns in the fish (Table 3).

Table 3. Effect of two different salinity levels (10 and 25 ppt) and two different ration levels (4% and 6% BW d^{-1}) on proximate carcass composition (% wet weight basis), viscero-somatic index (VSI) and hepato-somatic index (HSI) in *Chanos chanos* under field conditions–100 day treatment

Salinity level –ppt (ration level %)	Moisture	Protein	Fat	Ash	Phosphorus	Energy (kJ g^{-1})	Viscero-somatic index (VSI)	Hepato-somatic index (HSI)
Initial value	72.2±0.10	15.44±0.30	2.95±0.02	2.78±0.06	0.38±0.07	5.89±0.09		
10(4)	69.12±0.26a	19.69±0.29b	3.58±0.04b	3.60±0.06b	0.58±0.03b	6.76±0.11b	8.66±0.19c	1.14±0.05c
10(6)	69.41±0.27a	19.36±0.26b	3.45±0.04c	3.53±0.07b	0.54±0.04b	6.67±0.07bc	7.97±0.08d	1.00±0.06d
25(4)	67.98±0.29b	20.89±0.26a	3.95±0.05a	3.91±0.05a	0.71±0.06a	7.06±0.10a	10.73±0.20a	1.54±0.04a
25 (6)	68.85±0.22a	20.02±0.26b	3.63±0.03b	3.79±0.05a	0.62±0.04ab	6.80±0.12b	9.66±0.20b	1.29±0.04b

All values are mean ±SE of mean.
Means bearing different letters in the same column differ significantly (P<0.05)
VSI=Viscera-weight/total weight of fish ×100
HSI=Liver weight/total weight of fish ×100

Proximate carcass composition revealed a significant (P<0.05) increase in protein, fat, energy and phosphorus in fish fed at 4% ration level and the values of these parameters were higher in fish grown at 25 ppt (Table 3). Assimilated energy was also significantly (P<0.05) higher in fish maintained at 25 ppt salinity (Table 3) and fed on low ration level. With regard to fish weight (% Kcal g^{-1} fish), percentage assimilation varied significantly (P<0.05) with ration and salinity levels.

3.1.2. Water quality characteristics

Irrespective of the salinity levels, DO, BOD, pH and release of nutrients (total Kjeldahl nitrogen, NO_3-N, SO_4) were significantly (P<0.05) higher in treatment where the fish were fed at 4% ration level (Table 4). Higher values of chlorophyll *a*, Net primary productivity (NPP), and phytoplankton and zooplankton population coincided with the highest values of alkalinity, turbidity and TDS in ponds where the fish were fed at 4% ration level.

Multivariate analysis revealed a significant positive correlation of Kjeldahl nitrogen (r=0.64), turbidity (r=0.70), TDS (r=0.38), phytoplankton (r=0.58), zooplankton (r=0.32) and chlorophyll *a* (r=0.58) with fish growth. Productivity indicating parameters viz., NPP (r=0.58), GPP (r=0.61), No_3-N (r=0.50) also showed a significant positive correlation with fish weight gain. Plankton population remained significantly higher (Table

4) where the fish were fed on low ration level. Phytoplankton were represented by Chlorophyceae (5 Taxa), Bacillariophyceae (5 taxa) and Cyanophyceae (1 taxa). Zooplankton were represented by Copepoda (4 taxa) and Rotifera (3 taxa).

Table 4: Physico-chemical and biological characteristics of pond water stocked with *Chanos chanos* fry at two different salinity levels (10 and 25 ppt) and fed on two different ration levels (4% and 6% BW d-1) under field conditions - overall mean of three sampling dates.

Parameters	Salinity-ppt (ration levels-%)			
	10 (6)	10 (4)	25 (6)	25 (4)
Physico-chemical characteristics				
Conductivity dSm^{-1}	18.52±0.30b	18.20±0.84b	31.28±0.85a	31.76±0.94a
pH	8.32±0.02b	8.42±0.01a	8.37±0.01a	8.39±0.02a
Dissolved oxygen mg l^{-1}	5.88±0.14b	5.70±0.06b	5.78±0.11b	6.23±0.12a
BOD mg l^{-1}	3.90±0.15a	3.62±0.13a	3.05±0.12b	2.35±0.13c
Carbonates mg l^{-1}	17.67±0.51b	21.42±0.77a	14.50±0.68c	12.42±0.73d
Biocarbonates mg l-1	206.08±2.60a	207.42±2.56a	193.33±4.01b	182.67±4.02c
Total alkalinity mg l^{-1}	223.67±2.80a	228.75±2.81a	207.42±4.22b	194.17±4.23c
Chlorides mg l^{-1}				
Total hardness mg l^{-1}	3283.33±66.67c	3812.50±102.30b	5516.69±970.29a	5654.17±64.26a
Calcium mg l^{-1}	467.83±18.13ab	480.10±12.37a	411.85±21.74b	425.78±25.60ab
Magnesium mg l^{-1}	599.02±20.96b	701.28±10.98b	1553.90±112.72a	1572.39±107.91a
Total Kjeldahl nitrogen mg l^{-1}	3.21±0.16c	3.39±0.17bc	3.86±0.20b	4.67±0.21a
NO_3-N mg l^{-1}	0.72±0.03c	0.88±0.03b	0.97±0.04b	1.25±0.01a
NO_2-N mg l^{-1}	0.64±0.03a	0.56±0.02b	0.53±0.02b	0.41±0.02c
NH_4-N mg l^{-1}	0.56±0.04a	0.44±0.02b	0.41±0.03b	0.31±0.02c
o-PO_4 mg l^{-1}	0.06±0.01a	0.07±0.01a	0.07±0.004a	0.07±0.004a
SO_4 mg l^{-1}	83.27±1.55b	95.74±2.12c	119.79±3.88b	135.12±4.89a
Turbidity NTU	24.46±0.99b	26.89±1.14b	27.83±1.08b	32.17±1.68a
Total dissolveds olutes (TDS) mg l^{-1}	4672.00±0.99c	4922.92±86.18b	5425.00±111.19a	5502.08±80.62a
Biological characteristics				
NPP mg Cl^{-1} d^{-1}	0.43±0.03c	0.56±0.03b	0.60±0.03b	0.79±0.04a
Gross primary productivity mg Cl^{-1} d^{-1}	1.58±0.04d	1.71±0.04c	1.84±0.04b	2.07±0.04a
Phytoplankton numbers l^{-1}	8239±286d	9260±370c	10468±316b	11708±344a
Zooplankton numbers l^{-1}	4687±107c	6114±269b	5906±205b	7033±227a
Phytoplankton (d)	1.86±0.25b	2.01±0.20b	2.22±0.16b	2.24±0.13a
Zooplankton (d)	0.69±0.07b	0.84±0.10b	1.07±0.08a	1.19±0.17a
Chlorophyll *a* µg l^{-1}	2.98±0.13c	3.25±0.11c	3.69±0.13b	4.38±0.15a
Pheophytin *a* µg l^{-1}	0.94±0.07b	1.16±0.08a	1.32±0.08a	1.32±0.05a

All values are mean±SE of mean. Mean bearing different letters in the same row differ significantly (P<0.05).
Water temperature during the experimental period ranged from 26.4~ 32.8^{o}C.

3.2. Experiment 2: Effect of five different (10, 15, 20, 25 and 30 ppt) salinity treatments at low (4% BW) and high (6% BW) ration levels on growth performance and feed conversion efficiency in milkfish fry under laboratory conditions

Differences in survival rate among different salinity treatments within each trial were very low (data not shown). In contrast, differences in growth rate were observed among various salinity treatments and the two ration levels. Growth performance of milkfish increased with each increase in the salinity from 10-25 ppt and thereafter a depression in growth with reduced digestibility and feed conversion efficiency were observed in fish maintained at 30 ppt. Among the two ration levels, growth performance, nutrient retention and digestibility parameters were significantly higher in the group fed at 4% BW d^{-1} than the groups fed at 6% BW d^{-1} (Table 5). Even excretion of metabolites (N-NH^{+}_4 and o-PO^{-}_4) remained low in fish fed at low ration level and more so at 25 ppt (Table 5).

The concentration of metabolites (N-NH^{+}_4 and o-PO^{-}_4) excreted in the holding water decreased significantly with increase in salinity. Low values in total ammonia excretion and reactive phosphate production (mg kg^{-1} BW day^{-1}) were recorded in fish maintained at 25 ppt. Peak values in N-NH^{+}_4 excretions occurred approximately 7 h post-feeding and the levels gradually declined thereafter. The concentration of reactive phosphate (o-PO_4^{-}) in the water was high when the samples were analysed 2h post-feeding, declining to the lowest level thereafter and reaching a peak approximately 20 h post-feeding. Thereafter, the levels again declined and remained low.

Table 5: Effect of five different salinity levels (10, 15, 20, 25 and 30 ppt) and two different ration levels (4% and 6% BWd^{-1}) on growth performance, nutrient retention, digestibility and excretion of metabolites in *Chanos chanos* fry under laboratory conditions (LD 12:12 at 25±1°C) – 100 day treatment.

Parameters	Salinity (ppt)					
	Control (0)	10	15	20	25	30
Low Ration Initial weight (g)	0.18±0.02a	0.18±0.01a	0.17±0.02a	0.18±0.02a	0.17±0.02a	0.18±0.01a
Initial length (cm)	2.89±0.07	2.87±0.08	2.88±0.09	2.85±0.06	2.86±0.07	2.88±0.08
Final weight (g)	2.68±0.16e	4.62±0.09d	5.25±0.02c	5.61±0.05b	6.32±0.04a	5.29±0.03c
Final length (cm)	4.48±0.6	8.63±0.04	9.18±0.06	9.71±0.04	10.73±0.04	9.27±0.04
Live weight gain (g)	2.51±0.17e	4.44±0.08d	5.07±0.03c	5.42±0.05b	6.15±0.04a	5.11±0.02c
Growth (% gain in BW)	1633±274	2545±197	3212±360	3272±375	3954±409	2935±236
Specific growth rate (SGR)	2.76±0.16c	3.26±0.07b	3.46±0.12ab	3.47±0.11ab	3.67±0.10a	3.39±0.01ab
Feed conversion ratio (FCR)	2.02±0.04a	2.02±0.04a	2.00±0.02a	1.95±0.04ab	1.86±0.03b	1.99±0.04a
Gross energy retention (GER)	18.46±0.25b	18.86±0.46b	16.68±0.20b	19.32±0.39b	20.94±0.27a	19.01±0.33b
Gross protein retention (GPR)	25.03±0.33bc	24.76±0.91c	25.18±0.46bc	26.75±0.59ab	28.38±0.61a	26.01±0.60bc
Protein efficiency ratio (PER)	1.24±0.03b	1.25±0.04b	1.25±0.02b	1.29±0.03ab	1.35±0.02a	1.26±0.03b
Apparent protein digestibility (APD)	80.98±0.06f	81.40±0.09e	81.76±0.09d	82.88±0.07b	83.57±0.13a	82.35±0.09c
Total ammonia excretion (mg kg^{-1} BW d^{-1})	1346±7.37a	1223±7.53b	1096±8.77c	797±8.90e	609±7.70f	938±7.26d
High ration Initial weight (g)	0.18±0.02a	0.19±0.01a	0.19±0.02a	0.18±0.02a	0.17±0.02a	0.18±0.01a
Initial length (cm)	2.89±0.09	2.84±0.07	2.86±0.07	2.88±0.08	2.85±0.07	2.89±0.08
Final weight (g)	2.23±0.03f	3.73±0.04e	4.29±0.03d	4.93±0.05b	5.53±0.03a	4.75±0.04c
Final length (cm)	4.24±0.05a	7.59±0.08e	8.22±0.04d	8.73±0.07b	9.61±0.05a	8.57±0.08c
Live weight gain (g)	2.06±0.04f	3.54±0.05e	4.11±0.05d	4.75±0.06b	5.36±0.04a	4.57±0.04c
Growth (% gain in BW)	1291±157d	2000±190cd	2485±328bc	2870±335ab	3438±360a	2611±204bc
Specific growth rate (SGR)	2.59±0.12d	3.02±0.08c	3.20±0.13bc	3.35±0.12ab	3.53±0.10a	3.28±0.07abc
Feed conversion ratio (FCR)	2.04±0.03a	2.07±0.03a	2.03±0.03a	1.93±0.02bc	1.90±0.02c	1.99±0.03ab
Gross energy retention (GER)	17.46±0.25	17.28±0.29	17.85±0.29	19.00±0.24	19.79±0.22	18.31±0.33
Gross protein retention (GPR)	23.72±0.69c	23.11±0.67c	23.78±0.52c	25.55±0.45ab	26.40±0.45a	24.49±0.55bc
Protein efficiency ratio (PER)	1.23±0.02c	1.22±0.02c	1.24±0.02bc	1.30±0.02ab	1.32±0.02a	1.26±0.02abc
Apparent protein digestibility (APD)	80.79±0.07e	81.48±0.11d	81.71±0.09d	82.69±0.05b	82.97±0.07a	82.29±0.10c
Total ammonia excretion (mg kg^{-1} BW d^{-1})	1477±7.05a	1371±6.74b	1329±6.65c	1125±9.44e	994±19.60f	1245±7.30d
Total phosphate production (mg kg^{-1} BW d^{-1})	493±6.39a	457±3.31b	401±5.24c	335±6.56e	308±6.88f	377±5.72d

All values are mean±SE of mean.
Means bearing different letters in the same row differ significantly (P<0.05).

Body composition revealed significantly (P<0.05) higher values for protein, fat, energy and phosphorus, while those of moisture and ash contents remained significantly (P<0.05) lower in fish maintained at 25 ppt salinity and at low ration level (Table 6).

Table 6. Effect of five different salinity levels (10, 15, 20, 25 and 30 ppt) and two different ration levels (4% and 6% BW d^{-1}) on proximate Carcass composition (% wet weight basis) in *Chanos chanos* fry under laboratory conditions (LD 12:12 at 25±1OC) – 100 day treatment

Carcass composition (%)	Initial values	Salinity (ppt)					
		Control (0)	10	15	20	25	30
Low ration (fish fed at 4% BW d^{-1})							
Moisture	72.2±0.10	68.41±0.03a	68.26±0.06ab	68.19±0.06bc	67.73±0.07d	67.04±0.06e	68.05±0.08c
Crude protein	15.44±0.30	18.82±0.29c	19.03±0.22c	19.47±0.22bc	20.13±0.23ab	20.46±0.23a	19.91±0.22ab
Crude fat	2.95±0.02	3.66±0.02c	4.18±0.07a	3.79±0.03bc	3.66±0.02c	4.18±0.07a	3.86±0.03b
Ash	3.97±0.03	3.92±0.04e	4.02±0.04de	4.09±0.04cd	4.24±0.03b	4.57±0.03a	4.17±0.03bc
Phosphorus	0.40±0.01	0.65±0.03d	0.68±0.03cd	0.68±0.02cd	0.82±0.03b	0.99±0.03a	0.14±0.03c
Gross energy (kJg^{-1})	5.75±0.06	6.79±0.02d	6.93±0.02b	6.87±0.02c	6.93±0.02b	7.13±0.01a	6.92±0.02b
High ration (fish fed at 6% BW d^{-1})							
Moisture	72.2±0.10	70.04±0.04a	69.78±0.05a	69.30±0.09b	68.73±0.13c	68.10±0.17d	69.00±0.07c
Crude protein	15.44±0.30	17.72±0.22d	18.05±0.23cd	18.38±0.23bcd	18.93±0.23ab	19.36±0.26a	18.11±0.23abc
Crude fat	2.95±0.02	3.62±0.08bc	3.54±0.03c	3.64±0.03bc	3.72±0.02b	3.87±0.03a	3.68±0.03b
Ash	3.97±0.03	3.69±0.03d	3.74±0.02d	3.84±0.03c	4.02±0.04b	4.16±0.03a	3.96±0.04b
Phosphorus	0.40±0.01	0.58±0.02d	0.63±0.02c	0.65±0.01c	0.73±0.02b	0.78±0.01a	0.66±0.02c
Gross energy (kJg^{-1})	5.75±0.06	6.46±0.02d	6.51±0.02d	6.61±0.02c	6.73±0.03b	6.88±0.03a	6.68±0.02bc

All values are mean±SE of mean. Means bearing different letters in the same row differ significantly (P<0.05)

Determination of proteolytic, amylolytic and cellulolytic enzyme activity also revealed significantly (P<0.05) higher values (Table 7) in the digestive tract of fish maintained at 25 ppt and at low ration level. Muscle protein, HSI and VSI values also followed a similar trend. However, muscle glycogen and liver glycogen remained significantly (P<0.05) lower in fish maintained at 25 ppt and at low ration level, their levels increased with further increase in salinity (30 ppt, Table 7).

4. Discussion

Growth performance (growth day^{-1} and SGR) increased significantly (P<0.05) with each increase in water salinity more so in fish fed at low ration level. Highest values however, were observed at 25 ppt salinity. High APD (83.57 at 4% ration level), nutrient retention, feed conversion efficiency and high intestinal enzyme activity coupled with high growth were also observed at 25 ppt salinity even in fish maintained under controlled conditions at low ration level, thus also supporting our field results. Low excretory levels of N-NH^{+}_{4} and o-PO^{-}_{4} by fish maintained at 25 ppt, further support high nutrient retention and less energy expenditure by the fish at 25 ppt salinity level.

Table 7: Effect of five different salinity levels (10, 15, 20, 25 and 30 ppt) and two different ration levels (4% and 6% BW d^{-1}) on muscle protein, muscle glycogen, liver glycogen, enzyme activities (protease, amylase, cellulase and lipase), viscero-somatic index (VSI) and hepato-somatic index (HSI) in *Chanos chanos* fry under laboratory conditions (LD 12:12 at 25±1^{0}C) – 100 day treatment.

Parameters	Salinity (ppt)					
	Control (0)	10	15	20	25	30
Low Ration Muscle glycogen (mg g^{-1})	1.77±0.04a	1.73±0.03ab	1.62±0.04bc	1.50±0.06cd	1.46±0.03d	1.62±0.04bc
Liver glycogen (mg g^{-1})	1.88±0.04a	1.77±0.03b	1.65±0.03c	1.52±0.03d	1.40±0.04e	1.58±0.03cd
Muscle protein (mg g^{-1})	102.77±3.49d	105.07±3.48d	117.40±3.31c	137.21±2.06b	152.31±2.66a	123.33±3.35c
Total protease enzyme activity (mg g^{-1} h^{-1})	4.90±0.03f	5.04±0.04e	5.23±0.04d	5.81±0.05b	6.02±0.05a	5.63±0.03c
Specific protease enzyme activity[1]	1.39±0.03f	1.57±0.03e	1.84±0.03d	2.30±0.03b	2.58±0.03a	2.17±0.04c
Total amylase activity (mg g^{-1} h^{-1})	3.19±0.03e	3.36±0.03d	3.41±0.03d	3.75±0.04b	3.95±0.05a	3.54±0.04c
Specific amylase activity[2]	1.09±0.03e	1.20±0.03d	1.32±0.04c	1.63±0.03b	1.87±0.05a	1.54±0.04b
Total lipase activity (mg g^{-1} h^{-1})	1.66±0.04e	1.71±0.04e	1.81±0.03d	2.19±0.03b	2.81±0.03a	2.00±0.05c
Specific lipase activity[3]	0.69±0.03e	0.75±0.04de	0.80±0.03d	1.17±0.04b	1.59±0.03a	0.98±0.04c
Total cellulase activity (mg g^{-1} h^{-1})	0.88±0.05e	0.92±0.05e	1.07±0.05d	1.53±0.04b	1.77±0.05a	1.28±0.05c
Specific cellulase activity[4]	0.51±0.02cd	0.49±0.02d	0.57±0.02c	0.73±0.02b	0.86±0.03a	0.67±0.02b
Viscero-somatic index (VSI)	8.12±0.10e	8.75±0.14d	9.45±0.17c	10.06±0.18b	10.87±0.18a	9.96±0.16bc
Hepato-somatic index (HSI)	.51±0.05d	1.62±0.04cd	1.69±0.04cd	2.08±0.03b	2.70±0.13a	1.74±0.04c
High ration Muscle glycogen (mg g^{-1})	2.02±0.04a	2.02±0.04a	1.96±0.03ab	1.88±0.04b	1.68±0.03c	1.88±0.05b
Liver glycogen (mg g^{-1})	2.10±0.09a	1.99±0.03ab	1.89±0.04bc	1.80±0.03c	1.64±0.03d	1.87±0.03bc
Muscle protein (mg g^{-1})	95.52±2.62d	99.30±2.56cd	105.64±2.68bc	117.70±2.60a	123.46±3.21a	109.46±3.04b
Total protease enzyme activity (mg g^{-1} h^{-1})	4.38±0.03e	4.66±0.06d	4.88±0.04c	5.21±0.06b	5.37±0.06a	5.01±0.05c
Specific protease enzyme activity[1]	1.21±0.04f	1.34±0.03e	1.43±0.03d	1.70±0.03b	1.86±0.04a	1.57±0.03c
Total amylase activity (mg g^{-1} h^{-1})	2.64±0.03f	2.73±0.03e	2.89±0.03d	3.18±0.02a	3.48±0.02a	2.97±0.03c
Specific amylase activity[2]	0.80±0.05d	0.99±0.03c	1.14±0.02b	1.22±0.03b	1.35±0.03a	1.12±0.04b
Total lipase activity (mg g^{-1} h^{-1})	1.76±0.04e	1.80±0.03e	2.02±0.05d	2.30±0.04b	2.44±0.03a	2.18±0.04c
Specific lipase activity[3]	0.69±0.03d	0.77±0.03cd	0.93±0.04bc	1.24±0.14a	1.20±0.02a	1.00±0.04b
Total cellulase activity (mg g^{-1} h^{-1})	0.72±0.03d	0.81±0.03cd	0.94±0.04bc	1.09±0.03ab	1.22±0.02a	0.88±0.11c
Specific cellulase activity[4]	0.39±0.02c	0.40±0.02c	0.41±0.04bc	0.49±00.02b	0.60±0.03a	0.47±0.03bc
Viscero-somatic index (VSI)	7.64±0.15e	7.97±0.07de	8.30±0.07d	9.64±0.12b	10.71±0.12a	8.68±0.15c
Hepato-somatic index (HSI)	0.93±0.08d	1.05±0.06cd	1.21±0.06c	1.62±0.05ab	1.77±0.04a	1.48±0.04b

[1] mg of tyrosine liberated/mg of protein/minute, [2] mg of maltose liberated/mg of protein/minute, [3] micromole fatty acid liberated/mg of protein/hour, [4]mg ofglycose liberated/mg of protein/minute.
All values are mean±SE of mean. Means bearing different letters in thes ame row differs significantly (P<0.01).

Growth performance of milkfish in inland saline groundwater at 25 ppt salinity in a culture period of 60-100 days is comparably rather higher than most of the studies reported in the literature [7, 25]. Walsh *et al.* [26] were of the view that when natural food is supplemented with artificial/supplementary feed, higher growth is obtained. They also reported that formulated diet containing 50% crude protein enhanced weight gain in milkfish in brackish water ponds after 28 days. Studies of Jana *et al*. [25], however, have suggested that milkfish require about 40% dietary protein for high growth irrespective of the protein source (fishmeal/processed full fat soyabean). Higher growth in the present study may, therefore, be attributed either

to the favourable salinity levels of inland saline groundwater and/or to the optimum protein (40%) or fat (9%) contents [25] of the diet.

Condition factor 'k' indicative of the well-being of the fish varied between 0.66 and 0.68 at the end of 100 days; however, significantly ($P<0.05$) higher values (0.68) were observed in fish maintained at 25 ppt. Bishara [27] reported that the values of condition factor decreased with age. In the present studies, highest values of 'n' (3.32) in LWR were observed in fish maintained at 25 ppt further support the highest growth attained by the fish at 25 ppt salinity at low (4%) ration level.

Feeding levels and protein contents appear to affect the amount of energy assimilated by fish [28]. In the present study, two ration levels (4% and 6%) were used and thus the assimilated energy appears to be a function of the both ration and salinity of the water. These results are in agreement with the observations of Sumagaysay and Borlongan [28] and Jana *et al.* [25] in milkfish and other fish species.

These studies have further revealed that exposure of milkfish to higher salinity (>25 ppt) not only repressed growth but also affected digestibility and other physiological parameters such as feed conversion efficiency, digestive enzyme activity, liver and muscle glycogen levels, VSI and carcass composition. As the intestine plays a major role in osmoregulation, salinity-mediated decrease in digestibility may therefore be due in part to a higher rate of food movement in the fish maintained at high salinities and thus reducing the time required for more and complete digestion and absorption of nutrients. These results are similar to those reported by Ferraris *et al.* [14] and Jana *et al.* [7] in milkfish. De Silva and Perera [13] found that food intake in mullets increased in high salinities, a finding which might be related to a higher rate of food movement and lower digestibility under these conditions. As marine fish drink water for osmoregulatory reasons, it is possible that digestive efficiency is compromised in sea water because of the food motility changes necessitated by osmoregulatory processes. A fish exposed to higher salinities has to expend more energy to meet the metabolic cost for ionic and osmotic regulation [29], it is thus presumed that growth and feed conversion might be improved if the external environment is manipulated by maintaining optimum salinity levels in order to reduce these costs. Growth retardation at high salinity treatments in milkfish can probably also be related to habitat preference, since the wild fry cease their pelagic mode of life and start migrating toward the estuarine environments where the salinity is lower than in the open sea.

Fish maintained at 25 ppt salinity and fed at low ration level had large accumulations of fat, as revealed by high VSI. Low values in muscle and liver glycogen in fish at 25 ppt salinity indicate utilization by the fish thus sparing protein for accumulation. High values of muscle and carcass protein and also of fat in fish at 25 ppt salinity also lend support to these findings. High retention of nutrients and low excretion of metabolites ($N\text{-}NH^{+}_{4}$ and $o\text{-}PO_4^{-}$) at 25 ppt salinity treatments further support the field results. Although milkfish is a euryhaline species, higher salinity treatments (>25 ppt) not only repressed growth performance but also affected FCR, digestibility and other physiological parameters. These results are similar to those obtained on milkfish [25] and gilthead sea bream, *Sparus auratus*, which are euryhaline species [15]. Their studies indicated better daily growth rate and feed conversion efficiency in sea bream grown at 28 ppt salinity then at low or at high salinities. Deacon and Hecht [30] also observed low growth rate and low feed conversion in juvenile spotted grunter, *Pomadasys commersonii*, at low salinity (5 ppt) than in iso-osmotic or hyper-osmotic (>12 ppt) salinity. Partridge and Jenkins [16] also obtained high SGR and improved FCR in juvenile black bream when exposed to 24 ppt than to 60 ppt.

The effects of salinity are also well reflected in the physico-chemical and biological characteristics of the pond water. Although no marked variations in DO concentrations were observed at the end of 100 days. DO concentrations deceased with increased salinity and statistically showed a significantly negative correlation with fish weight gain. Statistically, fish weight gain, growth percentage gain and SGR showed a significantly positive correlation with most of the water quality parameters including nutrients and productivity indicating parameters (NPP and GPP) except with BOD_5 ($r= -0.64$), carbonates ($r= –0.53$), bicarbonates ($r=-0.48$), chlorides ($r= -0.3$), magnesium ($r= -0.21$) and NH_4-N ($r= -0.55$), clearly revealing that high salinity (upto 25 ppt) of inland saline groundwater favours high growth of milkfish.

Available nitrogen (NO_3-N), NO_2-N, NH_4-N, turbidity and NPP, in general, increased significantly ($P<0.05$) with increased water salinity and showed a positive correlation with fish weight gain (growth percentage gain and SGR), indicating that fish growth is also influenced by the trophic status of the ponds. Many other studies [31] also showed that fish growth is positively correlated with the trophic status of pond waters.

Application of multiple regression models indicated that fish growth (weight gain) is significantly ($P<0.05$) correlated with NO_3-N, Kjeldahl nitrogen, $o\text{-}PO_4$, SO_4, TDS, hardness, conductivity, alkalinity and

turbidity, clearly revealing that milkfish is a euryhaline fish species, highly tolerant to high hardness and thus its growth appears to be significantly correlated with the trophic status of the ponds.

5. Conclusion

From aquaculture point of view, present studies have revealed that inland saline groundwater of high salinity can be profitably utilized for the culture of milkfish. Highest growth and feed utilization were observed when the fish were cultured at 25‰ salinity and fed at 4% ration level.

Competing Interests

None declared.

Authors' Contributions

UKB: Looked after the experiment, collected samples and analysed physico-chemical characteristics; SKG: Planned the experiment, arranged the basic requirements, and drafted the manuscript; AB: Carried out analysis of biological characteristics, performed statistical analysis and finalized the manuscript.

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