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Effect of stocking density on growth and survival of *Cirrhinus reba* (Hamilton, 1822) during spawn to fry nursing (outdoor)

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Abstract
To study the effects of stocking density of *Cirrhinus reba* (Reba carp) in nursing of spawn for 15 days on growth performance, survival and digestive enzyme's activities were studied from 7th to 22nd July, 2015. The present experiment was conducted in 20 m^3 tanks in triplicate at the College of Fisheries, Lembucherra. Three different treatments @ T1: 3.0; T2: 5.0 and T3: 7.0 lakh spawn ha^{-1} and fed with 30% protein diet. Significantly ($p<0.05$) highest mean final weight (0.77±0.00 g), final length (3.74±0.21 cm), survival (55.80±0.81%), protease activity (1.32 units/ mg protein/ min), lowest AFCR (0.57±0.02) and highest APER (5.80±0.17) was found in the in T1 with lowest stocking density (3 million ha^{-1}) in nursery rearing of spawn. There were no significant difference ($p>0.05$) observed in condition factor, hepato-somatic index and relative fatness. Thus, 3 million spawn ha^{-1} appear to be most suitable stocking density for nursing of Reba.

Keywords: AFCR, *Cirrhinus reba*, digestive enzyme's activities, relative fatness, spawn

1. Introduction

Reba carp, *Cirrhinus reba* is an important food fish, which is distributed over Indian sub-continent [27, 34, 42]. It is locally known as bhagna bata, raik or tatkini, and considered as one of the most important indigenous minor carp species. The natural production of Reba has declined considerably due to increased fishing pressure and various anthropological activities leading to siltation, aquatic pollution, and loss of natural habitat for spawning and growth [23]. Reba carp mainly inhabits in rivers and clear streams but is also found in tanks, canals, ponds, beels and inundated fields [33, 34, 42]. It is primarily plankton feeder but also feeds on detritus, mud, vegetables, crustaceans and insect larvae [42]. Spawning season of Reba carp is April to August with peak spawning occurring during the monsoon season [1, 23]. Relative fecundity of Reba carp was documented between 2.0-2.5 lakh egg kg^{-1} body weight of female [23]. Stocking density is related to number of fishes stocked in per unit of volume. It is an important parameter in aquaculture, since it has direct effect on the growth and survival and hence production [4]. Stocking density is an important indicator that determines the economic viability of the culture system. Positive effect of stocking density on growth is reported in some species and it is critical factor for many aquatic animals for their growth and survival [37, 45]. Decrease in stocking density results in decrease stress, which leads to lower energy requirements, causing higher growth rate and food utilization in the aquaculture production system. Stocking density is directly related with the competition for food and space in ecosystem [37]. Generally, fish needs to compete less for food and space in lower stocking density than the higher this minor carp is an eco-friendly fish and an important source of proteins, fats, carbohydrates, vitamins and minerals [43]. Its flesh is oily, tasteful and people would like to eat due to its lucrative size, high nutrition, and attractive flavor and less spines [31, 33, 39]. It is a congruous species for aquaculture but the wild population of Reba is rapidly declining due to sundry causes [31, 39]. Hence to meet the increasing demand of protein in India adaptation of semi- intensive and intensive culture practice on some important species of fishes is very important. The present investigation was undertaken to develop a practical and economically viable methodology for mass seed production and nursing of spawn of *C. reba* under controlled nursery management system in cemented tanks.

2. Materials and Methods
2.1 Experimental site and design
The present study was carried out in the cement cistern tanks of 20 m^3 (5m×4m×1m) each which are present at the College campus, Department of aquaculture, College of Fisheries, Lembucherra, Tripura the experiment were during monsoon season of 2016. The cemented tanks were selected in completely randomized design (CRD), divided into three treatments namely T1, T2 and T3 each was having three replications. Three different stocking densities of spawn of Reba viz. 3 (T1), 5 (T2) and 7 (T3) million ha^{-1} were stocked in nursing of spawn to fry. The experiment was conducted for 15 days rearing.

2.2 Tank preparation, stocking and fertilisation
The tanks were provided with a red soil bed of 8 cm thickness. Before starting the experiment, tanks were well exposed to sunlight. The tanks were prepared following standard management practices [27, 32]. Tanks were initially cleaned, dried and treated with lime (100 g $Ca(OH)_2$ $tank^{-1}$) @300 kg ha^{-1} and then filled with ground water. After one week of liming, all tanks were fertilized with mustard oil cake @750 g $tank^{-1}$ in two split doses each, *i.e.*, @ 750 kg ha^{-1} for the production of live feed. Oil cake was soaked in water for 24 hours before its application. Manuring and fertilization were done before and after stocking of seed at periodic interval as per the carp culture-package of practices to enhance production [2]. Nylon net was put over the tanks in order to prevent the bird predation. The spawn of *C. reba* were collected from department of Aquaculture, College of Fisheries, Lembucherra, Tripura and acclimatized before stocking and stocking was done in morning hours. The experimental ponds were stocked with 4 days post-hatch *C. reba* having an initial length and weight of 0.05± 0.004 cm and 0.002±0.0004 g, respectively.

2.3 Diet preparation and feeding
The proximate composition of formulated diet is presented in Table 1.

Table 1: Proximate composition of diet on dry weight basis.

Parameters	Value (%)
Crude protein	30.17±0.02
Crude lipid	5.73±0.05
Crude fibre	5.04±0.08
Ash	10.11±00.12
NFE	48.95±0.27

*Data expressed as mean ± SE, n=3

The diet for the experimental fry was formulated with 30% protein by using soybean meal, mustard oil cake, fish meal, wheat bran, broken wheat, corn flour and vitamin-mineral mixture in appropriate proportion. Spawn were fed the rate of 200% of the body weight initially and then after quantities of feed were adjusted after 7 days on the basis of increase in the average body weight of the stocked biomass. Spawn was fed 3 times in a day.

2.4 Physico-chemical, soil quality parameters and primary productivity
Selected soil quality parameters like texture, pH, nitrogen, organic carbon and phosphorus were measured before and after completion of the experiment following standard methods described [7]. Physico-chemical parameters of water were monitored fortnightly. Water temperature was recorded using a Celsius thermometer. Dissolved oxygen and pH were measured directly using Optical DO Probe (ProODOTM, YSI Environmental) and digital pH meter (HI 991001, HANNA). Total alkalinity, hardness, free carbon dioxide, NH^+_4-N, NO_2-N, and NO_3-N were determined by standard titrimetric method [3]. Primary productivity (mg C m^{-2} h^{-1}) was estimated by Light and Dark bottle method at definite intervals [14].

2.5 Estimation of growth, survival, production and feed utilization
At the end of experiment, growth in terms of length and weight, mean daily gain (ADG), specific growth rate (SGR), survival (%), condition factor (K), hepato-somatic index (HSI), relative fatness (RF), net yield (g), total biomass (g), apparent feed conversion ratio (AFCR) and apparent protein efficiency ratio (APER) were evaluated.

2.6 Digestive enzyme's activities
Protease, lipase and amylase activities were analysed by standard method [12, 8, 38] respectively.

2.7 Statistical analysis
The data obtained was analysed statistically through one way analysis of variance (ANOVA) and interpreted by suitable statistical method with Statistical Package for Social Sciences (SPSS, version 16.0 for windows). Software following Duncan's New Multiple Range test was used to determine whether any significant difference existed among treatment means [13, 46]. In all cases, value of P<0.05 was considered significant.

3. Results
3.1 Physico-chemical, soil quality parameters and primary productivity
The value of different physico-chemical, soil quality parameters and primary productivity presented in the Table 2, 3, 4 and 5.

Table 2: Water quality range during experimental period.

Parameter	Range
Water temperature (^0C)	26.20-29.37
pH	7.65-8.00
DO (mg l^{-l})	6.67 to 9.33
Total alkalinity (mg l^{-l})	55.33-118.67
CO_2 (mg l^{-l})	0.00-3.33
Hardness (mg l^{-l})	58.67-41.33
NH_4^+-N (mg l^{-l})	0.01-0.04
NO_2-N (mg l^{-l})	0.09-0.94
NO_3-N (mg l^{-l})	0.15-1.48
PO_4-P (mg l^{-l})	0.07-0.13

Table 3: Water quality parameters during nursing.

Parameter	Initial (0 Day)			Sampling (7th Day)			Final sampling (15th Day)		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Temperature (ºC)	**29.37±0.16^{a}**	29.23±.036^{a}	29.07±0.24^{a}	28.37±0.14^{a}	28.10±0.35^{a}	28.50±0.10^{a}	26.40±0.15^{a}	**26.20±0.17^{a}**	26.60±0.05^{a}
pH	7.70±0.32^{a}	8.00±0.17^{a}	7.99±0.32^{a}	7.86±0.14^{a}	7.93±0.09^{a}	7.65±0.18^{a}	7.95±0.04^{a}	7.89±0.20^{a}	7.94±0.19^{a}
DO (mg l^{-l})	8.53±1.04^{a}	8.40±1.80^{a}	9.33±2.54^{a}	7.20±0.23^{a}	7.87±2.14^{a}	6.67±1.48^{a}	7.53±0.76^{a}	7.47±0.24^{a}	7.80±0.83^{a}
Total alkalinity (mg l^{-l})	60.00±3.46^{a}	68.00±2.00^{ab}	73.33±4.80^{b}	89.33±5.45^{a}	110.67±6.76^{b}	118.67±4.05^{b}	55.33±4.66^{a}	56.00±5.03^{a}	60.00±5.77^{a}
CO_2 (mg l^{-l})	1.99±1.99^{a}	3.33±1.66^{a}	2.00±1.99^{a}	2.99±0.00^{a}	2.66±1.44^{a}	2.99±1.52^{a}	2.32±0.33^{b}	0.00±0.00^{a}	0.00±0.00^{a}
Hardness (mg l^{-l})	41.33±4.30^{a}	50.00±2.00^{ab}	55.33±1.30^{b}	42.00±3.05^{a}	50.00±1.15^{b}	56.00±2.30^{b}	43.33±4.05 ^{a}	48.67±2.90^{ab}	58.67±1.76^{b}
NH_4^+-N (mg l^{-1})	0.01±0.003^{a}	0.03±0.003^{ab}	0.07±0.020^{b}	0.04±.00^{a}	0.04±.01^{a}	0.03±.00^{a}	0.03±0.00^{a}	0.04±0.01^{a}	0.04±0.00^{a}
NO_2-N (mg l^{-1})	0.09±0.03^{a}	0.50±0.18^{a}	0.31±0.09^{a}	0.49±0.13^{a}	0.71±0.06^{a}	0.94±0.25^{a}	0.45±0.06^{a}	0.24±0.14^{a}	0.40±0.21^{a}
NO_3-N (mg l^{-l})	0.23±0.01 ^{a}	1.38±0.43^{b}	0.26±0.05 ^{a}	0.66±0.36^{a}	1.48±0.91^{a}	0.66±0.33^{a}	0.15±0.06	0.16±0.10	0.18±0.02
PO_4-P (mg l^{-1})	0.13±0.04^{c}	0.12±0.03^{b}	0.11±0.02^{a}	0.07±0.00^{a}	0.10±0.04^{a}	0.12±0.01^{a}	0.07±0.00^{a}	0.10±0.00^{ab}	0.11±0.01^{b}

*Data expressed as mean ± SE, n=3
*Mean value in the same column with different superscripts vary significantly (P<0.05)

Table 4: Soil quality parameters during nursing.

Parameter			T1	T2	T3
Initial	pH		6.15±0.15^{a}	6.25±0.09^{a}	6.21±0.02^{a}
	Nitrogen (%)		0.16±0.00^{a}	0.22±0.01^{a}	0.19±0.07^{a}
	Organic carbon (%)		0.51±0.05^{a}	0.82±0.05^{b}	0.80±0.9^{b}
	Phosphorus (%)		0.68±0.02^{a}	0.96±0.07^{a}	0.81±0.17^{a}
	Soil texture	Sand (%)	90.67±0.66^{b}	85.67±1.67^{a}	84.33±2.02^{a}
		Silt (%)	4.33±0.33^{a}	6.67±0.33^{b}	7.67±0.88^{b}
		Clay (%)	5.00±0.58^{a}	7.67±1.33^{ab}	8.00±1.15^{b}
Final	pH		6.19±0.12^{a}	6.33±0.16^{a}	6.23±0.10^{a}
	Nitrogen (%)		0.16±0.00^{a}	0.22±0.01^{a}	0.20±0.04^{a}
	Organic carbon (%)		0.68±0.10^{a}	0.87±0.02^{a}	0.92±0.4^{a}
	Phosphorus (%)		0.71±0.03^{a}	0.98±0.05^{a}	0.88±0.18^{a}
	Soil texture	Sand (%)	90.00±0.58^{a}	85.00±1.52^{a}	84.00±1.73^{a}
		Silt (%)	4.00±0.00^{a}	7.00±0.58^{a}	7.67±0.88^{a}
		Clay (%)	6.00±0.58^{a}	8.00±0.00^{a}	8.33±0.88^{a}

*Data expressed as mean ± SE, n=3
*Mean value in the same row with different superscripts vary significantly (p<0.05)

Table 5: Primary productivity (mg C m^{-2} h^{-1}) during rearing.

Parameter		T1	T2	T3
Initial	NPP (mg C m^{-2} h^{-1})	27.08±3.18^{a}	40.28±5.00^{a}	29.17±2.40^{a}
	GPP (mg C m^{-2} h^{-1})	57.64±4.22^{a}	56.94±3.67^{a}	50.00±4.16^{a}
Final	NPP (mg C m^{-2} h^{-1})	43.75±3.60^{ab}	53.13±4.77^{b}	39.58±2.08^{a}
	GPP (mg C m^{-2} h^{-1})	66.67±5.51^{a}	68.75±3.60^{a}	60.42±2.08^{a}

*Data expressed as mean ± SE, n=3
*Mean value in the same column with different superscripts vary significantly (p<0.05)

3.2 Growth, survival, production and feed utilization

The growth parameters are presented in Table 6. The mean initial length of was 0.05±0.00 cm and the weight was 0.002±0.00 g of all three Treatments. The mean final length and weight of spawn was found to be significantly (P<0.05) different in all three treatment at the end of the experiment. At the end of 15 days rearing period of spawn, the final mean length of T1, T2 and T3 was 4.24±0.12 cm, 3.85±0.18 cm and in 3.33± 0.16 cm, respectively and the final mean weight of T1, T2 and T3 was 0.76±0.01 g, 0.52±0.03 g and in 0.46±0.03 g, respectively.

From the Table 7, the final biomass (g) was found significantly highest (p<0.05) in T3 (3117.8±166.72) whereas the lowest biomass (g) found in the T1 (2567.3±21.29). The net fish yield (g) found significantly highest (p<0.05) in T3 (3109.8±166.71) whereas, the lowest in T1 (2563.3±21.36). The Hepato Somatic Index (HSI) was also found in the order of T1 (0.49±0.011), T2 (0.40±0.008) and T3 (0.37±0.017). The relative fatness for the different treatments was found significantly highest (p<0.05) in T3 (1.18±0.10) whereas the lowest in T2 (0.86±0.11). The condition factor (K) was observed at diminishing order from T3 (1.30±0.20), T1 (1.01±0.135) and T2 (0.94±0.209).

Three apparent feed utilization parameters are presented in Table 8. The Apparent feed conversion ratio (AFCR) did not show any significant different (p>0.05), it was found highest in T3 and lowest in T1. The apparent protein efficiency ratio (APER) was showed significant difference (p<0.05), It was significantly highest in T1.

Table 6: Growth performance of *Cirrhinus reba* (Reba carp) in different treatments

Parameter	Treatment		
	T1	T2	T3
Initial mean length(cm)	0.05±0.00^{a}	0.05±0.00^{a}	0.05±0.00^{a}
Initial mean weight(g)	0.002±0.00^{a}	0.002±0.00^{a}	0.002±0.00^{a}
Initial mean stock (nos.)	6000±0.00^{a}	10000±0.00^{a}	14000±0.00^{a}
Mean final Length (cm)	4.24±0.12^{b}	3.85±0.18^{ab}	3.33± 0.16^{a}
Mean final weight (g)	0.77±0.00^{b}	0.53±0.03^{a}	0.47±0.03^{a}
Mean length gain (cm)	3.74±0.21^{b}	3.35±0.18^{ab}	2.83±0.16^{a}
Mean weight gain (g)	0.76±0.01^{b}	0.52±0.03^{a}	0.46±0.03^{a}
Mean final stock (nos.)	3347.6±4.85^{a}	5120.6±5.17^{b}	6558.3±3.70^{c}
Mean body weight gain (%)	76610 ±7.66^{b}	52766 ±2.98^{a}	47450 ±2.61^{a}
Specific growth rate (SGR) (%)	44.28±0.07^{b}	41.78±0.37^{a}	41.07±0.38^{a}
Mean daily weight gain (MDWG) (g)	0.05±0.000^{b}	0.03±0.003^{a}	0.03±0.000^{a}
Mean daily weight gain (MDWG) (%)	5.12±0.05^{b}	3.52±0.20^{a}	3.16±0.17^{a}

*Data expressed as mean ± SE, n=3
*Mean value in the same row with different superscripts vary significantly (p<0.05)

Table 7: Yield parameters of Reba in different treatments.

Parameter	Treatment		
	T1	T2	T3
Condition factor (K)	1.01±0.135^{a}	0.94±0.209^{a}	1.30±0.20^{a}
Net fish yield (g)	2563.3±21.36^{a}	2694.2±126.39^{b}	3109.8±166.71^{b}
Total biomass (g)	2567.3±21.29^{a}	2704.2±126.39^{b}	3117.8±166.72^{b}
Hepato Somatic Index (HSI)	0.49±0.011^{a}	0.40±0.008^{a}	0.37±0.017^{a}
Relative Fatness(RF)	0.94±0.07^{a}	0.86±0.11^{a}	1.18±0.10^{a}
Overall Survival (%)	55.80±0.81^{c}	51.21 ±0.57^{b}	46.85±0.26^{a}

*Data expressed as mean ± SE, n=3
*Mean value in the same row with different superscripts vary significantly (p<0.05)

Table 8: Mean feed utilization parameters for different treatments

Parameter	Treatment		
	T1	T2	T3
Apparent Feed Conversion Ratio (AFCR)	0.57±0.02[a]	0.58±0.06[a]	0.62±0.05[a]
Apparent Protein Efficiency Ratio (APER)	5.80±0.17[b]	5.04±0.9[a]	5.34±0.19[ab]

*Data expressed as mean ± SE, n=3
*Mean value in the same row with different superscripts vary significantly ($p<0.05$)

3.3 Digestive enzyme's activities
The mean final digestive enzyme's activities (Protease, Lipase and Amylase) are presented in the Table 9. The protease, lipase and amylase activity of Reba spawn did not show any significant variation ($p>0.05$) among different treatments. The highest protease activity was found in treatment T1 (1.32 units/ mg protein/ min) while lowest was obtained in treatment T3 (1.09 units/ mg protein/ min). Highest lipase activity was found in T1 (0.47 units/ mg protein/ hr) and lowest in T3 (0.39 units/ mg protein/ hr). In case of amylase activity all treatments showing same result (0.03 units/ mg protein/ min) and lowest in both T4 and T1 (0.04 units/ mg protein/ min).

Table 9: Digestive enzyme's activities of fry

Parameters	Treatment		
	T1	T2	T3
Protease (units/ mg protein/ min)	1.32±0.16[a]	1.16±0.21[a]	1.09±0.16[a]
Amylase (units/ mg protein/ min)	0.03±0.01[a]	0.03±0.03[a]	0.03±0.01[a]
Lipase (units/ mg protein/ hr)	0.47±0.21[a]	0.42±0.20[a]	0.39±0.22[a]

*Data expressed as mean ± SE, n=3
*Mean value in the same row with different superscripts vary significantly ($p<0.05$)

4. Discussion
Physico-chemical parameters play a significant role for the production of natural fish food organisms and also maintain a healthy ecosystem for fish. Growth, feed efficiency and feed consumption of fish are normally dependent on environmental factors [6, 16]. In the present study the rearing practices was carried out in the suitable temperature range of 26.20-29.37 ^{0}C which is closed to observations made by [19, 20, 30]. The pH value was suitable for nursery rearing (7.65-8.00) as also observed by [30, 35]. The dissolved oxygen (6.67-9.33 mg l^{-1}) and alkalinity (55.33-118.67 mg l^{-1}) was also within the acceptable range indicating medium level of productivity. All the other studied water quality parameters (Table 3) were in permissible range during nursing of spawn. Similar observations were made by [5, 34, 35]

Gross and net primary productivity (mg C^{-1} m^2 hr^{-1}) of experimental water was in the range of 50.00±416[a] to 68.75±3.60[a] and 27.08±3.18[a] to 53.13±4.77[b] respectively. There was a trend of increment in primary productivity once the tanks were fertilized time to time which reflected with higher growth of the experimental fishes. Similar observation were made by [20, 30, 35, 40] in various carps and barb nursery ponds.

The pH of the red soil was 6.15-6.33, sandy (84.00-90.67%) in texture, low in organic carbon (0.51-0.91%), nitrogen (0.16-0.22%) and phosphate (0.68-0.98%) indicating poor in productivity and acidic in nature. However, use of lime and manure improved the productivity status suitable for seed rearing practices reflected by higher survival and growth rate.

It is well established in culture of fish that the stocking density is a critical factor for their growth and survival for many aquatic animals [37, 45]. It is directly related with the competition for food and space [29, 37]. In the present study, the growth parameters were observed and compared to three different stocking densities at the end of 15 days of experiment on spawn rearing period. The results showed that the mean final length, mean final weight and specific growth rate was found significantly difference ($p<0.05$) in different stocking densities although the same feed was applied at an equal ratio and same feeding frequency. The significantly, ($p<0.05$) highest mean final weight (0.77±0.00 g) and mean final length (3.74±0.21 cm) was found in the in T1 and lowest mean final weight (0.47±0.03 g) and lowest mean final length (2.83±0.16 cm) in T3. The results also exhibited that the growth found to be increased with the decreasing stocking density. It might be due to the less competition for feed and space in treatment T1 and unavailability of proper feed and space in treatment T3 or due to more stress in high stocking or vice versa. The other growth parameters like specific growth rate (SGR), mean weight gain and mean length gain were also found to be inversely proportion to stocking density. It has been observed that 11.20% and 61.45% additional growth gain was observed in T2 and T1 respectively in compared to T3. A competitive interaction happened when the larvae are stocked at the high densities and also create stressful situation in rearing system with the presence of abundant food interaction [20, 21, 35]. Current study is in agreement with the observation made by [26].

In the current study, the survival percent was found significantly different ($p<0.05$) in different stocking density. Significantly ($p<0.05$) highest survival was found (55.80±0.81) in T1 and lowest survival percent was (46.85±0.26) in T3. In the present study, the survival percent found to be increased with the decreasing stocking densities. The causes of comparatively low survival in treatment T3 and T2 might be due to the more competition for food and space as well as enhanced stress due to high stock of fry. Additional survival was noticed in lower stocking densities in T1 (7.81%) and T2 (1.72%) as compared to T3. This study is also in conformity with the research findings of [19, 20, 30, 34, 35, 40, 44] during seed rearing experiments of various indigenous/exotic carps and barb species. In the present study, the survival percent was high (69.53±2.23 to 88.33±2.68) which in accordance with findings of [36] in their study on *C.ariza* (67.84±2.71 to 85.76±2.09). Generally, fish needs to compete less for food and space in lower stocking densities than the higher stocking densities.

The total biomass was found to be increased with increasing of stocking density. it was found significantly highest ($p<0.05$) in T3 (3117.8±166.72 g) whereas the lowest was in the T1 (2567.3±21.29 g). Hepato somatic index (HSI) is known indices of well-being fish in storing energy level in the fish liver. Fish grows in poor environment usually have a smaller liver (with less energy reserved in the liver). In the present study, significantly higher ($p<0.05$) HSI value was noticed in T1 (0.4882±0.011) compared to other treatments. In case of condition factor (k) there was no significance difference was found among the treatments during rearing. The highest condition factor was noticed in treatment T3 (1.0147±0.135. It is well known that a high condition factor reflects good environmental quality [17]. The values obtained from this study were high and hence suggested that the species was reared in good environmental condition. Relative fatness or coefficient of condition is widely used in fish biology as an index to illustrate the environmental suitability. In the present study, there was no significant difference in relative fatness among the treatments during rearing. Hence, the current study is in accordance with the findings of [22], who observed that condition factor is not affected significantly by stocking density.

The apparent feed conversion ratio (AFCR) was did not show any significant different (p>0.05). The lowest AFCR was noticed in T1 (0.57±0.02) and highest in T3 (0.62±0.05). The results of the present study indicated that utilisation of feed; higher growth was obtained with low stocking density. It may be due to smaller size of ration, higher digestibility feed and proper utilization of feed. The present study is also in close proximity with the AFCR values reported by [10, 11, 24, 25, 34, 36], stated that digestibility plays an important role in lowering the AFCR value by efficient utilization of food. Digestibility depends on daily feeding rate, frequency of feeding, and type of food used [9]. [10] observed increasing trends of AFCR values with increasing ration size in the growth trial of Indian major carp (*Labeo rohita*). [18] found increasing AFCR values with increasing ration size by feeding common carp (*Cyprinus carpio*) with supplementary feed. However, the lower AFCR value in the current study indicates better food utilization efficiency where the value of AFCR decreased with the decrease of stocking densities. The Apparent protein efficiency ratio (PER) was a measure to show as to how well the protein sources in the diet could provide essential amino acid requirement of the fishes. In the present study, the results showed significantly (p<0.05) highest protein efficiency ratio in T1 (5.80±0.17) as compared with lowest in T2 (5.04±0.9). Highest APER in T1 was correlated with its highest growth and high intestinal protease activity during the experimental period. The present study was found close agreement with the findings [41] where APER ranged from 0.12-2.24 in common carp.

While studying the digestive enzyme activity of the experimental fishes, the protease, lipase and amylase activities did not show significant differences (p>0.05) during nursing. Regardless of no statistical difference, protease activity was highest in T1 (1.32 units/ mg protein/ min) while lowest was obtained in T3 (1.09 units/ mg protein/ min). Same trend was also noticed in lipase activity. Amylase activity in all the treatments was same (0.03 units/ mg protein/ min). The enzymatic activities were within the normal range exhibiting tendency of higher activity in the treatments where stocking was low and exhibited higher growth. Present findings is in agreement with the findings of [15] who elaborately studied on the ontogeny of digestive enzymes in common carp larvae as well as with the findings of [28] who revealed that in general, highest enzyme activities were observed among larvae of *Mystus nemurus*, which fed on a combination diet.

5. Conclusion

From the present study, it can be concluded that the growth, survival of *C. reba* spawn were inversely related to the stocking densities of hatchlings. Stocking density of 3.0 million hatchlings ha^{-1} may be desirable for nursing of *C. reba* for 15 days. Production of adequate quality seeds through application of our present findings might be extremely helpful towards the protection of *C. reba* from extinction as well as for its conservation and rehabilitation in north east India.

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