Journal of Entomology and Zoology Studies
Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(3): 608-612

Received: 24-03-2018
Accepted: 25-04-2018

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Carbon budget for nursing of *Cirrhinus reba* (Hamilton, 1822) with different stocking density in north-eastern India (Tripura)

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Abstract
Carbon budget was performed for the nursing of spawn of *Cirhhinus reba* in 9 tanks for 15 days and each of 20 m^2 water area. Three different treatments @ T1: 3.0, T2: 5.0 and T3: 7.0 million ha^{-1} spawn were maintained. The experimental spawn were fed with 30% protein diet in powder form and it was formulated by using soybean meal, mustard oil cake, fish meal, wheat bran, broken wheat, corn flour and vitamin mineral mixture in appropriate proportion. The tanks were fertilized uniformly by application of lime @300 kg ha-1 and mustard oil cake @750 kg ha^{-1}. The recovery of carbon was highest in T1 (44.58%) followed by T3 (41.84%) and T2 (40.02%). At the time of harvest discharged water accounted for 44.37- 38.58% OC of the total inputs. Organic carbon accumulation increased in both the discharged water with increase in stocking density.

Keywords: *Cirrhinus reba*, nursery, carbon budget, growth, spawn

Introduction

Aquaculture is defined as "the planned and purposeful production of aquatic animals and plants". Activities of the Aquaculture involve a variety of inputs for Indian major carps as well as in minor carp production including manures, fertilizers, feed and a combination of all these things. In a pond ecosystem, most of the nutrients are distributed in the water, fish biomass and sediment of the pond. Sediment of the pond plays an important role in the balance of culture system; it can act as a buffer in water nutrient concentration [8]. It has been observed that the sediment layer of few centimetres depth contains more nutrients than the content of the water column [5]. Generally farmers believed that a large proportion of nutrients received in ponds end up in pond mud and discharged effluents. It is essential to estimate the nutrient budget to assess the fate of nutrients added to the pond culture systems and to reduce the nutrient losses in discharged water. Nutrient budget could permit quantification of potential pollution impact of a specific pond management strategy. Nutrient budget has been formulated for experimental ponds containing channel catfish [6], striped bass [10], tilapia [14], high densities of shrimp [14, 26] and shrimp [35]. Nitrogen and phosphorus budgets were formulated for commercial shrimp ponds in Thailand [7, 36], extensive shrimp ponds in Bangladesh [38], semi-intensive shrimp farms in Mexico [29] and intensive shrimp farms in Australia [21]. It has been observed that the recovery of organic carbon decreases with the increase in stocking densities of experimental carps in earthen pond systems [1]. *Cirrhinus reba*, reba is an important food fish [23, 30], and considered as one of the most important indigenous minor carp species in North East India. It is a good source of protein, calcium and low fatty acid, as well as an ideal dietetic food for human consumption [2]. The nutrient budget for Indian major carps as well as in minor carp's culture is scanty. Hence, it is proposed to assess the nutrients organic carbon (OC) uptake by the reba seed at three different stocking density levels.

2. Materials and methods

2.1 Experimental site and design

The study was carried out in the cement cistern tanks of 20 m^3 (5m4m×1m) each which were at Department of Aquaculture, College of Fisheries, Lembucherra, Tripura, the experiment were conducted during monsoon season of the year, 2016. The cemented tanks were selected in completely randomized design (CRD), divided into three treatments namely T1, T2 and T3, each having three replications. Different stocking densities spawn of Reba *viz.* 3 (T1), 5 (T2) and 7 (T3) million ha^{-1} were taken. The experiment was conducted for 15 days.

2.2 Tank preparation, fertilisation and stocking
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 [23, 28]. 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 [3]. The seed of *C. reba* were collected from department of Aquaculture College of Fisheries, Lembucherra, Tripura and acclimatized before stocking and it was done in morning hours. The experimental ponds were stocked with an initial length and weight of 0.05 cm and 0.002 g respectively.

2.3 Diet preparation and feeding
The diet for the experimental seed was formulated with 30% protein 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 Growth performance
Growth in terms of length and weight, specific growth rate (SGR), survival (%), total biomass (g) and apparent feed conversion ratio (AFCR) were evaluated at the end of experiment.

2.5 Carbon budget
Total organic carbon (OC) were calculated based on input, output, uptake and accumulation in the culture system during the nursing period were measured by following method.

2.5.1 Organic carbon in soil sediment
Total organic carbon (OC) of the soil and in the fish was determined using rapid titration method [39].

2.5.2 Organic carbon in Water
The total organic carbon in water was determined by using chromic acid rapid titration method [4].

2.5.3 Organic carbon input in the form of feed and fish was calculated as follows:
Organic carbon (OC) in feed= nutrient concentration in feed × total amount of feed supplied.
Organic carbon (OC) in fish = nutrient concentration in fish carcasses × total fish biomass [27].

2.6 Analytical method and 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 (Duncan, 1955; Zar, 1999). In all cases, value of $P<0.05$ was considered significant.

3. Results
3.1 Growth performance
Seed rearing and production details are presented in Table 1. The survival rate of the seed varied from 46.85 to 55.80% with an average of 77%. At the end of experiment, the average weight of seed ranged from 0.47 to 0.77 g. The apparent feed conversion ratio (AFCR) ranged from 0.57 to 0.62.

Table 1: Detailed growth performance

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}
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}
Specific growth rate (SGR, %)	44.28±0.07^{b}	41.78±0.37^{a}	41.07±0.38^{a}
Total biomass (g)	2567.3±21.29^{a}	2704.2±126.39^{b}	3117.8±166.72^{b}
Overall Survival (%)	55.80±0.81^{c}	51.21 ±0.57^{b}	46.85±0.26^{a}
Apparent Feed Conversion Ratio (AFCR)	0.57±0.02^{a}	0.58±0.06^{a}	0.62±0.05^{a}

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

3.2 Organic carbon budget during spawn rearing
Organic carbon budget input, output and accumulation of the carbon in experimental tank was measured and are presented in the Table 2. The total input of OC was found 449.06±8.49 g, 526.16±2.20 g and 580.00±5.49 g in treatments T1, T2 and T3 respectively. Organic carbon output in the form of fish was found 200.00±1.66 g, 210.66±9.84 g and 242.88±1.29 g in the treatments T1, T2 and T3 respectively. The organic carbon output in the form of discharged water in the treatments T1, T2 and T3 was found 160.00±9.23 g, 153.60±4.88 g and 152.00±3.33 g respectively**.** The total output of organic carbon was found 360.00±1.05 g, 364.26±4.97g and 394.88±9.88 g in treatments T1, T2 and T3 respectively. Significance different ($P<0.050$) were found in the recovery of organic carbon in between the three different stocking densities.

Table 2: Organic carbon budget in seed rearing of reba

Parameters		Organic carbon (g)			Organic carbon (%)		
		T1	T2	T3	T1	T2	T3
Input	Feed	240.64±8.97[a]	317.74±2.25[b]	376.37±8.75[c]	53.55±0.98[a]	60.24±1.74[b]	64.88±0.91[c]
	Water	76.27±0.53[a]	76.27±0.53[a]	71.47±3.73[a]	17.00±0.42[c]	14.55±0.69[b]	12.33±0.72[a]
	Fertilizer (MOC)	132.16±0.0[a]	132.16±0.0[a]	132.16±0.0[a]	29.45±0.55[b]	25.21±1.05[a]	22.79±0.23[a]
	Total input	449.06±8.49[a]	526.16±2.20[b]	580.00±5.49[c]	100.00	100.00	100.00
Output	Fish	200.00±1.66[a]	210.66±9.84[ab]	242.88±1.29[c]	55.63±1.28[a]	57.78±1.89[a]	61.42±1.81[a]
	Water	160.00±9.23[c]	153.60±4.88[b]	152.00±3.33[a]	44.37±1.28[a]	42.22±1.89[a]	38.58±1.28[a]
	Total output	360.00±1.05[a]	364.26±4.97[a]	394.88±9.88[b]	100.00	100.00	100.00
Nutrient retention		89.07±1.89[a]	161.91±1.71[b]	185.12±4.59[b]	19.69±3.85[a]	30.61±1.97[b]	31.94±1.07[b]
Sediment accumulation		42.89±1.67[a]	30.32±8.70[a]	33.93±3.161[a]	9.42±3.54[a]	5.92±1.88[a]	5.85±0.52[a]
Others		46.18±3.42[a]	131.58±25.85[b]	151.19±7.40[b]	10.27±0.67[a]	24.69±3.85[b]	26.09±1.45[b]
Nutrient recovered by fish		200.00±1.66[a]	210.66±9.84[ab]	242.88±1.29[b]	44.58±1.14[a]	40.02±0.37[ab]	41.84±1.84[b]

Data expressed as mean ± SE, n=3

Mean value in the same row with different superscripts vary significantly ($P<0.05$)

4. Discussion

In aquaculture, it is well established that the stocking density is a critical factor for their growth and survival for many aquatic animal [33, 40]. Stocking density is related to the volume of water per fish in pond. It has been observed that the increase in stocking density results in increased stress, which leads to higher energy requirement, causing a reduction in growth rate and food utilization [24]. It is directly related with the competition for food and space [24, 33]. The results of present study showed that the mean final length, mean final weight and specific growth rate was varied significantly difference ($P<0.05$) in different stocking densities. Although the same feed was applied at an equal ratio and same feeding frequency. The specific growth rate (SGR) was also found to be inversely proportional to stocking density. The results also exhibited that the growth found to be increased with the decreasing stocking density. The survival % was found significantly different ($P<0.05$) in different stocking densities during spawn rearing period. 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. 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 [18, 16, and 31]. Current study is in agreement with the observation made by Jena *et al.* [22} who experimented on the growth of Indian major carp for 15 days where found highest growth with lowest stocking density (2.5 million ha^{-1}) in all three Indian major carps. This study is also in agreement with the research findings of [37, 34, 15, 16, 25 and 31] during fry/fingerlings 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 [32]. The lowest FCR was noticed in T1 (with less stocking density and highest in T3 with higher stocking density. The results of this 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. This study is also in close proximity with the FCR values reported by authors [11, 19, 20, and 32]. [12] Author stated that digestibility plays an important role in lowering the FCR value by efficient utilization of food. Digestibility depends on daily feeding rate, frequency of feeding, and type of food used [9]. It has been observed increasing trends of FCR values with increasing ration size in the growth trial of Indian major carp (*Labeo rohita*)[11]. However, the lower FCR value in the current study indicates better food utilization efficiency where the value of FCR decreased with the decrease of stocking densities.

Organic carbon budget analysis was carried out in all the treatments in replicates during rearing period of Reba seed to understand the nutrient recovery pattern in close system. The total input of organic carbon in the form of feed, water and fertilizer was noticed 449.06±8.49 g, 526.16±2.20 g and 580.00±5.49 g in treatments T1, T2 and T3 respectively during spawn rearing. The total output of organic carbon in the form of fish and water was found 360.00±1.05 g, 364.26±4.97 g and 394.88±9.88 g in treatments T1, T2 and T3 respectively. Significantly highest ($P>0.05$) recovery of organic carbon was observed in T1 (44.58±1.14%) with lowest stocking density (3 million ha^{-1}) followed by T3 (41.84±1.84) and T2 (40.02±0.37). An additional recovery in T3 and T1 was noticed @ 4.54% and 11.38% respectively compared to T2. The findings of present study confirmed the outcome of [1] where authors stated that the recovery of organic carbon decreases with the increase in stocking densities of experimental carps in earthen pond systems.

5. Conclusion

From the present investigation it is revealed that significantly ($P<0.05$) highest organic carbon (44.58±1.14%) recovery (with improved growth, survival and AFCR) was found in the in treatment T1 with lowest stocking density (3 million ha-1) in nursery rearing of spawn of C. reba. The proper stocking density with good quality feed, fertilizers and better management practices could help to reduce the OC loss through the harvested water and bottom soil sediment. However the further research is needed in aspect of nutrient budget utilization in carp culture system. Hence, it is recommended that the stocking density of the Reba spawn for nursery rearing could be maintained @ 3 million ha-1 for obtaining higher recovery of OC, survival and growth.

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