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Comparative study on growth performance and survival of Indian major carps and male monosex tilapia in recirculating aquaculture system

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ABSTRACT

An experiment was performed in a recirculatory aquaculture system (RAS) to carry out a comparative study on the growth performance and survival of Indian major carps (*Labeo rohita* and *Gibelion catla*) and monosex tilapia (*Oreochromis niloticus*) fingerlings for a period of 90 days. Ten fingerlings each of *L. rohita* (average weight 30±0.1 g), *G. catla* (average weight 28±0.1 g) and *O. niloticus* (average weight 31.5±0.2 g) were distributed randomly into twelve cages, in quadruplicates. *L. rohita* and *G. catla* were fed commercial floating feed (30% crude protein and 5% crude lipid) *ad libitum* while *O. niloticus* were initially fed at 3% of the body weight upto 100 g size and was changed to 2% of the body weight. There was significant ($p<0.05$) increase of daily weight gain, percentage weight gain and specific growth rate (SGR) in *O. niloticus* followed by *L. rohita* and *G. catla*. Significantly lower ($p<0.05$) feed conversion ratio (FCR) and higher ($p<0.05$) protein efficiency ratio (PER), protein productive value (PPV) and lipid productive value (LPV) were found in *O. niloticus* followed by *L. rohita* and *G. catla*. At the end of the experiment, survival was similar for all three fish species, however total biomass was significantly ($p<0.05$) higher in *O. niloticus* followed by *L. rohita* and *G. catla*. The findings of this study showed the potential of RAS as an alternative to the pond culture of monosex tilapia and rohu.

Keywords: Growth performance, Indian major carps, Monosex tilapia, Recirculatory aquaculture system, Survival

Introduction

Aquaculture is still the fastest-growing food-producing sector with a contribution of 114.5 million t to the total fish production having 263.6 billion USD value. This industry, which accounts for 46% of the total production and 52% of fish for human consumption (FAO, 2020), still has a lot of scope to grow in terms of income for fisheries and fish farmers. The global population is increasing at a rate of 1.1% each year and is projected to hit 8.5 billion by the end of 2030. According to the FAO, *per capita* intake of food fish will rise 1.8% by 2030. This will place additional strain on farm production, as marine capture fisheries are projected to decline over time due to overfishing. Farmers have used a variety of aquaculture systems, including cage culture, pen culture, irrigated or flow-through systems, tanks and raceways and so on. However, since these systems and methods necessitate a larger land area, a marginal farmer with a smaller land area would be unable to follow these farming systems. As such, to achieve maximum production from an equivalent area of land, sustainable intensification approaches are needed while keeping the effects on environment in check (Godfray *et al.*, 2010).

The recirculating aquaculture system (RAS) is regarded as the future sustainable technology for land-based fish farming because of the many advantages over traditional methods of open and land-based aquaculture systems. In RAS, the water sources are mostly borehole where the chances of bacterial contamination are extremely low. Artificial feed is fed to the fish and all parameters are artificially controlled, so the influence of natural conditions is negligible. RAS is environment friendly due to its low waste generation, effective feed consumption and water reuse. Therefore, in areas where land and water are scarce, small-scale fish farmers, women and entrepreneurs may use this system to promote fish production, increasing their income and sustaining their livelihood.

India is the second-largest aquaculture fish producer and third largest fish producing country in the world. Fish production increased from 10.76 million t in 2015-16 to 14.73 million t in 2021-22 with production from inland sector being 11.25 million t (GoI, 2022). As compared to other protein sources, Indians consume a lot of fish with a *per capita* consumption of 10.5 kg (GoI, 2020), however to meet the demands of the ever-growing population, fish supply in the country still remains low. Thus, species diversification is the need of the hour in today's world, especially for a country like India where people are gradually opting different fish species for consumption instead of a single species based on its taste, nutritional value, price, availability and quality which pushes the drive to produce multiple species on a greater commercial scale.

Carps form the mainstay of this sector contributing 6.43 million t to the total fish production with rohu (*Labeo rohita*) and catla (*Gibelion catla*) comprising the bulk of the catches. Their higher growth rate, compatibility with other carps, specific feeding habit, easy availability of seeds and high consumer preferences have made them suitable candidate species for aquaculture especially in India where carps dominate the fish markets with catla being the second most important species after rohu. On the other hand, tilapias (*Oreochromis* spp.) are second only to carps in terms of table size fish production globally having some distinctive characteristics that drive their continued progression and might exceed carp production within a short time span. Monosex tilapia culture is gaining prominence in recent years in RAS due to its quicker growth, resistance to overcrowding and poor quality of water (Appiah-Kubi, 2012). Therefore, the present study was aimed at comparing the culture potential of rohu, catla and monosex tilapia, when reared in RAS.

Materials and methods

Experimental site

The experiment was performed over a period of 90 days in twelve 1.0 m^3 hapa (made of high density polythene) within recirculating aquaculture system (RAS) at College of Fisheries, Dholi, Muzaffarpur, Bihar, India. During the trial, dissolved oxygen was kept above 85% of saturation, ammonia-N was below 0.1 mg l^{-1} and pH was in the range 7.9 and 8.5. The RAS unit included an overhead sump tank, two 1.0 hp centrifugal pump, four air injectors and a trickling biofilter containing bio-balls. In order to prevent any fish from jumping out and escaping, the cages were covered with polyethylene net having 0.5 mm diameter mesh size.

Fish and feeding

Rohu (*L. rohita*) and Catla (*G. catla*) fingerlings having average weight of 30±0.1 and 28±0.1 g respectively were procured from Patahi Fish Farm, Muzaffarpur, whereas monosex tilapia (*Oreochromis niloticus*) fry were procured from M. M. Fish Farm, Raipur, Chhattisgarh and reared upto fingerling size (average weight 31.5±0.2 g) at College of Fisheries, Dholi. Ten fingerlings per cubic meter of either *L. rohita* or *G. catla* or *O. niloticus* were stocked randomly in quadruplicates into twelve cages installed in RAS. Commercial floating feed (1.8 mm diameter) containing 30% crude protein and 5% crude lipid were fed *ad libitum* to *L. rohita* and *G. catla* while *O. niloticus* were initially fed at 3% of the body weight upto 100 g size and thereafter the feeding rate was changed to 2% of the body weight. The feeding frequency of *O. niloticus* was divided into five equal parts and the daily ration was fed at every two hour interval from 08:00 to 16:00 hrs.

Water quality measurements

Alkalinity, hardness, ammonia, dissolved oxygen, nitrite, nitrate, temperature and pH were measured in each cage weekly throughout the tenure of the experiment according to the conventional techniques specified in APHA (1998).

Total bacterial load analysis

Total bacterial load in the inlet freshwater, RAS water and bio-ball present in biological filter was determined weekly throughout the tenure of the experiment, according to Malik (1992). Samples of water were collected from inlet water and RAS water, while bacterial film scrapped from bioballs were collected and diluted in 100 ml posphate buffered saline (PBS) for further analysis. Serial dilution and plating technique were used for enumeration of bacterial load per ml of sample.

Fish sampling and analysis

Body weight of all fishes was assessed at every 15-days intervals to monitor the growth performance. All the fishes were starved one day before measurement of body weight. Five fish from the stock of each fish species were randomly selected and sampled at the beginning prior to stocking, while three fishes from each cage were weighed and sampled at the end of the experiment for proximate evaluation. Proximate analysis of fish carcass was done in the Fish Processing Laboratory of COF, Dholi, following the conventional techniques detailed in AOAC (1995). Moisture was determined by drying sample at 105^{0}C to a constant weight. Nitrogen content of the sample was analysed using Kjeltec semi-automated system [(KELPLUS CLASSIC DX VATS (P)] and crude protein (CP) was calculated by multiplying nitrogen (%) by 6.25. Crude lipid (CL) was determined using Soxtec system (SCS06 AS DLS TS) using diethyl ether (boiling point 40-60^{0}C) as a solvent and ash content was estimated by incinerating the sample in a muffle furnace at 600^{0}C for 6 h. Total carbohydrate was calculated by differences as carbohydrate (%) = 100 - (CP% + CL% + ash%). The digestible energy value of diets was calculated as described by Halver (1976).

Growth parameters

The following indices were used to assess the growth performance of the fishes:

Weight gain (WG, %) = $(W_t - W_0)/W_0 \times 100$

Daily weight gain (g day^{-1}) = $(W_t - W_0) / t$;

Specific growth rate (SGR) (g %) = $[(\log_e W_t - \log_e W_0)/t] \times 100$;

where W_t , W_0 are final and initial weights of the fish; *t* is the feeding duration (in days).

Nutrient utilisation parameters

The following indices were used to calculate the nutrient utilisation:

Feed conversion ratio (FCR) = FI / (W_t-W_0)

Protein efficiency ratio (PER) = (W_t-W_0) / Protein fed

Protein productive value (PPV) = ($P_t \times W_t$) - ($P_0 \times W_0$)/ Total protein fed

Lipid productive value (LPV) = ($L_t \times W_t$) - ($L_0 \times W_0$)/ Total lipid fed

where P_t , P_0 are final and initial body protein; L_t, L_0 are final and initial body lipid and FI is feed intake.

Survival (%)

Survival (%) was calculated at the end of the experiment by counting the number of fish in each cage and was calculated as:

Survival (%) = (Total number of fish in each cage/Initial stocked number of fish) × 100

Total biomass production

At the end of the experiment, total fish harvested from each cage were weighed and total biomass production was calculated as:

Total biomass production (kg m^{-3}) = Final weight of all fishes in one cage/Volume of cage

The feeding trial and subsequent handling and sampling of the experimental fish were carried out as per the guidelines of Dr. Rajendra Prasad Central Agricultural University, Bihar, India.

Statistical analysis

The treatment effects on the mean values of all parameters were subjected to one-way ANOVA ($p<0.05$) in IBM SPSS Statistics 26 software. For *post-hoc* comparisons between treatments, Duncan's Multiple Range Test (DMRT) was performed and data presented as mean±standard error.

Results

Total bacterial count

Total plate counts (TPC) differed significantly among rearing water, ground water and bioballs and ranged from 1.96×10^7 to 2.72×10^7 CFU ml^{-1} (Table 1).

Proximate composition of feed and fish carcass

The proximate composition of commercial diet fed to experimental animals during the experimental period is given in Table 2. Carcass composition of experimental fish was significantly ($p<0.05$) different among the treatment groups (Table 3). The moisture content was significantly ($p<0.05$) higher in *G. catla* (72.67±0.23) as compared to *L. rohita* (71.56±0.23), but similar to *O. niloticus* (71.45±0.25). The crude protein content of *G. catla* (14.27±0.12) was significantly ($p<0.05$) lower as compared to *O. niloticus* (15.54±0.21) and *L. rohita* (15.18±0.09). The crude lipid content was significantly ($p<0.05$) higher in *O. niloticus* (6.60±0.03) than *L. rohita* (6.11±0.13) and *G. catla* (5.87±0.17). The total ash content values were significantly ($p<0.05$) lower in *O. niloticus* (2.91±0.07) than *G. catla* (3.57±0.04) and *L. rohita* (3.35±0.12).

Growth performance

The growth performance differed significantly ($p<0.05$) among species (Table 4). Body weight in the three species increased linearly with time until termination (Fig. 1). The initial body weights of *O. niloticus*, *G. catla*,

Table 1. Total bacterial load at different sites

Site of sample collection	Total aerobic plate count (CFU ml^{-1})
Bio-balls	$2.72^a \times 10^7$
RAS water	$1.85^b \times 10^7$
Inlet freshwater	$1.96^b \times 10^7$

Data expressed as mean±SE; Mean values in the column with different superscripts vary significantly ($p<0.05$).

Table 2. Proximate composition of commercial diet fed to experimental animal (% Dry weight basis)

Parameter	Mean±SE
Organic matter (%)	92.90±3.15
Crude protein (%)	29.45±1.12
Crude lipid (%)	4.68±0.27
Ash (%)	7.10±0.24
Carbohydrate (%)	58.77±2.16
*Digestible energy (KJ 100 g^{-1})	124.06±6.55

Calculated digestible energy, DE (KJ 100 g^{-1}) 5 [(CP% x 4) + (EE% x 9) + (TC% x 4)]/3.184

Table 3. Proximate composition of carcass of *O. niloticus*, *G. catla* and *L. rohita* fingerlings (% wet weight basis)

Parameter	Treatment groups		
	Tilapia	Catla	Rohu
Moisture	71.45^b±0.25	72.67^a±0.23	71.56^b±0.23
Crude protein	15.54^a±0.21	14.27^b±0.12	15.18^a±0.09
Crude lipid	6.60^a±0.03	5.87^b±0.17	6.11^b±0.13
Ash	2.91^b±0.07	3.57^a±0.04	3.35^a±0.12
Carbohydrate	3.50±0.12	3.61±0.17	3.80±0.14

Data is expressed as mean±SE; Mean values in the same row with different superscripts vary significantly ($p<0.05$)

Table 4. Growth performance of *O. niloticus*, *G. catla* and *L. rohita* fingerlings reared in RAS

Parameter	Treatment groups		
	Tilapia	Catla	Rohu
WG (%)	782.61^{a}±8.05	119.84^{c}±6.51	271.94^{b}±4.92
DWG (g day^{-1})	2.73^{a}±0.02	0.37^{c}±0.01	0.91^{b}±0.01
SGR (g %)	2.42^{a}±0.01	0.87^{c}±0.03	1.46^{b}±0.01

Data expressed as mean±SE; Mean values in the same row with different superscripts vary significantly ($p<0.05$). WG: Weight gain, DWG: Daily weight gain, SGR: Specific growth rate

L. rohita were 31.50±0.20, 28.0±0.40 and 30.25±0.48 g respectively. At the end of 90 days of experimental period, the final body weight in *O. niloticus* was significantly ($p<0.05$) higher (278.0 g) than *L. rohita* (112.5 g). *G. catla* had the lowest final mean body weight (61.5 g). Percentage weight gain of *O. niloticus* (782.61±8.05) fingerlings was the highest ($p<0.05$), followed by *L. rohita* (271.94±4.92) and lowest in *G. catla* (119.84±6.51). Daily weight gain was significantly ($p<0.05$) higher in *O. niloticus* (2.73±0.02 g day^{-1}) than in *L. rohita* (0.91±0.01 g day^{-1}) and *G. catla* (0.37±0.01 g day^{-1}). Specific growth rate (SGR) was significantly higher ($p<0.05$) for *O. niloticus* (2.42±0.01) followed by *L. rohita* (1.46±0.01) and *G. catla* (0.87±0.03).

Nutrient utilisation

Nutrient utilisation of the commercial diet by experimental fish is presented in Table 5. Feed conversion ratio (FCR) was significantly ($p<0.05$) lower in *O. niloticus* fingerlings (0.94±0.00) than *L. rohita* (1.19±0.02) and *G. catla* (1.69±0.02). Protein efficiency ratio, protein productive value and lipid productive value were significantly higher ($p<0.05$) in *O. niloticus* fingerlings followed by *L. rohita* and lowest in *G. catla*.

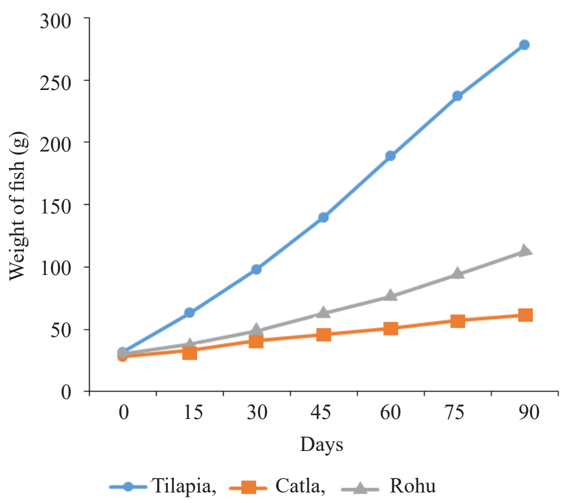

Fig. 1. Body weights of *O. niloticus*, *G. catla* and *L. rohita* fingerlings reared in RAS, during the experimental period

Survival

No significant ($p>0.05$) difference in survival was observed among species (Fig. 2). However, fingerlings of *O. niloticus* (92±0.91) registered highest survival (%) than *L. rohita* (91± .71) and *G. catla* (89±0.82).

Total biomass production

The total biomass production of each species are presented in Fig. 3. Total biomass production was significantly ($p<0.05$) higher in *O. niloticus* (2.55±0.03 kg m^{-3}) than *L. rohita* (1.02±0.02 kg m^{-3}) and *G. catla* (0.54±0.01 kg m^{-3}).

Discussion

The water quality parameters during the culture period were comparable to the optimal range for tilapia and carp farming in ponds (Appiah-Kubi, 2012; Jena *et al.*, 2015). No marked variation was recorded for different water quality parameters *viz.* water temperature, pH, dissolved oxygen, alkalinity, hardness, ammonia-N, nitrite-N and nitrate-N in RAS during the experimental period. This might be due to continuous water recirculation and efficient nitrification by the nitrifying bacteria (Tanjung *et al.,* 2019; Irhayyim and Fotedar, 2019) which prevented

Table 5. Nutrient utilisation of *O. niloticus*, *G. catla* and *L. rohita* fingerlings reared in RAS and fed with commercial diet

Parameter	Treatment groups		
	Tilapia	Catla	Rohu
FCR	0.94^{c}±0.00	1.69^{a}±0.02	1.19^{b}±0.02
PER	3.55^{a}±0.02	1.98^{c}±0.02	2.82^{b}±0.05
PPV	52.18^{a}±1.00	25.98^{c}±0.50	40.00^{b}±0.81
LPV	135.54^{a}±0.98	46.14^{c}±3.92	90.04^{b}±3.48

Data expressed as mean±SE; Mean values in the same row with different superscripts vary significantly ($p<0.05$). FCR: Feed conversion ratio, PER: Protein efficiency ratio, PPV: Protein productive value, LPV: Lipid productive value

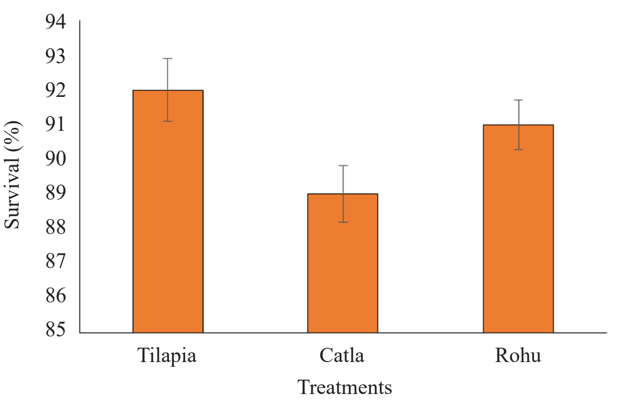

Fig. 2. Survival (%) of Tilapia, Catla and Rohu fingerlings reared in RAS

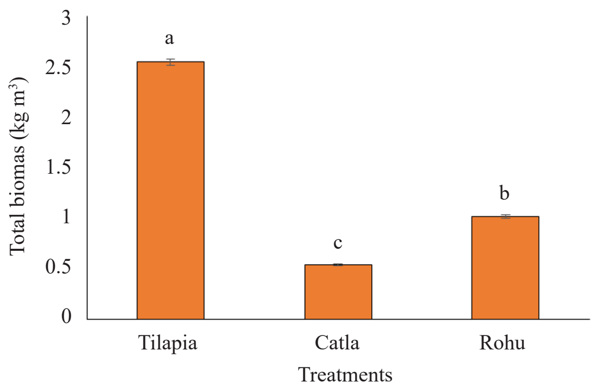

Fig. 3. Total biomass production (kg m^{-3}) of Tilapia, Catla and Rohu fingerlings reared in RAS and fed with commercial diets

the accumulation of metabolites and dissolved organic matter in the RAS.

The results obtained from TPC analysis of samples indicated that the bioballs (2.72×10^7 CFU ml^{-1}) had developed higher bacterial colonies as compared to ground water (1.96×10^7 CFU ml^{-1}) and fish-reared water (1.85×10^7 CFU ml^{-1}). However, significantly similar ($p>0.05$) TPC was found between ground water and fish reared water. From the results, it is evident that the bioballs formed a perfect filter media for the growth of bacteria which further helped in the effective conversion of toxic ammonia into least toxic form through nitrification process. Similarly, Irhayyim and Fotedar (2019) also reported that bioball was effective in achieving higher ammonia removal rates in RAS. Tanjung *et al.* (2019) also found that a combination of cotton, bioball and charcoal as a filter media was effective in control of ammonia concentration in RAS.

The proximate composition of fishes varies with species and ranges between 65-90% water, 10-22% protein, 1-20% lipid and 0.5-5% ash (minerals) (Nair and Mathew, 2000). The results recorded in the present study correspond to these values. Moisture and ash content decreases with the increase in body weight (Silva *et al.,* 2015; Edea *et al.,* 2018) while crude protein, lipids and dry matter content increases with the increase in body weight (Salam *et al.,* 2001; Silva *et al.,* 2015; Edea *et al.,* 2018). The results of the present study are in accordance with the above literature and results reported by Ogunji *et al.* (2008) and Thongprajukaew *et al.* (2017) with Nile tilapia (*O. niloticus*), since a decrease in the moisture and ash content and an increase in the crude protein and dry matter content was observed in *O. niloticus*, *L. rohita* and *G. catla* as body weight increased. The dry matter, protein, lipid and ash content found in the present study were comparable to that reported by Khan *et al.* (2012) with catla (*G. catla*), rohu (*L. rohita*) and mrigal (*C. mrigala*) in pond monoculture and polyculture systems. Size of fish significantly affects their proximate composition. Small size fishes have lower meat:bone ratio, which leads to higher whole body ash content compared to large size fishes. Therefore, smaller size of catla compared to rohu and monosex tilapia at the end of experiment may have led to higher ash content in whole body composition of catla compared to rohu and monosex tilapia.

The higher percentage weight gain, daily weight gain and SGR of monosex tilapia and rohu might be due to greater feed intake, feed acceptance and better efficiency of converting ingested feed into body mass. The results of the present study with monosex tilapia (*O. niloticus*) are in agreement with the study by Luo *et al.* (2014) who reported 472.14% weight gain of GIFT (*O. niloticus*) in RAS. The present result also concurred with Kawser *et al.* (2016) who reported 952.94% weight gain in a recirculating aquaponic system with monosex tilapia (*O. niloticus*).

Similar SGR as found in the current study was observed earlier with Nile tilapia (*O. niloticus*) in RAS (Luo *et al.,* 2014). On the contrary, Kawser *et al.* (2016) found lower SGR of monosex tilapia cultured in a recirculating aquaponic system. Due to lack of literature available on culture of rohu (*L. rohita*) and catla (*G. catla*) in RAS, a direct comparison with other culture systems could not be made. However, lower SGR of 1.14 was reported for common carp (*Cyprinus carpio*) cultured in RAS by Tabarrok *et al.* (2020) compared to 1.46 of the present study with rohu (*L. rohita*). In pond polyculture system, rohu showed lower SGR while catla showed comparatively higher SGR (Jena and Das, 2011; Jena *et al.*, 2015; Verma *et al.*, 2018) than that found in the current study with RAS. This reflects that catla is more suited to pond conditions and shows good growth in open water area in comparison to RAS.

Feed conversion ratio is one of the major factors for cost effective fish production and economics of RAS highly depends on FCR. In the present study, lower FCR was observed in tilapia than rohu and catla. Tilapia eats supplementary feed more aggressively than carp of a similar age (Hossain, 1995). Therefore, the lower FCR obtained in tilapia is due to the more efficient feed utilisation by the tilapia compared to rohu and catla, thus contributing in reducing feed wastage. This reflects RAS to be a viable option to effectively culture monosex tilapia and rohu in confined condition. Higher PER, PPV and LPV were recorded in tilapia compared to rohu and catla, which indicated more effective utilisation of the dietary proteins and lipids by tilapia compared to rohu and catla in RAS. Feeding diet that provides maximum PPV will lower the potential of nitrogen loss to the environment (Allan and Booth, 2004) and increase its availability for protein buildup (Raven *et al.*, 2006).

The lower growth performance and nutrient utilisation of catla in RAS in the present study might be due to the fact that, culture within confined closed area that restricted its movement and the stocking density of catla used might be higher. When the stocking density is above the threshold level, a lack of sufficient space acts as an independent stress that reduces growth (Woiwode and Adelman, 1989). High stocking density can affect fish growth performance and nutrient utilisation even though water quality in aquaculture systems is well maintained (Schram *et al.,* 2006; Karakatsouli *et al.,* 2007; Tolussi *et al.,* 2010). The crowding effects on catla can be considered as a result of physiological stress rather than of other environmental and chemical stressors (Yin *et al.,* 1995). Crowding stress can lead to an inhibition of muscle growth in fish, through muscular atrophy modulation (Yarahmadi *et al.,* 2016; Valenzuela *et al.,* 2018).

The high survival of all experimental fish species (tilapia, rohu and catla) cultured in RAS could be attributed to the optimum water quality. The net fish biomass obtained after three months of experimental period showed tilapia as best suitable fish species for culture in RAS among the three experimental species. However rohu produced higher biomass than catla. According to FAO (2005-2021), the net biomass obtained from tilapia in pond culture systems generally range from 8-10 t ha^{-1} *i.e* 0.8-1.0 kg m^{-3}, while in the present study, biomass of 2.55 kg m^{-3} was obtained from monosex tilapia culture in RAS. Similarly, the net biomass generally obtained from Indian major carps in carp polyculture systems ranges from 4-6 t ha^{-1} (FAO, 2005-2021), *i.e* 0.4-0.6 kg m^{-3}, whereas the total biomass production obtained from rohu and catla in the present study was 1.02 kg m^{-3} and 0.54 kg m^{-3} respectively. These results suggest that monosex tilapia and rohu gives higher total biomass production when cultured in RAS compared to pond culture system.

The findings of this study thus unveiled the potential of RAS as a suitable alternative to existing land based farming systems in terms of efficiently culturing monosex tilapia and rohu with commercial feed application. Water quality was maintained at optimum levels with continuous water recirculation and efficient nitrification by the nitrifying bacteria in RAS. Rohu showed prominent growth performance, which proved it to be an ideal candidate in RAS among the Indian major carps (IMCs), whereas poor growth performance of catla at stocking density of 10 nos. m^{-3} in RAS referred for further studies to establish its optimum stocking density in RAS.

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