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Full Length Research Paper

Effect of stocking density on growth, maturity, fecundity, reproductive behaviour and fry production in the mouth brooding cichlid *Oreochromis mossambicus* (Peters)

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Growth and reproductive performance of *Oreochromis mossambicus* (Peters) in relation to stocking density was evaluated in indoor aquaria. Juveniles weighing 978 ± 370 mg were held in densities of 10, 20, 30 and 40 aquarium-^{1} (429, 858, 1287 and 1716 juveniles/m^{3}) in aquaria (size: 46 × 23 × 23 cm) each containing 12 L freshwater. They reared on dry *Tubifex tubifex* worms and dry fish pellets for over 25 weeks. Growth and specific growth rates were inversely proportional to stocking density (P=0.0555, r=0.944; P=0.0395, r=0.960 respectively). At the highest stocking density (40 juveniles aquarium-^{1}), magnitude of decrease in daily growth was 51.21%. Increase in stocking density resulted in reduction in size at maturity in both the sexes (male: 2 to 7.5%; female: 1.37 to 5.57%), reduction in fecundity (25 to 31%), fry production (18.58 to 28.58%) and inter-spawning interval (25 to 40%). At a living space of 597 cm^{3}/fish (40 fish aquarium-^{1}), though males constructed the nest, females failed to spawn as post vitellogenic oocytes had become atretic and/or resorbed.

Key words: *Oreochromis mossambicus*, stocking density, size at maturity, fecundity, spawning, fry production.

INTRODUCTION

The commercial production of tilapia has increasingly gained expansion in many countries due to its suitability to variety of pond farming conditions, resistance to diseases, high survival and growth rate (Onumah et al., 2010). Tilapias have certain reproductive strategies that set them apart from the vast majority of fish species (Lorenzen, 2000). The reproductive biology of tilapia has been widely investigated from different parts of the globe (Hatikakoty and Biswas, 2002). In many species of fishes reproductive success has been shown to be influenced by brood stock, sex ratios, stocking density age, size, nutrition and feeding regime (Tahoun et al., 2008). Introduction of *Oreochromis mossambicus* and *Oreochromis niloticus* into freshwater habitats of India took place in 1952 and 1970 respectively (John, 2003). Since then they have established in many lakes and reservoirs. Reproductive potential of Tilapia has been reported to be influenced by temperature (Hyder, 1970), salinity (Chervinsky, 1982), food (Miranova, 1977) and dietary protein level (De Silva and Radampola, 1990; Gunasekara et al., 1995). Similarly, the effect of stocking density on growth, sex ratio, fecundity and fry production in Nile tilapia, *O. niloticus* has been studied from time to time (Ridha and Cruz, 1998; Al-Herbi and Siddiqui, 2000; Yousif, 2002; Abdel Tawwab et al., 2005; Osofero et al., 2007; Khalfalla et al., 2008; Offem et al., 2009; Onumah et al., 2010; Chakraborthy et al., 2010). As compared to this, in *O. mossambicus* hitherto, the effect of stocking density on growth alone has been reported (Al–Jerian, 1998). In intensive aquaculture practices, the economic viability of production is dependent on the density at

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which a fish species can be stocked (Papst et al., 1992; Huang et al., 2002). While extreme limits of growth are species specific, the obvious upper limit of the number is space, which can harbor not more than a population specific density (Backiel and Le Cren, 1978). Since in India *O. mossambicus* (Peters) is more widely distributed than *O. niloticus* (Linnaeus), the aim of this study was to examine the effect of stocking density on survival, growth, fecundity, reproductive behaviour and fry production in *O. mossambicus* within aquarium.

MATERIALS AND METHODS

Juveniles of *O. mossambicus* were collected through drag net from Mallathahalli lake, (12º 57' 53'' N to 77º 29' 40'' E), Bangalore, India. After collection, juveniles were transferred into inert polyethylene containers with lake water and transported to the laboratory. In the laboratory, juveniles were held in stock tanks (size: 60 × 30 × 30 cm) in 30 L of water and acclimated to laboratory conditions for five days. During this period, juveniles were fed on dry *Tubifex tubifex* worms and dry fish food pellets. Water was aerated continuously and changed once every two days. From this stock, juveniles ranging in size of total length from 2.0 to 3.5 cm (Mean: 2.20 ± 0.453) were selected and were held at densities of 10, 20, 30 and 40 individuals aquarium^{-1} (46 × 23 × 23 cm) in 12 L of water. A constant water level was maintained and with the help of an aqua pump fixed to each aquarium, water was aerated continuously. The bottom f each aquarium was spread uniformly with sand (thickness: 2.5 cm) and over this, an artificial grass mat was placed as substrate. A sub-sample of juveniles in the size range of experimental individuals were taken from the stock and weighed using an electronic digital balance (make: Orion; precision: 1 mg) to determine the initial live body. The initial total length of these juveniles ranged from 2.0 to 3.5 cm and live body weight varied from 450 to 1295 mg. Differences in total length and body weight of these juveniles were analyzed with a non-parametric analysis of variance (Kruskal–Wallis test, 1952). No significant differences were found in the initial total length and body weight of the fishes used for the experiments (Kruskal–Wallis; H = 1.44, P = 0.18) suggesting that the sample taken for the experiments had normal distribution. Juveniles of *O. mossaambicus* weighing 1.014 to 1.154 g are found to consume live *T. tubifex* worm equivalent to 20% of their initial body weight (Vishwanath, 2009). Taking this into consideration, every day, juveniles were fed twice (9 and 16 h) at 12% initial body weight with dried *T. tubifex* worms and at 14% of their initial body weight with dry fish food pellets (Manufacturer: Taiyo pet products Pvt. Ltd; Composition: crude protein 32%, crude fat 4% crude fiber 5% and moisture 10%). Feeding was continued for 180 days. All feeding experiments were conducted using laboratory mesocosm (aquarium), wherein the temperature of water; was maintained at 25.6 ± 3.00°C and a fluorescent light was provided well above each aquarium to maintain constant photoperiod. Dead fish were removed and recorded daily. Two replicates were maintained at each stocking density. Observations were made daily to record size at maturity (mm), initiation of nest construction and pre and post reproductive behaviour. A Digital canon camera (resolution 5.0 mega pixels) was used to record the reproductive behaviour. At the end of 180 days, changes in body length and body weight of each fish was recorded. Following the procedure of Bagenal (1978), fecundity was estimated in the gonads of females in the final maturation stage. Growth of the fish in each density was calculated following the equation:

Average weight gain (AWG) = Average final weight – average initial weight.

Daily growth (g/fish day^{-1}) = Final weight – initial weight/time (days).

Growth rate (mg/g fish day^{-1}) = Final weight – initial weight/days/initial weight.

Specific growth rate (SGR: % day^{-1}) = 100 [Ln Wt1 – Ln Wt0/t] (Degani et al., 1989) where, Ln is the natural log, Wt0 is the initial weight (g), Wt1 is the final weight (g), and T is the time or number of days reared. Each fish was dissected to confirm sex and maturity stages. Ovaries were excised, condition of the ovaries were noted, then fixed in Gilson's fixative and used for fecundity estimation. Data on gonado-somatic index (GSI), size at maturity, fecundity, duration to initiate first nest construction, spawning, inter spawning interval and number of fry produced by each female were collected. The results obtained were subjected to one way analysis of variance (ANOVA) using SPSS (ver. 12) (Statistical Package for the Social Sciences). Differences between means were compared by multiple range tests at significance level of 5% (Duncan, 1955).

RESULTS

Table 1 presents the final body weight of the fish as indication of influence of stocking density (no/12L water), volume of water/fish (ml) and space (cm^3/fish) under laboratory experimentation. It is obvious from the experimental design that as the population density increased, volume of water and space available per fish decreased and as a result, the biomass (g/L) increased.

The juvenile survivorship showed an inverse relationship with stocking density (r=0.095, P <0.01). At density of 10 fish aquarium-^{1}, we observed 100% survivability and the rate steadily decreased with increase in stocking density (90, 70 and 62.5% survival at stocking density of 20, 30 and 40 fish aquarium-^{1} respectively).

Growth

Final live body weight, gain in body weight and daily growth of *O. mossambicus* in relation to the four population densities are given in Table 2. The initial live body weight of fry was 0.979 ± 0.37g. Stocking density had an effect on the size variation among individuals of initially uniform size in each of the tested population densities. In stocking densities of 10, 20 and 30 fish aquarium^{-1}, minimum to maximum body weights of fish ranged from 2.25 to 17.98 g. On the other hand in 40 fish aquarium^{-1}, it varied from 1.69 to 9.24 g respectively.

The daily growth of fish at stocking density of 10 fish aquarium^{-1} averaged 50.18 mg/fish day^{-1} and with increase in stocking density to 20 and 30 fish aquarium-^{1}, daily growth of fish decreased to 41.74 and 40.35 mg/fish day^{-1} respectively. At the highest stocking density of 40 fish aquarium^{-1}, daily growth decreased by 51.21% (24.48 mg fish day^{-1}). Mean differences in daily growth observed between treatments were tested for significance using Duncan Multiple Range test (Duncan, 1955). Though there was a reduction in growth amounting to 16.81% (20) and 19.60 % (30 fish aquarium^{-1}), difference in

Table 2. Changes in live body weight and growth of *O. mossambicus* in relation to stocking densities.

Group	Stocking density (number/aquarium)	Final body weight (g)	Gain in body weight (g)	Growth (mg live/fish day^{-1})
I	10	10.02 ± 4.24^{a}	9.04 ± 4.46^{a}	50.18 ± 24.83^{a}
II	20	8.50 ± 4.91^{a}	7.52 ± 4.84^{a}	41.74 ± 27.32^{a}
III	30	8.25 ± 5.05^{a}	7.27 ± 5.05^{a}	40.35 ± 28.07^{a}
IV	40	5.39 ± 2.29^{b}	4.41 ± 2.29^{b}	24.48 ± 12.73^{b}

Mean ± SD values followed by different superscripts in a column differ significantly (P< 0.05).

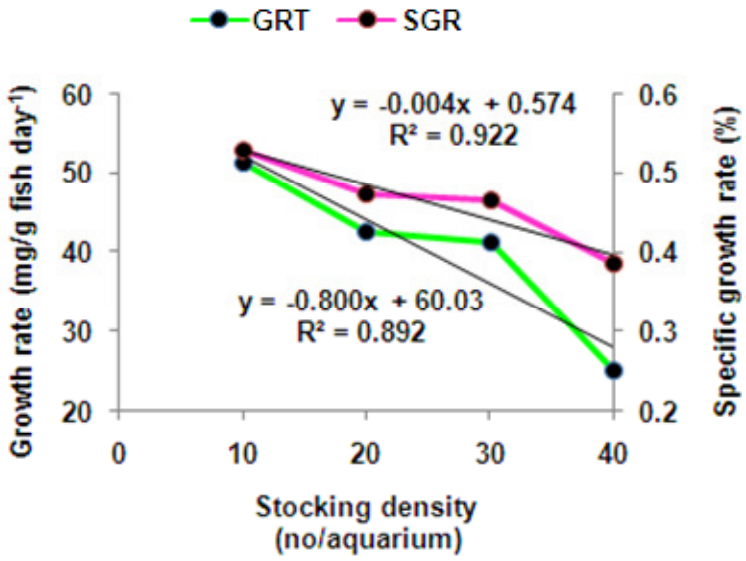

Figure 1. Inverse relationship between stocking density and growth and specific growth rates in *O. mossambicus.*

growth between groups I and II, and between I and III and II and III were insignificant. However, differences in growth between groups I and IV, II and IV and III and IV were found to be highly significant (P = < 0.05).

Elliott (1975a) and Brett and Groves (1979) described the relationship between specific growth rate (G) and body weight (W) of fish by an equation of the form: Log G = a + b log W. Figure 1, describes the relationship between population density and growth rate and population density and specific growth rate in *O. mossambicus* respectively. It is evident that both growth and specific growth rates in *O. mossambicus* displayed an inverse relationship with stocking density. The corresponding equations are;

Growth rate: Y = -0.800x + 60.03 R^2 = 0.892

Specific growth rate: Y = -0.004x + 0.574 R^2 = 0.922

Length at sexual maturity

The length at which 50% of a fish population reaches sexual maturity (L_{50}) is considered to be the length at onset of sexual maturity (Pitt, 1970). The percentage of mature fish was significantly higher in stocking densities of 10, 20 and 30 than in 40 fish aquarium^{-1}. Males were found to mature earlier than females in all population densities. In either sex, size at maturity was found to decrease with increase in population density. The smallest size at attainment of maturity in male and female *O. mossambicus* was found to differ in relation to stocking density (Figure 2) and at each population density, the observed difference in size at maturity between male and female was found to be statistically significant (P < 0.05).

Gonado-somatic index (GSI)

Irrespective of stocking density, GSI in both the sexes was found to increase with progressive development of gonads. In males (Figure 3) GSI varied from 0.346 (40 fish aquarium^{-1}) to 0.915 (20 fish aquarium^{-1}), while in females it ranged from 1.597 (40 fish aquarium^{-1}) to 5.620 (30 fish aquarium^{-1}). Though the GSI of female at 30 fish

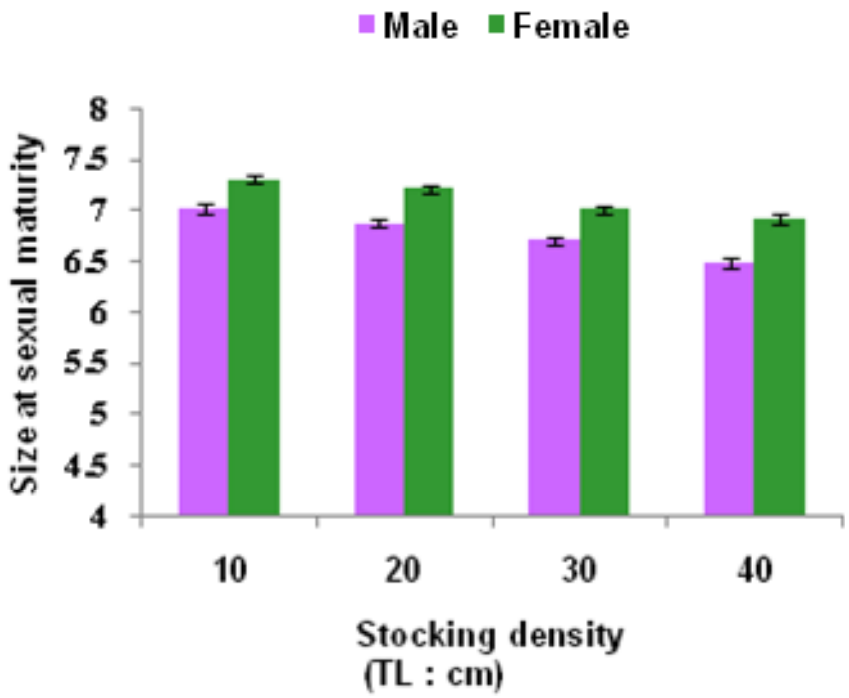

Figure 2. Influence of stocking density on size at sexual maturity in male and female *O. mossambicus.*

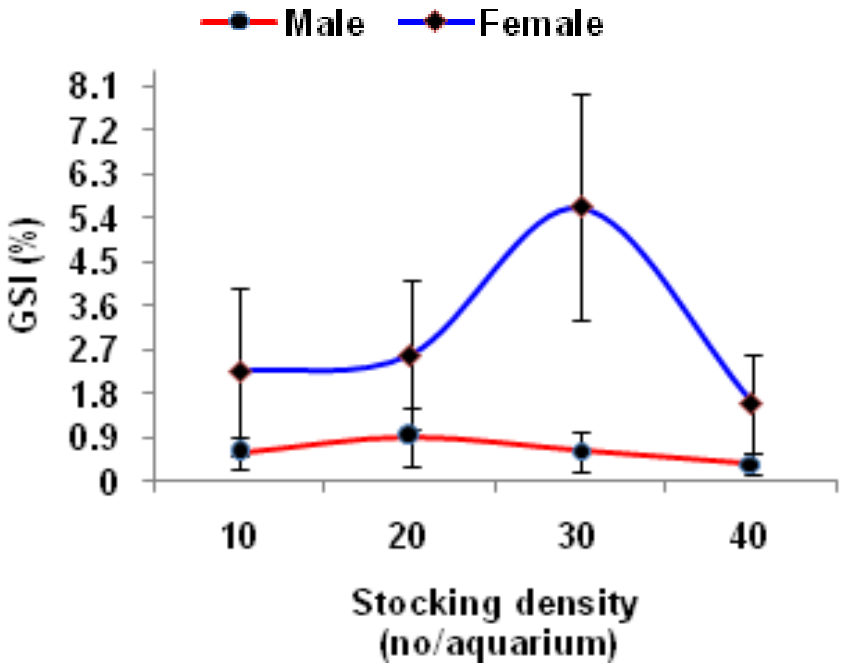

Figure 3. Gonadosomatic index (GSI) of male and female *O. mossamabicus* at different stocking densities.

aquarium^{-1} remained high, differences in GSI of fish between the four stocking densities were found to be insignificant in both sexes (P< 0.889).

Fecundity

All the females reached maturity (6/6; Table 3) at the

Table 3. Changes in the number of mature females and fecundity of *O. mossambicus* in relation to different stocking densities.

Group	Stocking density (Number fish aquarium^{-1})	Number of mature females	Fecundity (number/fish)
I	10	6 ± 0.7	100 ± 2.07^{a}
II	20	7 ± 0.6	75 ± 1.35^{b}
III	30	8 ± 0.9	69 ± 0.89^{c}
IV	40	5 ± 0.4	-

Mean values followed by different superscripts in a column differ significantly (P< 0.0001).

Table 4. Effect of stocking density on duration and minimum size required (TL) to initiate nest construction in male *O. mossambicus*.

Stocking density (Number/aquarium)	Size at nest construction (TL: cm)	Duration required to initiate nest construction (days)
10	7.2^{a}	118
20	7.1^{a}	105
30	6.9^{b}	105
40	6.6^{c}	98

Mean values followed by different superscripts in a column differ significantly (P<0.001).

lowest stocking density. This number increased till 30 fish aquarium^{-1} (8/11). With further increase in population density to 40 fish aquarium^{-1}, number of mature females decreased (5/9). At 10 fish aquarium^{-1}, mature females produced more seed per clutch and more seed per unit weight than in higher stocking densities. Fecundity of *O. mossambicus* in stocking density of 10 fish aquarium^{-1} was found to be 100 ± 2.07 eggs/female (relative fecundity RF: 12), while at 20 fish aquarium^{-1} it was observed to be 75 ± 1.35 eggs/female (RF: 10). Fecundity was further reduced to 49 ± 0.89 eggs/female (RF: 8) at 30 fish aquarium^{-1}. The differences were found to be significant (F 89.981, P< 0.0001). In fish stocked at 40 aquarium^{-1}, fecundity could not be estimated as eggs were observed to be either in stage II (early developing) or had undergone atresia.

Breeding behaviour and spawning

Observations on breeding behaviour of *O. mossambicus* under different stocking densities revealed an interesting aspect of male and female body colour and behaviour. In males, at all tested densities, the breeding activity begins with appearance of white colouration on the neck, red on the margin of the fins and black on the rest of the body, meanwhile, female develops an enlargement of genital openings and red tinge on the tip of fins. Dominant males in all the stocking densities established breeding territory for constructing the nest and during this period they were aggressive and chased away intruders to the nest area. Dominant males were observed to dig a shallow bowl shaped pit in the sand on one side of the aquarium just below the artificial mat. The male scoops out mouthful of sand and spits it out over the top edge of bowl. This is carried out until a pit with diameter two times of its length is achieved. The size of male (total length) at nest construction did not differ in stocking densities of 10 (7.2. cm) and 20 (7.1 cm) fish aquarium^{-1} (Table 4). With further increase in stocking density to 30 fish aquarium^{-1}, males were found to construct the nest at 6.9 cm while males in 40 fish aquarium^{-1} not only constructed nest at smaller size (6.6 cm) but also initiated nest construction after 90 days of rearing. On the other hand, fish stocked at 10 fish aquarium^{-1} took 118 days of rearing to initiate nest construction (Table 4). Differences observed in size at nest construction between groups I and III, I and IV, II and III and II and IV were found to be significant (F=56.203; P< 0001). Nest construction was followed by attraction of mate, pair formation and courtship behaviour. This was observed in densities of 10, 20 and 30 fish aquarium^{-1}. Courtship behaviour involved circling the female, body tilting and leading the female to the nest. In the spawning, site pairs circled each other and performed a variety of shakes and nudge each other till the female begins to lay eggs. At stocking density of 40 fish aquarium^{-1} though breeding male constructed the nest and attracted the female, other males interfered by chasing away the ovulatory female and inhibited the breeding male to display the courtship behavior.

Spawning and fry production

Stocking density strongly influenced the spawning activity. An inverse relationship between stocking density

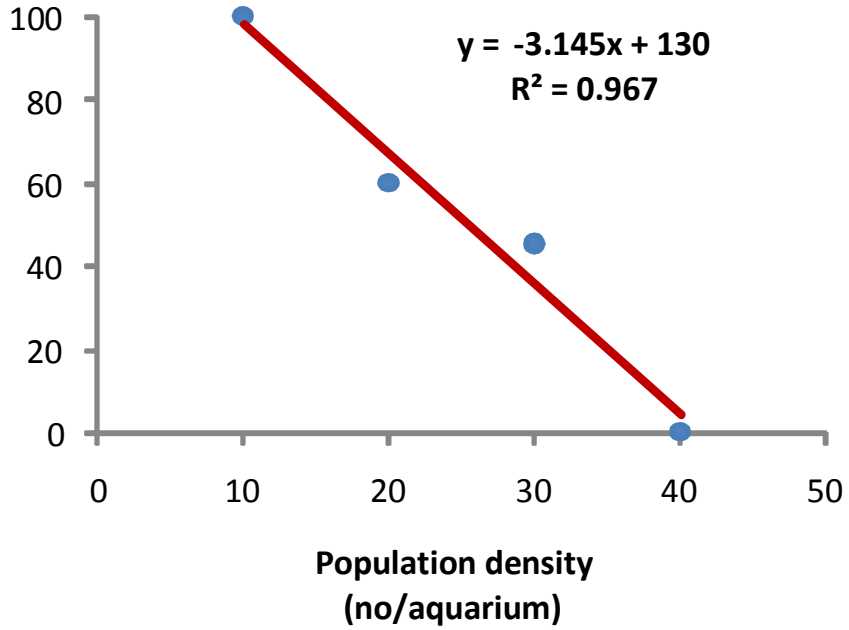

Figure 4. Inverse relationship between stocking density and percent spawning females *(%* calculated in relation to total number of females in the respective stocking densities).

Table 5. Effect of stocking density on duration and minimum size required to initiate mouthing brooding, swim up fry production and inter-spawning interval in *O. mossambicus*.

Population density (No/aquarium)	Duration to initiate first mouth brooding (days)	Size at first mouth brooding (cm)	Swim-up fry (Number/fish)	Inter spawning interval (ISI) (days)
10	121 ± 2.081[a]	7.8 ± 0.08[a]	70 ± 8.08[a]	20 ± 1.527[a]
20	108 ± 1.707[b]	7.6 ± 0.05[b]	57 ± 5.77[b]	15 ± 0.899[b]
30	100 ± 1.825[c]	7.4 ± 0.07[c]	50 ± 6.69[c]	12 ± 0.955[c]
40	Nil	Nil	Nil	Nil

Mean values followed by different superscripts in a column differ significantly (P< 0.001).

and percent spawning females was observed (Figure 4). At 10 fish aquarium^{-1}, all the mature females (6/6) spawned. With increase in stocking density to 20 and 30 per aquarium, reduction in number of spawning females (6/7 and 5/8 respectively) was noticed. On the other hand at 40 fish aquarium^{-l}, mature females (5/5) failed to spawn. The differences were found to be significant. In the hybrids of tilapia (*O. niloticus* × Oreochromis *hernorum*), Bhujel (2000) has also observed an inverse relationship between stocking density and percentage of spawning females. Female was found to lay eggs in batches. After each batch of eggs was laid, male swam over the eggs and fertilized them. In the three stocking densities, the whole process lasted for 60 to 80 min. During this spawning activity, other males tried to sneak into the spawning nest and these were chased away by the spawning male. Once the fertilization process by male was complete, females picked up the fertilized eggs into their mouth for incubation. To ensure fertilization of eggs, females were observed to suck the genital papilla of males. Fertilized eggs were found to hatch after 2 to 3 days of incubation. Hatched individuals are the brooding fry with yolk sac. The yolk sac fry were brooded in the oral cavity until they attained a length of 1.1 cm in 20 to 22 days. During brooding, the mother frequently rotated the developing eggs to provide ventilation. From Table 5, it is seen that time taken to initiate mouth brooding by female was found to decrease with increase in stocking density. At stocking density of 10 fish aquarium^{-1}, females took 121 days to initiate mouth brooding and this duration decreased to 108 and 100 days at stocking densities of 20 and 30 fish aquarium^{-1} respectively. The differences observed between the groups was significant (P<0.001). Similarly, the size at which mouth brooding was initiated

was also observed to decrease with increase in stocking density. At 40 fish aquarium^{-1} though male constructed the nest, female failed to spawn and exhibited mouth brooding.

Maximum number of swim-up fry (70) was observed in 10 fish aquarium^{-1}. Production of swim-up fry was found to decrease with increase in stocking density and the differences were found to be significant. This observation is similar to the reduction in number of young produced by *Oreochromis aureus* with increase in stocking density (Allison et al., 1979). From the table, it is further evident that stocking density had a significant bearing on inter spawning interval. In females at 10 fish aquarium^{-1}, the inter spawning interval (ISI) was observed to be 20 days, while that in females at 20 fish aquarium^{-1}, ISI was found to be 15 days. Further reduction in ISI to 12 days was apparent in stocking density of 30 fish aquarium $^{-1}$. The differences in ISI between the three groups were statistically significant ($P < 0.05$).

DISCUSSION

Optimum utilization of space for maximum production in intensive fish culture practices is known to improve the profitability of fish farm (Chakraborthy et al., 2010). In this study, though the survival rate was observed to be density dependent, it was generally remained high. This high survival rate (62.5 to 100%) could be attributed to sufficient food supply, continuous aeration of water (Al-Herbi and Siddiqui, 2000) and prevention of accumulation of unconsumed food/fecal matter. Although the initial size of fish was homogenous and the food supply was in *ad libitum*, at the end of the experiment, significant differences in length and weight of fish were noticed in all treatments. Jobling (1982) has opined that size variation could result from natural variability and/or induced by competition and/or social interactions. Crowding may lead to the formation of social hierarchy in fish with the appearance of dominant and subordinate individuals (Koebele, 1985). In the four stocking densities, *O. mossambicus* was found to establish social hierarchy. At 40 fish aquarium^{-1}, establishment of social hierarchy resulted in smaller fishes been inhibited from feeding by dominant and aggressive fish. Disproportional food acquisition in *Tilapia zilli* has been attributed to size hierarchy effect (Koebele, 1985). Aggressive behaviour is required to establish and maintain hierarchies among fish and the degree of aggressive behaviour may be influenced by social factors such as fish density and physical environment factors such as aquarium size (Evans et al., 2008).

Calculating the increase in biomass per liter of the available water during 180 days of experimental period, it becames clear that the suppression of growth is related to density rather than biomass (Table 1). Therefore it is very likely that restriction of space at higher stocking densities is a kind of conspecific population stress that could make the fishes to spend more energy for homeostatic process (Schreck, 1982; Aksungur et al., 2005). In *Oryzias latipes* decrease in the amount of space by four times did not affect the growth rate but increase in population density by four times resulted in significant changes in the growth rate (Magnuson, 1962). This observation on growth is in agreement with earlier observation on *O. mossambicus* (Al-Jerian, 1998). Reduction in growth with increase in population density has also been reported for *O. niloticus* and hybrids of *O. niloticus* × *O. aureus* by several authors (Liu and Chang, 1992; Al-Herbi and Siddiqui, 2000; Yousif, 2002; Abdel-Tawwab et al., 2005; Oumah et al., 2010; Chakraborthy et al., 2010). However, Osopheros et al. (2007) have recorded insignificant differences between daily weight gain and stocking density and between specific growth rate and stocking density in *O. niloticus*.

GSI was observed to be different for male and female in the different stocking densities. However, in the hybrid tilapia *O. niloticus* × *O. aureus*, GSI was found to remain similar for fish stocked at four different densities (Siddiqui et al., 1997).

In all the densities, males were found to mature earlier than females. It is very likely that males spend much of their energy for somatic growth than for reproduction and females spend much of their energy for reproduction (Boliver et al., 1993). At the highest stocking density (40 fish aquarium^{-1}) due to stunted growth, fish were observed to mature earlier than those held at10 aquarium^{-1}. Hatikakoty and Biswas (2002) indicated females maturing at smaller size than their male counterparts. On the other hand, in the hybrid tilapia (*O. niloticus* × *O. aureus)* no apparent differences in the total length of smallest mature male and female were found at different densities (Siddiqui et al. 1997).

Increase in stocking density significantly reduced fecundity (eggs/female). Reduction in fecundity with increase in stocking density has been reported for the related species *O. niloticus* (Tahoun et al., 2008). The maximum fecundity (100 eggs/female) as observed presently in stocking density of 10 fish aquarium^{-1} is significantly lower than the fecundity reported for the field population of the same species by earlier workers (315-900/fish, Nijaguna,1989; 2020/fish, Jayaraj, 2000). On the other hand, the present observed value falls within the values (100-850 eggs/female) reported for the same species by Hatikakoty and Biswas (2002). It appears that in high stocking density, competition for space and social stress may be responsible for low utilization of food and poor growth leading to low fecundity.

Stocking density had no effect on the nest constructing behavior of male. However, at 40 fish aquarium^{-1}, despite the initiation of courtship by male, ovulatory females failed to visit the nest and spawn. This could be due to the hindrance by the dominant males whose density at 40 fish aquarium-^{1} remained high (♂ = 14; ♀ = 9). In *O.*

niloticus, nest deprivation did not reduce mating success (Mendonca and Gonocalves-de-Freitas, 2008). Starvation during critical period of gonad growth and environmental stress has been indicated to bring about atretic oocytes (Gaber, 2003). It is very likely that degenerative change as observed in the different stages of development of oocytes as also atretic state of oocytes due to population stress might have resulted in the failure of spawning in females. Fry production of blue tilapia (*O. aureus*) has been shown to be affected by different brood sex ratio (Khalafalla et al., 2008). Presently, when fish were stocked at 10 fish aquarium^{-1} with an observed sex ratio of 1:1.5 (M/F) highest numbers of fry was produced. While fry production was found to decrease with increase in stocking density, failure to produce fry at a living space of 597 cm^3/fish as observed in 40 fish aquarium^{-1} would certainly affect production of tilapia under intensive culture system. Therefore in intensive production of *O. mossambicus*, minimum living space of 2389 cm^3/juvenile would ensure good growth and fry production.

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