EFFECT OF STOCKING DENSITY ON GROWTH AND SURVIVAL OF HORMONE TREATED TILAPIA (Oreochromis niloticus) FRY REARED IN HAPAS-IN-POND SYSTEM

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ABSTRACT
An experiment was performed in hapas-in-pond to evaluate the effect of stocking density on the growth performances and survivability of monosex male Tilapia (Oreochromis niloticus) fry for a period of 28 days. Fry were collected, weighed and stocked inside a 1 m² hapa in a 0.24 ha pond with stocking densities of 1200 fry/hapa (T1), 1700 fry/hapa (T2) and 2200 fry/hapa (T3). Each stocking were made in triplicate. They were fed with a 32% crude protein plus hormone (17 α-methyl testosterone) mixed feed 5 times a day. The physico-chemical parameters were recorded and found to be within suitable range for fish culture. The growth performances and survival of Tilapia (O. niloticus) fry were significantly (P<0.01) higher in T1 than those obtained from T2 and T3, respectively. The FCR (feed conversion rate) were calculated lowest (1.56) in T1 compared to T2 and T3, respectively. However, these results indicate that stocking density had a significant effect on growth performances and survival rates of monosex Tilapia (O. niloticus) fry.

KEY WORDS: growth performances, Oreochromis niloticus, stocking density, survival rate.

INTRODUCTION
Tilapia is regarded as the second most cultured aquaculture fish species globally because of its easy to adapt in tropical and sub-tropical regions of the world (Shelton, 2002). It is the most significant fish species which can reduce the gap of increasing worldwide demand for protein sources from fish, crustaceans and molluscs (Shelton, 2002). It is the most significant fish species which can reduce the gap of increasing worldwide demand for protein sources from fish (Ng and Romano, 2013). Its production worldwide has also increased from 1,099,268 tons in 1999 to about 3,500,000 tons in 2010 but production is still low to meet demand (FAO, 2012). Farming of Tilapia (Oreochromis niloticus) has received considerable attention in Bangladesh because of its promising aquaculture potential. Tilapia has good resistance to poor water quality and diseases, tolerance to a wide range of environmental conditions, ability to convert efficiently the organic and domestic waste into high quality protein, rapid growth rate as well as tasty flavor (Balarin and Hallar, 1987). Despite having many advantages as an aquaculture fish species, one of the main obstructions in tilapia farming at commercial scale is its precocious reproduction. There are a number of ways to control reproduction in mixed sex population. One of these is the culture of all-male tilapia. Culturing of all male Tilapia is preferred because of their faster growth as well as proper management. However, the most effective method to control its reproduction by treating synthetic androgens (17 α-methyl testosterone) for producing monosex Tilapia as male tilapia grows approximately 30% as fast as the females. The effect of stocking density for cultured monosex Tilapia is very important for the maximization of its production, profitability and sustainability. As, stocking density is considered to be one of the most important factors that directly or indirectly affect fish growth, feed utilization and finally the total fish production (Liu and Chang, 1992). Fry are most commonly stocked at densities of 3000 to 4000 per m² of hapa, or flowing water tank. Vera Cruz and Mair (1994) was used O. niloticus to compare stocking densities of 1000, 3000 and 5000 per m² of hapa and found best sex reversal at 3000 and 5000 per m² of hapa but lower survival at 5000 per m² of hapa. Pandian and Vardaraj (1987) found that fry can establish a hierarchy in feeding order resulting in small fish not consuming adequate quantities of hormone treated feed for successful sex reversal. Moreover, physico-chemical parameters are considered to have primary importance in
fish culture (Chakraborty and Banerjee, 2010) but also greatly affect the stocking densities. In most of the cultivated fish species, growth is inversely related to stocking density and this is mainly attributed to social interactions (Haylor, 1991 and El-Sayed, 2002). Therefore, in the preliminary stage hormone treated (monosex) Tilapia farming, the fish farmers must have adequate knowledge about a proper stocking density to get more production. However, the present experiment has been designed initially to understand the effect of stocking density on the growth, FCR as well as survival in hapas-in-pond system.

MATERIALS AND METHODS

**Study site:** The experiment was conducted in a private fish farm named Ghinuk Fish Farm situated at Shambhuganj, Mymensingh, Bangladesh from 20 June to 18 July, 2012.

**Description of the experimental hapa:** The experimental hapa (size of each hapa 1m\(^2\)) were set at the corner of 0.24 ha pond and rest space of the pond was used by the hatchery owner for commercial purposes. All the hapas were rectangular of synthetic netting of mesh size 1.5 mm closed from all sides except the top. There was well organized inlet and outlet facilitates in the pond which hapas were set up.

**Design of the experiment:** The experiment was conducted for a period of 28 days with three treatments each having three replicates. For convenience, hapas were arbitrarily numbered was 1 to 9. A total of 15300 *O. niloticus* fry (0.012±0.002 g) were collected and stocked in 9 hapas at three stocking densities such as 1200 fry/hapa (T\(_1\)), 1700 fry/hapa (T\(_2\)) and 2200 fry/hapa (T\(_3\)).

**Hormonal feed preparation and feeding:** A hormone treated fish feed was prepared according to Killian and Kohler (1991). The 17 α-methyl testosterone was the hormone used in the experiment. A stock solution was prepared by dissolving 0.06 g of hormone in 750 cm\(^3\) of 95% ethanol. Treatments were made by taking the accurate amount of the hormone from stock solution and brought up to 100 ml by addition 95% ethanol. This solution was evenly sprayed over 1 kg of 32% crude protein formulated feed (Quality feed) and mixed properly. The stocked fry in hapas were then fed with 17α-methyl testosteron hormone mixed formulated feed according to Guerrero (1975) technique 5 times a day at an initial rate of 25% of their body weight and adjusted 10-12% of their body weight towards the end of experimental period.

**Growth measurement:** Random sampling method was accomplished at an interval of 7 days to assess the growth and health status as well as for feed adjustment. At least 50 Tilapia fries were sampled from each hapa with the help of a scoop net.

**Physico-chemicals parameters:** The physico-chemicals parameters such as water temperature, dissolved oxygen, pH, total alkalinity and ammonia-nitrogen (NH\(_4\)-N) was measured weekly from each hapa using a celsius thermometer, a portable dissolved oxygen meter (HI 9142, Hanna Instruments, Portugal) and a portable pH meter (HI 8424, Hanna Instruments, Portugal) and a portable ammonia test kitr (Hanna test kit, Portugal), respectively. Total alkalinity was determined following the titrimetric method according to the standard procedure and methods (Clesceri et al., 1992).

**Harvesting of experimental fry:** After 28 days of rearing, *O. niloticus* fry were caught from each hapa with the help of a scoop net. The fry were counted for estimated survival rate and at least 50 fingerlings individually weighted to assess final growth in terms of weight, specific growth rate (SGR % day) and feed conservation ratio (FCR). Specific growth rate (SGR % day) and feed utilization efficiency were calculated according to Ricker (1975) respectively as follows:

**Weight gain (g) = Mean final weight - Mean initial weight**

SGR (%/day) = \[\ln(W_{sub.2}) - \ln(W_{sub.1}) / (T_{sub.2} - T_{sub.1}) \times 100\]

Where, \[W_{sub.1}\] = initial live body weight (g) at time \[T_{sub.1}\] (day)

\[W_{sub.2}\] = final live body weight (g) at time \[T_{sub.2}\] (day).
Survival (%) = No. of fish harvested/No. of fish stocked × 100
Feed Conversion Ratio (FCR) = Dry weight (g) of feed supplied / Live weight (g) of fish gained

Data analysis: Comparison of treatment mean was carried out using one-way analysis of variance (ANOVA), followed by testing of pair-wise differences using Duncan’s Multiple Range Test (Vann, 1972). Significance was assigned at the 1% level. All statistical analysis was done by using the SPSS (Statistical Package for Social Science) version-13.5.

RESULTS
Physico-chemical properties: The experimental hapas (all treatments) were set in same pond and the physico-chemical parameters were more or less same for each of the 3 treatments. The temperature, dissolved oxygen, pH, total alkalinity and ammonia-nitrogen (NH₄-N) were ranged from 29.06 - 30.45 °C, 5.97 - 6.83 mg/l, 7.98 - 8.62, 122.76 - 125.5 mg/l and 0.003 - 0.006 mg/l, respectively during the experimental period. However, there were no significant differences (P > 0.01) of physico-chemical parameters among the treatments (Table 1).

Table 1. Physico-chemical properties of weekly samples over the 28 days experiment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₁ (12000 fry/hapa)</td>
</tr>
<tr>
<td>Water temperature (°C)</td>
<td>29.06±0.89</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/l)</td>
<td>6.83±0.75</td>
</tr>
<tr>
<td>pH</td>
<td>8.43±0.23</td>
</tr>
<tr>
<td>Total alkalinity (mg/l)</td>
<td>124.17±10.05</td>
</tr>
<tr>
<td>Ammonia-nitrogen (NH₄-N) (mg/l)</td>
<td>0.003±0.001</td>
</tr>
</tbody>
</table>

Mean± SD (Standard deviation) and range in parentheses; Figures in the same row having the same superscript are not significantly different (P > 0.01).

Growth performances of monosex Tilapia fry under different stocking densities: The weight of mono-sex Tilapia (O. niloticus) fry under different stocking densities at the end of the experiment in weekly sampling is shown in Fig 1 which indicates that the improvement of weight were always higher in T₁ than T₂ and T₃. However, the mean final weight was 0.37±0.26 g in T₁ significantly higher (P<0.01) than the other treatments. The weight gain (g) attained under T₁, T₂ and T₃ were 0.36±0.16, 0.27±0.07 and 0.18±0.04 g, respectively. The result revealed that significantly (P<0.01) the highest weight gain was recorded in T₁ while lowest was in T₃. Furthermore, the highest mean values of specific growth rate (SGR) (% per day) was 12.24±0.26 in T₁ and the lowest was 9.90±0.43 in T₃. The SGR in T₁ was significantly (P<0.01) higher differences than other treatments when ANOVA was performed (Table 2).
The mean FCR value of T_1, T_2 and T_3 were recorded 1.56±0.25, 1.72±0.4 and 1.85±0.25, respectively. The FCR value of T_1 was found to be significantly (P<0.01) lowest which indicates that lower amount of feed was needed to produce one unit fish biomass and highest was found in T_3. The Survival rates were 92.48±3.41, 81.42±2.56 and 78.56±2.14 in T_1, T_2 and T_3, respectively. However, the survival rate was also significantly (P<0.01) higher in T_1 where the stocking density was 12000 fry/hapa than other treatments.

Table 2. Growth performances, feed utilization and survival of Tilapia (*O. niloticus*) fry after 28 days rearing

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>T_1 (12000 fry/hapa)</td>
</tr>
<tr>
<td></td>
<td>T_2 (17000 fry/hapa)</td>
</tr>
<tr>
<td></td>
<td>T_3 (22000 fry/hapa)</td>
</tr>
<tr>
<td>Initial weight (g)</td>
<td>0.012±0.002^a</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>0.012±0.002^a</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>0.012±0.002^a</td>
</tr>
<tr>
<td>Specific growth rate (SGR) (%)</td>
<td>11.25±0.61^b</td>
</tr>
<tr>
<td>Feed conversion ratio (FCR)</td>
<td>12.24±0.26^a</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>9.90±0.43^b</td>
</tr>
</tbody>
</table>

Mean± SD (Standard deviation) and range in parentheses; Figures in the same row having the same superscript are not significantly different (P > 0.01).

**DISCUSSION**

Growth, feed efficiency and feed consumption of fish are normally governed by few environmental factors (Fry, 1971). Environmental factors exert an immense influence on the maintenance of a healthy aquatic environment as well as production of food organism. The water temperature was recorded between 29.06 and 30.45 °C during the study period. Rahman *et al.* (2013) recorded water temperature ranged from 26.93 to 27.41 °C in nursing of Thai koi (*Anabas testudineus*). The present findings agree with the finding of Wahab *et al.* (1995) and Kohinoor *et al.* (1998). Dissolved oxygen content was varied from 5.97 to 6.83 mg/l among the treatments in the experiment. Rahman *et al.* (2013) was found dissolved oxygen 4.13 to 4.71 mg/l, while Kohinoor *et al.* (2012) recorded 4.23 to 5.32 mg/l in *H. fossilis*.
cultured ponds. However, according to Wahab et al. (1995) dissolved oxygen content of a productive pond should be 4 mg/l or more. The pH in all pond water was slightly alkaline throughout the experimental period. According to Swingle (1969), the range of pH 6.5 to 9.0 is suitable for fish culture. Boyd (1982) reported that the suitable range of ammonia-nitrogen in fish culture less than 0.1 mg/l. However, in the experiment the level of ammonia-nitrogen content ranged from 0.003 to 0.006 mg/l in different treatments which is not lethal for the Tilapia (*O. niloticus*) fry (Rahman and Monir, 2013).

**Growth performance:** Growth performances (final weight, weight gain and specific growth rate) and survival rate of Tilapia (*O. niloticus*) in hapa revealed that T₁ was significantly higher (*P*<0.01) where the stocking density of fry (1200 fry/hapa) was low compared to those of T₂ (1700 fry/hapa) and T₃ (2200 fry/hapa) although the same feed with equal ratio was applied among all the treatments during the experiment. The present results agreed with the findings of Mensah et al., (2013) who studied the effect of stocking density (1000, 1500 and 2000 fry/m²) on the growth performances of Nile Tilapia and found that final body weight gain was significantly higher at a density of 1000 fry/m². Moreover, El-Saidy and Gaber (2002) were found that mean final weight and SGR of Tilapia (*O. niloticus*) significantly (*P*<0.01) higher at the lower stocking density. However, the significantly lower growth performances and survival were in T₃ and T₂ than T₁ that might be due to voluntary appetite suppression, more expenditure of energy because of antagonistic behavioral interaction, competition for food and habitat for higher number of fry (Diana et al., 2004 and Ouattara et al., 2003).

**CONCLUSION**

From the experiment, it can be concluded that stocking density had a significant effect on growth performances and survival of Tilapia (*O. niloticus*) fry. The growth and survivability were increased with the decreasing of density. Further studies are necessary for a longer period to determine the optimum stocking density for monosex Tilapia fry production at farm level.

**REFERENCES**


