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Effects of brood stock density and hapa net material on the production of Nile tilapia (*Oreochromis niloticus* L. 1758) fry at Shoa Robit integrated development project site, Ethiopia

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Abstract
In this study, the effects brood stock density and hapa net material on the fry output of Nile tilapia was conducted with the objectives of identifying the appropriate brood stock density for maximum fry production and ease of management and evaluating the effectiveness of three types of locally available shade nets as breeding hapas. The experiment was run with two brood stock densities (4fish/m^2 and 8fish/m^2) and three types of shade net materials (shadow net 30%, nursery plantation net and khaki fish net) with six treatments in duplicates. The result showed that higher brood stock density (8fishes/m^2) gave significantly (P<0.05) higher total mean fry production (980.01±35.80 fry/m^2), while, the lower brood stock density (4fishes/m^2) gave lower total mean fry (596.5±16.20 fry/m^2).on the other hand, The mean number of fry/female was inversely correlated with density in which the lower broods tock density (4fish/m^2) gave significantly higher (198±5.40 fry/female) compared to the higher density (8fishes/m^2) with 163.33±5.96 fry/female. No significant difference (p>0.05) was observed in total mean fry production among the agricultural shade nets used for both densities. The first clutch (day21) gave significantly higher (p<0.05) mean fry production of 390.33±39.93compared to the second (day 42) with mean fry production of 204.9±22.46 and third clutch (day 63) with mean fry production of 193±19.2. Whereas, there was no significant difference between the second and third clutches in this study.

Keywords: fry, hapa, brood stock density, shade net

Introduction
Fish has a critical dietary protein and micro nutrients for millions of people in Sub-Saharan Africa [6, 14]. Fish flesh contains Omega – 3 (Eicosapentaenoic acid (EPA) and Omega – 6 (Docosahexaenoic Acid (DHA)) highly unsaturated fatty acids which are lacking in human body. These fatty acids are especially important to the development of the brain and the body. Moreover, cheap source of protein is required to support the increasing population in Ethiopia. On the other hand the capture fisheries production for such valuable product is stagnating due to improper management and irresponsible exploitation coupled with limited potential of the water bodies [23, 10]. Tilapia is the most preferred species for aquaculture in its adaptability to basic culture systems. Tilapias are hardy fish species with great tolerance to poor water quality, overcrowding and disease. Tilapia species are also preferred for their ease of reproduction throughout the year [18, 9, 12]. However, inadequate supply of quality fingerlings have been remained the major challenge for tilapia aquaculture in the world [16]. This is partly due to its intrinsic behavior of producing a few eggs at a time. Therefore, different techniques have been developed in different parts of the world to solve this fry and fingerling shortage. There are mainly three techniques for *O. niloticus* fry and fingerling production in the world. These techniques include, open pond method, tank method and hapa (net enclosure) method.
The hapa method of fry and fingerling production of *O. niloticus* is probably the most widely used method in the world. This system is especially popular in Southeast Asian countries. Hapas (net enclosures) are a fine mesh cage made up of polyethylene netting material with nylon thread in various mesh sizes. Hapa nets resemble an inverted mosquito net. 3x3x1.5m is the most common size used to produce *O. niloticus* fry and fingerlings [8]. The hapa method of fingerling production allows higher output per unit area than the pond method, and produces

more consistent and uniform size of fingerlings. The pond does not have to be drained and prepared repeatedly for stocking between each breeding cycle. The hapa method is the most efficient and economical of the three methods. It does, however, require more management effort. Adequate water exchange is needed, and use of supplementary feed is essential [21]. Brood stock density is one of the important biological factors that have significant influence on fry production in tilapia culture [22]. Moreover, the exploitation of brood stock density is one of the several techniques applied to improve mass production of tilapia fry [1]. As it is known that tilapias are cannibalistic fish, lower brood stock densities gave better fry production than higher densities in several researches [3, 26]. Moreover, in ponds, the low production of tilapia fry has been ascribed to a suboptimal brood stock density [19].On the other hand, under intensive hatching systems, brood stocks are often stocked at high densities in small and confined breeding units such as aquaria, tanks and net enclosures (hapas), resulting in aggression and fighting between males, and thus, affecting fry production [5]. The low fecundity and asynchronous breeding habit of the maternal mouth-brooding tilapia of the genus *Oreochromis,* represents one of the major constraints that hinder expansion in tilapia reproduction. Therefore, optimum stocking rate of brood stock per unit area [3, 15, 26], brood stock exchange and conditioning at regular intervals [15, 17], frequency of periodic seed removal from the brooding females [15, 17] and temperature management [4] have been developed to overcome this problem. Commercial hatcheries for Nile tilapia are still none-existent in Ethiopia. It is in this context that the current proposal on the effect of brood stock density and hapa net material on fingerlings production of Nile tilapia (*Oreochromis niloticus*) at Shoa Robit integrated development project site is prepared with the objective sidentifying the appropriate brood stock density for maximum fry production and ease of management and to evaluate the effectiveness of three types of locally available agricultural shade nets as breeding hapas.

Materials and Methods

The study site

This study was conducted at Shoa Robit aquaculture-Agriculture integrated project site of Debre Berhan University which is located 225km from the capital city Addis Ababa. The agro-ecology of the area is lowland with average altitude of 1280m.a.s.l and with minimum and maximum temperatures of 21 and 31.75^{0}c respectively. Average Annual rainfall of the area is 1119mm [2].

Experimental design

The experiment was designed as a fractional factorial designs with two factors (brood stock density and hapa net material) one at three levels and the other at two levels. 18 male and 54 female brood stock fishes with average weight of 100g were used. The experiment was conducted in duplicates using 12 agricultural shade nets of locally available materials with an average mesh size of 2mm and 1.5x1.5x1m size set in plastic lined ponds. Two brood stock densities (4fish/m^2 and 8fish/m^2) and three types of hapa materials (Shadow net 30%, Nursery plantation net and Khaki fish net) locally available agricultural shade nets were evaluated in this experiment (figure.1). The hapa nets were suspended on wood and the volume of the water in each hapa was adjusted to be 1m^3. Fine mesh mosquito net was used at the bottom of the khaki fish net to avoid loss of eggs since the mesh size of the khaki net was larger. Water quality parameters (pH, dissolved oxygen and temperature) were measured daily. The experimental ponds were fertilized with poultry manure at a rate of 1500kg/ha/week following the procedure described by [21]. After selection, brood stocks were conditioned for 10-12 days before the beginning of the experiment in a separate hapa nets). Experimental diet were formulated to contain 30% crude protein which was optimum for *O. niloticus* as suggested by [11], and was prepared from locally available materials; Niger (*Guizotia abyssinica*) seed cake, mill sweeping, meat and bone meal, wheat bran and wheat flour. The feed was administered at the rate of 3% of their body weight twice a day. The extruded feeds (pellet) was made in Zoology Laboratory of Debre Berhan University in the ratio of Niger seed cake (20%), mill sweeping (16%), meat and bone meal (28%), wheat bran (32%) and wheat flour (4%). *Win feed 2.8 software* was used to analyze the supplementary crude nutrients of the composition. These proportions were sieved, mixed thoroughly with boiled water and the damp "dough-like" mixture was then pelleted by electric extruder machine. Boiled water provided lubrication for compression, extrusion and caused gelatinization of raw starch present in ingredients of plant origin, resulted in adhesion. The pellet was dried under shade condition for 48 hours. After the conditioning period is over, the breeding hapa nets were stocked with a sex ratio of 1 male to 3 females at a stocking density of 4 or 8 brood stocks per m^2.After spawning, the sac fry and swim up fry from the breeding hapa nets were collected and counted using fine mesh scoop nets and were transferred to nursery hapa nets.

Table 1: Experimental designations

Treatments	Brood stock density	Hapa net material
T1	4	Shadow net 30%
T2	4	Khaki fish net
T3	4	Nursery plantation net
T4	8	Shadow net 30%
T5	8	Khaki fish net
T6	8	Nursery plantation net

Fig 1: experimental set ups

Table 2: Description of the agricultural shade nets used in this study

Name of the shade net in this experiment	Factory/default name	Size
White shade net	Shadow net 30%	Monofilament: 0.28mm
Green shade net	Nursery Net/Nursery Plantation Net	-
Brown shade net	Khaki Fish Net Airtex Mesh Fabric Polyester	Elastane stretch 58" width 5mm holes

Data coding and analysis

The data obtained from the experiment was coded into SAS 9.1 and GL was used to analyze the data at 95% confidence interval. Tukey HSD test was used for the mean separation.

Since the data obtained was not normally distributed, square root transformation was made to normalize the data. Hence, according to Kolmogorov-Smirnov normality test the data was normally distributed ($p>0.05$). Therefore, the p-value presented in the tables below is of the transformed data. However, the standard error and the means are of the original data set.

Results

Water quality parameters

Water quality parameters were in an acceptable range for tilapia reproduction where the pH varied from 9 to 9.53, while the DO of the culture water varied between 6.5 and 8 mg/l. The maximum and minimum water temperatures during the experiment were 27.9 and 27.5 respectively (table 3).

Table 3: Average water quality parameters during the experiment

Treatments	pH	Dissolved oxygen (mg/l)	Temperature (^{0}C)
T1	9.33	6.7	27.9
T2	9.29	7.1	27.8
T3	9.32	7.0	27.5
T4	9.28	8.0	27.9
T5	9.00	6.6	27.7
T6	9.53	6.5	27.6

Effect of brood stock density on the mean total fry production

As indicated in table 4, the treatment with 8 brood stocks/m^2 gave significantly higher total fry production after 63 days of culture period accounting to 980.01±35.80 fry/m^2. On the other hand, the second treatment (4 broods tocks/m^2) gave 596.5±16.20 fry/m^{2}which is much lower compared to the first treatment value.

Table 4: Effect of brood stock density on the mean total fry production (three clutches)

Density/m^2	No of fry (mean±SE)	P value
4	596.5±16.20^a	P<.0001
8	980.01±35.80^b	

Means in the same column having the different superscript letter are significantly different ($p<0.05$)

Effects of the hapa material on mean total fry production

With a mean fry number of 659±19.12, 523.5±35.81 and 606.5±28.65 for Shadow net 30%, Nursery plantation net and Khaki fish nets respectively in the lower density (4fishs/m^2) and with a mean fry number of 1066.5±49.58, 981±68.39 and 892±74.83 for Shadow net 30%, Nursery plantation net and Khaki fish nets respectively in the highest density (8fish/m^2), no significant difference was observed in the number of fry production between the hapa net materials (table 5).

Table 5: Effects of the hapa material on mean total fry production

Hapa net material	Density 4	Density 8	P value
	No of fry (mean±SE)	No of fry (mean±SE)	
Shadow net 30%	659±19.12^a	1066.5±49.58^a	0.1829
Nursery plantation net	523.5±35.81^a	981.5±68.39^a	
Khaki fish net	606.5±28.65^a	892±74.83^a	

Means in the same column having the same superscript letter are not significantly different ($p>0.05$)

Effects of density on number of fry/female

Significant difference ($P<0.05$) was observed on the mean total fry/female production between fish densities of 4 and 8 fishes/m^2. However, in contrast to the higher mean total fry production of the 8fish/m^2 density (163.33±5.96), the lowest density (4fish/m^2) gave better female efficiency (198.33±5.40fry/female (table 6)).

Table 6: Effects of density on Fry/female

Density (brood stock/m^2)	No of fry/female (mean±SE)	P value
4	198.33±5.40^a	0.0104
8	163.33±5.96^b	

Means in the same column having different superscript letter are significantly different ($p<0.05$)

Effects of hapa net material on fry/female

No significant difference was observed on fry/female between the different hapa net materials in this experiment (table 7).

Table 7: Effects of hapa net material on the mean fry/female

Hapa net material	No of fry/female (mean±SE)	P value
Shadow net 30%	198.79±5.40^a	
Nursery plantation net	169.04±7.88^a	0.1157
Khaki fish net	175.42±7.95^a	

Means in the same column having the same superscript letter are not significantly different ($p>0.05$)

Effects of clutch on mean fry production

Significant difference was observed on mean fry production between the different clutches where, the first clutch gave the highest mean fry production of 390±39.93 followed by second clutch with mean fry production of 204.9±22.46 (table 8).

Table 8: Effects of clutch on mean fry production

Clutch (days)	No of fry (mean±SE)	P value
1st clutch (day 21)	390.33±39.93^a	
2nd clutch (day 42)	204.9±22.46^b	<.0001
3rd clutch (day 63)	193±19.21^b	

Means in the same column having different superscript letter are significantly different ($p<0.05$)

Discussion

The present study showed that higher brood stock density (8fish/m^2) gave significantly higher number of fry after 63 days of culture in three consecutive clutches. This result is in agreement with [15] where maximum daily seed production was significantly higher at a density of 8fish/m^2 whereas, lower density was related to lower daily seed production. However, the results of the present study is in disagreement to [13] who suggested that 5 fish/m^3 for the optimum seed production of Nile tilapia in suspended hapa. In addition, [27] found that 4 fish/m^3 had better seed production and spawning synchrony than 8 and 12 fish/m^3. The justifications as to why the lower sex ratio gave better results in these experiments was attributed to the better water quality in lower densities compared to higher brood stock densities. On the other hand, the significantly higher mean fry per female production in the lowest density (4fish/m^2) might indicate better spawning synchrony as reported by [7] where only 29% of Nile tilapia females reared in hapas in ponds in Thailand spawned when brood stock density was 10 fish/m^3, compared to 39% and 42% at 5 and 2.5 fish/m^3. The present study also is in agreement with [24] where percent of female spawned was statistically higher at a density of 4fish/m^2 (24.69%) than at a density of 8fish/m^2 (19.33%).

Therefore, the significantly higher fry output in the higher density in the present study may be attributed to better water quality parameters and regular fry harvesting every 21 days to avoid fry being eaten by brood stock fish. The hapa materials and regular scrubbing of the hapas to avoid clogging may also have contributed to the better quality parameters favoring the higher stocking density to give better fry production. The sex ratio used also may contribute to the betterment of the fry production in the higher stocking density where 1:3 male to female ratio was used. Several experiments have been conducted on the effect of sex ratio on fry production and most of them found that sex ratio of 1 male to 3 females gave better reproductive performances than other sex ratios. [20] Found no significant difference (p>0.05) in seed production of red Tilapia between the 1:1 and 1:3 sex ratios in the 1st and 3rd clutches. However, in the 2nd clutch, seed production from 1:3 sex ratio was significantly higher (p<0.05) than the 1:1 sex ratio. However, sex ratio of 1:3 male to female produced higher mean seed production than the sex ratio of 1:1 even though they were statistically indifferent. Regarding the effects of hapa net material, in this experiment, since the standard hapa used in Asia is expensive and limited in Ethiopia, using alternative cost effective shade net materials was one of the main objectives of this experiment. To this end, three types of shade net materials were used (Shadow net 30%, Nursery plantation net and Khaki fish net). In terms of water quality parameters no significant difference was observed between the materials as the mesh size is nearly similar except the Shadow net 30%and Nursery plantation net were tighter than the Khaki fish net. Fouling of hapas was also observed more on the Shadow net 30%, compared to the Nursery plantation net and Khaki fish net. Generally, the three materials showed no significant difference in fry production per m^2 of water area in both brood stock densities. However, depreciation rate of the Khaki fish net is very high followed by Nursery plantation net and Shadow net 30%.

Regarding the effects of clutch (harvesting), the first clutch gave significantly higher fry output than the second and third clutches. This result is in disagreement with [20] where the second clutch gave significantly higher fry production than the first and third clutches. This may be attributed to the deterioration of water quality as fouling of hapas may increase with time.

Conclusion

This study showed that it is possible to produce significant amount of Nile tilapia fish fry using locally available shade nets comparable with the intensive indoor incubation hatchery system in Ethiopia. The study by [25] also confirmed that Nile tilapia fingerlings produced in incubation units, hapas or ponds exhibited similar grow out performance. These treatments produced the greatest yield of fish, and a greater proportion of harveste danimals fell in larger size categories. Moreover, the higher stocking density gave better performances in terms of fry production perm [2]; therefore, it is advisable to use the higher stocking density for broods tocks. On the other hand, Shadow net 30% has better durability and hardiness than the Nursery plantation net and Khaki fish net. Water exchange and brood stock re-conditioning must be considered after the third clutch to have optimum fry production.

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