Ghana J. Sci. **64** (2), 2023, 1 - 11 *Original scientific article* https://dx.doi.org/10.4314/gjs.v64i2.1

EFFECTS OF BROODSTOCK SEX PAIRING RATIOS AND REST PERIODS OF THE NILE TILAPIA *OREOCHROMIS NILOTICUS* ON FRY PRODUCTION

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ABSTRACT

This study evaluated the effects of varying male to female brood stock pairing ratios of 1:1, 1:2 and 1:3 with resting intervals of 0, 3 and 7 days on fry production of the Nile tilapia at the Aquaculture Demonstration Centre (ADC), Ashaiman, Ghana. Nine (9) treatments were arranged according to a 3 x 3 factorial design with three replicates giving a total of 27 spawning hapas (1 m^3). Male and female brooders of mean body weight (229 ± 5.43 g and 171.6 ± 21.7 g respectively) were paired at the different sex ratios. Water temperature and DO were taken daily whiles pH, salinity and ammonia were measured weekly. All the water quality parameters were within suitable range for Nile tilapia fry production. The total mean fry output for the sex pairing ratios (1:1, 1:2, 1:3) were 716 ± 344, 973 ± 325 and 723 ± 577 respectively. The total fry output for 0, 3 and 7 days of resting were 1503 ± 868, 2030 ± 637 and 3051 ± 1054 respectively with an observed 35% (3 days rest) and 103% (7 days rest) fry production increment over the 0-resting treatment. With respect to the combined effect of varying sex ratios and resting periods, the highest mean fry production (701 ± 405) was attained under the 7 days resting period at a pairing ratio of 1:2. The high reproductive performance of broodstock pairing ratio 1:2 and resting period of 7 days under this study confirms its efficient as a broodstock management technique for an increased Nile tilapia fry production.

Keywords: Broodstock, Fry, Ghana, Nile Tilapia, Sex pairing ratio

Introduction

The human population is well in excess of 9 billion with the accompanying challenge of provision of food and support of livelihoods (Garcia, 2010; Daszkiewicz, 2022). The demand for seafood has increased because it is deemed a healthier animal protein source. However, the world's fishing areas have reached their maximum potential for capture fisheries production and aquaculture currently accounts for appreciable growth in fish supply for human intake and reduced overreliance on wild fish stocks. The global aquaculture industry is expected to produce more than 102 million metric tonnes annually by 2050 to sustain the global per capita consumption (Merino *et. al*., 2012; FAO, 2022).

Ghana is a net consumer of fish with a per capita consumption of 21.9 kg in 2022. The annual total fish requirement exceeds supply, with a fish self-sufficiency of 54.8%. Aquaculture production has seen a steady growth from 44,610 mt in 2015 to 132,652.39 mt in 2022 (MoFAD, 2023), and increased intensification has been identified as a channel to bridge the wide gap between demand and supply. A major limitation to increased aquaculture production inter alia is the availability and access to high quality fish seed (Ragasa *et al*., 2022) despite the existence of a few government and privately-owned fish hatcheries in the country (MoFAD, 2023).

The Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) is the dominant fish cultured in Ghana and contributes about 80% to the total annual aquaculture production in the country (MoFAD, 2023). Nile tilapia production is inhibited by uncontrolled reproduction resulting in the production of low-priced fish below marketable sizes during harvest. Mating techniques in Nile tilapia ranges from lone pair to multiple sex pairing in order to produce fry (Fessehaye *et al.,* 2006) but the reproductive success is influenced by brood stock conditioning, sex ratios, resting periods, stocking density, stage of development, size, diet and feeding rate (Tahoun *et al*., 2008; Abou-Zeid, 2015). Low production of Nile tilapia seeds has also been blamed on very low brood stock density, poor spawning techniques, inadequate brood stock nutrition, high fry mortality, improper resting periods and unsuitable sex ratios. The use of appropriate brood stock pairing ratios and resting periods could enhance fry production and subsequently increase profit (Mires, 1982; Salama, 1996; Siddiqui & Al-Harbi, 1997; Phelps & Popma, 2000; Nour *et al*., 2008; Khalfalla *et al*., 2008; Rurangwa *et al*., 2015; Nasr-Allah *et al.*, 2021). The steadily growing importance of Nile tilapia culture calls for improvement in brood stock and hatchery management techniques necessary for the production of quality fingerlings in adequate quantities to meet production goals of farmers. Fry production at the Aquaculture Demonstration Centre of the Ministry of Fisheries and Aquaculture Development in Ashaiman, Ghana is done using a sex pairing ratio of 1:3 male to females under a no rest system with a production efficiency of 20% in 2018 (Lutterodt, 2018). The primary aim of this work was to investigate the efficiency of different sex pairing ratios and resting periods on Nile tilapia fry production at ADC, Ashaiman, Ghana.

Experimentation

Study area

The study was carried out at Ashaiman Aquaculture Demonstration Centre (ADC), a government fish hatchery situated in the Greater Accra Region of Ghana. The Centre lies between Longitude N 05.669 77 0 and Latitude W 000.05 394 (Fig 1).

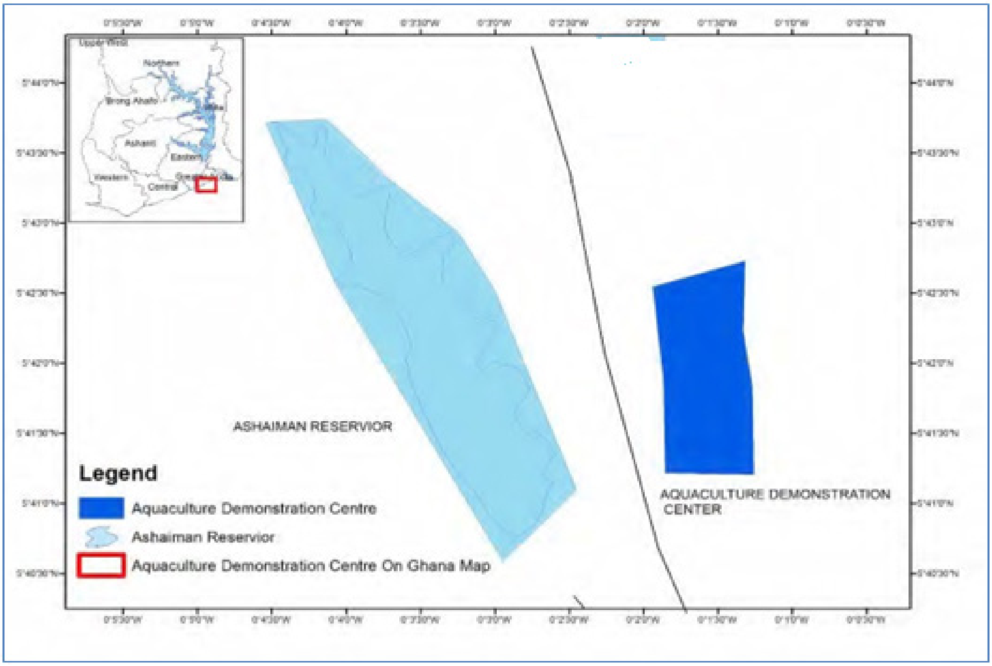

Fig. 1: Map of Ghana showing the Aquaculture Demonstration Centre at Ashaiman

The Centre was set up about 46 years ago under the Fisheries Directorate of the Ministry of Food and Agriculture but is now run by Ministry of Fisheries and Aquaculture Development (MOFAD). It forms about 5% of the irrigational dam area which was earmarked for agricultural activities. The dam is the main source of water supply for this breeding center and the surrounding community.

The Center's culture facilities include sixteen (16) nursery tanks of 50 m^2 each, four (4) earthen ponds of 150 m^2 for holding brooders, incubation room for egg hatching, and 5 (five) earthen ponds (1500 m^2 each, the largest being 2100 m^2) for grow-out and fingerling production. The core mandate of ADC is the multiplication and dissemination of tilapia and catfish fingerlings. The center also provides extension services and on-farm training to beneficiary fish farmers and students.

Experimental design

Nine (9) treatments were done to ascertain their effect on seed production. The treatments were male to female pairing ratios of 1:1, 1:2 and 1:3 at resting intervals of 0, 3 and 7 days. Each treatment had three replicates. The Akosombo strain of the Nile tilapia (*O. niloticus*) brood fish obtained from the Aquaculture Research and Development Centre (ARDEC), Akosombo, Ghana were used for this experiment.

Nile tilapia conditioning and stocking

The brooders were selected, hand sexed and separated into 6 m^3 hapas and fed with commercial pellet feed of 4.5 mm at 2% of body weight (BDW) twice daily for two weeks to condition them prior to the commencement of the experiment. The brooder population was 54 females of an average weight (171.6 ± 21.7 g) and 27 males (229 ± 5.43 g). After conditioning, the Nile tilapia brood fish were selected and weighed according to the desired sizes to eliminate bias, hand sexed and paired into twenty-seven (27) hapas. The hapas were mounted in a 1500 m^2 pond of depth 1.5 m. The hapas were supported with sticks and kept in position against the wind and current by using sand filled bottles as weights. Male and female brooders were paired in the male to female ratios of 1:1, 1:2 and 1:3 with resting intervals of 0, 3 and 7 days. This design was to ascertain the treatment that yields the highest quantity of fry. Each hapa was fed for 14 days at 2% BDW. The paired brooders were harvested for fry after 14 days and rested for 3 and 7 days.

Feeding of Brooders

The brooders were fed twice daily (09 hours and 16 hours GMT) at 2% body weight with a commercial feed of particle size 4.5 mm and 37% crude protein level.

Water quality measurement

Water quality parameters such as temperature, dissolved oxygen, pH, and, salinity was taken from each hapa twice a day throughout the experiment with a multi parameter probe Horiba water quality probe U-51(Horiba Ltd., Kyoto, Japan). Water samples were collected and taken to the laboratory for the determination of ammonia and nitrite and nitrate concentrations with a spectrophotometer.

Harvesting of fry

Fourteen days after stocking of brooders, fry were harvested from the surface of the water with a drag net. This process of fry harvest was repeated until all fry were collected. Fry were harvested from the hapas before the brooders were collected (Plate 4). This prevented mortality of fry due to aggressive movement of the broodstock. The mouth of the female brooders was examined for naturally spawned eggs or yolk sac fry. This was done for all the 3 treatments at 14 days inter harvesting period. The fry was put in a basin of water, cleaned and counted using gravimetric estimation. The gravimetric estimation was done by weighing a spoonful of fry and multiplying it by the number of spoons of fry scooped to obtain the total number of fry produced in each treatment.

Production parameters

The following production parameters were estimated based on the mathematical relationships:

Percentage hatching

This was done to estimate the hatching success of the brooders. It was expressed mathematically as:

Percent hatching = (No. of fry harvested / Total no. of eggs expected) x 100 (Abdullahi *et al.*, 2018) (1)

Fulton's condition factor

This provides information on the state of well-being of the experimental fish.

W/L^3 x 100 (Froese, 2006) (2)

Where K is the condition factor, W is the final weight and L is the final body length (cm).

Percentage survival (SR)

This was estimated to reveal the rate of survival of the fry.

SR (%) = N_2/N_1 x 100 (Ridha, 2006) (3)

Where N_1 is the total number of stocked fish and N_2 is the total number of fish surviving.

Data analysis

The data was subjected to one-way ANOVA to analyze the means and post hoc analysis using Duncan's multiple range test to test for significant differences ($p < 0.05$) between the treatment means.

Results

Water quality in hapas

Table 1 indicates the mean values of water quality parameters measured during the study in the breeding hapas. Mean water temperature ranged from 29.03 ± 0.51°C in the morning to 34 ± 0.5°C in the afternoon. Dissolved oxygen (DO) concentration varied from 7.56 ± 0.04 mg/L in the morning to 12.73 ± 0.15 mg/L in the afternoon. The levels of the ammonia (0.1 ± 0.06 mg/L), nitrite (0.04 ± 0.3 mg/L), salinity (0.25 ± 0.01‰) and pH (7.8 ± 0.2) were within acceptable range for production of Nile tilapia fry.

TABLE 1

Mean values of water quality parameters in breeding hapas during the study period.

Parameter	Morning	Afternoon	Evening
Temp (°C)	29.03 ± 0.51	34 ± 0.50	32 ± 0.20
DO (mg/L)	7.56 ± 0.04	12.73 ± 0.15	10 ± 0.50
pH	7.8 ± 0.2		
Ammonia (mg/L)	0.1 ± 0.06		
Nitrate (mg/L)	0.17 ± 0.05		
Nitrite (mg/L)	0.04 ± 0.3		
Salinity (‰)	0.25 ± 0.01		

Broodstock growth parameters

The brooders' parameters are presented in Table 2. The initial average weight of female brooders was 171.6 ± 21.7 g and that of the males was 229 ± 5.43 g. The final mean weight for females was 184 ± 22.4 g and 266.5 ± 20 g for the males. The condition factor of the female brooders (3.42 ± 0.24) was lower than that of the male brooders (3.46 ± 0.34) at the end of the experiment. The male brooders had a higher survival rate (99 %) than the female brooders (95 %).

TABLE 2

Production parameters of Nile tilapia brooders at the Aquaculture Demonstration Centre.

Parameters	Female	Male
Initial body weight (g)	171.6 ± 21.7	229 ± 5.43
Final body weight (g)	184 ± 22.4	266.5 ± 20
Initial condition factor (g/cm)	3.26 ± 0.24	3.07 ± 0.35
Final condition factor (g/cm)	3.42 ± 0.24	3.46 ± 0.34
% Survival	95	99

Fry production

Fourteen days after stocking of brooders, fry were harvested from the surface of the water. The production indicators of the Nile tilapia fry are presented in Table 3. Fry production showed variations with the sex pairing ratios and length of the period of rest of the brooders after a spawning cycle. The highest fry production (4087 ± 235) was observed for 1:2 sex ratio and 7 days period of rest whiles the lowest quantity of fry produced occurred with 1:1 with no rest (715 ± 25). Generally, fry production was lowest in all the sex ratios where the brooders were not given any rest (0 resting period) with no significant difference ($P = 0.1524$). There were significant differences in the quantities of fry produced between all the 3 sex pairing groups where brooders were not rested (0 resting period) and those that were rested for 3 and 7 days as shown in Table 3. Based on the results from Table 3, the spawning parameters, including percent hatching were estimated for the highest performed sex ratio of 1:2 as shown in Table 4. Resting brooders for 7 days after a spawning cycle produced the highest hatching success (91%) whiles the least percent hatching of 65 was obtained when brooders were not made to rest.

TABLE 3

Fingerling quantities harvested from the different sex pairing ratios (male: female) and resting periods. Values with same superscripts in a row or column are not significantly different.

Resting period (Days)	**Mean number of fry produced per sex ratio**			
	1:1	**1:2**	**1:3**	**P value**
0	715±25[a]	969±78[a]	723±28[a]	0.1524
3	3160±374[b]	2104±322[c]	2057±360[c]	0.0420
7	2565±321[b]	4087±235[d]	3723±710[d]	0.0391
P value	0.0023	0.0004	0.0019	

TABLE 4

Spawning parameters of Nile tilapia brooders paired at a sex ratio of 1:2.

Parameter		**Resting period (days)**	
	0	**3**	**7**
No. of females	3	6	9
Estimated no. of eggs/female	500	500	500
Total no. of fry harvested	969	2104	4087
Average no. of fry/female	323±54	350±24	454±38
Percentage hatching	65	70	91

Discussion

Water quality parameters

The levels of the water quality parameters obtained during this study were within the acceptable range for spawning and growth of the Nile tilapia as reported by Magid & Babiker (1975), Ross (2000), El-Sayed (2006) and El-Sherif (2008). The optimum temperature range suitable for reproduction and survival of Nile tilapia occurs at a temperature of 27 ^{o}C to 30 ^{o}C (Azaza *et al*., 2008). The water temperature values (29-32 ^{o}C) in this study were higher comparatively possibly because the experiment was conducted during the warmest season of the year (December-March, 2019), although it was within the acceptable range for reproduction. According to Yadav (2006) reproductive performance has been found to be very poor at temperatures higher than 35 ^{o}C and lower than 22 ^{o} C. Thus, the water temperature range (29-32 ^{o}C) during this study was within the recommended tolerable range (22-35 ^{o}C) which is acceptable for breeding and fry production for the Nile tilapia. The diurnal fluctuations in oxygen levels with supersaturation during the day and oxygen depletion at night are due to photosynthesis, respiration and diel fluctuations (Boyd, 2020). The mean value of 7.56±0.04 mg/L obtained in the morning showed that oxygen levels dropped during the night to a range which could still support Nile tilapia growth and reproduction. According to Boyd (2010, 2020), low DO value recorded in the morning is due to bacterial activities which consume oxygen and the lack of photosynthesis during the night to introduce or add on oxygen. The lowest limit of the dissolved oxygen for optimum fish production in ponds has been suggested to be 5 mg/l (Das, 2000, 2001) as cited by (Choudhary & Sharma, 2018). (El-Sayed, 2006) reported that optimum growth of fish occurs at pH ranges between 7.5 and 8.5 whiles (Nandlal & Pickering, 2004), and (Peterman, 2011) reported acceptable range of pH from 6 to 9. As indicated by (Choudhary & Sharma, 2018), tilapia generally can survive in pH ranging from 5 to 10 but do best in pH range of 6 to 9. This confirms that the mean level measured during the study (7.8 ±0.2) is acceptable and suitable for reproduction and spawning. According to (Nandlal & Pickering, 2004), suitable salinity levels for Nile tilapia culture in fresh water medium could rise from 5 to 10 ‰. Fineman-Kalio (1988) indicated that gonadal development and spawning of Nile tilapia could occur at salinities as high as 17-29‰ but stops completely at salinity above 30‰. The mean salinity (0.25±0.01‰) obtained from this work was acceptable and suitable for reproduction.

Fry production performance

The lower number of fry observed in the brooders that had zero resting period as compared with the higher numbers recorded for those that had 3 and 7 days of rest was probably caused by exhaustion of the broodstock (Ridha & Cruz, 1998) from continuous use of the same broodfish. This could possibly be the reason for a higher percent hatching of eggs in the rested brooders than those that were not rested in this study. Reproductive performance was enhanced in hapas where broodstock were rested indicating that resting possibly replenished or renewed the spent energy of the fish from earlier spawning occurrence. Although the 7 days of rest showed higher fry numbers across the three sex pairing ratios, than the 3 days, the numbers were not significantly different and this could be as a result of interruption of their social interaction. The separation, resting and re-pairing of broodstock for subsequent experimentation after an initial phase could lead to interruption in social interaction and affect fry production (Basiita, 2020). Little *et al*. (1993) noted that interruption of social interaction could affect fry production performance of brooders. Cannibalism and incomplete harvest cannot be ruled out as possible contributors to mortalities and the concomitant low fry production (Bhujel *et al*., 2000).

Pairing broodstock at lower ratios of 1:1 across the 3 periods of rest in this study was the least efficient It revealed that the ratio of 1:2 produced more fry than stocking at a higher ratio of 1:3 with or without rest. This indicates better performance of male brooders with fewer females than higher sex ratios or density. This may be due to the single male's ability to consistently interact with each female to trigger breeding. The higher fry production of 3160±374 and 2565±321 respectively for days 3 and 7 at a pairing ratio of 1:1 could have resulted from the male consistently pressuring the female to breed without any competition from other males in the hapa. The low density may also have enabled the female to establish a "safe territory" in which to reproduce consistently but the quality of fry produced could be compromised due to the low reproductive strength of the male from continuous breeding as it was for the zero-rest group. Therefore, to maximize fry production per female at a pairing ratio of 1:1, 3 days of rest is recommended. The relatively lower fry output at the highest pairing ratio of 1:3 and longest resting period could be due to higher stocking density of 4 fish/m2 coupled with a prolonged resting period. At higher densities there is competition for space which eventually affects reproductive efficiency whilst ovulation and reabsorption of eggs may also occur during long period of rest. According to Peters (1983), The reabsorption of well-developed ova in the ovary is related to the lack of opportunity to spawn due to a higher resting period (Peters, 1983; Basiita, 2020).

On the other hand, higher resting period with a lower stocking density (3 fish/m^2) appeared more favourable, probably due to increased mating frequency. (Lovshin & Ibrahim, 1988) found a 16% increase in egg and fry production over a 105-day period by resting *O. niloticus* males and females every 21 days. This may be due to the active roles of rested males in courtship and restored vitality of females to produce more quantity and quality of eggs (Lovshin & Ibrahim, 1988; Ambali, 1990; Little *et al*., 1993; Basiita, 2020). Glenney (2002) suggested that the breeding process be terminated once a peak production period of 17 to 20 days after stocking had been reached, and that these breeders be replaced. The continuous breeding under a higher stocking density reduced the reproductive strength of the males and the regenerative capacity of the females to produce eggs (El-Khasheif *et al*., 2013).

Conclusion

A broodstock sex pairing ratio of 1:2 combined with a resting period of 7 days after each successful spawning cycle was the best option for the management of the current Nile tilapia broodstock used at the Aquaculture Demonstration Centre for fry production. Fry production for brooders rested for 3 and 7 days had higher hatching success than those that were not rested.

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Received 30 Jun 23; revised 16 Jan 24.