EFFECT OF BROODSTOCK SEX RATIO ON GROWTH AND REPRODUCTIVE PERFORMANCE OF BLUE TILAPIA *OREOCHROMIS AUREUS* (STEINDACHNER) RERED IN HAPAS

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Abstract

The aim of the present study was to investigate the effects of different broodstock sex ratios on growth and seed out-put of blue tilapia *Oreochromis aureus* under the conditions of hapa in-pond- tilapia hatchery system. Three different broodstock sex ratios:- 1:1, 1:2 and 1:3 (male ♂: female ♀) of blue tilapia were tested using spawning hapas each measuring 4 (2X2X1 m^3) suspended in an earthen pond (10,000 m^2) at a depth of approximately 0.5 m height to attain a net volume of 2 m^3 per hapa). Broodfish were stocked at a density of 6 fish/ m^2 equivalent to 12 (6♂: 6♀), 12 (4♂:8♀) and 12 (3♂:9♀) fish per each spawning hapa, respectively for the 1st, 2nd and 3rd experimental sex ratios. Body weights ranged between 146.67±2.33 and 147.67±1.54 g and 118.67±1.86 and 135.0±1.73 g, respectively for male and female blue tilapia broodstock. Tilapia broodstock were fed diet containing 35%CP/ 3760 K cal ME/ kg of diet). The three experimental sex ratios were subjected to be studied in one way analysis of variance with three replicates/ treatment giving total number of nine (2 m^3) spawning hapas. Female broodstock growth performance in terms of average weight gain (AWG), average daily gain (ADG) and specific growth rate (SGR) was not significantly ($p>0.05$) affected by different broodstock sex ratios, while the male ADG and SGR were significantly ($P\leq0.05$) affected by the different experimental broodstock sex ratios. Male and female tilapia broodstock survival rates did not significantly ($p>0.05$) affected by the experimental treatments and all treatments had 100% survival rates. Seeds (egg, sac fry and swim-up fry) were collected every 3 weeks. The experiment lasted for a total 110 days. The results also showed that, Female broodstock group of the 2nd sex ratio (1▯ :2▯) produced more seed ($P\leq0.05$) per female (911.17±10.93 seeds) as compared to the 1st (854.63±17.32 seeds) and the 3rd (767.04±11.06 seeds) sex ratios. The absolute seed production (seed/ female), relative seed production (seed/ g female) and (seed/ female/ day) were in favor of the second sex ratio. Under the conditions tested in this study, fry production of blue tilapia was affected by different broodstock sex ratios and improved at a sex ratio 1:2 (male: female).

Key words: Blue tilapia, *Oreochromis aureus*, broodstock, sex ratio, reproductive performance and seed out-put.

INTRODUCTION

Reproductive success in many fish species has been shown to be influenced by, among other factors, the broodstock, sex ratio, stocking density, age, size, nutrition and feeding regime (Ridha and Cruz, 1989; Smith *et al*., 1991; Salama, 1996; Izquierdo *et al*., 2001; Chong *et al*. 2004; Tahoun, 2007; Hammouda *et al*., 2008 and Ibrahim *et al*. 2008). The use of fine-mesh cages or net enclosures (hapas) is one of the alternatives for producing fry. Hapas have the advantage in that they can be placed in existing bodies of water where other fish species are present and do not require that the pond be drained before the fry can be harvested (Guerrero and Garcia, 1983; Hughes and Behrends, 1983; Middendorp and Verreth, 1991; Maluwa and Costa-Pierce, 1993; Bhujel, 2000; Bimbao *et al*., 2000; Bhujel *et al*., 2001 and Bocek 2004 and Tayamen, 2007). The operational sex ratio (ratio of fertilizable females to sexually active males at a given time) is a principal factor influencing the intensity of sexual selection. The overall adult sex ratio is a key factor affecting sexual competition. If the adult sex ratio is biased, potential rates of reproduction are not sufficient to predict the direction of sexual competition. The sex ratio can also be influenced by the distribution of individuals in time and space, temperature and precopulatory guarding of multiple mates (Debuse *et al*., 1999). Brooders are generally stocked at one male: <2 females at a density of 4 to 5 fish/ m^2 of hapa or 0.2 to 0.6 kg/m^2 of hapa. Salama (1996) found that the highest fry production of Nile tilapia *O. niloticus* obtained with the lower sex ratio (1 male: 2 females). The differences among the sex ratio 1:2 and the other experimental sex ratios (1:3, 1:4 and 1:5 male to female ratio) were attributed to male fertilization efficiency, as it sometimes happens that more than one female is ready to spawn at the same time, while available males are not sufficient to fertilize the eggs, resulting in unfertilized eggs. Also crowding may have an effect. Siddiqui and Al-Harbi (1997) stated that, hybrid tilapia (*Oreochromis niloticus X O. aureus*) broodfish should be stocked at a density of 2 fish/ m^2 with a sex ratio of 1: 2 or 1: 3 (male: female) to maximize seed production in concrete tanks. Moreover, Ridha and Cruz (1998) compared the seed production of the tilapia *O. spilurus* at male to female ratio of 1:3, 1:4 and 1:5 under ambient and controlled (water temperature of 30 ± 1.0 ºC and photoperiod of 14n h/ day) conditions. They found that, sex ratios have no significant effect on the seed production under both breeding conditions. No significant differences were observed in the overall seed composition (eggs, yolk sac fry and swim-up fry) of female under both breeding conditions. Sex ratio of brood-fish can have both economic and technical impacts, sub-optimal ratios, in which either males or females are unable to spawn clearly waste resources and increase costs. In terms of maximizing genetic variability, a ratio of 1:1 is desirable but in practice, sub-ordinate fish may be excluded from spawning. Female to male brood-fish sex ratio ranges among 5:1 in ponds and 10:1 (female: male ratio) in hapas and tanks. Fewer than 5 females: 1 male appear to be within the optimal ranges in most situations, especially when synchronized spawning is required, less than 3 females: 1 male is optimal (Little and Hulata, 2000). Although, individual male can court and spawn with females successively, research shows that fertilization rates drop rapidly. Khater (2002) did not find any significant differences between mass spawning of *O. niloticus* and *O. aureus* reared in circular earthen ponds (each of 175m^2 and water depth of 0.75 m) at different sex ratio of 1: 1, 1: 2 and 1: 3 in number fry/ g female body weight. The sex ratio of 1 male to 3 females is more economical for fry production.

MATERIALS AND METHODS

Experimental fish

An over-wintered blue tilapia *O. aureus* broodstock were obtained from a commercial fish farm located in Kafr El-Sheikh Governorate. Broodstock were netted from earthen ponds, manually selected, sexed and transferred to conditioning happas (measuring 6X3X1 m^3),where they were held and kept separately for 20 days for adaptation to the new environment until starting the experiment (1 July, 2006). A total number of 69 females and 39 males were counted, batch weight and stocked in each spawning hapa (measuring 2X2X1) at a rate of 12 broodfish per spawning hapa (6 fish/ m^2). At the beginning of experiment, random samples of approximately 10 females and 10 males from each size classes were taken, individually weighed and immediately killed and kept frozen at-18°C until proximate analysis at the end of experiment.

Experimental diets

Broodstock were fed the experimental diet containing 35%CP and 3760 K cal/ Kg at a feeding rate of 3 % of fresh biomass in each hapa (six days per week). Broodstock were fed two times daily at 9.0 am and 2 pm with feed amounts adjusted at approximately 15–20 days intervals in response to weight gain.

Growth performance parameters

The growth performance parameters are calculated according to the following equations:

-Average Weight Gain (AWG) = Average final weight (g) – Average initial weight (g)

-Average Daily Gain (ADG) = [Average final weight – Average initial weight]/time (days).

-Specific Growth Rate (SGR %/day) = 100 [Ln Wt1 –Ln Wt 0 / t]

Where: - Ln: natural log Wt 0: initial weight (g), Wt 1: final weight (g) and T: time of days.

Water quality parameters

Air and water temperatures were determined four times weekly at 6.00 am and 2.00 pm by using a thermometer. Water dissolved oxygen (DO) content and water pH were measured weekly at 2.00 pm using a digital dissolved oxygen meter (Jenway model 9070) and a digital pH meter (model checker1 produced by Hanna Instrument Co.), respectively. Water salinity (mg/L) was determined biweekly using a digital conductivity meter (Jenway model 4075). Water alkalinity and total ammonia nitrogen (TAN mg/ L) were weekly determined following the methods described by Chattopadhyay (1998).

Statistical analysis

Statistical analysis of the experiment was done using SAS Version 9 (SAS Institute, 2002) statistical package. Data were statistically analysed in one analysis of variance. Mean of treatments were compared by Duncan (1955) multiple range test. Duncan test ($p<0.05$) was used to compare means and ($F<0.05$) was considered for the variance analyses.

RESULTS AND DISCUSSION

Means of water quality parameters measured throughout the experimental period are summarized in Table 1. With regard to ponds water parameters, pH, pre-sunrise and afternoon dissolved oxygen (DO), total ammonia nitrogen (mg/ L) and total alkalinity (mg/ L) in the present work. All values of the above-mentioned parameters were suitable for the normal growth and reproduction of tilapia and warm water fish (Tahoun, 2002 and 2007).

Table 1. Means of water quality criteria of the experimental earthen pond.

Month	Temperature (ºC)			pH	Dissolved oxygen (mg/ L)		Total ammonia Nitrogen (mg/ L)	Total Alkalinity (mg/ L)
	Air	Water (pm)	Water (am)		am	pm		
June	31.0	28.3	23.8	7.6	2.03	8.6	0.060	158
July	31.5	27.3	25.2	7.5	2.04	7.5	0.020	167
August	31.1	27.1	23.7	7.6	2.05	8.3	0.025	195
September	28.2	25.9	22.7	8.2	2.50	8.2	0.040	165

Data on the effects of different broodstock sex ratios on male blue tilapia growth performance are shown in Table 1. No significant differences were found in average initial body weight, average weight gain and average daily gain among different sex ratios while, the differences found in average final body weight and specific growth rate (%/ day) were significant (P≤0.05). Broodstock sex ratio did not significantly affect male survival rates and all treatments had 100% survival rates.

Table 2 . Effect of sex ratio on growth performance of male tilapia *Oreochromis aureus* broodstock.

Treatments/ Sex ratio	Initial weight	Final weight	Average weight gain	Average daily gain	SGR (%/ day)
1♂:1♀ 12 fish (6♂:6♀)/ m^2	147.67 a ±1.453	214.0. a ± 2.082	66.33 a ± 3.48	0.533 a ± 0.029	0.412 a ± 0.021
(1♂:2♀) ١٢fish (4♂:8♀)/ m^2	146.67 a ±2.186	208.33 b ±2.088	61.667 a ± 2.404	0.514 a ±0.020	0.390 a ± 0.017
(1♂:3♀) 12 fish (3♂:9♀)/ m^2	146.67a ± 2.333	205.33 b ± 1.453	58.67a ± 3.756	0.489 b ± 0.031	0.374 b ± 0.025

Means in the same column having different letters are significantly different (P≤0.05).

As shown in Table 3. No significant differences were found in female initial body weight, final body weight, average weight gain average daily gain and specific growth (%/ day) rate among different broodstock sex ratios. Broodstock sex ratio did not significantly affect female survival rates and all treatments had 100% survival rates.

Table 3 . Effect of sex ratio on growth and survival of female tilapia *Oreochromis aureus* broodstock.

Treatments/ Sex ratio	Initial weight	Final weight	Average weight gain	Average daily gain	SGR (%/ day)
1♂:1♀ 12 fish (6♂:6♀)/ m^2	135.0 a ± 1.73	170.33 a ± 5.17	35.33 a ± 2.91	0.294 a ± 0.02	0.270 a ± 0.022
(1♂:2♀) fish (4♂:8♀)/ $m^2$12	128.67 a ± 1.86	164.0 a ± 2.08	35.33 a ± 5.55	0.294 a ± 0.05	0.257 a ± 0.038
(1♂:3♀) 12 fish (3♂:9♀)/ m2	131.67 a ± 1.76	131.67 a ± 2.60	37.67a ±2.73	0.313 a ± 0.02	0.279 a ± 0.019

Means in the same column having different letters are significantly different (P≤0.05).

Table 4 shows the effect of different sex ratios on subsequent 3 seed clutches during the spawning period. At the first seed harvest (clutch 1), seeds per female at the third (sex ratio 1:3) the were significantly (P≤0.05) lower than those of the first (sex ratio 1:1) and the second (sex ratio 1:2), while the differences among broodstock groups in terms of seed produced per female at the 2nd and the 3rd seed harvests (clutches) did not significantly affected by the experimental sex ratios. The data also showed that, Female broodstock group of the 2nd sex ratio (1♂:2♀) produced more seed (P≤0.05) per female (911.17±10.93 seeds) as compared to the 1st (854.63±17.32 seeds) and the 3rd (767.04±11.06 seeds) sex ratios.

Table 4. Effect of sex ratio on seed out-put (seeds/ female) of female blue tilapia *Oreochromis aureus* broodstock.

Treatments/ Sex ratio	Clutch 1	Clutch 2	Clutch 3	Total seeds
1♂:1♀ 12 fish (6♂:6♀)/ m²	294.79 a ±0.14	271.75a ±21.53	288.08 a ± 12.13	854.63 b ± 17.32
(1♂:2♀) fish (4♂:8♀)/ m²١٢	306.39 a ±10.37	٢٩٩,١١ a ±5.07	٣٠٥,٦٧ a ±5.86	٩١١,١٧ a ±10.93
(1♂:3♀) 12 fish (3♂:9♀)/ m²	238.52 b ± 8.85	260.00 a ± 9.25	٢٦٨,٥٢ a ±12.96	767.04 c ±11.06

Means in the same column having different letters are significantly different (P≤0.05).

As shown in Table 5, One way analysis of variance showed no significant differences in absolute (total seed/ female), relative fecundity (seeds/ g female) and system productivity (expressed as seeds/ female/ day) of brood-fish groups at different sex ratio. The highest seed per female (911.17± 10.93) was in favor of the second (sex ratio 1 male: 2 females) followed by the first (sex ratio 1 male: 1 female) and the third (sex ratio 1 male: 3 females), respectively. Consequently, the system productivity (seeds per female per day) recorded the same trend as the total seed produced. The third treatment (sex ratio of 1male" 3 females) exhibited the lowest (P≤0.05) seed/ g female number (3.55±0.06) as compared to the first and the second experimental sex ratios (1male :1 female and 1:2 females). The difference in relative fecundity between the sex ratios 1:1 and 1:2 was insignificant (P>0.05).

Table 5. Absolute and relative fecundity of Blue tilapia as affected by broodstock sex ratio.

Treatments/ Sex ratio	Female Initial weight	Female Final weight	Female Mean weight	Total seeds/ Female	seeds/ g Female	Seeds/ Female/ day
1♂:1♀ (6♂:6♀)	128.67 a ± 1.86	164.00 a ± 2.08	210.67 ab ± 4.19	854.63 b ± 17.32	4.06 a ± 0.09	9.50 b ± 0.19
1♂:2♀ (4♂:8♀)	135.00 a ± 1.73	170.33 a ± 5.17	220.17 a ± 3.03	٩١١,١٧ a ±10.93	4.14 a ± 0.1	10.12 a ± 0.12
1♂:3♀ (3♂:9♀)	131.67 a ± 1.76	131.67a ± 2.60	216.33 b ± 2.54	767.04 c ± 11.06	3.55 b ± 0.06	8.52 c ±0.12

Means in the same column having different letters are significantly different (P≤0.05).

The results of the present study may be confirmed by those of Hughes and Behrends, 1983 who reported maximum seed production of female Nile tilapia stocked at a sex ratio of 1 male: 2 females). In this connection, Siddiqui and Al- Harby, 1997 tested the following four sex ratios (1:2, 1:3, 1:4 and 1:5 male to female hybrid tilapia, respectively) and found no significant difference in seed production, but better performance of females stocked at higher sex ratios (1 : 2 and 1 : 3 male to female, respectively than those of 1 : 4 and 1 : 5). Nevertheless, Bautista et al., 1988 found the best seed production of Nile tilapia at a sex ratio of 1male: 4 females at a broodstock density of 4 fish/ m^2. Torrans and Hiott (1990) reported higher production of bait-or forage-sized blue tilapia at the highest female density than those at lower densities. Recently, Nour *et al.* (2008) evaluated the spawning results of three broad tilapia species, (*Oreochromis niloticus* , *O. aureus* and *O. galilaeus*) fed on two dietary protein levels (25% and 35% CP) and stocked with two sex ratios {1 : 2 or 1:3 ; male (M): female (F)} on the criteria's of fry production. After spawning, the newly hatched tilapia fry were individually counted and stocked in twelve rearing concrete ponds (8.0 m long X2.0 m wide and 0.7 m depth) for two months in order to compare its performances. The results indicated that *O. niloticus* broad with 1:2 M/ F sex ratio fed on 35% CP produced the highest number of fry. The values of total production fry/ female, number of fry/ female per day, and number of fry/ g female showed the same trends. The highest total fry production in *O. niloticus* followed by *O. galilaeus* and the lowest was in *O. aureus*, however average number of advanced fry per gram female was (4.2) , (4.7), (4.6) for *O. niloticus*, *O. aureus* and *O. galilaeus* respectively. On the other hand, Khater (2002) studied the effect of different sex ratio on fry production of both *O. niloticus* and *O. aureus* brood-fish. No significant differences between mass spawning of *O. niloticus* and *O. aureus* at the different sex ratio 1: 1, 1: 2 and 1: 3 in number fry/ g female body weight. He found that, the sex ratio of 1 male to 3 females is more economical for fry production in circular earthen ponds (each of 175 m^2 and water depth of 0.75 m). M'Hango and Brummet (1998) compared fry production of tilapia *O. shiranus* stocked at sex ratios of 1:1 or 1:3 (male to female) and found that fry production per female was significantly higher at 1:1 sex ratio (111 fry/ female) compared to 66 fry/ female at 1:3 ratio. The authors recommended broodstock sex ratio of 1:1 when producing O*. shiranus* fry at a density of 1.25 fish/ m^2 in earthen ponds. Competition among females is a possible cause of reduced fry production in density stocked brood ponds. Silvera (1978) showed the sex ratio of 1:6 male to females to be optimum for mass production of *O. niloticus* and reported that the number of females is the detrimental factor as they can continue to be sexually active over a long period. It appears that, broodstock sex ratio and ratios to be used depend on the readiness of the females and some factors other than sex ratios as broodstock age and size (El- Gamal, 1987; Al-Ahmed *et al.*, 1988; Ridha and Cruz, 1989 Smith *et al.*, 1991; Little and Hulata, 2000 and Tahoun, 2007), broodstock nutrition and feeding regime (Santiago et al., 1985; Izquierdo *et al.*, 2001; Toguyeni *et al.* 2002; El-Sayed *et al.*, 2003 and 2005; Tahoun, 2007; Hammouda *et al.*, 2008 and Ibrahim *et al.*, 2008), broodstock stocking density (Bautista *et al.*, 1988; Msiska and Costa-Pierce, 1997 and Ridha and Cruz, 1999), broodstock exchange and replacement (Little, 1989 and Bimbao *et al*., 2000), hatchery system (Balarin and Haller, 1982; Gurrero and Garcia, 1983 and Bautista *et al*. 1988), frequency of removing seed (Snow *et al*. 1983). From the results of the present study, it is recommended to stock blue tilapia brooders in spawning hapas at a sex ratio of 1 male to 2 females in order to obtain the highest seed production.

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تأثير نسب جنس مختلفة علي أداء النمو و الأداء التناسلي لقطعان التفريخ في أسماك البلطي الأوريا المرباة في هابات

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أجريت هذه التجربة في مفرخ ومزرعة تجارية بمحافظة كفر الشيخ و استخدم في هذه التجربة حجمين من ذكور وإناث أسماك البلطي الأوريا حيث تراوحت متوسطات الوزن الابتدائي للإناث (0.90 ± 69.31و 1.32 ± 143.81) بينما كانت أوزان الذكور (1.32 ± 143.81 و ± 166.28 1.49). كما استخدمت ثلاثة نسب جنس مختلفة (male ♂: female ♀) 1:1, 1:2 and 1:3 من ذكور أسماك البلطي الأزرق المرباة في هابات تفريخ من النايلون أبعادها2X2X1 م[٣] و المثبتة في حوض ترابي مساحته ٢ فدان ونصف (١٠٠٠ م[٢]) وتم تخزين أسماك القطيع بكثافة تخزين ٦ سمكات/م[٢] تعادل ١٢ (٦ذكور:٦ إناث)/هابة التفريخ،١٢ سمكة (٤ ذكور:٨ إناث)/ هابة التفريخ و١٢ سمكة (٣ ذكور:٩ إناث)/ هابة التفريخ ويساوي حجمها ٢ م[٣] للمعاملة الأولي والثانية والثالثة علي الترتيب. وتمت تغذية الأسماك علي عليقة اسماك تحتوي علي ٣٥% بروتين خام و ٣٧٦٠ كيلو كالوري طاقة ممثلة/ جم من العليقة.

بينت النتائج أن متوسط الزيادة الحادثة في الوزن AWG ومعدل النمو اليومي ADG ومعدل النمو النوعيSGR لمجموعات الإناث لم تتأثر معنويا (علي مستوي احتمال٠,٠٥) باختلاف نسب الجنس. بينما تأثرت معنويا قيم معدل النمو اليومي و النوعي للذكور باختلاف نسب الجنس (المعاملات التجريبية) ولوحظ عدم تأثر معدلات الحياة (%) Survival rates في كل من الذكور و الإناث باختلاف نسب الجنس بين الذكور والإناث.

وبالنسبة لمعايير الأداء التناسلي لإناث القطيع لوحظ تفوق اسماك المجموعة الثانية ١٢ سمكة (٤ ذكور:٨ إناث)/ هابة التفريخ في عدد الزريعة الناتجة (911.17±10.93 seeds) مقارنه بأداء المجموعة الأولي (854.63±17.32 seeds) (٦ذكور:٦ إناث)/هابة التفريخ و المجموعة الثالثة (767.04± 11.06 seeds)(٣ ذكور:٩ إناث)/ هابة التفريخ علي الترتيب. وكذلك بينت نتائج التجربة تفوق اسماك المجموعة الثانية (نسبة الجنس ١ ذكر:٢ أنثي) في كل من قيم الخصوبة النسبية (عدد الزريعة الناتجة/ جم من وزن الانثي) وكذلك في معيار إنتاجية نظام التفريخ معبرا عنه (عدد الزريعة الناتجة/أنثي/ يوم).

تحت الظروف التجريبية لهذا العمل يوصي باستخدام نسبة الجنس ١ ذكر:٢ أنثي للحصول علي أفضل أداء تناسلي في قطعان التفريخ في اسماك البلطي الأزرق.