



Reproduction performance of female Nile tilapia under different environments and age classes

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ABSTRACT. Current assay verifies the reproductive performance of females of Nile tilapia in different age classes and reproductive environments. Four hundred and fifty-one females were evaluated in two different Brazilian environments, namely in Maringá, Paraná State, Brazil (Site 1 - hapas of 10 m³ and Site 2 - hapas of 1 m³) and in one environment in Alfenas, Minas Gerais State, Brazil (Site 3 - hapas of 14 and 28 m³). The females were divided into three age classes, corresponding to animals under one year (age 1); between one to two years (age 2); and over three years old (age 3). Spawning was verified once a week, from October 2012 to March 2013, corresponding to a 23-week assessment. The statistical analyses were performed with Proc Genmod from the statistical program SAS. Site 2 had the lowest results when compared to the other sites for multiple spawning, spawning frequency and volume of eggs/female for animals of age 2. Statistical differences were not detected between ages 1 and 2 for the same assessment site, although females at age 3 in Site 3 provided higher results than those at age 2. Results show that, precluding the use of animals in individual hapas (1 m³) and maintaining older females in the stock, may improve reproduction results.

Keywords: *Oreochromis niloticus*, spawning, volume of eggs.

Performance reprodutiva de fêmeas de tilápias do Nilo em diferentes ambientes e classes de idade

RESUMO. Objetivou-se verificar a performance reprodutivas de fêmeas de tilápia do Nilo em diferentes classes de idade e ambientes reprodutivos. O conjunto de dados foi composto por 451 fêmeas avaliadas em dois ambientes na cidade de Maringá, Estado do Paraná (hapas de 10 m³ - Local 1 e hapas de 1 m³ - Local 2) e em um ambiente em Alfenas, Estado de Minas Gerais (Local 3 - hapas de 14 e 28 m³). As fêmeas foram divididas em três classes de idade, correspondendo a animais de até um (Idade 1), entre um e dois (Idade 2) e com mais de três anos (Idade 3). A verificação de desovas foi realizada uma vez por semana no período de outubro/2012 a março/2013, correspondendo a 23 semanas de avaliação. Realizaram as análises estatísticas utilizando o procedimento Proc Genmod do programa estatístico SAS. O Local 2 apresentou resultados inferiores aos demais locais para todas as características estudadas ao utilizar animais de Idade 2. Diferenças estatísticas não foram observadas entre Idade 1 e 2 para o mesmo local de avaliação, no entanto a Idade 3 presente no Local 3 apresentou resultados superiores ao comparar com fêmeas de Idade 2. Desta forma, evitar o uso de animais em hapas individuais (1 m³) e manter fêmeas mais velhas no plantel podem melhorar os resultados reprodutivos.

Palavras-chave: *Oreochromis niloticus*, desova, volume de ovos.

Introduction

There are several biological characteristics that back the culture of the tilapia *Oreochromis niloticus*, and enhance the species as second in rank of the most cultivated freshwater fish in the world (MPA, 2011). It is the most cultivated species in Brazil and accounts for 46.6% of total produced (MPA, 2011). However, its pattern of asynchronous spawning and low fertility, associated with environmental factors

in the reproductive cycle such as photoperiod, temperature, nutrition, salinity and rainfall (Coward & Bromage, 2000) are a challenge for fingerling supplies. Fish-breeding farmers actually have to keep a large number of bloodstock in the squad to guarantee constant production.

Tilapia reproduction in Brazil is usually undertaken in earthen ponds, cages (also called hapas), concrete tanks and vinitanks. Despite the

high costs in acquiring hapas due to problems with regard to clogging and low oxygen levels, the hapas system is by far the commonest because diseased animals and improvement on spawning synchrony may be easily identified (Adel, 2012). However, the use of high density hapas may reduce the frequency of spawns and the number of eggs/spawning, suggesting that the density would vary between 2 and 6 fishes m^{-2} (Barman & Little, 2006; Trùng et al., 2013; Tsadik & Bart, 2007b), or rather, the adequate density to obtain the greatest fingerlings production.

Since a large amount of broodstock should be maintained due to the species' low fecundity, several techniques have been developed to improve the reproductive performance of the tilapia, such as water and air temperature, photoperiod (Biswas et al., 2005; Campos-Mendoza et al., 2004), oxygen levels (Kolding et al., 2008), nutritional requirements and supplementation (Bombardelli et al., 2009; Pereira et al., 2009) and different tilapia varieties (Abdel & Kamel, 2011; Mair et al., 2004; Tsadik & Bart, 2007a).

Although the evidence suggests that the best reproductive performance occurs in younger females (Dimmlich et al., 2009; Tsadik, 2008), little is known about the effect of age of breeding females. Results from a limited number of animals in recirculation water systems with controlled environmental factors differed from those in large-scale production, and the genetic improvement process aimed to increase the speed of weight gain may mask results and change the reproductive pattern in the tilapia (Mair et al., 2004).

Since the effect of age in females is not well-established on reproductive performance in genetically improved Nile tilapia, current assay evaluates the best age and reproductive environment in which the animals achieve their best performance and investigates the optimal use of reproductive females.

Material and methods

The broodstock used in current assay comprises animals from the fourth and fifth generations of the Genetic Improvement Program of Nile Tilapia, Tilamax variety, of the State University of Maringá, Maringá, Paraná State, Brazil, evaluated in 2010, 2011 and 2012. The animals were individually identified by 'Passive Integrated Transponder Tags' (PITT), average weight 10 grams, and evaluated in cage system in Diamante do Norte, Paraná State, Brazil (22°39'21"S and 52°51'36"W) for approximately seven months. The best specimens

from each family (full-sibs group) were selected and their daily weight gain at the end of the growing season used as selection criteria. The reproductive performance in females was obtained from this selection at three different sites:

Site 1 – 159 females and 56 males (2.84 females:1 male) were distributed in 5 polyethylene hapas, measuring 10 m^3 (1 m deep \times 2 m wide \times 5 m long), open mesh of 1 mm^2 , with an approximate density of 4.3 fishes m^{-2} , maintained in a 360 m^2 earthen pond and an average depth of 1 m.

Site 2 – 127 females and 127 males (1 female:1 male) were individually distributed in polyethylene hapas measuring 1 m^3 (1 m deep \times 1 m wide \times 1 m long), open mesh of 1 mm^2 , with a density of 2 fishes m^{-2} maintained in a 400 m^2 earthen pond covered by an agricultural greenhouse, and an average depth of 1 m. Sites 1 and 2 were located at the Fish Culture Experiment Station of the State University of Maringá (CODAPAR), in the District of Floriano (23°31'25"S and 52°03'12"W), Maringá, Paraná, Brazil.

Site 3 – 165 females and 64 males (2.6 females:1 male) were distributed in 4 polyethylene hapas: two measured 28 m^3 (1 m deep \times 2 m wide \times 14 m long) and two hapas measured 14 m^3 (1 m deep \times 2 m wide \times 7 m long), open mesh of 1 mm^2 , with an approximate density of 2.8 fishes m^{-2} maintained in a 360 m^2 earthen pond and an average depth of 1 m, located in Alfenas, Minas Gerais State, Brazil (37°25'19,1"N and 122°05'06"W).

The females were separated into three age classes, corresponding to animals up to one year, from one to two years, and more than three years old. Females aged 1 and 2 years were distributed in Sites 1 and 2, while Site 3 contained females 2 and 3 years old (Table 1). Males with the same female age classes were used to be mated at the three sites.

Table 1. Minimum (min) and maximum (max) age (days) of females separated in categories and the quantity (n) of animals at their respective sites, evaluated as to reproduction.

	Age 1		n	Age 2		n	Age 3		n
	Min	Max		Min	Max		Min	Max	
Site 1	289	343	86	609	651	73	-	-	-
Site 2	289	343	61	585	662	66	-	-	-
Site 3	-	-	-	594	651	95	986	1033	70

The animals were fed at three locations with specific commercial extruded feed for Nile tilapia breeding, corresponding to 1% of body weight once daily, in the morning and always after collections. The diet contained a minimum of 38% crude protein, 33,400 kcal kg^{-1} digestible energy, 10% ether extract, 1% phosphorus, 500 mg kg^{-1} vitamin C and a maximum of 2% calcium. The average water

temperature was 29.60, 31.50 and 27.80°C respectively for Sites 1, 2 and 3.

Spawning was verified in all the sites once a week, between October 2012 and March 2013, or rather, a 23-week assessment. Collections were conducted at Sites 1 and 3 by restricting the broodstock to a small area in the hapa; the animals were captured one by one and the presence of eggs in the fish mouth was verified. When eggs were detected in the mouth, a mouthwash was used to remove the eggs with plastic bowls and the number of the microchip that corresponded to that female spawning was noted.

The broodstock in Site 2 were maintained in individual hapas, allocated in trios inside the tank, with one male for two females. Mating and sampling were performed with dip nets and evident signs of spawning (bulging belly; reddened and swollen urogenital papilla) were verified. When these signs were observed, the male was placed in the hapa with the female and the couple was kept together until spawning or until better conditions for spawning by another female which composed the trio were perceived. The sampling and microchip number were performed in the same way as described for the other sites. However, coupled to the spawning information in the case of Sites 1 and 2, the volume of eggs produced per female was measured with a 100 mL-graduated beaker.

The data set for the statistical analyses contained information on 451 females and estimated the spawning probability (presence or absence of spawning), multiple spawning (females with more than one spawning) and spawning frequency (number of spawning obtained per female during the evaluation period). The probability of spawning and multiple spawning were threshold characteristics, with 1 if positive (spawning occurrence and multiple spawning occurrence) and 0 if otherwise. The spawning frequency was considered as a continuous characteristic, with rates ranging from zero to ten spawning. For the average volume of eggs/female (total volume of eggs/female ÷ number of spawning/female) and total volume of eggs/female, information from the 145 females of Sites 1 and 2 was employed. The statistic model for all estimates was:

$$Y_{ijk} = \mu + L_i + I_j(L_i) + e_{ijk}$$

where:

Y_{ijk} = observed rate for female k, at Site i and age j;

μ = mean;

L_i = effect of spawning Site i (i = Site 1, 2, and 3 for spawning probability, number of spawning and multiple spawning; and Site 1 and 2 for average volume and total volume of eggs/female);

$I_j(L_i)$ = effect of Site i nests age j (j = age 1, 2, and 3 - Table 1);

e_{ijk} = residual random effects.

Statistical analyzes were performed with Proc Genmod of SAS statistical software (SAS, 2004). The Generalized Linear Models were implemented taking into consideration the binomial distribution for the variables under analysis, except for the spawning frequency, average volume and total of eggs/female, which were performed by the normal distribution with logarithmic function. The orthogonal contrast analysis between younger and older females at each site was also included.

Results and discussion

Assessment of Sites 1 and 2 did not show any differences ($p \geq 0.05$) when ages 1 and 2 were compared for spawning probability, multiple spawning probability and spawning frequency. However, the rates for age 3 at Site 3 were higher than those for age 2 in all the characteristics under analysis (Table 2).

Table 2. Spawning probability (%), multiple spawning probability (%), spawning frequency and orthogonal contrast coefficients in Nile tilapia at different sites according to age.

	Spawning probability			Multiple spawning			Spawning frequency		
	1	2	3	1	2	3	1	2	3
P1	70.93 ^{Aa}	58.90 ^{Aa}	-	44.19 ^{Aa}	43.84 ^{Aa}	-	1.49 ^{Aa}	1.53 ^{Aa}	-
P2	67.21 ^{Aa}	50.77 ^{Aa}	-	36.07 ^{Aa}	18.46 ^{Ab}	-	1.10 ^{Aa}	0.80 ^{Ab}	-
P3	-	61.05 ^{Ba}	87.14 ^A	-	43.32 ^{Ba}	70.00 ^A	-	1.63 ^{Ba}	3.49 ^A
C1	Ns			ns			ns		
C2	Ns			ns			ns		
C3	p < 0.05			p < 0.05			p < 0.05		

P1: Site 1; P2: Site 2 and P3: Site 3.

Estimates with different uppercase letters in the same line differ at $p < 0.05$ for the same site at different ages. Estimates following different lowercase letters in the same column differ at $p < 0.05$ for the same age at different sites.

Orthogonal contrast coefficients: C1 (co = 0, Site 1 Age 1 = 1, Site 1 Age 2 = -1); C2 (co = 0, Site 2 Age 1 = 1, Site 2 Age 2 = -1); C3 (Site 3 Age 2 = 1, Site 3 Age 3 = -1). ns = not significant; p < 0.05 significant by orthogonal contrast analysis.

There was no difference ($p \geq 0.05$) between sites with regard to animals of the same age for spawning probability. In the production systems using hapas installed in earthen ponds, similar to those in current study, Bombardelli et al. (2009) obtained a percentage of spawning females ranging between 44 and 66% when using the Thai variety fed on diets with different energy levels. Coward and Bromage (1999) conducted an indoor (laboratory) reproduction system to verify the timing of spawning, fecundity and egg size of *Tilapia zilli*. These authors reported spawning in 66% of females,

whilst 31% of females had more than one spawning during the 80-day experimental period. Results are consistent with those obtained for spawning probability and multiple spawning respectively at ages 1 and 2.

When multiple spawning probability and spawning frequency were analyzed, the performance at Site 2 was reported lower than the other sites under analysis (Table 2). Rates for multiple spawning probabilities were 43.84, 18.46, and 43.32 at Sites 1, 2, and 3, respectively. Spawning frequency rates were 1.534, 0.80 and 1.623 at Sites 1, 2, and 3, respectively (Table 2). Age 3, present at Site 3 only, provided the greatest number of spawning (3.486), consequently different ($p < 0.05$) from age 2 (1.632).

Campos-Mendoza et al. (2004), Pereira et al. (2009) and Tsadik and Bart (2007a) reported mean rates 9.09, 0.65, 2.74 and 3.72, respectively, for spawning/female Nile tilapia in experiments with different objectives, such as different ages and assessment periods. The above rates agree with those estimated in current analysis. The high spawning frequency observed by Campos-Mendoza et al. (2004) is justified because the environmental conditions were close to those required for the Nile tilapia, coupled to different photoperiods. The latter suggested that long periods of light (18 hours of light: 6 hours of darkness) may increase spawning rates.

Size and body weight are important indexes of the animal's age (Coward & Bromage, 1999; Oliveira et al., 2013). Actually in most studies which evaluate reproduction characteristics, the body weight is used as a parameter to select animals for experiments due to the standardization and the difficulty of accurate age measurement. Thus, (Almeida et al., 2013) and Moura et al. (2011) reported a lower spawning frequency in larger females, coupled to the preference for small animals for better management. Females with more than 300 grams are generally discarded although the opposite was done in current study. The above revealed that certain assumptions on the best age of dam must be studied further and deeply.

Greater spawning frequency and volume of eggs produced by older females observed in current analysis disagree with results by Coward and Bromage (1999) who observed spawning in only 31% of females with body weight over 350 grams when compared to 100% of spawning in females weighing less than 49 grams. In studies on the investigation of the effect of age on the Nile tilapia reproduction, Tsadik (2008) registered a decrease from 6 to 3 spawning/female when comparing

broodstock respectively 4 and 24 months old. The author concluded that females over 30 months have the greatest fecundity rates (eggs/spawning), size and weight of eggs, although spawning frequency, fertilization and hatching percentages decrease in older females. Woodhead (1978) suggests that advanced age causes the filling of the ovaries with connective tissue, wall thickening and a reduction of germinal tissue, or rather, natural features of senescence which affect the reproductive capacity.

Current study demonstrated that Site 2 (in 1 m³ hapas) had a lower spawning frequency than the other sites, especially with regard to age 2. Females from this site may have been underestimated because they were used for the production of fries (group of full-sibs and half-sibs) to compose the new generation of animals to be evaluated in the Genetic Improvement Program of Nile Tilapia of the State University of Maringá. Consequently when the female got over three spawning from different males, it was replaced by a female who had never spawned so that the number of families would increase. Another factor that may have affected the reproduction of this group of animals is the inclusion of males in the female hapa to maintain the ratio of one male to one female. The above scheme contributed towards an increase in aggressiveness, resulting in injuries in females and sometimes in death, with the need to replace the animal.

Rates of the greatest volume of eggs/female values ($p < 0.05$) were observed at Site 1 and age 2, corresponding to average volume and total volume of 16.219 and 43.395 mL female⁻¹, respectively. No difference was observed at Site 2 between ages for the two characteristics. When the two sites were compared, differences were observed only at age 2 for the average volume (Site 1: 16.219 and Site 2: 12.158) and total volume of eggs (Site 1: 43.395 and Site 2: 16.105), indicating that older females at Site 1 had the greatest egg production rates. These contrasting results corroborated with those previously obtained and enhanced the high average and total volume of eggs in females at age 2 in Site 1 (Table 3).

Table 3. Average volume (mL), total volume (mL) of eggs/female and orthogonal contrast coefficients at two different sites according to age.

	Average volume		Total volume	
	Age 1	Age 2	Age 1	Age 2
P1	11.205Ba	16.219Aa	24,650Ba	43,395Aa
P2	10.772Aa	12.158Ab	13,435Aa	16,105Ab
C1	p < 0.05		p < 0.05	
C2	ns		ns	

P1: Site 1; P2: Site 2 and P3: Site 3. Estimates with different uppercase letters in the same line differ at $p < 0.05$ for the same site at different ages. Estimates with different lowercase letters in the same column differ at $p < 0.05$ for the same age in different sites. Orthogonal contrast coefficients: C1 (co = 0, Site 1 Age 1 = 1, Site 1 Age 2 = -1); C2 (co = 0, Site 2 Age 1 = 1, Site 2 Age 2 = -1); ns = not significant; $p < 0.05$ significant by orthogonal contrast analysis.

When analyzing female Nile tilapia, GIFT variety, weighing approximately 660 grams and reproduced in indoor systems, Nascimento et al. (2013) registered an increasing volume of eggs with increasing levels of vitamin E in the broodstock diet, ranging between 6.64 and 11.08 mL of eggs/female. The above results are consistent with those estimated for the age 1 at Sites 1 and 2 for average volume, similar in weight as in the above-mentioned study.

Owing to the lack of statistic differences between ages 1 and 2, orthogonal contrasts analysis were performed to identify possible differences between ages. However, the contrasting results corroborated with those previously obtained and underscored the best reproductive performance of females at age 3 (Table 2). In fact, the best reproduction performance of older females is more evident when the superiority of 58 and 114% is verified for multiple spawning and spawning frequency, respectively, when compared with the age with the second best performance. The above disagrees with results testing the reproductive performance for tilapia performed by Trong et al. (2013); Tsadik (2008).

The process of genetic improvement of Nile tilapia to increase growth rate may be adversely affecting fertility and production of tilapia fingerlings of the GIFT variety. Taylor et al. (2008) observed that the IGF-I levels accurately reflected growth rate prior to elevations in sex steroids, suggesting that IGF-I may provide an endocrine signal between the somatotropic and reproductive axes that growth rate and/or size are sufficient to initiate gonad development. This result corroborates the positive correlation observed between the greatest growth and early maturation (Longalong et al., 1999). The relative and absolute low fertilities of the GIFT variety tilapia were confirmed by Mair et al. (2004) who carried out studies to check the growth and reproduction of different varieties of Nile tilapia and the absence of males and females with developed gonads in the age at which was expected more than 50% of mature animals. This fact indicated that these animals were sexually late, disagreeing with Longalong et al. (1999). Animals with late sexual development may be one of the reasons for the better reproductive performance of older females, since the beginning of the breeding occurs at an older age. It is thus possible that the production peak also occurs later.

It may be underscored that some environmental factors may be more important than others with regard to tilapia reproduction. However, the best results are obtained when environment and

genetics are considered together, trying to offer the best conditions for obtaining maximum performance. Thus, if one decides to keep the females longer on the squad, some factors must be taken into account, such as the need of a larger area for the allocation of females (Moura et al., 2011) since they may easily weigh more than two kilograms. Management may be more difficult and special attention must be given on the renovation of the squad with the emergence of animals with best genetic potential (Bhujel et al., 2007). Furthermore, it is well-known that the identification of the female groups with the best reproductive performance, regardless of age, may have great economic importance. In their maintenance of animals with these characteristics only, fish farmers may reduce feed costs; reduce the number of broodstock and use females and management more efficiently.

Conclusion

A better reproductive performance may be obtained by using females aged two and three years in Sites 2 and 3, respectively. Further, reproductive performance improves by avoiding the breeding of one female to one male in small hapa volume.

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