Effects of Maternal Age on Reproductive Performance and Growth of Nile Tilapia, *Oreochromis niloticus* (L.) Fry

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ABSTRACT

This study aimed to investigate the effects of maternal age on spawning frequency, egg quantity and quality, and growth performance of Nile tilapia, *Oreochromis niloticus* (L.) fry. The study was conducted on two age classes of Nile tilapia broodstock, 6 and 24 months old, stocked in 3 replicate concrete tanks. Eggs were collected from mouth of females once a week. Number of spawned female, total weight and number of eggs collected from each tank as well as water quality were monitored and recorded weekly. Comparison study on growth performance of fry produced from female broodstock of different ages was consecutively carried out for five weeks. The study on reproductive performance revealed that the weight of 6-month old females (279±7 g) was significantly lower than that of 24-month old females (909±37 g) ($P<0.05$). The young female broodstock spawned more frequently, 13.6±3.3 female $\cdot$ week^{-1} compared to 5.8±1.9 female $\cdot$ week^{-1} of the old female broodstock. The two maternal ages produced not significantly different total weight of eggs per week, 98.9 ± 19.7 and 75.2 ± 28.8 g $\cdot$ week^{-1}, respectively. However the young female broodstock could produce better relative weight of eggs at 26.4 ± 2.8 g $\cdot$ kg^{-1}, week^{-1} compared to 14.0±2.1 g $\cdot$ kg^{-1}, week^{-1} of the old female broodstock ($P<0.05$). The young female broodstock also produced significantly higher number of eggs per week (44,793±12,318 eggs $\cdot$ week^{-1}) but smaller in size (2.25±0.23 mg $\cdot$ egg^{-1}) compared to the old female broodstock (21,368±10,125 eggs $\cdot$ week^{-1} and 3.07±0.20 mg $\cdot$ egg^{-1}, respectively), ($P<0.05$). Water quality by mean DO and TAN in the old broodfish tanks (3.9±0.6 and 2.92±0.23 mg $\cdot$ l^{-1}, respectively) were inferior to those in the young broodfish tanks (5.0±0.6 and 0.55±0.13 mg $\cdot$ l^{-1}, respectively). Poor water quality in the old broodfish tanks was possibly caused by the higher quantity of feed given due to the high biomass of the old broodfish and insufficient water exchange. Different-sized eggs apparently hatched out fry of different sizes. Fry produced from small eggs weighed less than those hatching from large eggs, 0.363 ± 0.001 and 0.438 ± 0.031 g, respectively ($P<0.05$) by the fifth week of nursing. The Average Weight Gain (0.314 ± 0.008 and 0.375 ± 0.027 g) and Average Daily Gain (0.012 ± 0.000 and 0.014 ± 0.001 g $\cdot$ d^{-1}) further reaffirmed that different sized fry hatched out from different sized eggs. Specific Growth Rate of fry from small- and large-sized eggs (7.4 ± 0.8 and 7.2 ± 0.4 % $\cdot$ d^{-1}, respectively), however, was not significantly different ($P>0.05$). Results of these studies suggested that Nile tilapia seed production efficiency could be improved by using younger (six months old) rather than older (24 months old) females.

Keywords: maternal age, reproductive performance, growth performance, Nile tilapia, *Oreochromis niloticus*.

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INTRODUCTION

Nile tilapia was introduced into Thailand in 1965 and has become the most productive inland farmed fish. Fish seed is an essential element among inputs for farmed fish production. Nile tilapia is a precocious species. Its sexual maturity is a function of age, size, and environmental conditions (Duponchelle and Jacques, 1998). Environmental factors such as high level of precipitation and dissolved oxygen could enhance the fecundity of tilapia species in natural environments (Issa, 2005).

The species matures at about 10 to 12 months (350 to 500 g) in several East African lakes. The same population under conditions of near maximum growth will reach sexual maturity in farm ponds at an age of 5 to 6 months (150 to 200 g) (Popma and Lovshin, 1995). Tilapia hatchery normally uses a large number of broodfish to produce eggs due to its low fecundity and asynchronous spawning between females in captive conditions (Tsadik and Bart, 2007, cited by Mohamed *et al*. (2013), Tahoun, 2008). Broodstock management plays a crucial role on seed supply in terms of quantity and quality, for commercial tilapia production. Therefore knowledge on factors regulating broodstock productivity is crucial in seed production. Age and stocking density significantly affected the natural spawning performance of Nile tilapia broodstock (Tahoun, 2008). The recommended optimum age range of Nile tilapia broodstock was 6-18 months old due to its short reproductive cycle, high egg production, relatively homogeneous egg weight and size, and high fertilization and hatching rates (Tsadik, 2008). Many studies reported the association of age and quality of gametes. Thus, to obtain good quality offspring, the age of the breeders must be taken into account (Valentin *et al*., 2015). At Kamphaeng Saen Fisheries Research Station, Nakhon Pathom province, Thailand where tilapia fry was regularly produced for sale, we observed that older female broodstock (larger size) produced larger eggs but smaller in number. This study aimed to examine the effects of different ages, 6 and 24 months old Nile tilapia broodfish on 1) reproduction performance including spawning frequency, number, and size of eggs, and 2) the subsequent growth performance of fry. It was expected that results from these studies will be useful for hatcheries to improve efficiency of seed production.

MATERIALS AND METHODS

The experiments were conducted at Kamphaeng Saen Fisheries Research Station, Faculty of Fisheries, Kasetsart University, Kamphaeng Saen Campus in Nakhon Pathom province, Thailand, from August to December 2015. Different-aged Nile tilapia broodstock (Chitralada 3 strain) had been raised in rectangular concrete tanks and they regularly produced fry for sale. The study included two consecutive experiments aimed to investigate the effects of two maternal ages, 6 and 24 months, on reproductive performance and the subsequent growth performance of fry.

Experiment 1: Comparison of reproductive performance

Six concrete tanks (size 1.9 x 5.0 x 0.6 m^3) retaining two maternal age classes of Nile tilapia broodstock were selected for the study. These tanks had been regularly used to produce fry for sale. The six tanks retained the same sex ratio of male:female broodstock at 1:4 with a density of 6 fish $\cdot m^{-2}$. Therefore 45 females and 15 males of the same age were stocked together in a 10 m^2 concrete tank. The fish in the six tanks could be categorized into two groups, based on their age with corresponding size. Tanks B1, B2 and B3 contained 6 months old broodstock whereas tanks C1, C2 and C3 contained 24 months old broodstock. Fish were fed with commercial feed pellets (30% protein) at 1% body weight $\cdot day^{-1}$. Water was changed 100% once a week when collecting eggs and another 30% exchange was done by continuous flow during the week.

Size of broodfish

Mean weight of female broodstock was determined at the beginning by weighing a sample of 30 females from each tank. Mean weights of the two groups were compared by two-independent T-test at 95% confidence interval.

Reproductive performance

Three parameters including spawning frequency, size and number of egg were used to describe reproductive performance of the two groups of female broodstock. Spawning frequency was assessed by opening the mouth of every female in each tank once a week to see whether they were incubating eggs or not. The number of females with eggs in their mouths in each tank was recorded as spawned females. Eggs in the mouth of the females from each tank were collected and pooled together, cleaned, weighed and counted. Average weight of an individual egg was calculated by dividing the total weight of eggs by the total number. Water quality parameters such as DO, pH, temperature and TAN in each tank were monitored every other day in the morning. Different means of spawn frequency, total weight of eggs, total number and mean weight of individual egg were tested for statistical differences by two-independent T-test at 95% confidence interval.

Experiment 2: Growth performance of fry

Six aquaria with dimensions of 25 x 50 x 31 cm were cleaned and filled with 40 liters of clean water. Each aquarium was equipped with a sand stone as oxygen diffuser. Eggs collected from young females (B tanks) and old females (C tanks) in Experiment 1 after cleaning, weighing and enumerating, were incubated separately for three days. The hatchlings (yolk sac fry) were then nursed for another 3 days in the tray until they have absorbed their yolk, turning into swim-up fry. A sample (120 fry) was taken from each tray. Mean initial weight of hatchlings was determined by using a scoop net to collect fry from the trays, allowing the residue water in the scoop net to drain as much as possible, and transferring the fry into a container with a known amount and weight of water on a digital balance. The increment increase in weight after adding the fry was the total weight of fry. Mean individual weight was obtained by dividing the total weight with the total number of fry. The hatchlings were handledwith caution since they were very small and delicate.

A combination of fishmeal and fine rice bran at a ratio of 1:3 was fed to the fry 5 times $\cdot$day^{-1} or every 2 hours during daytime. The given amount of feed varied from 10 to 40% during the culture period based on age and feeding demand of the fry. Weight of the fry was monitored once a week and the amount of feed was adjusted accordingly. About 80% of water exchange was done every two days in the first two weeks, then every day from week 3 to 5 when the fry were growing and consuming more feed. Water quality parameter such as DO, pH and temperature were monitored every day. TAN was analyzed every two days by standard methods (APHA, 1998). Different means of fry weights were tested for statistical differences by two-independent T-test at 95% confidence interval.

Growth performance parameters

Growth performance parameters were calculated using the following formulae.

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

Average Daily Gain (ADG) = [Average final weight (g) – Average initial weight (g)] /T

Specific Growth Rate (SGR %/day) = [Ln Wt_f – Ln Wt_0 / T] 100

Where: - Ln: natural logarithm, Wt_0: initial weight (g), Wt_f: final weight (g), T: time (days) between Ln Wt_0 and Ln Wt_f.

RESULTS

Experiment 1: Comparison of reproductive performance

Mean weights of 6 and 24-month old female were different (P<0.05), 279±7 g and 909 ±37 g, respectively (Table 1). The six-month old females spawned more frequently (13.6±3.3 spawned female • week^{-1}) with a more absolute number of eggs (44,793±12,318 eggs • week^{-1}) than 24-month old females (5.8±1.9 spawned female • week^{-1} and 21,368±10,125 eggs • week^{-1}, respectively) (P<0.05) (Table 1). The absolute total weight of eggs in g • week^{-1} of the two maternal aged females was not different (P>0.05). The older females, however, produced a significantly larger egg, 3.07±0.20 mg • egg^{-1} compared to 2.25±0.23 mg • egg^{-1} of the younger females (P<0.05) (Table 1). Regarding the differences in spawning frequency, when taking the number of spawned females into account, it was found that the relative number of eggs per young female fish was not significantly different from that of the old individual female, i.e. 3,282 ± 253 and 4,173 ± 728 eggs • female^{-1} • week^{-1} (P>0.05), respectively. The old females, however, spawned a significantly high relative weight of eggs than the young female, 12.8 ± 1.9 and 7.4 ± 0.8 g • female^{-1} • week^{-1} (P<0.05), respectively. However when taking the biomass of the two different maternal ages into account, it was found that one kilogram of young female fish could produce significantly more eggs by weight, than one kilogram of the old female, 26.4 ± 2.8 and 14.0±2.1 g • kg female^{-1} • week^{-1}, respectively. Moreover, the young female could also produce a significantly high number of eggs, 11,764.9 ± 905.2 and 4,146.5 ± 1,482.3 eggs • kg female^{-1} • week^{-1}, respectively (P<0.05) (Table 1).

Water quality parameters by mean DOs and TAN in the tanks of younger female broodstock were superior to those in the tanks of the older female broodstock (DO = 5.0±0.65 and 3.9±0.6 mg • l^{-1}, and TAN = 0.55±0.13 and 2.92±0.23 mg • l^{-1}, respectively) (P<0.05) (Table 2). Mean DO levels of water in the tanks retaining young broodfish were significantly higher than that in the older broodfish tanks during the study period, but *vice versa* was found for TAN (Figures 1 and 2, respectively). Temperature and pH were not significantly different.

Table 1. Summary of reproductive performance of two maternal age females (mean ± SD).

Parameter	Age of female broodstock	
	6 months	24 months
Weight of female broodstock (g)	279 ± 7^{a}	909 ± 37^{b}
Spawning frequency (spawned female • week^{-1})	13.6 ± 3.3^{a}	5.8 ± 1.9^{b}
Egg number (eggs • week^{-1})	44,792.6 ± 12,318.2^{a}	21,368 ± 10,125^{b}
Weight of eggs (g • week^{-1})	98.9 ± 19.7^{a}	75.2 ± 28.8^{a}
Mean weight of egg (mg • egg^{-1})	2.25 ± 0.23^{a}	3.07 ± 0.20^{b}
Egg number per female (eggs • female^{-1} • week^{-1})	3,282 ± 253^{a}	4,173 ± 728^{a}
Weight of eggs per female (g • female^{-1} • week^{-1})	7.4 ± 0.8^{a}	12.8 ± 1.9^{b}
Relative weight of eggs (g • kg female^{-1} • week^{-1})	26.4 ± 2.8^{a}	14.0±2.1^{b}
Relative egg number (eggs • kg female^{-1} • week^{-1})	11,764.9 ± 905.2^{a}	4,146.5 ± 1,482.3^{b}

Note: means in the same row with different superscripts are significantly different (P<0.05; T-test).

Table 2. Water quality conditions in broodfish tanks (mean ± SD)

Parameters	Age of female	
	6 months	24 months
DO (mg $\cdot l^{-1}$)	5.0 ± 0.6^{a}	3.9 ± 0.6^{b}
TAN (mg $\cdot l^{-1}$)	0.55 ± 0.13^{a}	2.92 ± 0.23^{b}
pH	7.0 ± 0.2^{a}	7.0 ± 0.2^{a}
Temperature (^{o}C)	27.7 ± 0.7^{a}	27.6 ± 0.7^{a}

Note: means in the same row with different superscripts are significantly different (P<0.05; T-test).

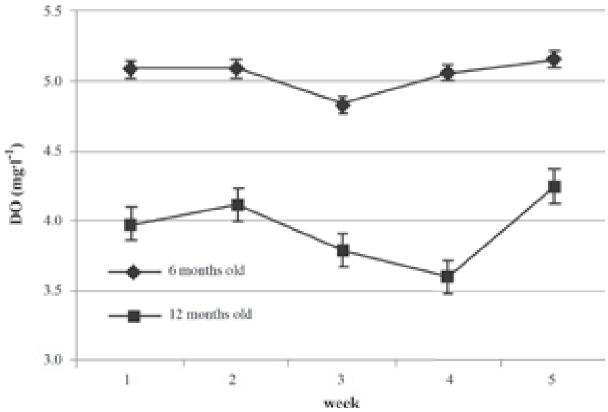

Figure 1. Mean DO during study period

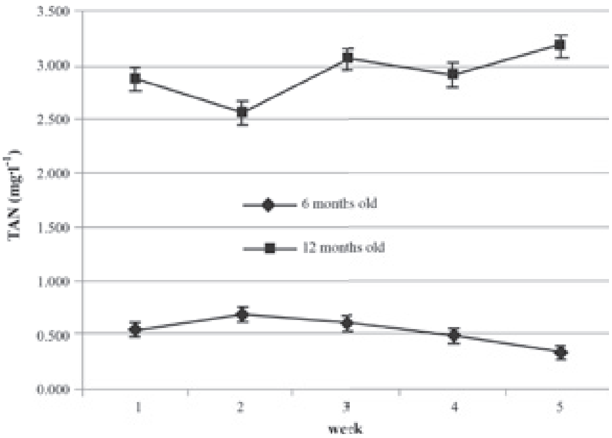

Figure 2. Mean TAN during study period

Experiment 2: Growth performance of fry

Mean weight of fry from different maternal ages was not different in the first week. The difference, however, was significant from week 2 to 5 (P<0.05). Fry hatched out from small eggs were smaller than those hatching out from large eggs. Mean weights of fry from small and large eggs on the fifth week were 0.363±0.001 and 0.438±0.031 g, respectively (Table 3). AWG (0.314 ± 0.008 and 0.375 ± 0.027 g) and ADG (0.012 ± 0.000 and 0.014 ± 0.001 g $\cdot$ d^{-1}) of fry produced from small and large eggs during the 5-week experimental period were significantly different (P<0.05). The SGR of 7.4 ± 0.8 and 7.2 ± 0.4 % $\cdot$ d^{-1}, respectively however, were not significantly different (Table 3). Dissolved oxygen, a crucial factor for fish growth at a favorable level of 6.2 mg $\cdot$ l^{-1} of the two treatments was not significantly different (Table 3).

Table 3. Descriptive growth performance of fry and water quality (mean ± SD).

Items	Age of female	
	6 months	24 months
W_{t1} (g)	0.049 ± 0.008^a	0.063 ± 0.009^a
W_{t5} (g)	0.363 ± 0.001^a	0.438 ± 0.031^b
AWG	0.314 ± 0.008^a	0.375 ± 0.027^b
ADG (g $\cdot$ d^{-1})	0.012 ± 0.000^a	0.014 ± 0.001^b
SGR (% $\cdot$ d^{-1})	7.4 ± 0.8^a	7.2 ± 0.4^a
DO (mg $\cdot$ l^{-1})	6.2 ± 0.3^a	6.2 ± 0.3^a

Note: means on the same row with different superscripts are significantly different (P<0.05; T-test).

DISCUSSION

Experiment 1: Comparison of reproductive performance

The weight of the 24-month old female was about 3 to 4 times that of the 6-month old female, which simply marked the significant size difference of the two different maternal age broodstock. Small size (young) female Nile tilapia was more preferable in producing seed due to the difficulty in handling larger female when collecting eggs. Tilapia broodfish with a weight of 150-300 g was recommended as the best size for breeding and preferred by tilapia hatcheries due to ease in handling (Bhujel, 2000; Nandlal and Pickering, 2004).

Reproductive performance of the younger female was superior to the older female broodstock when considering the number of spawned females per week. This could be the representative of spawning frequency since the same number of female broodstock was stocked in the same size tank. In addition, young female produced more than two fold of the number of eggs from the older female. Spawning intervals were also different between the young and the old female brookstock since the older fish have reduced reproductive activity and/or a greater inter-reproductive period. Decrease in relative fecundity, egg production, and fertilization rates were found in females as age progresses (Valentin *et al*., 2015). Tsadik (2008) reported that eggs per spawn of 4-month old increased by two-fold from that of the 24-month old female Nile tilapia. Eggs per female per day declined concurrently with per spawn per female after 18 months old. In most tilapias, fecundity varies considerably among fish of the same species, and even among female of similar sizes, especially in large fish classes (Coward and Bromage, 1999 cited by El-Sayed, 2006). Small Nile tilapia produced many more eggs, with shorter spawning intervals than large fish (Little, 1989 cited by El-Sayed, 2006; El-Saidy and Gaber, 2005).

Difference from the absolute number of eggs, the absolute weight of eggs (grams • week^{-1}) of the two different aged female broodfish was not different. The difference could be explained by different-sized eggs. It was apparently seen that the young female spawned smaller size eggs but in larger numbers compared to the larger size eggs but smaller number from the old female. Variation in egg sizes could be influenced by the age and size structure of the broodfish, genetic stocks and strains, and seasonal changes. Superimposed on these may be compounding intraspecific association between egg size, fecundity and female nutritional status (Rana, 1986).

Apart from the absolute results of reproductive performance, the relative performance should also be analyzed to see the actual reproductive performance of individual females of different ages. We found from the analysis that the number of eggs per week obtained from a young individual and an old female fish was not significantly different, but the young female produced relatively lower egg weights than the old female. Even if this study did not determine fecundity, the number and size of eggs could be associated with fecundity. Popma and Lovshin (1995) reported that the young Nile tilapia (usually less than 6 months) spawned frequently (every 4 to 6 weeks) under appropriate environmental conditions but the overall fecundity was low.

Because of the apparently different size/ biomass of the two differently aged females, the reproductive performance should also take into account the relative weight of the female. The analysis found that a one kilogram young female could produce almost twice the weight of eggs and almost three times the egg number versus the one kilogram old female. Again fecundity was not studied in this current research, but the number of eggs obtained per week could be more or less associated with the fecundity of females. Fecundity and egg size are directly related to fish size, length or age but with high variability (Rana, 1986; Little, 1989; Rhidha and Cruz, 1989 cited by El-Sayed, 2006). Females with low body weight have more relative fecundity (Mohammed *et al*., 2014).

Stocking 6 broodfish • m^{-2} with male: female ratio of 1:4 has been successful in Nile tilapia seed production at Kamphaeng Saen Fisheries Research Station of Kasetsart University. Feed was given at 1% of body weight once a day. Consequently more feed was applied to the old broodfish in relation to biomass. Water quality parameters such as DO and TAN in particular indicated that certain water exchange scheme was possibly insufficient and caused poor water quality conditions (low DO but high TAN), mainly in the old broodfish tanks during the 5-week study period. Broodfish survived well under these water quality conditions but could have an impact on reproductive performance in addition to age. Kolding *et al*. (2008) reported the effect of low DO, wherein smaller females exposed to low oxygen produced smaller and fewer eggs than larger females in high dissolved oxygen situation. Therefore, the influence of DO and TAN on number of eggs produced should be further studied. Reproductive performance parameters such as the relative weight of eggs and egg number produced from one kilogram of 6-month old and 24-month old female broodstock strongly suggest that 6-month old broodfish could be employed for seed production due to its high efficiency of inputs (water and feed) utilization.

Experiment 2: Growth performance of fry

Size of offspring is generally associated with size of egg. The bigger the egg, the larger the offspring is. Data from Experiment 1 revealed that size of eggs is related with age and inevitably size of female broodstock. The older female broodstock produced larger size egg. Difference in egg size from different maternal ages could be easily observed. In Table 1, however, it confirmed the difference scientifically. Mean initial weight of fry produced from the two different egg sizes did not show any significantly difference at the beginning (t_1) possibly due to the fact that it is difficult to weigh the minute yolk sac fry. Mean weight of fry was different (P<0.05) from weeks 2 to 5 when it became bigger. AWG and ADG were also significantly different (P<0.05) hence these parameters strongly supported the size differences of the two groups fry.

The larger fry can grow and survive better than the smaller fry since the former contain more yolk and have more stored energy for growth before feeding on external feed (Fuiman, 2002). Size of offspring is also related to environmental conditions, wherein poor conditions result in fewer and larger offspring (Johnston and Leggett, 2002). Bigger eggs resulting in longer and heavier fry have been reported in many fish species including brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), chinook salmon (*Oncorhynchus tshawytscha*), chum salmon (*Oncorhynchus keta*), coho salmon (*Oncorhynchus kisutch*), channel catfish (*Ictalurus punctatus*), herring (*Clupea harengus*) and jack mackerel (*Trachurus symmetricus*). They have all shown that the growth of fry was linearly related to their yolk reserves and hence to the egg size (Rana, 1986). The effect of the interrelated egg size, the amount of yolk, and fry size on survival and growth of fry, could be the cause of wild conditions when the hatched out fry has to seek external feed where there is sometimes scarcity of food. However survival difference under experimental conditions was not observed when sufficient feed was applied.

The differences in SGR of the two groups of fry was not significantly different ($P>0.05$). This means that whether fry is produced from small or large eggs could have the same specific growth rate under sufficient feed condition.

CONCLUSION

The reproductive performance of the 6 months old female was apparently better than that of the 24 months old female when parameters such as spawning frequency, relative weight of eggs ($g \cdot w^{-1} \cdot kg \cdot female^{-1}$) and relative number of eggs ($eggs \cdot week^{-1} \cdot kg \cdot female^{-1}$) are taken into consideration.

The offspring from larger sized egg was bigger than those from small sized eggs. Consequently, the weight, AWG and ADG of offspring from larger sized eggs were significantly higher than those of the small size egg. However, SGR in $\% \cdot d^{-1}$ of the two groups of fry was not significantly different.

It was strongly recommended that tilapia hatchery should use six-month old female to produce fry rather than the older, 24-month old female.

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