Effect of Different Levels of Water Depths on Spawning and Reproductive Performance of Red Tilapia Hybrid (*Oreochromis* sp.)

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Abstract: In this study we have investigated the effect of water levels (20, 40, 60 and 80 cm of water column) on spawning and reproductive behavior of red tilapia hybrid. Two spawnings were obtained during the experimental period except for fish of 20 cm of water depth treatment did not give any seed production. It was noted that there are significant differences ($P<0.05$) between the treatments for the two spawnings. The fish of third treatment were recorded the best results in most reproductive performance parameters while the fish of second treatment were recorded the least results. The results revealed that the fish of third treatment were the most numerous egg number 988.0±19.49 egg/fish and the fish of first treatment were recorded the fewest egg number 576.0±8.38 egg/fish.

Keywords: Red tilapia, broodstock, water column, seed production, spawning, reproductive behavior

INTRODUCTION

Spawning in earthen ponds is the oldest method used for seed production of tilapia. It is still widely used in different regions of the world, especially in developing countries (Little and Hulata, 2000). Tilapia can spawn easily in ponds, regardless of pond size and depth, once the environmental requirements (temperature, salinity, etc.) and biological criteria (stocking density, sex ratio, etc.) are met. However, pond size, shape and depth affect harvest efficiency and seed production (El-Sayed, 2006).

Spawning efficiency can be affected by tank size, dimensions, shape, color and depth (El-Sayed, 2006). Another important water level management consideration is that fish depend on acquiring appropriate temperatures for spawning. Temperature regimes change with water levels (Gertzen *et al.,* 2012).

Egg depositions have been recorded at depths of 15 cm to 4 m, with larger females laying eggs in deeper water (Schwanck, 1989). Variation in depth may also be related to availability of substrate and water temperature. The deposition site is prepared by clearing away debris (Annett *et al*., 1999), and egg deposition generally occurs within one day of territory establishment and courtship. In some cases, egg deposition can be delayed for six days, with a large proportion of breeding pairs even failing to lay eggs (Schwanck, 1987).

Legner (1978) studied the mass culture of *Tilapia zillii* in 18 and 34 cm deep, 5.5 X 7.6 m earthen ponds. He showed an optimum 9 pair initial density for the maximum production of fry after 104 days. Biomass increase was, nevertheless, greater at the 6 pair stocking density in the deeper ponds. Pond depth had no significant effect on size and weight gain of parental fish. Nest and brooding tube construction was variously influenced by pond depth and stocking density. Partial shading of ponds, while not affecting the maximum water temperature, did apparently reduce fish biomass production, the average depth of nests. An estimated 1 million fish could be produced in 104 days from 190 similar ponds with a water surface equivalent to 0.8 ha.

The objective of this study was to investigate the effects of different levels of water on spawning and reproductive performance of hybrid red tilapia (*Oreochromis* sp.).

MATERIALS AND METHODS

This study was conducted at Mariculture Research Center (MRC), Faculty of Environmental Agricultural Sciences, Arish University, North Sinai, Egypt. The study showed influencing of water depths on the reproductive performance and behavior of hybrid red tilapia (*Oreochromis* sp.) in tanks.

In this study the red tilapia broodstocks were placed in u-shaped fiberglass tanks (320L × 150W × 100H cm) full of well water and supplied with compressed air. Each tank was equally separated by fine mesh as a replicate and each part stocked with 4 fish (3 females and 1 male) with two replicates.

The study designed as four treatments as following, first treatment (T1) where water column was 20 cm, second treatment (T2) where water column 40 cm, third treatment (T3) where water column 60cm and fourth treatment (T4) where water column 80 cm. The bottom of the tanks consisted of a 10-15 cm sand layer to be used for nest building (a male activity in this species). Reproductive behaviors (nest building and mouthbrooding) up to reproduction or up to 10 days after the first nest construction were observed.

The experiment was carried out from June to July, 2015 for a period of 50 days. Where the water parameters were adjusted at suitable criteria for red tilapia spawning (temperature averaged 29.24±1.30°C, salinity averaged 20.36±0.14 ppt, dissolved oxygen concentration averaged 9.32±0.68 mg/l and pH averaged 6.97±0.37). All water parameters previous mentioned were suitable according APHA (1998). Twenty four females and eight males were used. Beginning females average weight were 97.9±4.2 g with average length was 17.9±1.0 cm. Average male length was 20.4±1.5 cm, and average body weight was 152.5±4.1 gall broodstock fish male and female obtained directly from MRC.

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Behavior observations were made daily at 10:00, 14:00 and 18:00 h. Reproduction and associated behaviors were analyzed by number of nests built divided by number of days after the first nest, latency (days) to initiate the first nest building and reproduction frequency detected by the occurrence of mouthbrooding incubation. During reproduction, male red tilapia build nest and court females. Both sexes spawn into the nest and the female catches the eggs into its mouth for mouthbrooding. The eggs in the mouth of females are easily observed by visual inspection from outside the tanks and are clear indicators of reproduction. Each nest consisted of an excavation in the bottom shaped as an inverted cone. At the end of experiment, the fry was collected after each spawning to count and weight. Eggs were taken from the female ovaries to calculate numbers of eggs and evaluate the difference of egg diameter under different water depths.

The parameters used to determine the productive performance were total number of fry/treatment, mean number of fry/spawning, total fry weight (g), mean fry weight (g)/spawning, number of fry/female, number of fry/female/day, number of fry/Kg female, number of fry/m3/day, rest period (days), number of spawning (spawning frequency), spawned females % and average egg diameter (mm) that was determined by the geometric mean of the larger and smaller diameters of each egg on a sample of 30 eggs per female according to Siraj *et al*. (1983). The relative production (seed/cm) = Absolute seed production/mean length (cm) and relative production (seed/g) = Absolute seed production/mean weight (g).

Broodstocks were fed at a rate of 2% of total biomass *ad libitum* diet containing 30.33% protein (Table 1).

Table (1): Ingredients composition and chemical analysis of the experimental diet (% on dry matter basis)

Ingredients composition	**(%)**
Fish meal (70% CP)	21
Soybean meal (44% CP)	21
Yellow corn	22
Wheat bran	10
Rice bran	20
Linseed oil	2
Vitamins premix(1)	2
Minerals premix(2)	2
Chemical analysis	**(%)**
Crude protein (CP)	30.33
Ether extract (EE)	5.62
Ash	5.61
Crude fiber (CF)	5.31
Nitrogen free extract (NFE)	53.13

(1): One kilogram from vitamin premix contained 10.000.000 / Iu, 2.000.000 / Iu, 10.000 g, 1000 mg, 1000 mg, 5000 mg, 2000 mg, 10 mg, 50 mg, 10.000 mg,30.000 mg, 1000 mg, 500 mg, 20.000 mg, 10.000 mg, 1000 mg, 1000 mg and 50 mg of Vitamin A, D3, E, K3, B1, B2, B6, B12, Biotin, C, Nicotinic acid, Pantothenic acid, Folic acid, D. L. methionine, L. lysine, L. threonine, L. tryptophan and propylene glycol.

(2): One kilogram from minerals premix contained 3000 mg, 100 mg, 5000 mg, 4000 mg, 100 mg, 1000 mg, 1000 mg, 76500 mg and 36000 mg of minerals Fe, Co, Mg, Zn, Se, Cu, I, P and Ca.

Statistical analysis:

The data obtained in this study were analyzed by one-way ANOVA procedure of SAS (1988). Means were compared by Duncan's new multiple range test (Zar, 1996).

RESULTS

Analysis of variance showed that significant differences ($P<0.05$) was detected in all reproductive performance parameters between different levels of water depth treatments in the first and second spawning (Table 2). In the first spawning T1 had no spawning occurred. While at T2 recorded the lowest values of mean of fry number (281.50±9.90), mean of fry weight, number of spawned fish and the shortest rest period (17.66±1.50 day). The highest value of mean of fry number (661.50±9.60) was found at T3. No significant differences occurred in the number of spawned fish and the rest period in the first spawning. The highest value of mean of fry weight (12.10±1.27 g) was recorded at T4 (Table 2).

In the second spawning, the minimum values of mean of fry number, mean of fry weight and number of spawned fish were recorded at T2 and the maximum one at T3. The shortest rest period (14.50±1.50 day) was recorded for T3. The longer one (20.00±2.25 day) was recorded for T2 in partnership with T4 (Table 2).

The results obtained in Table 3 showed significant differences ($P<0.05$) between treatments in all reproductive performance parameters. The results of total number of fry production, mean of fry number/spawning, number of fry/female, number of fry/female/day, number of fry/kg of female, number of fry/m^3 of water and number of fry/m^3/day showed that, T3 was the best treatment in all these reproductive performance parameters. The lowest values of total number of fry production, mean of fry number/spawning, number of fry/female, number of fry/female/day and number of fry/kg of female were recorded for T2. The lowest values of number of fry/m^3 (809.66±9.34) and number of fry/m^3/day (16.19±0.92) were recorded for 80 cm of water depth treatment (Table 3).

Table (2): Means (±SD) of reproductive performance during the first and the second spawnings for red tilapia hybrid at different levels of water depths in tanks (50 days)

Items	*Water depth (cm)	T1	T2	T3	T4
First spawning	Mean of fry No.	--**	281.50^{b} ± 9.90	661.50^{a} ± 9.60	619.00^{ab} ± 4.84
	Mean of fry weight (g)	--	6.50^{c} ± 0.53	10.90^{b} ± 1.82	12.10^{a} ± 1.27
	Number of Spawned fish	--	1.50^{a} ± 0.70	2.50^{a} ± 0.70	2.00^{a} ± 0.01
	Rest period (day)	--	17.66^{a} ± 1.50	20.40^{a} ± 3.15	18.25^{a} ± 2.10
	Spawned female %	--	50.00^{c} ± 3.57	83.33^{a} ± 3.57	66.66^{b} ± 0.01
Second spawning	Mean of fry No.	--	722.50^{c} ± 5.61	1325.00^{a} ± 19.74	935.50^{b} ± 12.02
	Mean of fry weight (g)	--	10.20^{b} ± 2.23	15.65^{a} ± 2.33	14.50^{a} ± 2.12
	Number of Spawned fish	--	1.50^{b} ± 0.70	2.50^{a} ± 0.70	3.00^{a} ± 0.01
	Rest period (day)	--	20.00^{a} ± 2.25	14.50^{b} ± 1.50	20.00^{a} ± 2.10
	Spawned female %	--	50.00^{c} ± 3.57	83.33^{b} ± 3.57	100.00^{a} ± 0.00

*Values in rows having the same superscript letters are not significantly different ($P \geq 0.05$); ** No spawning occurred

Table (3): Means (±SD) of reproductive performance during the experiment for red tilapia hybrid at different levels of water depths in tanks (50 day)

Items / *Water depth (cm)	T1	T2	T3	T4
Total fry production	--**	1004.00^{b} ± 30.51	1986.50^{a} ± 25.86	1554.50^{a} ± 19.86
Mean number of fry / spawning	--	502.00^{b} ± 15.25	993.25^{a} ± 17.93	777.25^{a} ± 14.43
Number of fry/ female	--	334.66^{b} ± 6.83	662.16^{a} ± 8.62	518.16^{a} ± 8.22
Number of fry/ female/ day	--	6.69^{b} ± 1.53	12.44^{a} ± 0.37	10.36^{a} ± 1.96
Number of fry/ kg female	--	3418.39^{b} ± 19.84	6763.64^{a} ± 16.52	5292.75^{a} ± 18.56
Number of fry/ m^3	--	1045.83^{ab} ± 8.04	1379.51^{a} ± 10.83	809.66^{b} ± 9.34
Number of fry/ m^3/ day	--	20.91^{ab} ± 1.60	27.59^{a} ± 0.25	16.19^{b} ± 0.92

*Values in rows having the same superscript letters are not significantly different ($P \geq 0.05$); ** No spawning occurred

Significant differences between treatments (P<0.05) and spawned female (%) were recorded. Significant differences were observed between treatment of total fry weight (g), mean of fry weight/spawning and rest period; minimum values were recorded for T2. The maximum values were obtained for T3 and T4 in spawned female (%) had (83.33%). Longer rest period and egg numbers were observed at T3 and T4 respectively (Table 4). The egg diameter and absolute fecundity of initial sample had (22.47±0.94 μm) and (865.00±12.54) respectively.

The diameter of hybrid red tilapia eggs taken from fish ovary after 50 days exposure to different levels of water depth; T1, T2, T3 and T4were investigated. There were significantly differences (P<0.05) between treatments. The smallest egg diameter (22.45±1.49) μm was recorded for T4 and the largest one (25.93±1.05) μm was recorded for T3 (Table 4).

Table (4): Means (±SD) of reproductive performance parameters during the experiment for red tilapia hybrid at different levels of water depths in tanks

Items / *Water depth (cm)	T1	T2	T3	T4
Spawned female %	--**	50.00^{b} ± 3.45	83.33^{a} ± 2.53	83.35^{a} ± 1.00
Total fry weight (g)	--	16.70^{a} ± 1.76	26.55^{a} ± 0.49	26.60^{a} ± 0.93
Mean of fry weight (g)/spawning	--	8.35^{a} ± 1.38	13.27^{a} ± 0.24	13.30^{a} ± 0.69
Rest period (day)	--	18.83^{a} ± 4.10	17.45^{a} ± 0.50	19.13^{a} ± 0.15
Egg diameter (μm)	25.02^{a} ± 2.52	22.68^{b} ± 1.72	25.93^{a} ± 1.05	22.45^{b} ± 1.49
Absolute fecundity	576.00^{c} ± 8.38	854.00^{b} ± 15.91	988.00^{a} ± 19.49	913.50^{ab} ± 14.25

*Values in rows having the same superscript letters are not significantly different ($P \geq 0.05$); ** No spawning occurred

Table (5) shows the relation between total length and body weight for female hybrid red tilapia and water depth. The analysis of variance shows significant differences (P<0.05) between treatments. The lowest values of absolute fecundity, relative seed production (seed/cm) and relative seed production for weight (seed/g) were recorded for T2 and the highest values for T3.

Table (5): Means (±SD) of total length and body weight for female hybrid red tilapia and seed production effected by water depths

Items / *Water depth (cm)	T1	T2	T3	T4
Mean length (cm)	18.75^{a} ± 1.06	17.00^{b} ± 0.47	18.75^{a} ± 0.11	19.41^{a} ± 0.11
Mean weight (g)	94.25^{c} ± 1.06	104.00^{b} ± 1.83	116.00^{a} ± 0.01	105.00^{b} ± 1.25
Absolute fecundity	--**	1004.00^{b} ± 30.51	1986.50^{a} ± 25.86	1554.50^{a} ± 19.86
Relative production (seed/ cm)	--	59.06^{c} ± 1.94	105.95^{a} ± 3.62	80.09^{b} ± 1.69
Relative production (seed/ g)	--	9.65^{c} ± 2.39	17.13^{a} ± 0.48	14.80^{b} ± 2.34

*Values in rows having the same superscript letters are not significantly different ($P \geq 0.05$); ** No spawning occurred

Latency periods after building first nest (day) were showed in Figure (1) with the shortest period was recorded for T4 and the longest one for T2. Figure (2) shows means of number of nests/day at different levels of water depths with the least number of nests were recorded for T1 and the maximum one was recorded for T4.

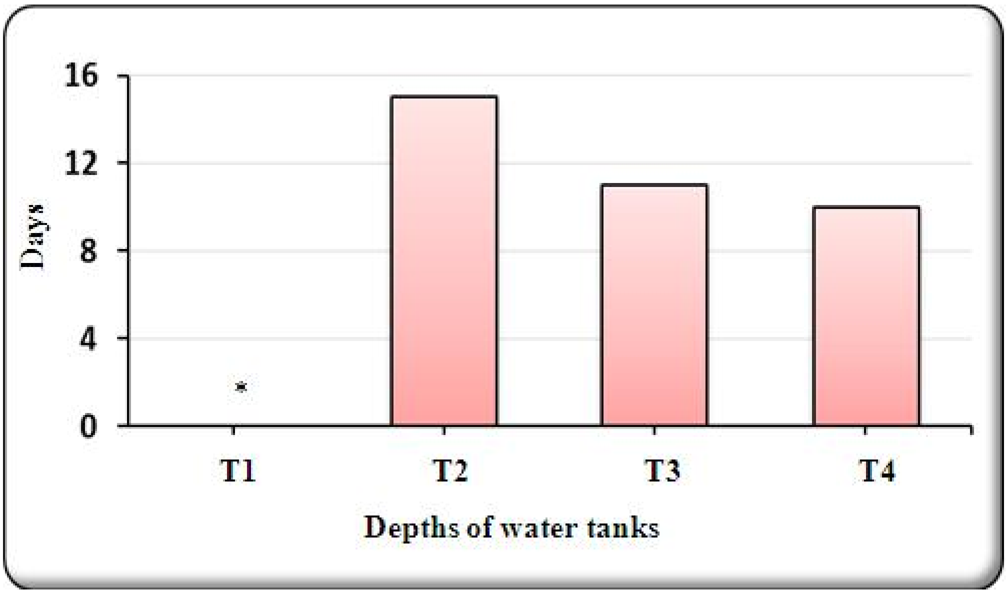

Fig. (1): Latency period to the first nest building (day) at different levels of water depths
* No spawning occurred

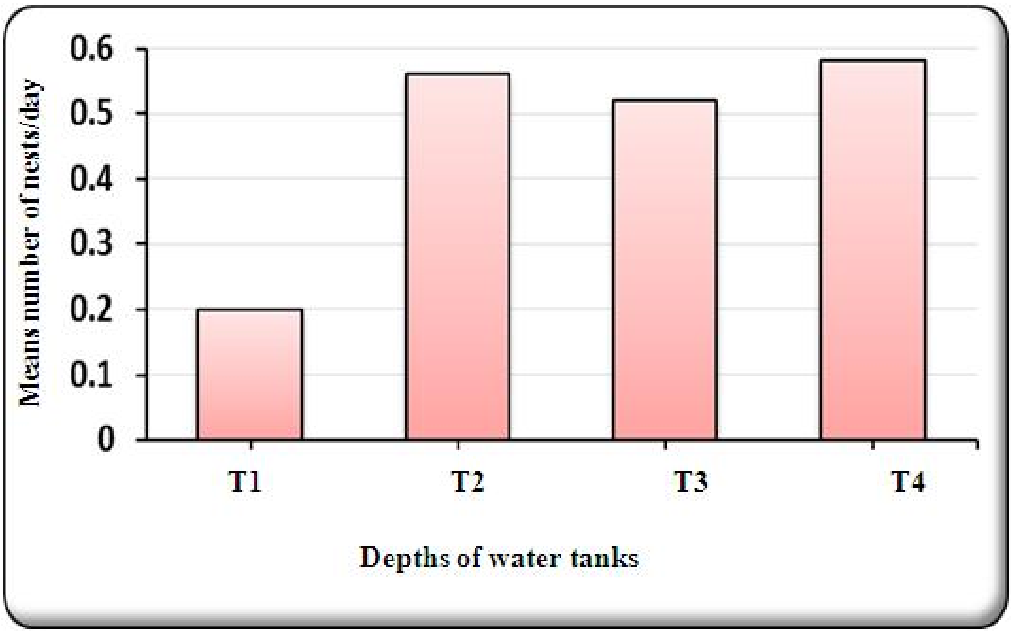

Fig. (2): Number of nests/day at different levels of water depths

DISCUSSION

The current study shows that increasing the water column from T3 to T4 increased the seed production when compared with low water depth T1 and T2. The height water column are maintain the stability of water temperature for long time than low water column that in agreement with Salem *et al.* (2005) who stated that increasing the water depth from 71 to 95 cm increased the number and weight of seed in concrete tanks.

In this study large size of eggs in red tilapia may be related to the parental care of eggs reported for the species. Large egg size enhances fry and larval viability due to its higher yolk content, and hence, food supply to the larva. Thus, large egg size in red tilapia may be an indication of better larval viability and a compensation for low fecundity in the species. The ripening of eggs and spawning are controlled by hormones, nutrition of the female and external (ecological) factors (Hempel, 1979). Usually maturation and spawning are controlled by a combination of endogenous and exogenous controls are not governed by any specific factor.

Egg size was negatively correlated to fecundity the lower number of eggs in tilapia, the larger the size of eggs. However, fecundity was independent of the size of the fish (Imevbore, 1970). Fecundity was also dependent on the size of fish and thus, the larger the fish, the higher its egg number and this may be due to more available visceral volume for holding the eggs. Fecundity exhibited higher correlation with gonad weight than with total length or total weight (Rheman *et al.,* 2002).

The present study demonstrated that the deepest water column T3 and T4 is the best treatments for nest buildings, seed production and the shorter latency days. The possible reason for this may be due to fish feeling with the territorial safe.

Reproductive performance of red tilapia was found to be better in deep water T3 –T4 than in shallow T1 in agreement with the results reported by El-Sayed *et al*. (1996) who stated that reproductive performance of Nile tilapia was found to be better in deep tanks (1–2 m) than in shallow (0.5 m) tanks. Courtship, spawning intensity and seed production of tilapia can also be related to artificial spawning shelters.

In this study the courted female cleaned the nest moving stones outside of the nest with its mouth. Spawning occurred only after the couple showed a very clearly intumesced genital papilla which was inclined downwards. When the male built more than one nest, spawning took place in the one built last. This was in agreement with the findings reported by Ves-De-Freitas and Nishida (1998).

The red tilapia male was positioned perpendicularly to the female, which waved vigorously its tail and laid an egg batch. The male then swam above the eggs and delivered milt on them. At this time, the female approached its mouth to the male's genital papilla, sucked milt and picked up the eggs into its mouth. After the spawning events ended, the male left the nest before the female and again started to attack to the other fish. The female's mouthbrooding was carried outside the nest. After spawning the genital papillae of both fish were reddish. This results in agreement with the results reported by Ves-De-Freitas and Nishida (1998).

At the start of the pre-courtship phase, both sexes will adopt breeding colors and the male will aggressively defend his territory from conspecific individuals and other species. Initially, both females and males show very similar levels of aggression towards each other, with a series of chases and butts and the female repeatedly fleeing the males' territories (Annett *et al*., 1999). Eventually, the female is increasingly tolerated by male and a courtship is takes place (Baldaccini, 1973).

Male courting is initially aimed at larger females but males will redirect their courting attention to females that show the most interest. During the actual courtship, the aggressive behavior displayed by the male and female during pre-courtship is rare or absent. When the pair is established, both members become aggressive to intruders and they defend their shared territory (Annett *et al*., 1999).

CONCLUSION

The present study concluded that the using of 40 to 80 cm of water depth in tanks will obtain large numbers of hybrid red tilapia fry. The decrement or increment of water depth from that mentioned depth adversely affects the production of seeds. Spawning at 20 cm of water level in tank had not occurred.

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تأثير مستويات مختلفة من عمق المياه على التفريخ ومظاهر التكاثر للبلطي الأحمر الهجين

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تم دراسة تأثير مستويات ٢٠، ٤٠، ٦٠ و٨٠ سم من عمق مياه التنك على التفريخ ومظاهر التكاثر لأسماك البلطي الأحمر الهجين. وتم الحصول على تفريختين خلال فترة التجربة باستثناء أسماك المعاملة ٢٠ سم من عمق المياه فإنها لم تُنتج زريعة. ولُوحظ أن هناك فروق معنوية ($P < 0.05$) بين المعاملات في التفريختين. وسجلت أسماك المعاملة الثالثة من عمق المياه أفضل النتائج في معظم عوامل الأداء التناسلي بينما سجلت أسماك المعاملة الثانية من عمق المياه أقل النتائج. وأن أسماك المعاملة الثالثة من عمق المياه كانت الأكثر في عدد البيض ٩٨٨.٠±١٩.٤٩ بيضة/سمكة وسجلت أسماك المعاملة ٢٠ سم من عمق المياه الأقل في عدد البيض ٥٧٦.٠±٨.٣٨ بيضة/سمكة.