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Deciphering the right age for high spawning performance in *Oreochromis niloticus*: Implications for beneficial fish seed production

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ABSTRACT

Studies decoding the most suitable age for high spawning performance in Nile tilapia are scant. The provision of cheap and sustainable tilapia seed is becoming increasingly challenging. The effect of age and critical water quality factors on the reproductive performance of Nile tilapia (*Oreochromis niloticus*) was examined from March to August 2015 in Northeastern Bangladesh. Three treatments were designed as T_1 (1-year-old), T_2 (1.5-years-old), and T_3 (2-years-old) with five replicates of each age group. The brooders were reared in 40 m^2 (8m x 5m) blue filter net hapas in a 33 decimal earthen pond. A total of 150 females and 50 males were maintained in each hapa. Eggs were collected every week from the buccal cavity of incubating females. The results indicated that the pond water quality was acceptable, supporting the formal productive requirements. The highest number of breeders (30%) was found in T_2, and the lowest (16.7%) was in T_1. Besides, the highest number of eggs was obtained from T_2 (1580474), followed by T_3 (1190566) and T_1 (661380). The average egg production was 1095±144, 1429±185, and 1579±250 in T_1, T_2 and T_3, respectively. The total number of spawned fishes was 604, 1106, and 754 in T_1, T_2, and T_3, respectively. In conclusion, the results showed that the breeding performance was the highest 1.5-years-old group (T_2), followed by the 2-years-old batch (T_3). These results suggested that egg production performance of Nile tilapia females increases with increasing age and somatic growth to a certain extent. These outcomes could be widely applicable to enhance tilapia seed production and help improve the sustainable supply of tilapia seed.

Introduction

Aquaculture has developed as the fastest food-producing industry due to the tremendous potential for high-quality protein products from marine and freshwater resources to satisfy the ever-growing global demand for aquatic food. Tilapia seizes the second-highest position after carps in the modern aquaculture industry (Waite et al. 2014), which alludes to the rampant increase in demand and productivity. Several biological characteristics support and attract the culture of *Oreochromis niloticus*. Tilapia culture is uniquely evolving since it occurs in almost every aquatic culture, whether fresh or saline waters. This species is also called 'aquatic chicken' for its rapid growth, comfortable farming system, tolerance to adverse environmental conditions, and resistance to diseases (El-Sayed 2006). Tilapia culture has expanded worldwide at an average annual growth rate of 14% since 1984, while the overall

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growth rate of aquaculture was 11% (Tsadik Getinet 2008). The global production of farmed tilapia exceeded 2,002,087 metric tonnes (Fitzsimmons 2006). Nile tilapia, *Oreochromis niloticus,* has been the undisputed leader among tilapia species, accounting for nearly 75% of the volume (Tsadik Getinet 2008). Although Bangladesh is a small country based on land, it occupies the 4th position globally for its inland fish production, while tilapia production having a considerable contribution (DoF 2014). In 2012-13, around 228450 metric tons of tilapia were produced in Bangladesh, which was 12.28% of the global tilapia production (DoF 2014).

Although tilapia culture is swiftly spreading, the shortage of tilapia fry production is one of the most pressing issues that hinder the sustainable fish seed supply from meeting seasonal demands (Tsadik Getinet 2008). If the trend continues, it can seriously jeopardize the second most crucial fish culture system globally. The low and inconsistent seed production is linked with low fecundity and asynchronous spawning cycles, posing significant constraints to developing sustainable commercial tilapia aquaculture and its expansion (Tsadik Getinet 2008). Even in a controlled environment (i.e., tanks), natural seed production rapidly decreased after an initial peak spawning product (Guerrero and Guerrero 1985). Such decrease in spawning has been chiefly attributed to the development of asynchronous spawning in captivity (Guerrero and Guerrero 1985; Macintosh and Little 1995).

Being an asynchronous spawner, tilapia produces significantly few eggs, causing a considerable gap between seed production and farmer demand. Besides, tilapia seed producers frequently face many complexities regarding broodstock management, including early maturity, feeding management, asynchronous spawning, and low fecundity for large-scale seed production (Bhujel 2000). Therefore, brood stock management and breeder number should be optimized to ensure the availability of quality seed to maximize tilapia production (Macintosh and Little 1995; Coward and Bromage 2000; El-Sayed 2006).

Several techniques have been tried to alleviate low fecundity and asynchronous spawning for improving tilapia seed production. The methods include manipulation of critical environmental factors such as temperature and photoperiod (Msiska and Costa-Pierce 1997; Ridha and Cruz 2000), age and size at first maturity (De Silva 1986; Duponchelle et al. 1998), density, and sex ratio (Siddiqui et al. 1997; Beveridge and McAndrew 2000), fecund type (Tsadik and Bart 2007), artificial incubation of eggs (Siraj et al. 1983), frequency of seed removal (Little et al., 1993; Ridha and Cruz 1998), and dietary protein and feeding levels (Gunasekera 1996; Siddiqui et al. 1997).

Different tilapia species attain the first maturity at varying ages and sizes (Macintosh and Little, 1995; Bhujel 2000). Trewavas (1983) stated that tilapia reaches sexual maturity at 20-30 cm (150-250 g) under suitable conditions. However, tilapia may mature at a smaller size under adverse environmental circumstances (Duponchelle and Legendre 2000). Under aquaculture conditions, tilapia becomes sexually mature at even smaller sizes than in the natural environment. According to Mansour (2001), farmed Nile tilapia attained the first maturity at 30 to 50 g. On the contrary, Bhujel (2000) reported that Nile tilapia gained sexual maturity within six months after hatching at 60 g body weight. Rana (1988) reported fecundity and seed production largely depended on female body size rather than age. An essential feature of mouth brooding tilapia is low fecundity with large egg size (Duponchelle and Legendre 1997). The number of egg production ranges from 100 eggs (average weight of fish 100g) to 1500 eggs (from 1 kg body weight fish) (FAO 2021). But De Graaf (1999) reported that absolute fecundity ranges from <100 to >3000, and the size of the eggs range from <2 to 7.9 mm. Besides, Little (1989) reported that petite females produced more eggs at shorter intervals than large ones. Alternatively, larger females generate more eggs per clutch. According to Tsadik and Bart (2007), the fecundity of Nile tilapia brood fish is influenced by genetic factors and ecological conditions.

Nevertheless, age is one of the most critical factors of any breeding activities for wild fish populations. Every fish species becomes sexually functional at a certain age. Therefore, to obtain a better growth-performing fish seed, the age of fish brooders is a crucial factor. Thus, this study was initiated to determine the optimum age ranges for reproducible and production of high-quality and large quantity eggs by comparing three age groups of Nile tilapia to ensure the sustainable availability of quality tilapia seeds.

Materials and methods

Study site

The experiment was conducted at Bangladesh Rural Advancement Committee (BRAC) Tilapia Hatchery and Farm at Sreemongal Upazilla in Moulvibazar district for six months from March to August 2015 in a 33 decimal earthen pond.

Fry collection and rearing

Ten thousand tilapia fries (average body weight 0.01 gm) were collected from BRAC Tilapia Hatchery and reared in a $5m^2$ filter net hapa (2.5m x 2m) for two months. Fry were fed with mega nursery feed containing 30% protein at the 30% body weight rate. At an average body weight of 0.5 gm, tilapia fry transferred to two same-sized hapas (2.5m x 2m) for another three months. The nursery stage was fed at the rate of 15% of their body weight. Subsequently, the fry (average body weight 2 gm) was kept in five hapas (8m x 5m) for rearing until 2015. The fish were fed on ready mega floating feed with 30% crude protein at 5% of biomass daily dispensed in two equal rations in the morning (9.30 am) and afternoon (3.30 pm).

Identification of male and female

Male and female tilapia were segregated by observing the genital papillae or the vent. Females are distinguished based on red and round vents with short papillae, while males have long and pointed papillae. Males have only one aperture in the papillae from where urine and sperms are released, while females have two openings; one for releasing ova/eggs and the other for excretion (Bhujel 2012). A total of 1000 females and 250 males were captured and reared until February 2015 to mature at two years of age on their growth performance. Similarly, another set of 1000 females and 250 males tilapia were grown from September 2013 to February 2015 to age 1.5 years. Furthermore, the third cohort of 1000 females and 250 male's tilapia was raised from March 2014 to February 2015 to reach 1.0 years age.

Pond preparation

An earthen pond of 33 decimal was dried up by draining out the water through an electric pump. Lime was liquefied and applied at a 1kg per decimal rate by spreading homogeneously on the pond bottom. The pond was filled with water once a week after liming. Urea and triple superphosphate (TSP) were applied at 200 g and 100 g per decimal, respectively. When the pond was ready with sustainable water quality, we installed 15 hapas (8m x 5m) suspended in earthen ponds. The net hapas were placed equidistantly along the longitudinal axis of the pond by the attachment with nylon twine to bamboo poles. The hapa net was anchored at the bottom of each corner, while half-bricks were placed inside the hapas to prevent them from floating.

Brood stocking and feeding

The brooders were transferred to the hapas to observe the egg production. Each treatment was designed with five replicates. A total of 150 females and 50 males were maintained in each hapa. A total number of 750 female and 250 male brooders in each treatment were stocked using one year (T_1), 1.5 years (T_2), and 2 years (T_3) old brooders of 150-250g, 250-400g, and 400-600g body weight, respectively. The brood fish were regularly fed on formulated mega floating feed pellets of 30% crude protein twice a day at 3% body weight in all three treatments.

Collection of eggs

Eggs and fry were collected at seven-day intervals from the buccal cavity of the incubating females for artificial incubation. The diverse varieties of fertilized eggs, near-hatched larvae, and yolk sac fry of females were graded according to their varied hues and appearance. We identified four colors, viz. tiny white, pale yellow, deep yellow, and reddish fry with yolk sac, and the obtained hatch was maintained in different plastic containers placed in a bamboo frame. Collected eggs were sterilized for 8-10 minutes at room temperature with a 5-7 ppt saline water solution. The eggs, larvae, and yolk sac fry were then sent to an incubator for incubation (Towers 2015).

Spawned females count

The number of spawned females was counted weekly by observing the incubated eggs in the buccal cavity. This process was carried for each treatment.

Analysis of spawning performance

Collected eggs were counted and weighed to evaluate the reproductive performance of the brood stock of different treatments. The number and average weight of eggs and fry from each hapa were estimated by weighing a known sample number of eggs/fries on an electron balance to two decimal places and then bulk weighed the entire batch. Sample eggs (200 eggs/fry of the same stage) from each hapa were counted manually and weighed separately three times after dabbing to remove moisture on a piece of dry absorbent tissue for 10 seconds while held in a fine-mesh nylon net. Batch samples were treated similarly after allowing 15 seconds for moisture removal (Little 1989).

Water quality parameters

Water quality parameters like water temperature (WT), transparency, dissolved oxygen (DO), and pH were measured every week throughout the trial. A digital thermometer was used for water temperature, while an oxygen meter and pH meter were utilized to measure DO and pH in pond water. Besides, a Secchi disc was used to measure the transparency of the water.

Statistical analyses

Univariate analysis along with Tukey's multiple range tests was used with a 5% significance level. The statistical analysis system (SPSS) software version 16.0 was used to analyze all the data.

Results

Water quality parameters

We recorded the most critical water quality parameters during the experiment, including WT, DO, pH, and transparency. The results showed that the lowest water temperature was recorded in March (24.8 °C), while the highest temperature was observed in June (36 °C) followed by August (34.85 °C), as shown in Table 1.

Table 1. Salient water quality parameters range for different month of the study period

Parameter	March	April	May	June	July	August
Temperature ^{0}C	24.8 - 28.8	28.7 - 31.3	30.2-32.85	32.9-36.0	27.4-33.95	25.8-34.85
DO (mg/L)	5.15 - 5.87	4.30 - 5.09	3.9-4.68	2.90-3.91	3.10-5.90	3.10-4.60
pH	7.13 - 8.20	7.65 - 8.80	6.90-8.30	7.30-8.35	6.88-7.50	7.0-8.0
Transparency (cm)	30.0 - 36.0	26.0 - 28.0	14.0 - 26.0	13.0 - 20.0	18.0 - 38.0	22.0 - 26.0

The DO level was observed contrary to the WT. The pH records exhibited the lowest level at 6.90 (May) to the highest pH level in April (8.80). The water was more turbid during May and clearer during March that could be linked with the intensity of rainfall events. Overall, the pond water quality was found satisfactory during the whole study period.

Spawning performance assessment

Table 2 presents the weekly spawning performance of the breeding tilapia from March to August 2015. Among the three treatments, the highest number of 1-year-old spawning females was 43 in the third week of July, while the lowest was observed as six females during the first and second week of June. In case of 1.5 years old cohort (T_2), the highest number of females was observed as 82 each in the last week of March and first of April while the lowest was 12 in the first week of June. Similarly, the 2-years old batch of the spawning tilapia females showed the highest number of 81 in the fourth week of March, while the lowest was 3 in the first week of June. The highest average number of spawning tilapia was recorded in April (70.25), followed by March (69.4) in the T_2 i.e., the 1.5 years old group of the brooders (Table 3). The highest average spawning number of tilapia females in the one-year-old group was 36.75, while the lowest was in June (13.8). The mean number of eggs was found 1095±144, 1429±185, and 1579±250 (Table 4).

The total spawned fishes were calculated as 604, 1106, and 754 in T_1, T_2, and T_3, respectively. But the total number of eggs counted during the whole experimental duration was 661380, 1580474, and 1190516 in T_1, T_2, and T_3, respectively (Table 4). On the other hand, the monthly highest number of spawned tilapias was observed as 1279 (June), 1649 (May), and 1875 (March) in T_1, T_2, and T_3. Overall, the spawning records have shown that the breeding performance was the highest in the 1.5 years-old group (T_2), followed by the 2-years-old group (T_3), and the lowest breeding performance was recorded for the 1-year-old group (T_1), as shown in Fig. 1. However, Fig. 2 displays the monthly spawning performance of female tilapia and exhibits a steep decline from the peak in April to the lowest point in June.

Impact of water quality on spawning performance

The overall comparison among the three age groups of the spawning female tilapia showed the comparable effect of WT on spawning performance (Fig. 3). The highest spawning performance was observed at 28.7 °C, while the lowest was observed at the highest (34.8 °C) during the experiment. Furthermore, the T_2 displayed the highest performance at the optimal WT increased spawning activity was observed in T2 at the DO level of 4.9 mg/L (Fig. 4).

Table 2. Weekly collected number of spawned tilapia females from each treatment

Date	T$_1$ (1 year)		T$_2$ (1.5-years)		T$_3$ (2-years) %	
	Number	**%**	**Number**	**%**	**Number**	**%**
02.03.2015	17	11	53	35	51	34
09.03.2015	19	13	61	41	59	39
16.03.2015	22	15	71	47	66	44
23.03.2015	23	15	80	53	81	54
30.03.2015	26	17	82	55	70	47
06.04.2015	32	21	82	55	61	41
13.04.2015	35	23	76	51	62	41
20.04.2015	27	18	62	41	52	35
27.04.2015	25	17	61	41	40	27
04.05.2015	22	15	52	35	32	21
11.05.2015	11	7	32	21	12	8
18.05.2015	12	8	25	17	11	7
25.05.2015	13	9	24	16	9	6
01.06.2015	6	4	12	8	3	2
08.06.2015	6	4	13	8.7	4	3
15.06.2015	11	7	13	8.7	4	3
22.06.2015	20	13	21	14	10	7
29.06.2015	26	17	23	15	8	5
06.07.2015	26	17	23	15	10	7
13.07.2015	42	28	47	31	13	9
20.07.2015	43	29	37	25	17	11
27.07.2015	36	24	34	23	15	10
03.08.2015	32	21	44	29	21	14
10.08.2015	35	23	46	31	24	16
17.08.2015	25	17	17	11	11	7
24.08.2015	12	8	15	10	8	5

Table 3. Monthly breeding performances of *Oreochromis niloticus* in three treatments

Months	T$_1$ (1 year)		T$_2$ (1.5-years)		T$_3$ (2-years)	
	ASN	**%**	**ASN**	**%**	**ASN**	**%**
March	21.4	14.2	69.4	46.2	65.4	43.6
April	29.75	19.75	70.25	47.0	53.75	36.0
May	14.5	9.75	33.25	22.25	16.0	10.5
June	13.8	9.0	16.4	10.88	5.8	4.0
July	36.75	24.5	35.25	23.5	13.75	9.25
August	26.0	17.25	30.5	20.25	16.0	10.5

ASN, average spawning number

Discussion

Information regarding the appropriate age for the best egg production performance by *O. niloticus* is scant worldwide. Therefore, this study was conducted to determine the suitable age group for the best egg production performance by experimenting on three age groups, including 1.0 years, 1.5 years, and 2.0 years using a considerable number of individuals (*n*>3000). Additionally, these three groups of brood fish were raised at the institution-owned fish farm.

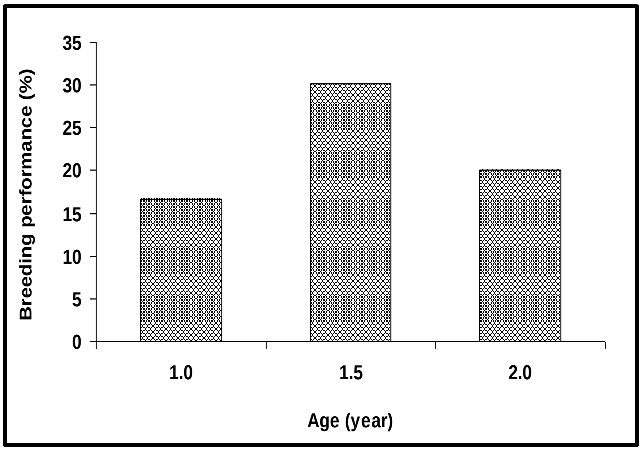

Fig. 1. Breeding performance (%) based on age of Nile Tilapia (*Oreochromis niloticus*)

The results of the current study showed that the 2-years-old group showed the highest number of eggs (1579±250) in each fish, while the 1.5-years-old group produced the medium (1429±185), followed by the lowest number of eggs (1095±144) in the 1-year-old cohort. Further, the frequency of laid females was higher in the 1.5-years-old group (1106), followed by 2-years-old (754), while the lowest was recorded in the 1-year-old (604) cohort. Brooks et al. (1997) has described that the age of females and the number of their eggs were positively correlated. Further, Bromage et al. (1992) reported that the absolute fertility increases in tandem with fish age and size. However, the intervals between spawning vary by age, suggesting that older fish have less reproductive mobility with an extended inter-reproductive break, similar to this study.

During this experiment, the 1.5-years-old group showed the highest number of eggs (1580474), while the 1-year-old cohort showed the lowest (661380). Further, the present study showed that reproductive performance was the best in 1.5-years-old fish during all seasons. An average 30% breeding performance was achieved in T_2. The 2-years-old fish showed good performance from March to April but declined the egg production performance during May to August. On the other hand, the 1-year-old group showed the lowest performance from March to April when the environmental conditions were relatively favorable. From July to mid-August, their performance increased due to age increase, and the environment was also encouraging as the rainy season commenced. Consequently, our results suggested that the 1.5-years-old batch could be the most suitable age group to breed for a profitable business.

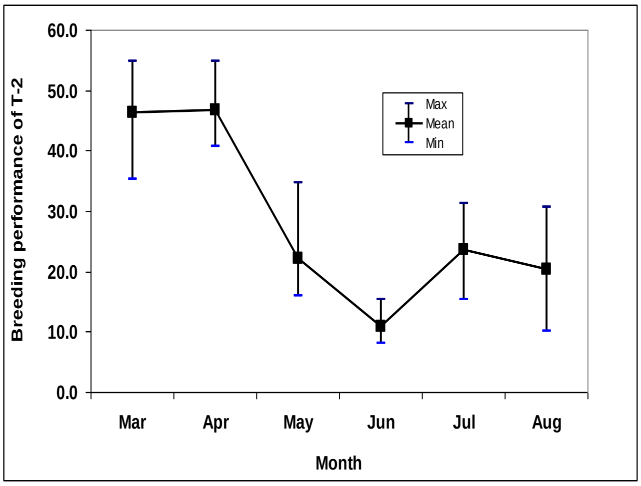

Fig. 2. Monthly comparison of the spawning performance of Nile tilapia (*Oreocromis niloticus*) observed in 1.5-years-old group of females

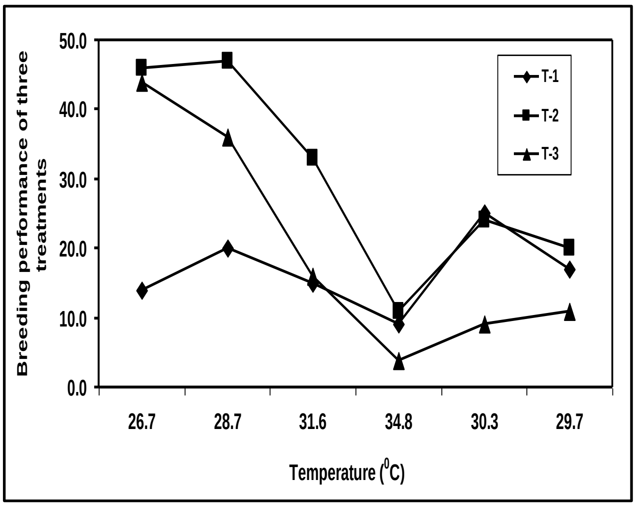

Fig. 3. Impact of water temperature on the spawning performance of female Nile *tilapia* (*Oreochromis niloticus*)

The reproductive performance of Nile tilapia is highly correlated with WT. WT is known to influence the physicochemical and biological factors of the water body (Paul, 1998). The present study showed that in

March, the WT range was 24.8 °C to 28.8 °C, considered the most suitable temperature range for tilapia spawning. On the other hand, the highest temperature was recorded in June ranged between 32.9 °C to 35.7 °C, when the egg production performance was the lowest. According to Philippart and Ruwet (1982), the suitable temperature for tilapia reproduction must be above 20 °C. Further, Bhujel (2000) reported that tilapia seed production increased with increasing WT from 25 °C to 31°C.

Except for anaerobic bacteria, DO is the most critical environmental factor for all aquatic life. Aquatic plants and animals use oxygen to regulate their metabolic processes. Tilapia can use atmospheric oxygen (Popma and Lovshin 1996) besides DO. Consequently, this fish can survive at a low concentration of DO in any aquatic environment. However, a consistently lower DO level may cause diseases and decreased reproduction performance (Bhujel 2000). In this research, DO was found to vary from 2.9 to 5.9 mg/L, which is satisfactory and suitable for the successful reproductive performance (El-Sayed 2006).

during this study. Consequently, the pH values were at a satisfactory level, indicating a suitable environment for better performance of tilapia egg production.

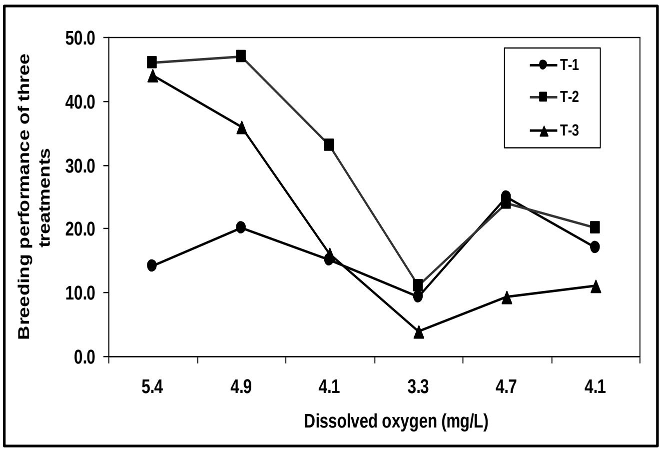

Fig. 4. Impact of dissolved oxygen level on the spawning performance of female Nile tilapia (*Oreochromis niloticus*)

Transparency plays a vital role in Tilapia egg production. Sechi disc reading between 20 and 30 cm means the water body to be productive. In the present

Table 4. Number of eggs per fish (*Oreochromis niloticus*) from different treatments

Month and spawned females	Average no. of egg/female		
	T_1	T_2	T_3
March	1206	1617	1875
April	1182	1488	1791
May	1279	1649	1733
June	988	1358	1435
July	962	1245	1335
August	950	1217	1305
Total egg	6567	8574	9474
Mean ± SD (No. of eggs)	1095 ± 144^a	1429 ± 185^b	1579 ± 250^b
Total no. of spawned females	604	1106	754
Total no. of egg during the experiment	661380	1580474	1190566

* Values in the same column with different superscripts are significantly different (p>0.05).

Similarly, pH is considered a critical abiotic factor of any aquatic environment. The growth and metabolic rate, including other aquatic organisms' physiological activities, could be troubled due to an acidic pH of water (Swingle 1967). Swingle (1967) also stated that the optimal pH value ranges from 6.5 to 9.0 for favorable reproductive results. Lower pH levels have been shown to harm plankton production as well as fish growth. The pH level ranged from 6.88 to 8.80

study, transparency varied from 20 to 38 during the study period. The highest and lowest values were 38 and 14cm in March and August, respectively. Rahman (1992) concluded that the transparency of productive water bodies should be 40 cm or less. In the present study, transparency shows a suitable water body condition that played a vital role in tilapia egg production. Overall, the optimal ranges of leading water quality factors provided a supportive environment for tilapia spawning.

Conclusion

Nile tilapia culture has become popular in the present era due to its rapid growth and adaptability to harsh environmental conditions. Low production cost and high profit make this species one of the most suitable culture options for aquaculture. The reproductive performance of Nile tilapia could be significantly improved by choosing the high-performing fish individuals of a particular age. This experiment concluded that the most suitable age group of Nile tilapia is 1.5 years-old to harvest the maximum number of fish eggs as well as the fish fry. In addition, the crucial environmental factors (WT, DO, pH, and water transparency) could have a critical effect on tilapia seed production under captive conditions. This study could provide vital insights into enhancing the reproductive performance in Nile tilapia.

Conflict of Interest

There is no competing interest that might influence the research work.

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