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EFFECT OF TEMPERATURE TREATMENT ON SOME REPRODUCTIVE AND PHYSIOLOGICAL PERFORMANCE IN FEMALE NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

(With 3 Tables)

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(Received at 6/12/2004)

تأثير المعالجة الحرارية على الأداء التناسلى والفسيولوجى لأنثى أسماك البلطى النيلى

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تمت هذالدراسة لايضاح تأثير المعالجةالحرارية على الأداء التناسلى ومستوى هرمون الأستروجين لأنثى أسماك البلطى النيلى. وقد وجد الأتى: ١- زاد المعامل النسبى للمناسل معنويا فى الأسماك بزيادة درجات الحرارة (١±١٦، ١±١,19±٢٥، ١±28، ١±32). ٢- زاد عدد البيض لكل وحدة جم معنويا فى المجموعات بزيادة درجات الحرارة (١±١٦ ، ١±١,19±٢٥، ١±28، ١±32).٣- زادت نسبة النضج فى البويضات معنويا فى المجموعات بزيادة درجات الحرارة (١±١٦، ١±١,19±٢٥، ١±28، ١±32). ٤- زاد مستوى هرمون الأستراديول- ب١٧ فى المجموعات بزيادة درجات الحرارة (١±١٦، ١ ±١,19±٢٥، ١±28، ١±32). ٥- لم يتم ملاحظة أى تناسل فى الأسماك المعالجة فى درجات الحرارة (١±١٦، ١±19). ٦- لم يكن تأثير درجة الحرارة قاصرا على معدل التبويض بل كان له تأثير على الأداء التناسلى لأنثى أسماك البلطى النيلى. حيث وجدت علاقة عكسية بين درجة الحرارة وكلا من فترة حضانة البيض، فترة رعاية الذريعة والفترة بين كل تبويضين متتاليين. ٧- وجد اختلافا معنويا بين فترة حضانة البيض، فترة رعاية الذريعة والفترة بين كل تبويضين متتاليين فى الأسماك المعرضة لدرجات الحرارة (١±٢٥، ١±28، ١±32). من كل النتائج السابقة تعتبر المعالجة الحرارية من أهم العوامل لتطوير الأداء التناسلى والفسيولوجى لأنثى أسماك البلطى النيلى.

SUMMARY

This study was carried out to investigate the effect of temperature on some reproductive performance and estrogen levels in female Nile tilapia (*Oreochromis niloticus*). An obvious significance increase in

gonado-somatic index (GSI) was shown by increasing temperature (16±1, 19±l, 25 ± 1, 28 ± 1 and 32±1) respectively. The present results provided empirical evidence that temperature increased egg production significantly by increasing temperature in fish treated groups (16±1, 19±l, 25 ± 1, 28 ± 1 and 32±1°c) respectively. Concerning oocyte maturation, the present results revealed a significant increase in the percentage of oocyte maturity by increasing temperature in treated groups at (16±1, 19±l, 25 ± 1, 28 ± 1 and 32±1°c) respectively. Moreover, results in this study showed significant increase in estrogen level in the groups under elevated temperature (25±1, 28±l and 32±l °c).On the other hand, no spawning was observed in groups of fish treated with water temperature at 16±1 and 19±l°c. Water temperature not only affects spawning frequency but also affects different reproductive performance in *O. nilotica* where an inverse relationship between water temperature and incubation period and inter-spawning interval was recorded. The differences between fish treated with water temperature 25±1, 28±l and 32 ±l °c respectively were statistically significant for incubation period, rearing period and interspawning interval. These results indicated that temperature consider the key factor for promoting the reproductive and physiological performance in female Nile tilapia (*O. niloticus*).

Key word: *Reproduction, Estrogen, Sexual behaviour and Female Nile tilapia (Oreochromis niloticus).*

INTRODUCTION

With the ever increasing need for a cheap source of animal protein to overcome the world over increasing population problem, more and more attention has directed to fish farming and aquaculture with emphasis on tilapia, which have a lot of advantages including fast growth, high acceptability to consumers, high density of stocking and tolerance of a wide range of culture conditions (Macintosh and Little, 1995 and Graaf *et al.,* 1999).

In fish farming, obtaining mature and viable gametes from male and female fish allover the year is one of the most serious problems to be solved, because of their influence by the aquatic ecosystem characteristics (Encina and Granado-Lorencio, 1997).

Fish are affected directly with changes in the aquatic environment that maintains their homeostasis, growth and reproduction. For successful management, it is necessary to know the physical

environmental limitations for each fish species. Temperature is considered the most important environmental factors in teleost fish reproduction (Van Der kraak and Pankhurst, 1997).

Temperature considered as an accurate indicator for gonadal maturation and reproductive performance (Tacon *et al.*, 1996). Exposure of female *O. aurea* to 28°C for two weeks resulted in an ovarian weight higher than that of those kept at 17°C (Terkatin –Shimony *et al.*, 1980). *O. mossambicus* maintained at temperatures between 26-28°C had gonads higher than those maintained at lower temperature (20-22°C) (Chmilevsky, 1995). *O. aurea* and *O. niloticus* females produced more eggs per unit body weight (relative fecundity) under better environmental conditions such as temperature (Payne and Collinson, 1983). Similarly, the favorable environmental conditions such as temperature leads to increased fecundity through their effects on hormonal mechanisms of hypothalamus-pituitary-gonads axis (El-Shazly, (1993;Rocha and Reis-Henriques, 1996).

Accordingly, the present study is a trial to shed a light on the effect of different temperature treatment on some reproductive and physiological performance in female Nile tilapia (Oreochromis niolticus).

MATERIALS and METHODS

The present study was carried out in Faculty of Veterinary Medicine, Suez Canal University, during the period from March to July 2004.

1) Fish: One hundred and fifty apparently healthy and sexually mature fish of both sexes (10 males and 140 females) were selected for the present investigation. They were purchased a live from the Fish Research Center, Suez Canal University, Ismailia, Egypt. Fish were transported in plastic tanks filled with water to our laboratory.The body weight ranged from 35 - 90 g and body length from 10-14 cm.

2) Aquaria: Fish were kept on rectangular glass aquaria (L100 x W50 x H50 cm). Each aquarium was supplied with aerated water using air pump allover the period of the experiment. Dissolved oxygen was maintained at 5-6mg/L by continuous aeration and was estimated by using oxygenmeter (The optimum dissolved oxygen for Nile tilapia is 4 mg/L) (Magid and Babiker, 1975). One third of water was daily replaced by fresh dechlorinated water according to El-Dessoky (2001).

3) Feeding: The fish were fed once a day at 10:00 AM on a ration containing 40% protein at a rate of 3% of the body weight according to Ufodike and Omoregie (1991).

4) Water temperature: The water temperature was adjusted using thermostatically controlled heaters allover the period of the study.

5) Experimental design: This work was conducted at the early spawning season (March to July). Fish were kept for 2 weeks before starting the experiment to acclimate the aquarium condition. Fish were sexed into males and females according to Huet (1986).

6) Effect of temperature: Fish were divided into 5 groups. Each contains 4 aquaria; two of them contained 16 females divided equally for reproductive performance. In addition to two more aquaria in each group for reproductive observation, each contain 1 male and 6 females. Water temperature was maintained according to the following schedule.

Fish group	Water temperature °C	No. of fish		No. of aquarium
		For reproductive performance	For behavioural observation/aquirium	
A	16 ± 1	16 (8 + 8) female	7 (1 male +6 female)	4
B	19 ± 1	16 (8 + 8) female	7 (1 male +6 female)	4
C	25 ± 1	16 (8 + 8) female	7 (1 male +6 female)	4
D	28 ± 1	16 (8 + 8) female	7 (1 male +6 female)	4
E	32 ± 1	16 (8 + 8) female	7 (1 male +6 female)	4

Control group (Group B) was kept under the normal climatic condition prevailing during the period of study where, the temperature ranged from 19-20°c.

Fish reared for reproductive performance in each aquarium was sacrificed at end of the experiment (after 30 days).

7) Fish marking: Fish in behavioural experiment were marked by using different figures of plastic sheets. These marks were fixed with a thread at the caudal fin peduncle behind the dorsal fins according to Earn (1997).

8) Observation time: Observations were performed as follow: Each breeding group was observed three times daily (20 minutes/aquarium) at circularly determined time from 8 AM to 5 PM for recording the spawning frequency, incubation period, rearing period and inter spawning interval of female.

Females were transported from the breeding aquarium in a plastic container containing water to another isolation aquarium.

The end of rearing period was detected by the development of the aggressive behaviour and cannibalism by the female toward its fry and by fry behaviour since it showed less response to their mother's mouth and become actively free swimming by the end of the maternal care period (Tacon *et al.*, 1996).

9) Sampling technique:

a- Gonado-somatic index (GSI): Gonads were dissected out of fish, wiped with filter paper and weighed; the fish was weighed without the gut (gutted weight). The gonado-somatic index (GSI) was calculated for each fish according to Nassr Allah (1998). GSI = (Weight of the gonads /gutted weight of the fish) x 100

b- Blood sampling: Blood samples were collected from caudal vein into dry clean centrifuge tubes without anticoagulant and left to clot at a room temperature, then centrifuged at 3000 r.p.m. for 20 minutes. The clear non-haemolyzed serum was transferred carefully to clean, dry rubber stopper and labeled glass vials. Finally, serum samples were stored at -20°C until analysis. Level of estrogen were measured by radioimmunoassay technique using kit obtained from Diasorin Co. Catalogue No. / REF.: CA-1558. The analysis was carried out according to Yelow and Berson (1971).

c- Oocyte sampling: Oocytes were collected to evaluate oocyte maturation according to the method described by Morehead *et al.*, (1998). Oocytes were placed in a petridish, rinsed in isotonic saline and the cytoplasm was cleared using clearing solution formed from Ethanol: Formalin: Glacial acetic acid (6:3:1). Few drops of glacial acetic acid were added to complete clearing of the cytoplasm.

Eggs were examined under dissecting microscope, differentiated into immature and mature oocytes. Immature oocyte appeared small in size with large central nucleus surrounded by a zone of cytoplasm, while mature oocyte appeared larger in size, concomitant with abundance of yolke in the cytoplasm and migration of the germinal vesicle (nucleus) towards the animal pole. The mature oocytes were counted and the percentages were calculated in each sample and expressed by oocyte maturation %.

d- Fecundity: Relative fecundity (defined as egg numbers per unit weight in the ovaries) was counted and expressed in eggs/g according to Munkittrick and Dixon, (1998).

10) Statistical analysis: Statistical analysis was done according to Sendecore and Cochran (1980). Analysis of variance (ANOVA) test was applied to compare between the trated groups.

RESULTS and DISCUSSION

GSI showed a significant increase by increasing water temperature (16±1, 19±l, 25 ± 1, 28 ± 1 and 32±1) respectively (Table 1). These findings are in consistence with those results of Tan (1985) studying milkfish, Michel (1991) and Mahfouz (1994) working on Glarias gariepinus, Dube and Portelance (1992) working on Cray fish, Foo and Lam (1993) working on *Tilapia mossambicus*. A possible explanation may be related to the metabolic rate of fish which varies directly with temperature (Cai and Summerfelt, 1992) and the natural relationship existed between temperature, amount of food consumed and GSI in poikilothermic animals. For these reasons, females direct all its nutritive supply toward its gonad during breeding season (Hahn, 1994).

Table 1: Effect of temperature treatments on GSI (%), fecundity (Oocyte/g) and oocyte maturation in female Nile tilapia (*Oreochromis niloticus*) (Mean ± SE).

Reproductive Parameters* / Sampling date	temperature treatments °C.	GSI (%)	Fecundity (Oocyte/g)	Oocyte Maturation (%)
After 30 days	16 ± 1	0.588±0.03^{e}	651.5±9.3^{e}	65±7.5^{d}
	19 ± 1	0.650±0.02^{d}	767.5±15.1^{d}	71.5±3.1^{c}
	25 ± 1	0.693±0.02^{c}	811.8±32.4^{c}	76.83±1.7^{b}
	28 ± 1	1.27±0.06^{b}	1136.3±68.1^{b}	87.17±1.6^{a}
	32 ± 1	1.365±0.02^{a}	1338.3±25.7^{a}	87.83±2.1^{a}
$LSD_{0.05}$		0.042	42.15	4.89

*Values having different letters within the same columns are significantly different.

Furthermore, the present results provided empirical evidence that temperature significantly increased egg production in fish treated groups (16±1, 19±l, 25 ± 1, 28 ± 1 and 32±1) respectively after 30 days (Table 1). These findings are in agreement with those of El-Shazly (1993). The concomitance between fecundity and GSI detected in the present work is in agreement with those of El-Agamy (1987) and Hop *et al.* (1995) in *Gerres oyena* and *Boreogadus saida* respectively. This positive correlation may be due to the fact that production of eggs tends to dominate the other functions of the body (El-Agamy, 1987). Moreover, since most physiological functions occur more rapidly with increasing

temperature, it is not surprising that vitellogenesis is also influenced by warm temperature in Indian murrel (Garg and Jain, 1984).

Concerning oocyte maturation, the present results revealed significant increase in the percentage of oocyte maturity in female groups by increasing temperature treatment (16±1, 19±l, 25 ± 1, 28 ± 1 and 32±1) respectively after day 30 (Table 1). The possible explanation was recorded by Biythe *et al.* (1994) who suggested that temperature might regulate the process of oocyt development by controlling metabolism. A higher temperature increase compresses the reproductive cycle of the fish, which may have resulted in a faster rate of oocyte development. Another explanation was recorded by Van der Kraak and Pankhurst (1997) who suggested that a specific sex steroid converting enzymes may be activated under various temperature treatments leading to final oocyte maturation or ovarian regression.

The present work revealed that there is a concomitant pattern between ovarian weight, fecundity and oocyte maturation. These results are in agreement with Lin *et al.* (1995) working on common carp and grass carp and Francisco *et al.* (2001) working on sea bass.

Estrogen (Estradiol 17-β) level increased significantly in groups under elevated water temperature (16±1, 19±l, 25 ± 1, 28 ± 1 and 32±1) respectively after 30 days (Table 2). This may be explained by the role of elevated water temperature in activation of ovaries to secrete estrogen which is necessary for gonadal development and oocyte maturation (Michel, 1991). This correlation may be due to stimulation of hypothalamo-pituitary-gonadal axis leading finally to secretion of gonadal steroids, which are responsible for acceleration of gametogenesis and vitellogenesis (Rocha and Reis-Henriques, 1996).

Table 2: Effect of temperature treatments on serum estradiol 17-β (ng/ml) in female Nile tilapia (*Oreochromis niloticus*) (Mean ± SE).

Sampling date	Temperature treatments (°C)	Estradiol 17-β (ng/ml)*
After 30 days	16 ± 1	3.83±0.24^{d}
	19 ± 1	4.16±0.22^{c}
	25 ± 1	4.43±0.24^{b}
	28 ± 1	6.78±0.21^{a}
	32 ± 1	6.97±0.15^{a}

$LSD_{0.05}$ = 0.269

*Values having different letters within the same columns are significantly different.

No spawning was observed in groups of fish treated with water temperature at 16±1 and 19±1°C (Table 3). On the other hand, spawning percentage observed in ascending rate with water temperature 25±1, 28±1 and 32±1°C were 50, 75 and 91.7 respectively. In fact, water temperature is a significant controlling factor of reproduction in *O. niloticus*, this may be due to the acceleratory effect of water temperature on the process of gametogenesis in *O. mossambicus* (Chmilevskiy, 1995).

Water temperature not only affects spawning frequency but also affects different reproductive performance in *O. niloticus* where an inverse relationship between water temperature and incubation period and inter-spawning interval were recorded. The differences between fish raised in water temperature 25±1, 28±1 and 32±1°c were statistically significant at ($P \leq 0.01$) for incubation period, rearing period and inter-spawning interval (Table 3). The significant differences in the incubation and rearing periods may be due to the fact that low rearing water temperature retards while high rearing water temperature accelerates the developmental rate of fish eggs and fry (Rana, 1990).

Regarding the inter-spawning interval, it has been found that water temperature affects gonadal development of fish directly and indirectly (Chmilevskiy, 1995). In the first case, the rate of metabolic processes and the rate of synthesis of individual compounds were accelerated with higher rearing temperature. The indirect effect is carried out through the influence on other organs and systems of the body. Stroganov (1962) mentioned that under the effect of reduced temperature, digestive enzyme function declines and the time taken for passage of food through the gastro-intestinal tract increases. Kazanskiy (1976) found that blood flow slows and the process of hormone secretion is disrupted under the effect of reduced water temperature so that nutrients are not effectively delivered to the developing gonads and their hormonal regulation is impaired. Finally as the incubation and rearing periods were shorter (7.43 and 2.5 days) at higher rearing temperature (32°C), the rematuration and recovery of the female ovary reared at 32°C group were faster than those reared at 25°C and 28°C, So the inter-spawning interval was shorter in group E (34.25 days) than in groups D and C (40.98 and 42.44 days) respectively. These results agreed with those obtained by Chmilevskiy and Lavarova (1990) who found that reduced water temperature led to slowing the development of the sex glands in mouth brooders Tilapia.

Table 3: Effect of temperature treatments on reproductive performance of female Nile tilapia (*Oreochromis niloticus*) (Mean ± SE).

Courtship Activity * / Temp. Treatments °C	Spawning %	Incubation period (Days)	Rearing period (Days)	Inter-spawning interval (Days)
16 ± 1	0	0	0	0
19 ± 1	0	0	0	0
25 ± 1	50	14.23±0.6^{a}	4.33±0.5^{a}	42.44±1.32^{a}
28 ± 1	75	10.5±0.14^{b}	3.17±0.4^{b}	40.98±1.35^{b}
32 ± 1	91.7	9.43±53^{bc}	2.5±0.5^{c}	34.25±1.55^{c}

*Values having different letters within the same columns are significantly different at p ≤0.01

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