Diskussionspapiere

Discussion Papers

August 2009 Nr. 09/08

Effects of stocking density and photoperiod manipulation in relation to estradiol profile to enhance spawning activity in female Nile tilapia

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ISSN 1865-2697

Abstract

This study investigated the effects of stocking density and photoperiod manipulation in relation to plasma estradiol-17β (E_2) profile to enhance spawning activity in female Nile tilapia (*Oreochromis niloticus)* using F1 clonal crosses. The fish were divided into experimental and control groups and subjected to a combination of stocking density and photoperiod treatments (40kg/m^3;6L:18D, 40kg/m^3;12L:12D and transferred into single compartments at 12L:12D; 14kg/m^3;12L:12D), respectively. Blood samples were taken by caudal puncture from experimental fish for estradiol profile analysis. Results showed that experimental fish exhibited significantly higher number of spawns per day, total fecundity and relative fecundity (p<0.05). Hatching and swim-up rates were also higher in the experimental than in the control group. Regression analysis revealed a significant positive relationship between fish size (body weight), total and relative fecundity (p<0.001). However, the relationship between fecundity and inter-spawning interval (ISI) and between ISI and fish weight were weak and insignificant. It was also revealed that E_2 levels demonstrated a pattern based on completed reproductive cycle. The study therefore established that a combination of stocking density and photoperiod treatments can be adopted to manipulate the timing of spawning activity in female Nile tilapia without having adverse effect on other reproductive parameters such as egg qualities and fecundity. Findings further suggested that the effects of exogenous factors on manipulation of spawning activities of female Nile tilapia are achieved as a result of hormonal changes including E_2 levels.

Key words: Stocking density, Photoperiod, Spawning, Estradiol levels, Nile tilapia

1. Introduction

Commercial production of tilapia is increasingly gaining expansion in many countries due to its suitability to variety of pond farming conditions, resistance to diseases, high survival and growth rate (Pullin et al., 1991). The economic and nutritional importance of tilapia species is well outlined in Altun et al. (2006). According to Josupeit (2007), world tilapia production reached 2.5 million tones in 2005. Ongoing research studies such as selective breeding, gynogenesis, androgenesis, hybridization or ploidy manipulation with tilapia is considered a major breakthrough for the development of unique broodstock or progeny with desirable qualities to boost its production optimisation. However, these research activities are constrained by

inadequate supply of eggs at required time due to the asynchronous spawning behaviour of tilapia (Jalabert and Zohar, 1982; Rana, 1988; Bhujel, 2000). Thus, the importance of conditioning spawning of tilapia species for a predetermined time is worth adopting. This innovation therefore calls for the necessity to induce tilapia seed production outside the regular spawning period (Ridha, 1998).

Findings of several studies have demonstrated that a variety of environmental factors such as photoperiod, temperature, rainfall, salinity; biological parameters such as nutritional status and size of fish species; and farm management techniques such as feeding rate and stocking density are significant in the timing of reproductive activities of some seasonal fish (Allison, 1951; Hazard and Eddy, 1951; De Vlaming, 1972; Jalabert and Zohar, 1982; Lam, 1983; Carrilo et al., 1989; Haddy and Pankhurst, 2000). In the tilapia species, Ridha et al. (1998) found a positive effect of photoperiod manipulation on seed production in *Oreochromis spilurus*. Results of Biswas et al. (2005) suggested that photoperiod manipulation can be used to arrest the spawning problems in tilapia (*Oreochromis niloticus)*. Allison et al. (1979) demonstrated an inverse relationship between stocking density and the number of young produced by *Oreochromis aureus*. Balarin and Halter (1982) noted unsatisfactory spawning of tilapia in tanks stocked at above 10 kg/m^3.

Nevertheless, Bromage et al. (2001) emphasized the importance of research to consider interactive effect of these enviro-physical and management techniques on spawning. Although some studies have been undertaken on combination of photoperiod and temperature (Paessun and Allison, 1984; Ridha et al., 1998) as well as photoperiod and light intensity (Ridha and Cruz, 2000), no comprehensive study has been conducted to assess the effect of interaction between photoperiod and stocking density on the reproductive activities of tilapia species. It is also reported that the effects of exogenous factors on manipulation of spawning are achieved by corresponding adjustments in hormonal levels (Van der Kraak et al., 1998; Jalabert, 2005; Biswas et al., 2005). However, very few studies have extended the effects of exogenous factors in relation to endocrine mechanisms such as oestradiol levels which are responsible for the control of reproductive cycle.

Against this background, this study was aimed to investigate a combination of stocking density and photoperiod manipulation on spawning activity and the corresponding estradiol levels in female Nile tilapia (*Oreochromis niloticus)* using F1 clonal crosses. Bromage et al. (2001) stated that environmental effects on spawning are coordinated by genetic make-up of the species. Therefore, use of F1 clones dismisses variation effect due to genetic heterogeneity.

2. Materials and methods

2.1 Recirculation system conditions

The study was carried out in the warm water recirculation system of the Department of Animal Science, University of Göttingen. Water temperature was maintained constant at 28±0.5 ^{o}C. Levels of pH, nitrite, ammonium, and dissolved oxygen were evaluated twice per week to keep the water quality within the following range: pH 6.5–7.5; NO^{-}_{2} <0.2 mg/l; NH^{+}_{4} <0.4 mg/l; Oxygen >7 mg/l. Fish were fed with

commercial pelleted diet (Skretting C-2 Pro Aqua K18, Norway; crude protein 36%) at a daily ration of 2% body weight.

2.2 Experimental fish

Nile tilapia (*Oreochromis niloticus*) F1 clonal crosses of two homozygous lines with Lake Manzala origin were used for the present experiment. Vivid description of the origin of this population, kept at the Department of Animal Science, University of Göttingen, was outlined by Jenneckens et al. (1999) and Mueller-Belecke and Hoerstgen-Schwark (1995, 2000). Fish were divided into control and experimental groups consisting of 36 twelve months old female F1 clonal crosses with initial average body weight of 328±51 g and 336±70 g respectively. All fish were tagged with Passive Integrated Transponder (PIT) tags to assess performance of individual fish over the period of the experiments.

2.3 Stocking density and photoperiod treatments

The control fish were kept in an 800 l glass-fiber tank for 7 days at an initial stocking density of 40 kg/m^3. They were then transferred into 300 l glass aquaria and kept in groups of 12 at stocking density of 14 kg/m^3. Normal photoperiod of 12 hours of light and 12 hours of darkness (12L:12D) was maintained throughout the control experiment. This is the standard method of treating females to induce spawning at the recirculation system.

Experimental fish were kept in an equal 800 l glass-fibre tank at an initial stocking density of 40 kg/m^3 under a photoperiod of 6 hours of light and 18 hours of darkness (6L:18D). After a 21 day period, 36 spawners were stocked individually into single 50 l glass compartments (about 7 kg/m^3 stocking density) and a photoperiod of 12 hours of light and 12 hours of darkness (12L:12D) was restored. The photoperiod manipulation was ensured using water tight fluorescent lamp, placed 50 cm above the holding tank at a light intensity of 2500 lux. The system was covered with a light-opaque polythene sheet to exclude all external source of light. All lights were controlled with electronic timer to achieve the desired photoperiod.

2.4 Spawning activity

At the end of the stocking density and photoperiod treatments, fish were carefully monitored twice daily (from 9 to 10 am and from noon to 1pm) for signs of first spawning after stocking over a 5 day period to determine total number of spawns per each observation day. Evidence of spawning activity of fish included reddish genital papilla, swollen abdomen, aggressive behaviour, change in body colouration of the lower half of each flank and the entire ventral side to red. Fish found ready to spawn were anaesthetized by immersion in a 1/10,000 (v/v) dilution of ethylene glycol monophenyl ether, manually stripped for eggs, weighed and all data recorded. Stripped eggs were washed with 0.9% saline solution and spread over a considerable extent in six 90mm diameter Petri dishes for scanning and counting with a tally counter (Figure 1). This method allowed ample time for egg counting. Random sampling of 100 eggs from each batch of eggs were artificially fertilized with mixed sperm from 3 males and incubated at 28 ^oC water temperature

until swim-up stage of larvae at 9 days post fertilization. Reproductive parameters estimated included: fecundity (number of eggs in a freshly spawned egg batch), relative fecundity (number of eggs per gram body weight of the spawner), hatching rate (number of hatched normal larvae x 100/number of fertilized eggs) and swim-up rate (number of swim-up fry 9 days after fertilization x 100/number of fertilized eggs). Fish that spawned within the five day observation period were monitored for signs of second spawning for a 21 day period to determine inter-spawning interval (ISI). Inter-spawning interval was defined as time elapsed from one spawn to the next based upon completed reproductive cycle of repeated spawning fish.

2.5 Blood sampling

Over a period of 21 days, blood samples were taken daily from two fish in the experimental group between 11 am and 12 noon by caudal puncture starting on day 1 post first spawning. Females that spawned on the third and fourth day post stocking (n = 20, Figure 2a) were considered for the blood sampling analysis. All the 20 experimental fish were blood sampled because it was not possible to take blood everyday from the same fish over the 21 day cycle. Experimental fish were bled at least twice before the end of the experiment. Sampled blood was mixed with sodium cyanide to final dilution of 10% to prevent coagulation and centrifuged at 3000 rmp for 10 minutes. Supernatant plasma collected was stored at -20 ^{o}C until analysis.

2.6 Plasma analysis

Enzyme-linked immunosorbent assay (ELISA) sensitive laboratory technique was used to evaluate plasma levels of estradiol-17β (Meyer and Gueven, 1986). Details of this competitive assay and antiserum characteristics have been outlined by Meyer et al. (1990) and Dhesprasith (1995). Plasma samples were diluted 1:25 fold in assay buffer during extraction with diethylether. The sensitivity of the assay was 1 pg/ml (lowest detectable value). The validity of the assay was assessed by demonstrating that plasma samples dilute parallel to standards and that added hormones were recovered in the correct amount.

2.7 Statistical analysis

Data for parameters were expressed as means±standard deviation (SD). Statistical comparisons of estradiol levels, fecundity, relative fecundity, hatching and swim-up rates of experimental and control groups were made using the *t*-test to determine significant differences. Mean difference between number of spawns per day of treatment and control groups were tested for significance using a 2 x 2 contingency chi-square test. Regression analysis was employed to investigate relationships between the following parameters: fecundity and fish weight, relative fecundity and fish weight, fecundity and ISI and between ISI and fish weight. Data was tested for heteroscedasticity (Breusch-Pagan/Cook-Weisber test) prior to analysis and were $\log_{10}$ transformed if necessary.

3. Results

3.1 Reproductive performance

Results indicated that no significant differences were found between the experimental and control groups in terms of fish weight at the beginning and the end of the experiment ($p<0.05$). Table 1 summarised mean spawns per day, fecundity, relative fecundity, hatching rate and swim-up rate of experimental and control groups based on five day observation period post stocking.

Table 1 highlighted the advantage of the experimental group over the control group in terms of all the reproductive parameters investigated. The mean value of fecundity (eggs per spawn) obtained in the experimental group (1169.7±316.2 eggs per spawn) was significantly higher than in the control group (896.5±320.2 eggs per spawn). Consequently, mean relative fecundity (number of eggs produced per gram body weight) was estimated to be 4.0±1.6 eggs per grams body weight in the experimental group which was significantly higher than in the control group (2.6±1.0 eggs per grams body weight). Although no statistical differences were found, the mean hatching rate (66.4±11.8%) and mean swim-up rate (64.7±11.9%) in the experimental group were slightly higher than in the control group (mean hatching rate: 64.2±15.4%, mean swim-up rate: 62.3±15.2%).

Figure 2a showed that both the control and the experimental groups recorded no spawning on day 1 post stocking. Spawning in both groups started on day 2 through to day 5. Highest number of spawns per day (n = 13) in the experimental group and (n = 4) in the control group were observed on day 3. In the experimental group, 23 females (64%) spawned within the entire five day observation period compared to 10 (27%) in the control group. Mean number of spawns per day (n = 6±5.6) in the experimental group was significantly higher than in the control group (n = 2.5±1.3).

No second spawning activity was observed in the control group within the 21 day observation. However, in the experimental group, 15 out of 23 females (65%) spawned the second time within five days during a 21 day observation period (Figure 2b). Mean inter-spawning interval (ISI) based on completed reproductive cycle was 18.4±1.6 days varying from 16 days to 20 days (Figure 2b). Positive correlations was found between ISI and fish size (weight), whilst a negative correlation was observed between fecundity and ISI, however, regression analysis failed to detect any significant relationships (Table 2). The present study also revealed that fecundity and relative fecundity increased significantly with fish weight in the experimental group (Table 2).

3.2 Plasma estradiol-17β levels

Plasma estradiol-17β (E_2) concentration demonstrated a pattern based on completed reproductive cycle (Figure 3). Estradiol-17β levels were low on day 1 after the first spawning (0.37±0.05 ng/ml). Thereafter, there was a steady increase in E_2 levels until a peak value was reached on day 16 (6.32±0.5 ng/ml). Estradiol-17β concentration afterwards decreased to a basal level on day 17 and remained low until day 20 when it appeared to increase again on day 21. Estradiol-17β concentrations were significantly higher between day 8 and 16 than between day 1 and 3 and day 17 and 19 ($P<0.01$). Reduction of estradiol levels after the peak on day 16 simultaneously coincided with second spawning activities as confirmed by Figure 2b. Both females sampled for blood analysis on day 21 including one female on day 11 did not spawn for

the second time over the observation period. This could explain the large variation in the E_2 concentration on day 11 and 21 as shown by the standard deviation.

4. Discussion and conclusion

Although many studies have separately considered the effect of stocking density (Siddiqui, 1997) or photoperiod manipulation (Biswas et al., 2005) on reproductive activities in tilapia, the present study has demonstrated that the timing of spawning activity in Nile tilapia (*Oreochromis niloticus)* can be modified by a combination of stocking density and photoperiod manipulation. Ridha and Cruz (2000) also found light intensity and photoperiod combination as a powerful tool for synchronous seed production in Nile tilapia. Tropical tilapia are territorial species, exhibit a strong social hierarchy and need daily light cycle and space for their reproductive activities. In this study, it appeared that the initial high stocking density (40 kg/m^3) and change of day length provided by the photoperiod (6L:18D) manipulation in the experimental group offered a schooling conduct (Balarin et al., 1986) which haltered the reproductive activities of the female fish. Transferring the fish into the single compartments with enough holding space and ambient photoperiod might have broken up the social hierarchies to induce the large number of spawns in the experimental group.

Nevertheless, in order to consider stocking density and photoperiod technique as an effective tool in controlling spawning of farmed stocks of Nile tilapia (*Oreochromis niloticus),* there must be little or no associated loss in egg quality (survival of eggs and fry) and the fecundity of the brood stock. Ridha and Cruz (2000) reported poorer quality of eggs (yolk sac-fry, swim-up fry and yolk sac and swim-up fry) from groups treated with combination of light intensity and photoperiod of 500 lux/18 h, 500 lux/15 h and 500 lux/12 h. A reduced seed kg/female/day was observed by Ridha et al. (1998) in the 29 °C/13 h temperature/light duration treatment compared to the ambient condition. Biswas et al. (2005) obtained poorer egg quality and low fecundity in fish exposed to the 6L:6D:6L:6D photoperiod manipulation. However, no loss in egg quality such as survival of eggs and fry was experienced with the stocking density and photoperiod manipulation in the present work. Consistent with Campos-Mendosa et al. (2004), the method in this study helped to improve some important reproductive traits in Nile tilapia. Fish from the experimental group exhibited significantly higher mean total and relative fecundity over the control group.

Fecundity was between 656 and 1778 eggs per fish with a mean of 1169.7±316.2 eggs per fish in the experimental group. These figures are slightly higher than the 306-1158 eggs per fish estimated by Rana (1988) in *Oreochromis niloticus* but lower than the values (2020±80 – 2408±70 eggs per fish) obtained by Campos-Mendosa et al. (2004) also in *Oreochromis niloticus*. However, the mean weight of fish used by Campos-Mendosa et al. (2004) was larger than the fish used in the present study. It is well documented that total fecundity is related to factors such as age and size (weight) of fish (Rana, 1988; Coward and Bromage, 1999). Fish used in the present study were of the same age. However, findings from the study confirmed that fecundity was significantly related to fish weight at a rate of 0.97 compared to 0.65 estimated by Coward and Bromage (1999). Although Coward and Bromage (1999) found a significantly negative relationship between inter-spawning interval (ISI) and fecundity in certain weight classes of *Tilapia zilli* and suggested

that ISI may in part control fecundity, the present study also observed an inverse but insignificant relationship between fecundity and ISI. Relative fecundity in this study was estimated to increase significantly with fish weight contrary to the finding of Coward and Bromage (1999) who observed a significantly inverse relationship between relative fecundity and fish weight in *Tilapia zilli*.

Mean inter-spawning interval in the experimental group was observed to be 18.4±1.6 days (n = 23). This compares with the 18.6±2.3 days found by Ridha and Cruz (2000) in the light intensity (2500 lux) and light duration of 18 hours per day treatments of *Oreochromis niloticus.* The shortest spawning interval (16 days) found in this study is almost identical to the 15 days in Campos-Mendosa et al. (2004) but considerably longer than the shortest cycle of 7 days found by Coward and Bromage (1999). However, mean weight of fish (336±70 g) used in this experiment is considerably larger than the mean weight of females (136.35± 9.8 g) used by Coward and Bromage (1999). Siraj et al. (1983) noted that inter-spawning interval is usually shorter in smaller tilapia. This assertion was confirmed by findings in the present study although the relationship was weak. Tacon et al. (1996) also suggested that ISI variability in aquaria-held O*reochromis niloticus* was probably due to genetic differences between females confirming an observation made by Duponchelle et al. (1997) that genetic make-up plays an important role in determining the reproductive performance of fish. However, this study considered F1 clonal crosses to discard variation effect due to genetic heterogeneity and argued that the longer spawning cycles could be due to the body size (weight) of fish used.

Further investigation in this study demonstrated that plasma estradiol-17β (E_2) concentration played an important role in relation to the effect of stocking density and photoperiod treatments on the spawning activity of female Nile tilapia. In tilapia *Oreochromis mossambicus*, Foo and Lam (1993) observed a delayed reproductive activity due to low level of E_2 after cortisol treatment. Consistent with Biswas (2005), plasma estradiol-17β levels in the present study were low immediately after the first spawning. The low levels in turn retarded the ovarian growth due to reduced vitellogenesis (Foo and Lam, 1993). As the estradiol levels increased, synthesis of vitellogenin by the liver for active exogenous vitellogenesis in the oocyte may have been induced (Nagahama, 1994). Tyler and Sumpter (1996) recognised the contribution of vitellogenin to oocyte development. A decrease in estradiol levels after a pre-ovulatory peak may have promoted the possible stimulation of gonadotropin (Vizziano et al., 1996). A surge in the gonadotropin levels was related to the final stages of oocyte maturation and ovulation by Jalabert (2005). Observation of individual fish based on completed reproductive cycle of second spawning in this study confirmed the coincidence of low estradiol levels at spawning.

In conclusion, the study demonstrated that a combination of stocking density and photoperiod treatments can be adopted to manipulate the timing of spawning activity in Nile tilapia (*Oreochromis niloticus)* without having adverse effect on other reproductive parameters such as survival of eggs or fry and fecundity. This would enable supply of eggs or fry to be made available to tilapia breeders and farmers at required time in a short day planning period. Findings further demonstrated that the effects of exogenous

factors on manipulation of spawning activity in female Nile tilapia are achieved as a result of hormonal changes.

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Table 1: Mean spawns per day, fecundity, relative fecundity, hatching rate and swim-up rate of experimental and control groups in a five day observation period post stocking.

Reproductive Parameters	Total spawns*	Spawns per day	Fecundity (eggs/spawn)	Relative fecundity[#]	Hatching rate (%)	Swim-up rate (%)
Control group	10	2.5 (1.3)	896.5 (320.2)	2.6 (1.0)	64.2 (15.4)	62.3 (15.2)
Experimental group	23	6 [a] (5.6)	1169.7 [b] (316.2)	4.0 [b] (1.6)	66.4 (11.8)	64.7 (11.9)

*Standard deviations (in bracket) are placed below respective mean values, * indicates the total value for the entire 5 day observation period, [#] eggs per gram body weight, [a] values significantly different from control values: χ^2 test ($p<0.05$), [b] mean value significantly different from control mean: t-test ($p<0.05$).*

Table 2: Regression and correlation analysis of fecundity, relative fecundity, fish weight and inter spawning interval (ISI)

Dependent variable	Independent variable	Sample size (n)	Correlation coefficient (r)	Regression equation	Coefficient of determination (r^2)
Fecundity (eggs/spawn)	Fish weight (g)	23	0.88**	$\log y = 0.97 \log x + 1.57$ ($p < 0.001$***)	0.773
Relative fecundity[#]	Fish weight (g)	23	0.71**	$\log y = 0.91 \log x - 3.81$ ($p < 0.001$***)	0.506
Fecundity (eggs/spawn)	ISI (days)	15	-0.08	$\log y = -0.29 \log x + 7.91$ ($p = 0.765$)	0.007
ISI (days)	Fish weight (g)	15	0.09	$y = 0.002 x + 17.82$ ($p = 0.754$)	0.008

, * *significant at levels of $p<0.01$, $p<0.001$, [#] eggs per gram body weight.*

Figure 1: Eggs in six 90mm Petri dishes for counting

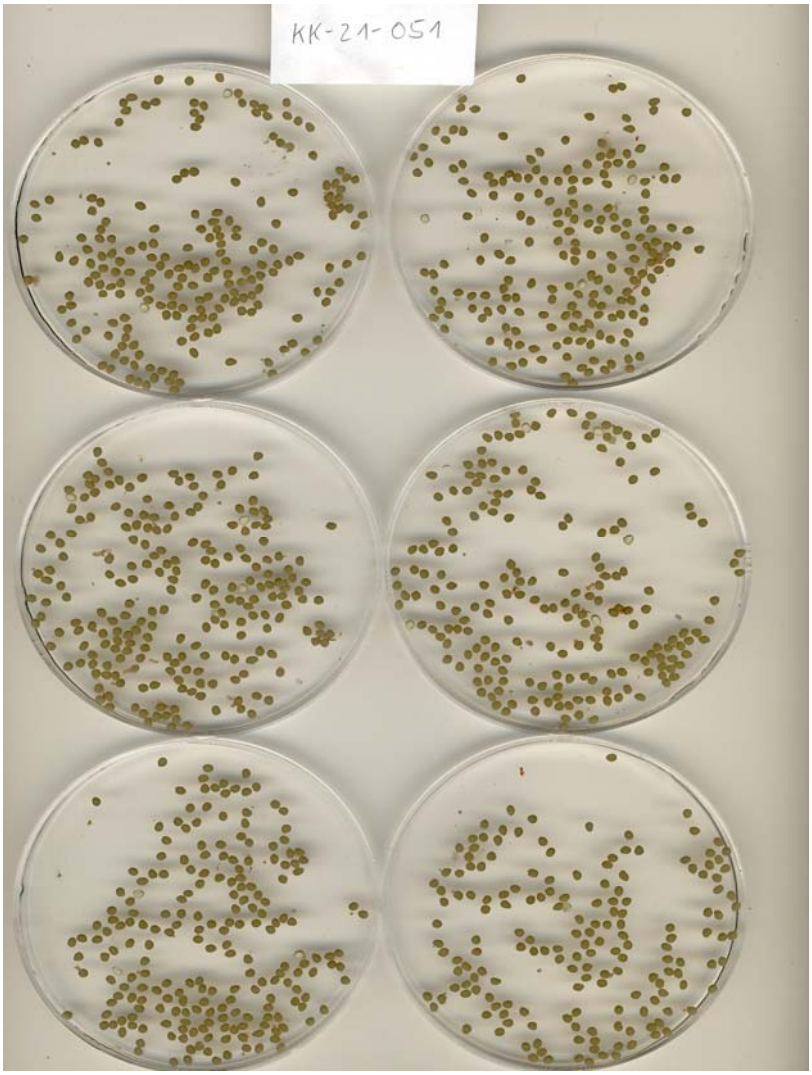


Figure 2: Distribution of first spawning (a) and second spawning (b) of experimental and control groups

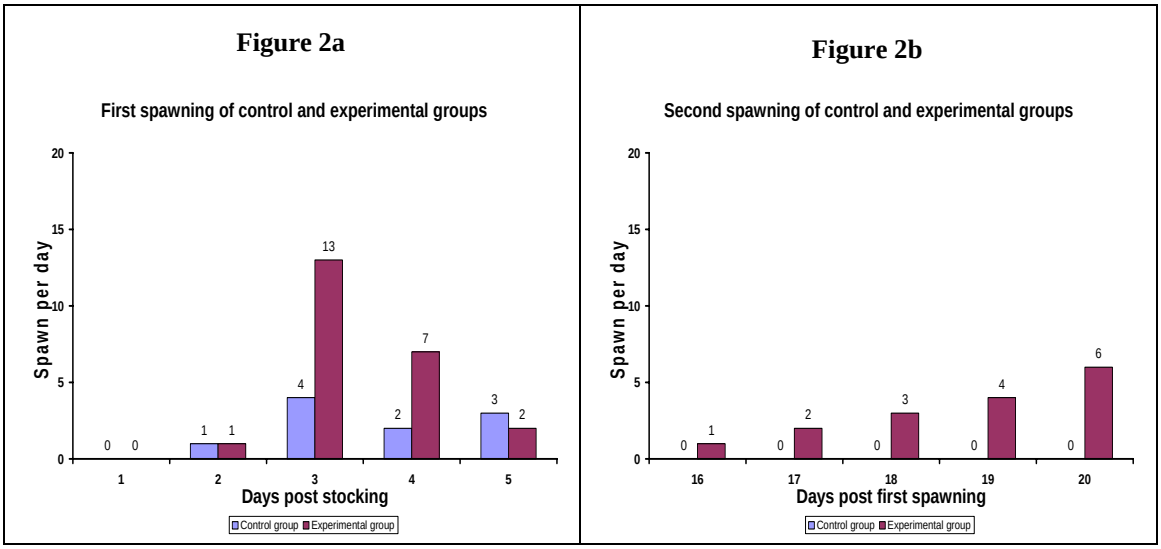
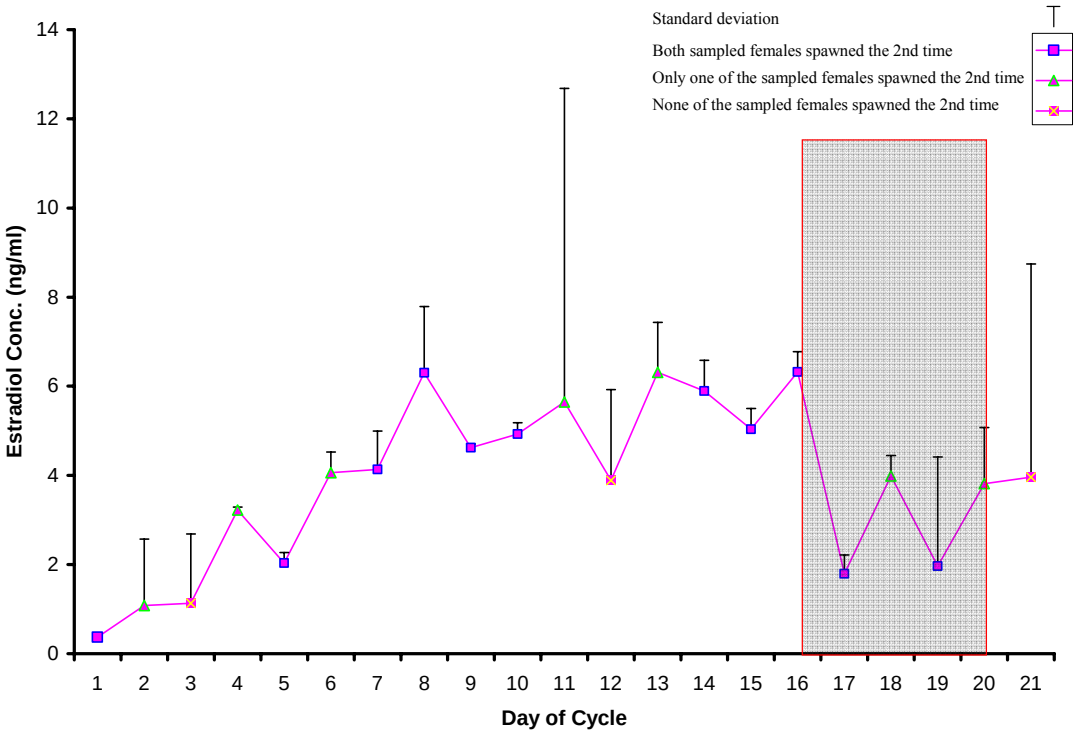


Figure 3: Changes in estradiol-17β concentration based on completed reproductive cycle of repeated spawning female Nile tilapia (*Oreochromis niloticus)*.



Data are presented as mean±standard deviation for the two fish sampled at each day of the cycle. Shaded portion corresponds to days of second spawning activities.

Haben Sie Fragen, wollen Sie an unserem Forschungsprojekt teilnehmen oder möchten Sie einen Kommentar zu diesem Beitrag geben? Wir würden uns über eine Nachricht von Ihnen freuen.

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