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Observations on the Seed Production of the Tilapia *Oreochromis spilurus* (Günther) under Different Spawning Conditions and with Different Sex Ratios

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Abstract

Two experiments were conducted to compare the seed production of the tilapia *Oreochromis spilurus* (Günther) under ambient and controlled (water temperature of 30.0 ± 1.0 °C and photoperiod of 14 h day^{-1}) conditions. Twelve 2.0 x 2.0 x 0.5 m fiberglass tanks were used and six tanks were assigned to each spawning condition.

In Experiment 1, each tank was stocked with 30 females. Males were added to ob-tain male to female sex ratios of 1:3, 1:4 and 1:5. Each sex ratio in each spawning condi-tion was duplicated and the experiment lasted for 14 months. In Experiment 2, twelve breeders were stocked per tank at 1:3 male to female sex ratio with two tanks per sex ratio. The experiment lasted for 10 months. In both experiments, seed collection was car-ried out every two weeks.

Results showed that sex ratios have no significant effect (P>0.05) on the seed produc-tion of *O. spilurus* under both breeding conditions. In both experiments, daily mean seed production under ambient conditions reached its lowest levels during the coldest (Decem-ber-January) and warmest (August) months of the year while, under controlled conditions, it was significantly higher (P<0.05) during the same months. Seed production was more evenly spread throughout the year. No significant difference (P>0.05) was observed in the overall seed composition (eggs, yolk-sac fry and swim-up fry) of females under both breed-ing conditions.

The results of this study indicate the potential of extending the restricted six-month spawning season of *O. spilurus* in Kuwait to a year with controlled water temperature and light duration.

Introduction

Kuwait, an arid land, has very limited supplies of freshwater and brackishwater but has an abundant supply of seawater. Thus, fish production is concentrated on the use of seawater. One of the candidate species for mariculture is tilapia. Screening of several tilapia species was conducted in Kuwait in the early 1980s and *Oreochromis spilurus* (Günther) was found to perform very well under high saline water conditions in Kuwait (Hopkins et al. 1989).

In general, tilapias can breed continuously throughout the year as long as the environmental conditions are suitable for spawning (Lowe-McConnell 1958). However, in places characterized by marked fluctuation in temperature between summer and winter months, the continuous spawning behavior of tilapia becomes interrupted by cold temperatures in winter and high temperatures in summer (Fishelson 1966).

The *O. spilurus*, a native to Kenya, is one of the tropical tilapia species that can breed throughout the year. Under ambient conditions in Kuwait, it has exhibited a well-defined breeding season that is limited to the warm months of the year (April-September), with little and occasional spawning outside this period (Al-Ahmad et al. 1988).

The restricted spawning season of *O. spilurus* limits fry production in Kuwait to only six months a year. For year-round production of tilapia in tanks and raceways using warm underground water, there is a need for continuous supply of fry throughout the year. It is, therefore, necessary to induce tilapia to spawn outside the restricted spawning period. Preliminary results showed the possibility of extending the spawning season of *O. spilurus* in Kuwait by controlling water temperature alone (Ridha et al. 1985). Hence, one of the objectives of this study is to determine the effect of controlled water temperature and light duration on the seed production and seasonal breeding of *O. spilurus* in Kuwait.

Broodstock sex ratio is considered to be one of the biological factors affecting seed production in tilapia. Most of the studies on the effect of sex ratios were carried out mainly on *O. niloticus* and *O. aureus*. Sex ratios of 1:1 to 1:10 (male:female) have been tested and recommended (Silvera 1978; Behrends 1983; Hughes and Behrends 1983; Bautista et al. 1988; M'hango and Brummett, in press). However, no information is available on the possible effect of sex ratio on the seed production of *O. spilurus*. Therefore, another objective of this study is to determine the effect of sex ratio on the reproductive performance of *O. spilurus*.

Materials and Methods

Two experiments were conducted consecutively using offspring of *Oreochromis spilurus* (Günther) originally from Kenya. Twelve 2.0 x 2.0 x 0.5 m (L x W x H) fiberglass tanks were used in each experiment and six tanks were assigned to each treatment. In Treatment 1 (ambient conditions), the tanks were placed inside a well ventilated green house covered only during summer with black polyethylene nets. The breeders were subjected to ambient water temperature and light duration throughout the experiment. Aerated underground brackishwater (2-5 ppt) stored in a 60 m^3 concrete reservoir and exposed to ambient conditions flowed through each tank at a rate of 0.5 l min^{-1}. In Treatment 2 (controlled conditions), the tanks were located inside rooms where light duration was controlled at 14 h day^{-1} (from 0600 h to 2000 h) using timer-controlled fluorescent tubes. Water temperature was maintained at 30.0 ± 1.0 °C throughout the experiment by recycling the effluent from the breeding tanks through a 2.0 m^3 header tank where immersion heaters connected to a temperature controller adjusted at 30 °C were installed. Room temperature was maintained at 28 ± 1.0 °C using heating/cooling air-conditioning units. Fresh brackishwater was added continuously to the header tank at a rate of 0.5 l min^{-1}. Following seed collection, the spawning tanks were refilled with preheated water.

Experiment 1. Two tanks were assigned to each of the following male to female ratios of 1:3, 1:4 and 1:5 per treatment. Each tank was stocked with 30 females having a mean weight of 85.9 g (57.1 - 111.8 g). Males with mean weight of 160.5 g (135.0 - 166.4 g) were stocked to achieve the desired male to female ratio in each tank. The experiment was conducted for 14 months.

Experiment 2. All tanks were stocked with 12 fish with a 1:3 male to female ratio for both treatments. The males had an average weight of 134.5 g (113.1 - 164.9 g) and the females had an average weight of 87.3 g (72.7 - 105.3 g). The experiment lasted for 10 months.

In both experiments, fish were fed with Aqualim sea bream pellets (45% crude protein) three times per day at a rate of 2% of the body weight. Initially, as seed collection can not be done in one day, it had to be staggered (starting 14 to 21 days after stocking), with each treatment represented in each seed collection. Thereafter, seed collection was carried out every two weeks. At seed collection, breeders were anaesthetized by adding 20 ppm of quinaldine. The seeds (eggs, yolk-sac fry and swim-up fry) were then removed from the buccal cavity of the brooding females (Ridha and Cruz 1989) and were counted. After each seed collection, the total weight and number of females and males in each tank were recorded.

Water temperature was recorded daily at 0900 and 1500 hr and the average of the two temperatures was recorded as the daily water temperature. Dissolved oxygen (DO) concentration was determined weekly using a YSI DO meter. Total ammonia and pH were measured biweekly.

Seed production was quantified as number of seeds kg^{-1} female day^{-1} and was analyzed for the entire duration as well as on a monthly basis. In Experiment 2, seed composition was determined as the percentage of unhatched eggs, yolk-sac fry and swim-up fry. The data on seed production for both experiments were subjected to a two-way analysis of variance and Duncan's New Multiple Range Test using the 5 % level of significance (Sokal and Rohlf 1981). When only two treatment means for each month and for the whole spawning period (ambient vs controlled) are compared, a one-way analysis of variance was used. Seed production in Experiment 1 at a sex ratio of 1:3 was compared to those in Experiment 2 using one-way analysis of variance.

Results

The DO and total ammonia concentrations were within optimum levels. DO concentration was always above 5 mg l^{-1} while total ammonia concentration ranged from 0.5 - 2.0 mg l^{-1}. The pH ranged from 6.5 - 7.5.

In both experiments, two-way analysis of variance revealed significant differences ($P<0.05$) in the mean monthly seed production of *O. spilurus* under both ambient and controlled spawning conditions (Tables 1 and 2). Under ambient conditions, the peak of seed production occurred one to two months after stocking. Peak production was in November (126 seed kg^{-1} female day^{-1}) in Experiment 1 while it was in April and May (112.2 and 112.5 seed kg^{-1} female day^{-1}, respectively) in Experiment 2. Under controlled conditions, the peak production in Experiment 1 was likewise at the start of the experiment in November (51.8 seed kg^{-1} female day^{-1}). This was followed by a gradual decline in

Table 1. Monthly water temperature and mean seed production (kg^{-1} female day^{-1}) of Oreochromis spilurus stocked at three sex ratios under ambient and controlled conditions (Experiment 1).

Treatment		Ambient Conditions				Controlled Conditions			
Sex Ratio		1: 3	1:4	1: 5	Mean+S.E.*	1:3	1:4	1:5	Mean+S.E. *
	Temp[B]								
October	26.0	89.0	30.0	55.5	58.2 ± 20.1^{bc}	16.0	15.5	23.5	18.3 ± 5.6^{cde}
November	23.0	151.0	87.5	139.5	126.0 ± 21.3^{a}	57.5	44.0	4.0	51.8 ± 7.5^{a}
December	21.0	42.5	42.5	47.5	44.2 ± 8.6^{cd}	48.5	52.0	32.5	44.3 ± 5.9^{ab}
January	19.0	5.0	0	7.0	4.0 ± 2.6^{e}	44.5	42.5	46.5	44.5 ± 4.9^{ab}
February	22.0	18.0	27.5	32.0	25.8 ± 9.8^{cde}	22.0	24.5	30.0	25.5 ± 4.2^{cde}
March	24.0	16.5	34.5	17.0	22.7 ± 7.4^{cde}	18.0	32.5	6.5	19.0 ± 5.8^{cde}
April	28.0	80.0	109.5	80.5	90.0 ± 20.1^{b}	39.5	31.5	35.0	35.3 ± 7.6^{abc}
May	30.0	42.5	62.0	74.5	59.7 ± 12.5^{bc}	27.0	26.5	21.5	25.0 ± 6.3^{cde}
June	31.0	13.5	9.5	25.0	15.9 ± 4.5^{de}	15.5	8.0	7.0	10.2 ± 2.2^{e}
July	32.0	14.0	12.5	14.5	13.7 ± 4.1^{de}	8.5	13.5	9.5	10.5 ± 2.2^{e}
August	34.0	17.5	0	0	5.8 ± 5.8^{de}	38.5	33.5	16.5	29.5 ± 5.4^{cd}
September	32.0	9.5	12.5	4.5	8.8 ± 2.4^{de}	29.5	6.5	11.0	15.7 ± 6.6^{de}
October	26.0	6.5	5.0	44.5	18.7 ± 5.9^{de}	11.0	5.5	9.0	8.5 ± 3.2^{e}
November	23.0	9.0	23.0	31.0	21.0 ± 6.0^{de}	4.5	13.0	6.5	8.0 ± 3.7^{e}
	Mean	36.7	32.4	40.9	36.7 ± 4.7	27.3	24.9	22.1	24.7 ± 2.0

[A]Means of duplicate groups.
[B]Ambient water temperature in °C.
*Means with different superscripts within a column differ significantly

Table 2. Mean weights of females and monthly mean[A] seed production (kg^{-1} female day^{-1}) of *O. spilurus* under ambient and controlled conditions (Experiment 2).

*	Temperature[B]	Ambient Conditions	Controlled Conditions
Stocking weight/female, g		87.90	89.30
Harvest weight/female, g		211.00	248.10
Growth rate, g/d		0.41	0.53
Survival rate, %		100.00	100.00
Monthly seed:			
April	28.0	112.16^{a}	108.50^{b}
May	30.0	112.50^{a}	93.33^{c}
June	31.0	85.50^{b}	67.08^{e}
July	32.0	34.91^{e}	101.16^{bc}
August	33.0	28.33^{e}	122.13^{a}
September	31.0	74.30^{c}	70.83^{d}
October	29.0	85.66^{b}	74.08^{d}
November	24.0	44.66^{d}	65.33^{e}
December	21.0	12.08^{f}	48.00^{f}
January	20.0	47.00^{d}	27.83^{g}
	Mean	59.75	75.45

[A]Means of six replicates.
[B]Ambient water temperature in °C.
*Means with different superscript within a column differ significantly ($P<0.05$).

seed production as spawning progressed with occasional seed recovery but at a lower rate. A similar trend was observed in Experiment 2 where the high production of 108.5 seed kg^{-1} female day^{-1} at the start of the experiment in April was followed by a gradual decline in seed production. Seed production peaked again in July and August (101.2 and 122.1 seed kg^{-1} female day^{-1}, respectively).

In both experiments, the lowest seed production rates under ambient conditions were observed during the coldest and warmest months of the year. In Experiment 1, the lowest seed production was obtained in January and August (4.0 and 5.8 seed kg^{-1} female day^{-1}, respectively). Under controlled conditions, the seed production rates of 44.5 and 29.5 seed kg^{-1} female day^{-1} for January and August, respectively, were significantly higher ($P<0.05$) than those under the other treatment (ambient conditions). A similar trend in seed production was observed in Experiment 2 but the lowest production was in December and August with 12.1 and 28.3 seed kg^{-1} female day^{-1}, respectively. Under controlled conditions, seed production rates for December and August were 48.0 and 122.1 seed kg^{-1} female day^{-1}, respectively, and were significantly higher ($P<0.05$) compared to those under ambient conditions.

For the entire period in Experiment 1, the mean daily seed production from females under ambient conditions (36.7 seed kg^{-1} female day^{-1}) was significantly higher ($P<0.05$) than that from females under controlled conditions (24.7 seed kg^{-1} female day^{-1}). On the other hand, for the entire period in Experiment 2, the mean daily seed production of 75.5 seed kg^{-1} female day^{-1} obtained under controlled conditions was significantly higher than the production of 59.8 seed kg^{-1} female day^{-1} obtained under ambient conditions.

No significant differences (P>0.05) were found among the daily mean seed production rates of females stocked at different sex ratios in either spawning condition in Experiment 1 (Table 1). Moreover, the combined seed production rates under both ambient and controlled conditions were not significantly different among the three sex ratios. However, the seed production with the 1:5 sex ratio under ambient conditions (40.9 seed kg^{-1} female day^{-1}) was significantly higher (P<0.05) than that under controlled conditions (22.1 seed kg^{-1} female day^{-1}). The first ten months seed production data at sex ratio of 1:3 in Experiment 1 were not significantly different (P>0.05) from those in Experiment 2 under either spawning conditions.

The data for *O. spilurus* seed composition (eggs, yolk-sac fry and swim-up fry) in Experiment 2 are presented in Table 3. No significant differences (P>0.05) were observed in the composition of seeds collected under both ambient and controlled conditions. Eggs in both spawning conditions constituted about 45% of the total seed. Variations in the monthly seed composition under ambient conditions were observed. Significantly higher mean percentages of eggs were produced in August with 87.3% and January with 100%. Significantly higher (P<0.05) mean percentages of yolk-sac fry and swim-up fry (combined) were obtained in April (86.8%), May (72.4%) and June (76.0%). The lowest percentage was obtained in August with 12.7% while no eggs were produced in January. Under controlled conditions, no significant differences in seed composition between months were observed (P>0.05). However, the percentages of yolk-sac fry and swim-up fry in August and January (56.0 and 29.6%, respectively) were significantly (P<0.05) higher than those obtained in the same months under ambient conditions.

Discussion

High seed production within the first two months after stocking of *O. spilurus* spawners under ambient conditions was observed in both experiments. This trend in spawning activity is in agreement with that observed by Guerrero and Guerrero (1985) and Bautista et al. (1988) for Nile tilapia (*O. niloticus*) and M'hango and Brummett (In press) for Shire tilapia (*O. shiranus*) where early spawning peaks were obtained after stocking, followed by a decline in seed production. Based on this observation, it is recommended that stocking of breeders be done at regular intervals throughout the year to optimize year-round seed production.

The very low seed production of females under ambient spawning conditions in January in Experiment 1 and December in Experiment 2 was due to the low water temperature (< 21.0 °C) during these months. Rothbard and Pruginin (1975) stated that tilapia, a warmwater species, can start spawning at 22 °C, with the optimum range at 25 - 29 °C. This demonstrates the adverse effect of low temperature on spawning activities in *O. spilurus*. Similarly, the high water temperatures in July, August and September also depressed reproduction and resulted in low seed production rates. A similar observation was reported by Al-Ahmad et al. (1988) for *O. spilurus* breeders in the first and second years of spawning when seed production decreased during August and September. Brummett (1995) reviewed the environmental factors which regulate maturation and reproduction in tilapia and stated that photoperiod, temperature and population density are predictive cues which affect the onset of sexual maturation and reproduction.

Table 3. Mean[A] seed percentages of eggs and yolk-sac + fry of *Oreochromis spilurus* under ambient and controlled conditions (Experiment 2). No significant differences between treatments ($P>0.05$) were observed.

Month	Ambient Conditions		Controlled Conditions	
	Eggs	Yolk-sac+Fry	Eggs	Yolk-sac+Fry
April	13.2	86.8*	70.4	29.6
May	27.6	72.4*	30.4	69.6*
June	24.0	76.0*	44.0	56.0
July	53.2	46.8	27.0	73.0*
August	87.3	12.7	44.0	56.0
September	55.2	44.8	43.8	56.2
October	43.0	57.0	36.1	63.9*
November	52.3	47.7	60.9	39.1*
December	32.1	67.9*	48.5	51.5
January	100.0	0*	70.5	29.5*
Mean	48.8	51.2	47.6	52.4

[A]Means of six replicates.

Note: For each spawning condition, monthly percentages of yolk-sac + fry with asterisks are significantly different from those for eggs ($P<0.05$).

Under ambient conditions, observed increases in seed production with increasing water temperature from February to May and decreasing temperature in September and October indicate the important role of water temperature in optimizing seed production in tilapia. Bautista et al. (1988) reported a similar effect of water temperature on the spawning of *O. niloticus* and observed an increase in its spawning frequency with an increase in water temperature. Furthermore, the results show that seed production throughout the year can be achieved by maintaining water temperature at the optimum range of 28 - 31 °C. However, since seed production under controlled conditions was significantly higher only during the hot summer months (July to September) and cold winter months (December to February), the advantage of controlling water temperature becomes more evident only at times where water temperatures are below and above the optimum ranges for spawning.

Seed production of female *O. spilurus* under controlled conditions in both experiments dropped to its lowest level after eight months despite the fact that water temperature remained at the optimum range for spawning. A similar observation was reported by Mires (1982) in females of *O. niloticus* maintained year-round at 25 - 28 °C. Such a decrease is probably caused by exhaustion of the breeding females. However, further research is required to test this hypothesis.

It appears that any improvement or maintenance of environmental conditions required for optimum seed production can only be effective for a certain period of continuous spawning. Lovshin and Ibrahim (1988) observed that male and female broodstock replacement resulted in a 16% increase in seed production when compared with female replacement only and no broodstock exchange. However, Little et al. (1993) observed that exchange of all females results in more than 50% productivity than exchange of spawned females only. Hence, it is recommended that broodstock be replaced after such period by a fresh batch.

For the entire period of the study, the low daily mean seed production rates of females under controlled conditions obtained in Experiment 1 compared with those in Experiment 2 may be due to the higher stocking density used in Experiment 1 (9-10 breeders m^{-2}). The higher density and number could have resulted in greater competition for spawning space and caused seed loss. Silvera (1978) found that when broodstock density reached 8 fish m^{-2} at a total weight of 526 g m^{-2}, seed production was reduced significantly. Behrends and Lee (1984) determined the minimum area needed for sexual activity to be 0.5-1.0 m^2. Broussard et al. (1983) and M'hango and Brummett (In press) explained reduced fry production under increasing broodstock density at a fixed sex ratio (1:3) in terms of the competition for both space and food among broodstock.

While seed production with ambient conditions was higher than with controlled conditions in Experiment 1, it was lower in Experiment 2. The exceptionally high seed production obtained in November (126 seed kg^{-1} female day^{-1}) with ambient conditions in Experiment 1 contributed to the contrasting results.

Seed production in both spawning conditions was higher in Experiment 2 than in Experiment 1. The difference in the starting month of the two experiments might have something to do with the differences in the results obtained. In Experiment 1, the broodstock were stocked towards the end of the spawning season in October while, in Experiment 2, broodstock were stocked at the beginning of the spawning season in April. The broodstock in Experiment 1 were older and had gone through the peak of spawning while those in Experiment 2 were younger and were just starting to spawn. Ridha and Cruz (1989) reported that fecundity of older spawners are significantly lower than younger spawners.

The effect of sex ratio on the seed production of *O. spilurus* was observed only in females with ambient conditions at the 1:5 sex ratio. With controlled spawning conditions, sex ratios appeared to have no effect on seed production in this study. Silvera (1978) showed the 1:6 sex ratio to be optimum for mass fry production in *O. niloticus* and concluded that the number of females is the determinant factor as they can continue to be sexually active over a long period. On the other hand, several investigators reported no significant differences in seed production of tilapia with various sex ratios (1:1 to 1:10) tested (Behrends 1983; Hughes and Behrends 1983; Guerrero and Garcia 1983; Bautista et al. 1988; M'hango and Brummett, In press). In tilapia, it appears that broodstock sex ratio in general might have no direct effect on seed production of tilapia and the ratios to be used depend on the readiness of the female and the space required for spawning. Tave (1993) cautioned the potential of inbreeding depression in populations stocked at higher stocking ratios (>1:1).

Normally, eggs constitute the majority of collected seeds (Chang et. al. 1988; Ridha and Cruz 1989). In this study, significantly more eggs were collected only in August and January when water temperature was above the optimum range. As eggs, especially those less than 48 hours post-fertilization, are more difficult to incubate artificially (Lee 1979) than yolk-sac fry and swim-up fry, collection of seeds with low percentage of eggs is preferred and the effort spent in hatching them is reduced. On the other hand, significantly higher percentages of yolk-sac and swim-up fry were obtained in April, May and June, when water temperature remained within the optimum range for spawning (28-31°C). These results clearly indicate that maintaining water temperature at about the optimum range has a positive effect not only on seed production but also on the composition and development of the collected seed as well.

The findings of this study indicate the potential of producing *O. spilurus* seeds throughout the year by controlling water temperature at 30°C and light duration at 14 h day^{-1}. Year-round seed production will efficiently utilize the resources of tilapia hatcheries and supply the required seeds for a more profitable year-round production of tilapia.

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