STUDIES ON AQUARIUM SPAWNING OF HORMONAL TREATED AND UNTREATED OREOCHROMIS AUREUS WITH REFERRING TO MICROSPORIDIAN INFECTION

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ABSTRACT

Genotypic male Oreochromis aureus previously treated for sex reversal to female state to prevent serial reproduction and prevent production of different sizes so to produce a uniform size in the same season and their sex reversed female (F_1) parent were stocked into aquaria with untreated females at various ratios. The reproduction of the two types of females with normal males was compared. Non of treated fish spawned; most were found to have ovetestes. The F_1 sex reversed female spawned repeatedly after she was paired with normal male in a large tank indicating that the presence of normal females under confined aquarium conditions may have a repressive effect upon estrogen sex reversed females. Duration of treatment in relation to the period of gonadal differentiation for the particular fish species The most effective treatment for estrogen sex-reversing male Oreochromis aureus appears to be oral administration of combination of 17 α methyl testosterone the source of hormone is Argenta Company.

Selecting broodstock from a hormone-treated population can result in pairing individuals of same gonadal sex, since the dimorphic urogenic papilion alone may be altered by exygenous hormones, also the number of parings of estrogen-treated fish that have resulted in spawns has been low in comparison to spawns from untreated fish under the same conditions. However spawning behaviour of functionally sex-reversed fish has not been examined. The primary objective of this study was to compare interaction behaviour and spawning success of normal and estrogen treated females stocked together and separately with normal male.

Also this study was designed to evaluate the Microsporidian infection and their effects among the genital organs of Tilapia aurea.

INTRODUCTION

Tilapias are widely cultured but excessive reproduction in ponds is a problem in culturing them for human consumption **(Bardack et al. (1972)**. Various methods of mono-sex have been used to control reproduction. The use of hormones to reverse the phenotypic sex is one mean of producing single sex population **(Guerrero, 1975)**.

Potential use of functionally sex-reversed fish as broodstock, to be spawned with normal (untreated) fish of the opposite phenotypic sex, was discussed by **(Shelton et al., 1978)**. Successful alteration of secondary sex characteristics and the gonads of an individual is dependent on the efficiency and dosage of the treatment in relation to the period of gonadal differentiation for the particular fish species **(Yamamato, 1969)**.

The most effective treatment for estrogen sex-reserving male Tilapia aurea appears to be oral administration of a combination of 17 α methynylestradiol and methalilbure **(Hopkins., 1979)**. Methalilbure is a chemical hypophyectomizing agent that has enhanced the effect of estrogen treatment on the species.

Several problems exist in the use of estrogen treated O. aureus as broodstock. Selection of broodstock from a hormone-treated population can result in pairing individual of the same gonadal sex. Since the dimorphic urogenital papillae alone may be altered by the exogenous hormones **(Hopkins, 1977)**. Also, the number of springs of estrogen-treated fish that have resulted in spawns has been low in comparison to spawns from untreated fish under the same conditions **(Liu, 1977)**. However, spawning behaviour of functionally sex-reversed fish has not been examined.

Parasites play an important role in determine the health status of fish, they may act as a primary source of diseases or predisposing factors or even secondary invadors for other infections **(Amin et al., 1985)** or may have a zoonotic importance **(Revenga, 1993)**. The parasitic infestation among fishes had been studied by several means; prevalence, distribution, species of parasites among some fishes and their pathological alterations **(Negm El-Din, 1987 and El-Gawady et al., 1992)**. The ovary was mottled with white spots and streaks; parasitic castration due to Microsporidian infection was very common especially at spawning season (May-June), **Noga (1996)**. The primary objective of this study was to compare interaction behaviour and spawning success of noraml and estrogen-treated females stocked together and separately with a normal male.

MATERIALS AND METHODS

1. The treatment hormone using 35 mg/L 17 α methyl testesterone and untreated female O. aureus were observed in aquaria when paired with untreated males. Each aquarium was 60 x 60 x 35cm and held about 100 L of pond water and stocked with 10 fish. The fish were collected from Idco Lack (Behera Governorate) We used 9 aquarium each aquarium contain 10 fish, the total number of fish is 90 in the whole experiment. Only the front panel was glass so that fish were not affected by activity in adjacent aquaria. To reduce losses caused by aggressive wielded wire with 5 x 7 cm opening was positioned inside about 15 cm from the front of the aquaria extending above the water line from side to side. The wire provided a place of partial refuge for females and visually delineated a territory for the male. Fish were fed daily adlibitum with pellets containing 40% protein. Water flow into each aquarium was about 2L/min. Aquaria were stocked in mid June when daily water temperature averaged 22°C. Neither the photoperiod nor the water temperature were controlled.

Experimental work :

One normal (untreated) male and three phenotypic females of approximately equal length (16-18 cm) were each marked with dorsal spine marking and placed in each aquarium either 0, 1, 2 or 3 by putting a mark on the dorsal spine, hormone-treated fish (genotypic males) presumably females were stocked with 3, 2, 1 or 0 normal untreated (genotypic) females, respectively. Each treatment was replicated three times. Each was examined upon introduction into the aquaria, untreated females had normal appearing papillae with an obvious transverse genital slit. Papillae were normal appearing in 46% of the estrogen-treated fish (genotypic males), slightly a typical in 24% and moderately to highly apparent in 30%. The number of fish in the experiment of treated females with moderately to highly apparent papillae were used in this experiment.

Two females (F_1) had been estrogen treated and previously identified by progeny testing to be functionally sex reversed (genotypic males); both had produced 100% male offspring when spawned with normal males. The other two females were stocked in aquarium 6 (Table 1) with two normal females and one normal male. The remaining treated fish used were offspring (F_2) of this surviving progeny tested female and had been estrogen treated for reversal of their potential male phenotype.

Surgical removal of the premaxillae **(Lee, 1979)** from each normal male reduced agonistically induced mortality of the females **(Rothbard, 1979)**. Females that died during the study were replaced with females from the appropriate progeny group. Two days after spawning occurred, eggs were removed from the mouth of incubating females and artifially incubated on a shaker ta-

ble following the recommendations of **Lee (1979)**.

Parasitological examination:

Fishes were collected alife and transported immediately to the laboratory for parasitological examinations. The genital organs of collected fish were carefully examined grossly (with naked eyes and by using hand lens), followed by microscopical examination of smears (mucus, blood, tissues and any suspected cysts). Pieces of tissues were compressed and examined. The detected parasites were collected and processed according to **Wessner (1968) and Lucky (1977)**. Identification of parasites were done according to **Woo (1995)**.

RESULTS AND DISCUSSION

Since none of the presumably sex-reversed F_2 females had previously spawned, the experiment was a progeny test for these fish. Thus, the treated F_2 group could contain some individuals incompletely affected by the estrogen treatment i.e., have a female-like papilla but retain testes or perhaps ovetestes.

Little prespawning aggessive behaviour was observed in the first 2 weeks of experiment when the water temperature was about 22°c±3

Spawning coloration and aggression intensified during the 3rd week when the water temperature increased to about 25°C. The typical spawning coloration of a O. aureus male includes a bright red margin on the fringes of the dorsal and caudal fins, a bluish coloration frequently showed characteristically male-like behaviour and spawning coloration on the operculi in the baseness of another males which interfered with establishment of a more ordered female hierarchy. On the operculi, but (in the absence of other males) no apparent vertical barring of the body after two more weeks the water temperature rose to 27 -28°C the male in each aquarium soon becomes assertive and aggressively chased the females throughout the aquarium. Later only the central area behind the partial barrier was actively defended by the male. However the male after chased a female that was in front of the barrier and occasionally allowed females to wonder behind the barrier.

Female-female interactions usually resulted in a recognizable hierarchy within 48 hours after stocking. The confrontation between two normal females usually involved frontal displays with operculi slightly flared, extension of the dorsal, pelvic, and pectoral fins, and prolonged and repeated open-mouth contact; body coloration was subdued. The dominant female usually occupied the lower level of the aquarium in front of the wire barrier, she pursued the other females

only for short distances. Subdominant females tended to occupy positions near the water surface, assuming a posture with the body angled upward. The dominance order was reinforced by periodic charges that usually include a feigned nip on the anterior portion of the body of a subdominant female. Occasionally, the aggressive female was least dominant of the three and was charged or nipped by other two females. The second female in the hierarchy was charged only by the dominant female. Subdominant females displayed a variety of color patterns that usually include vertical barring on the sides, the dominant females was generally lighter in color.

A complication in these interactions was caused by some of the estrogen-treated fish that were apparently incompletely sex reversed. Although they were considered females on the basis of the appearance of the papillae, their behaviour after stocking was often a more accurate indicator of their primary sex. Incompletely sex-reversed fish were rarely aggressive enough to displace the male, but frequently showed characteristically male like behaviour coloration which interfered with the establishment of more ordered.

Other incompletely sex reversed fish did not show male behaviour or coloration, and did not participate in the ordered female hierarchy previously described, most treated fish participated in the hierarchy establishment and eventually assumed subdominant female roles. Several females dies as a result of aggression from the normal male and/or treated fish; the treated fish were more capable of inflicting physical damage, since their premaxillae were intact. A treated fish showing this behaviour was removed and examined for milt; if the fish was a male it was replaced with another treated female. The sex treated fish first stocked into aquoria 7 and 8 (Table 1) were all replaced. One of the three fish originally stocked in aquarium 5 was, and the replacement fish was also exchanged when it produced milt.

During courtship the male displayed a continuously erect dorsal fin and the genital papilla become increasingly extruded. The pursuit of female by the courting male was more aggressive than the chasing observed between females. Nipping and nudging were directed towards the female's vent. Spawning occurred more frequently with the dominant female than with subdominant females.

Prespawning reproductive behaviour of the female including serveral attempts to either pick (imaginary) eggs up into her mouth or "clean out" a nest site on the bottom of aquarium behind the wire barrier. The male's agnostic behaviour would change rapidly to courtship behaviour during this period as the brooder pair would next stimulate egg deposition and fertilization After the termination of feigned spawning behaviour, the female would being egg deposition which was followed by fertilization .

Spawning by untreated (normal) female occurred in every aquarium except one (Table 1), al-

though not every normal female in each aquarium spawned. In contrast, none of the treated fish spawned. The mean number of spawns/untreated female was 0.44/week for the female stocking ratio (treated : untreated) of 1: 2 and 0.26 / week for those stocked at 0:3. The mean number of spawns/week was significantly higher in aquaria with a 0:3 and 1:2 female ratio (a = 0.05) than in those with other ratios. The mean number of spawns/untreated female/week was significantly higher (a = 0.05) in aquaria with a 1: 2 (treated/untreated) female ratio (but not in those with a 0:3 female ratio) than in aquaria with a 2:1 female ratio.

No spawning by treated fish had occurred in the aquaria by the end of July, including the F_1 female that had spawned successfully during the previous year; consequently, all fish were transferred outdoors to 4.200 L circular plastic pools. Each treated fish was individually stocked with an untreated male (premaxillae removed). Those untreated females that had not spawned in aquaria, each spawned at least once in the plastic pools. Pool spawns by untreated females are not included in the tabular data three spawns within a 6 week period occurred in the pool containing the progeny tested (F_1) sex-reversed female. All three spawns were fertilized by the same male and resulted in large numbers of viable and presumably 100% male offspring. One hundred fish sampled from the first spawn were all males. No spawning in the plastic pools containing other treated fish (F_2) was observed by 1 October, when the experimental was terminated. Gonadal examination of treated fish (F_2) showed that only two fish had ovaries of normal appearance. The remaining 14 fish had gonads that were apparent ovotestes.

Concerning to the parasitological examination, it was revealed a protozoan infested the ovaries, it was appeared as whitish pin-headed nodules embedded in ovary of Oreochromis aureus. It's spores were oval tiny (3x7 µm) and contained posterior vacuole (Fig. 1).

Progeny testing of estrogen-treated O. aureus has been previously accomplished and functionally sex-reversed females **(Guerrero, 1975)** were identified, but from pairings of one male with a single test females under less confined conditions than those provided by the aquaria in the present study **(Liu, 1977)**. Hormone treated females may be less aggressive than untreated females and therefore, may not be as competitive in spawning **(Hopkins, 1977 and 1979)**. The F_2 sex-reversed female that had previously been proven fertile spawned repeatedly when removed from confinement and the presence of two competing females. Each of these two untreated females spawned twice during the period of confinement. Also, normal (untreated females) that did not spawn in the aquoria spawned when moved to larger plastic pools. It appears that the stress of the additional crowding and the presence of competing females adversely affects individual spawning success.

Estrogen-treated fish of the F_2 generation that had been incompletely sex-reversed directed

their aggression towards the normal females or other treated fish **(Shelton et al., 1978)**, disrupting normal spawning interaction. Fish having ovotestes were usually co-dominant with untreated females, but were incapable of culminating any response to male courtship because ovulated oocytes would be unable to pass through testicular elements **(Yamamoto, 1969)**.

The number of spawns/untreated female was somewhat higher when one treated fish was stocked with two untreated female than when compared with other female ratios. However, the presence of two treated fish in an aquatum significantly reduced the spawning activity of the untreated female. Under these circumstances there may been too much agnostic behaviour from incompletely sex-reversed females. The low spawning success in progeny tests of estrogen-treated O. aureus has been previously reported **(Liu, 1977 and Hopkins, 1979)**. The presence of ovotestes in these estrogen-treated O. aureus not displaying obvious male characteristics indicates that the aberrant gonadal morphology associated with incomplete sex reversal may partially explain the low spawning rates.

In the present study, Microsporidian infection were noticed in O. aureus The same protozoa were reported previously in fresh water fishes **(Woo, 1995)**.

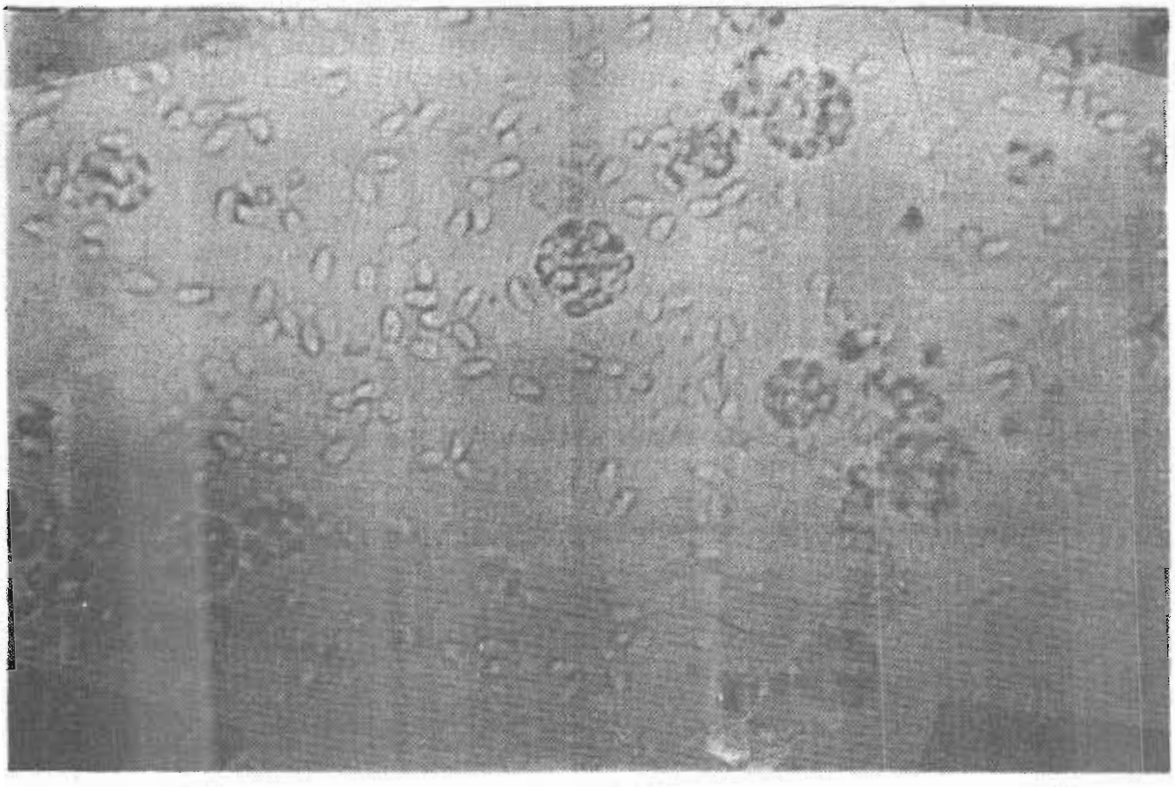

Fig. 1 : Wet mount of Microsporidium spp. spores.

Table (1): Spawning and mortality in aquaria stocked with untreated and estrogen-treated *Oreochromis aureus* females at various ratios.

Femal ratio[a] (Treated: untreated) and aquarium	Duration (weeks)	Spawns			Mortality		No. of treated fish yielding milt
		Total[b]	Weekly mean peraquarium	Weekly mean per untreated	Untreated females	Treated fish	
0:3							
9	7	4	0.57	0.19	2	-	-
10	8	8	1.0	0.33	0	-	-
1:2							
1	7	6	0.86	0.43	1	0	0
2	4	3	0.75	0.38	0	0	1
6[c]	4	4	1.00	0.5	0	0	0
2:1							
3	5	0	0	0	0	1	0
4	4	1	0.25	0.25	0	1[d]	1
3:0							
5	6	0	0	-	-	1	2
7	5	0	0	-	-	0	3
8	4	0	0	-	-	1	3

a: Each aquarium contained one untreated male. b: Spawns only by untreated females.
c: Aquarium 6 contained the F1 progeny-tested female and two untreated females.
d: Females; others had female papillae but with testes.

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الملخص العربى

دراسات عن السلوك التزاوجى للبلطى النيلى المعامل والغير معامل فى أحواض زجاجية بالإشارة إلى العدوى بالميكروسبروديان

أميمه عبود على خاطر

قسم بحوث أمراض الأسماك معهد بحوث صحة الحيوان

شارع نادى الصيد - الدقى - الجيزة

أجرى هذا البحث لملاحظة الخصائص الجينية لذكور البلطى النيلى التى عولجت بالهرمونات لتغيير جنسها إلى إناث وذلك لمنع خروج أجيال متتابعة وكذلك لمنع إنتاج مختلف الأحجام والهدف هو إنتاج حجم واحد فى الموسم الواحد. الذكور من جنس البلطى النيلى والتى سبق معاملتها للتحول الجنسى مع إناث من الجيل الأول منها F_1 الآباء قد تم وضعها فى أحواض زجاجية مع إناث غير معالجة بمعدلات مختلفة وقد تم مقارنة التكاثر فى الإناث المعاملية والغير معاملة مع ذكور غير معاملة.

لم تتكاثر أى من الأسماك المعاملة وكان معظمها تحتوى على Ovetests والجيل الأول F_1 من الإناث المعاملة لعكس الجنس تم تكاثرها بسرعة ولذلك فور وضعها مع الذكور الغير معاملة وهذا يعنى وجود إناث طبيعية فى الأحواض الزجاجية وقد يكون لها تأثر بسيط للإناث.

وإنتخاب الآباء من الأسماك المعاملة بالهرمون فإن القطيع يمكن أن يؤدى لتزاوج أسماك من نفس الجنس حيث أن المظهر الخارجى للأعضاء الجنسية يمكن للمعاملة الهرمونية، أيضاً عدد الأزواج الغير معاملة تحت نفس الظروف، ومع هذا فإن السلوك التزاوجى للأسماك المعاملة والتى نجحت فى التفريخ لم تدرس.

وقد لوحظ عدوى بعض هذه الأسماك بالميكروسبروديان، فتم دراسة هذه العدوى من حيث تأثيرها على الأعضاء التناسلية للبلطى النيلى.