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CROSS-BREEDING EXPERIMENTS ON SOME IMPORTANT FISHES OF FAMILY CICHLIDAE (GENUS *OREOCHROMIS*) AND EVALUATION OF THEIR HYBRIDS.

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ABSTRACT

The breeding characteristics, growth performance, survival rate and feed conversion ratio of the three species of Nile tilapia (*Oreochromis niloticus*), blue tilapia (*O. aureus*), red tilapia (*Oreochromis* sp.) and their different combination crosses were investigated. The present study emonstrates that: 1) the growth rates of *O. aureus* and red tilapia brood fish females were improved when they were crossed with males of *O. niloticus*; 2) the mean number of fry produced by females of *O. niloticus* was 1.2 and 1.3 times as that from *O. aureus* and red tilapia, respectively; 3) red tilapia inherited cold tolerance trait by back crossing with males *O. aureus*. This trait was transferred from *O. aureus* to a cold sensitive population of red tilapia; and 4) the hybrids of *O. niloticus* females x *O. aureus* males; red tilapia females x *O. niltoicus* males, *O. aureus* females x *O. niloticus* males, and red tilapia females x *O. aureus* males exhibited positive heterosis values (hybrid vigour) in growth parameters and survival rate. Also, these hybrids used less feed to produce one unit of gain in body weight than their parents. Thereby, these hybrids were judged to be of high potential commercial value in fish farming.

INTRODUCTION

Aquacultural organisms have very high reproductive rates and large phenotypic variance (Moav, 1976). This combination makes mass selection and hybridization for large size an easy task. Hybridization and selection are the principle means of fish improvement (kirpichnikov, 1971 and Zaki *et al.*, 1987). Hybridization is aimed to evolve a hybrid or strain of superior quality than the parent species. Thus the hybrids play an important role in fish culture (Essa, 1987).

During the past few years the genetic and breeding research on tilapia is limited to the four important mouth-brooding used in aquaculture: *Oreochromis niloticus, O. aureus, O. mosambicus* and *O. urolepis hornorum.* It covered research on: the inheritance of qualitative phenotypes; strains evaluations; heritabilities; in-breeding; environmental factors that influence genetic studies; manipulation of chromosome number; and interspecific hybridization to produce all male populations (Wohlfarth and Hulata, 1983; Behrends, 1983; Behrends and Smitherman, 1984; Tave, 1988; Doudet, 1992; El-Deeb and Essa, 1992; Gourene and Teugels, 1993 as well as Hulata *et al.*, 1993).

This study was undertaken to evaluate the value of hybridization in three important fishes of family Cichildae (Genus *Oreochromis*): Nile tilapia (*Oreochromis niloticus*), blue tilapia (*O. aureus*), red tilapia, *Oreochromis* sp. as well as their different combination crosses. The traits compared included in addition to growth performance, survival rate and feed conversion ratio during nursing period. The heterosis values were also considered.

MATERIAL AND METHODS

The present study was conducted for 90 days at Alexandria Governorate Fish farm, Fry Production Unit.

Three tilapia species were used for producing hybrid progenies:
1) Nile tilapia (*Oreochromis niloticus*): this is considered a fast growing species (Bardach *et al.*, 1972);
2) Blue tilapia (*O. aureus*): this is considered more cold tolerant than both *O. niloticus* and red tilapia (Behrends and Smitherman, 1984)
3) Red tilapia (*Oreochromis* sp.): a collective name for a large number of red, orange, gold and pink phynotypes. Red tilapia is a hybrid produced by the interbreeding between *O. niloticus* and the mutant of *O. mossambicus* (Liao and Chang, 1983). The stock in our study was introduced from Florida, USA (Florida strain) by Mariut Fish Farming Company, Alexandria.

Ripe females and males tilapias of different species were stocked into 6m^2 concrete ponds in April, 1997 at rate of 4 breeders/m^2. The sex ratio of the fish was three females to one male. Males and females of comparable size and weight were stocked together to reduce injuries to females caused by male aggressiveness during spawning behavior. Breeders of tilapias with mean weights of 47.29 ± 3.47 (female) and 72.79± 7.45 (male) were used during the present study. The spawners were supplementary fed with commercially available pellets (32.58% protein) at the rates of 1.5% of their body weight/day (Table 1). The temperature of the water in the spawning ponds was 22-31°C. the oxygen content was not less than 5.0 mg/l and the pH was on the alkaline side (7.6-8.5). The spawning period was 25 days.

Fry produced in each pond were collected daily or more often as they appeared at the water surface with a dip net and counted. Dip net collection was supplemented by use of a 2.0m long seine with a mesh size of 1.6 mm.

Six different hybrids were tested and evaluated during the present study:
1) Females *O. niloticus* x males Red tilapia.
2) Females Red tilapia x males *O. niloticus*.
3) Females Red tilapia x males *O. aureus*.
4) Females *O. aureus* x males Red tilapia.
5) Females *O. niloticus* x males *O. aureus*.
6) Females *O. aureus* x males *O. niloticus*.

Three replicated of each of the six hybrids and parents were stocked into concrete ponds. Complete random design was implemented in placement of fish groups in ponds. Ponds were 2.0m x 3.0m x 1.0m ($6m^3$). Daily food allowance for the tilapia groups was based on 10% of body weight twice a day with 49.47% crude protein fine commercial diet (Table 1). The duration of theexperimental rearing or nursing period was 90 days.

Young fry of different hybrids combinations were compared for their growth performance, survival percentage, feed conversion ratio and heterosis (hybrid vigour) according to Essa (1987), during nursing period. Samples were taken from each pond bi-weekly. At harvest, fish fingerlings were counted and weighed.

Data were analyzed with one-way analysis of variance (ANOVA), followed by Duncan's multiple range test when differences were indicated.

RESULTS AND DISCUSSION

1. Growth and survival percentage in brood fish under six different tilapia crosses conditions

The fish used as experimental subjects were hybrids of three Tilapia species *Oreochromis niloticus*, *O. aureus*, red tilapia (*Oreochromis* sp.). The weight and length gains of the brood fish were different in all six tilapia crosses conditions. The increase in individual weights and lengths in each of the experimental groups has being presented in Table (2). After 25 days spawning period, broodfish in group B (hybrids of red tilapia females and *O. niloticus* males) showed a fastest growth rate than the other five groups. They possessed average weights gain of 49.4 g and 93.6 g for females and males, respectively. While the corresponding results for lengths gain were 4.5 cm and 6.7 cm for females and males, respectively. It is obvious from Table (2) also that males grow faster than females in tilapias. Similar observations has been shown by Behrends (1983); Tave *et al.*, (1990) as well as Essa and Faltas (1997) for *O. niloticus* and *O. aureus*. This suggests that the differential growth rates have a genetic basis.

The present study demonstrates also that the growth rates of *O. aureus* and red tilapia brood fish females were improved when they were crossed with males of *O. niloticus*. This might be due to the compatibility behavioral patterns that may be existing between the three tilapia species.

From Table (2) it can be shown that, survival rate was 100% for all six groups. Therefore, the present data demonstrated the feasibility of using tilapias in fish farming. Wohlfarth *et al.*, (1983) showed that, summer and winter survival rate was over 80% in the parental species of *O. niloticus O. aureus* and *O. mossambicus*.

1. Fry production

The mean number of fry (swim-up stage) produced per female spawner in different hybrid combinations and the parental species is shown in Table (3). The mean number fry produced by the Nile tilapia (*O. niloticus*) females was 1.2 and 1.3 times as high as that from *O. aureus* and red tilapia females, respectively. While the differences in fry production of *O. aureus* and red tilapia females when spawned with males of different species are insignificant (ranged between 981- 1073 fry/female). This may be attributed to the differences in fecundity due more to the weight of fish stocked than the species of the male fish. These data suggest that female age and size could confound genetic differences when evaluating reproductive performance. Siraj *et al.*, (1983) examined the influence of female age and size on reproductive performance, egg size, fry size and early growth in *O. nilotiucs*. Female age and size were positively correlated with spawning rate, egg length, hatchability and sac fry length and were negatively correlated with number of eggs/kg female.Age of fry within the range of 7 to 10 days swim up tested in this experiment did not show a significant difference in the size attained, weight and length (Table 3). The average body weight and length of swim-up fry varied between 0.03-0.05g and 0.7-0.9 cm, respectively. A possible explanation to these results are that, the values of the coefficient of similarity and the genetic distance showed close similarity between *O. nilotcus, O. aureus* and red tilapia (El-Deeb and Essa, 1992).

3. Production performance

The different hybrids combinations were compared for their growth performance (mean weight and length gains per fish, mean daily weight and length gains as well as specific growth rate), condition factor, survival percentage and feed conversion ratio and the results are summarized in Tables (4 and 5).

3.1. Genetic improvement of red tilapia

The parental specie used for hybridization in this investigation may by divided into two groups, according to their relative tolerance to low water temperature and growth rate (Wohlfarth *et al.*, 1990, El-Deeb and Essa, 1992).

1) The two groups are: High growth and sensitive species or stain: *O. niloticus* and red tilapia

2) Normal growth and relatively resistant species: *O. aureus*.

Hybridization in fish is aimed to evolve a hybrid or strain of superior quality than the parent species. Therefore the following crosses were made:

(a) Back cross was made between red tilapia females and *O. nilotius* males. F1 progeny showed a superior growth rate in weight length and specific growth rate (Table 4) and survival percentage (83%) when compared with that of their parents (Table 5). Also, the F1 hybrids used less feed (1.09) to produce one unit of gain in weight than their parents (Table 5). But in case of condition factor, the F1 hybrids showed intermediate inheritance, 2.00 (Table 5).

(b) Back cross between female red tilapia and male *O. aureus* have been carried out in this study to increase red tilapia tolerance to low water temperature and improve growth rate of *O. aureus*. F1 progeny showed a superior growth in weight and length compared with that of the maternal species, *O. aureus* (Table 4). Table (5) proves that, the F1 hybrids was the most cold tolerant than their parents, thereby has the highest survival rate (85%), best feed conversion ratio and high condition factor (2.00) compared with those of their parents. Lagler (1956) stated that high values of condition factor indicated the suitability of the environment to the fish. It appears also that, cold tolerance is a dominant trait.

(c) In contrast, when red tilapia males and *O. niloticus* or *O. aureus* females were crossed only survival percentage and feed conversion ratio were improved

in the F1 progeny and the differences between the F1 hybrids and their parents for condition factor were insignificant (Table 5). The cross- breeding of red tilapia males and *O. niloticus* or *O. aureus* females resulted in a very poor growth in weight, length and specific growth rate when compared with those of their parents (Table 4) and the differences were significant ($P \leq 0.05$).

These data demonstrate that, it is easy to hybridize fishes related to each other. But in some cases, even reciprocal hybrids produced by crossing the same species by a male of another, and vice versa, may be different in fry production, growth in weight and length as well as survival percentage. According to Nikoljukin (1971) and Essa (1987) this might be due to the fact that differences of the reciprocal forms of hybrids are determined by cytoplasmic differences of the crosses species which will result in different interaction of the nucleus and the plasma in reciprocal crossing. This will lead to some viable hybrids and other may not be or at least differ in some quantitative phenotypes characters.

3.2. Genetic improvement of blue tilapia, *O. aureus*

A hybrid cross was made between females of Nile tilapia, *O. niloticus* and males of blue tilapia, *O. aureus*. Also, the reciprocal crossing have been carried out in the present study (females *O. aureus* x males *O. niloticus*). The F1 progenies were compared for the growth performance, condition factor, survival percentage and feed conversion ratio and the results are summarized in Tables (4λ5).

All such F1 progeny (female *O. niloticus* x males *O. aureus*) grew more rapidly (Table 4) and possessed highest survival percentage (91.00%) as well as best feed conversion ratio (1.13:1) than their parents (Table 5). But the differences in condition factor between the hybrids and their parents were insignificant ($P > 0.05$).

The F1 progeny of the reciprocal hybrids (females *O. aureus* x males *O. niloticus*) exhibited intermediate inheritance for growth in weight and length (Table 4). It has been reported that *O. aureus* is the most cold tolerant species

and *O. niloticus* is the fastest growth fish in genus Oreochromis (Bardach *et al.*, 1972 and Wohlfarth *et al.*, 1990), therefore it is not surprising to find that the cross-breeding of females *O. aureus* x males *O. niloticus* exhibited highest survival percentage (96.00%) and best feed conversion ratio (1.56) when compared with those of parents Table (5). Bautista *et al.* (1988) found that the progeny produced by the reciprocal crossing between O. niloticus and *O. aureus* had highest survival percentage than parents and did not differ significantly. In contrast, the hybrids have a less value of condition factor (1.83) than those of parents by 13.9%.

The reasons for the differences of the reciprocal forms of *O. niloticus* and *O. aureus* hybrids have been discussed in previous section (3-1) according to Nikoljukin (1971) and Essa (1987).

4. Heterosis

The term heterosis (hybrid vigour) characterized the increased ability of a hybrid as compared to the parental line species and this improvement is a result of action and interaction of allelic and non allelic dominance and very often linked genes (Demin, 1971). Growth performance and survival percentage are the most important for evaluation of fish culture and its productivity.

Table (6) shows the heterosis values of mean weight and length gains, specific growth rate and survival rate of crosses of *O. niloticus* (N) and *O. aureus* (A) and red tilapia (R) (*Oreochromis* sp.) during nursing period (90 days). Positive heterosis values of growth parameters and survival rate were recorded for the cross-bred fingerlings of R x N, R x A, N x A and A x N (the female is first), but negative heterosis values for growth parameters and positive heterosis values for survival rates were showed for the cross-breeding fingerlings of males red tilapia and females *O. niloticus* or *O. aureus*. Furthermore, females red tilapia and males *O. niloticus* backcross hybrids were

significantly heavier than the females red tilapia and males *O. aureus* backcross hybrids and exhibited higher positive heterosis in mean weighrt gain and specific growth rate. But females red titlapia and males *O. aureus* backcross hybrids showed higher positive heterosis in mean length gain and survival percentage than R x N backcross hybrids. These results might be attributed to 1) *O. nilotiucs* grow faster than *O. aureus* (Tave, 1988; El-Deeb and Essa, 1992); and 2) *O. aureus* was more cold tolerant than *O. niloticus* (Behrends and Smitherman, 1984). These results demonstrated that, parental heterosis was responsible for the increased heterosis in the backcross hybrids.

Although the hybrids exhibited heterosis, no hybrid was better than the hybrids produced from crossing females *O. niloticus* and males *O. aureus* (Table 6). Also, the reciprocal crossing A x N exhibited a good heterosis values. Similar observations and trends have been reported by Macintosh and Silva (1984). They found that, under equivalent conditions, *O. niloticus* female x *O. aureus* male fry had a higher rate of growth, feed conversion and were more uniform in size than the parents and *O. massambicus* fry. According to Hulata *et al.* (1993), the Nile strain hybrids of *O. niloticus* and *O. aureus* can be considered for commercial hybridization.

The differences among hybrids in the present study can be explained also according to SEAFDEC (1986), in tilapia crosses, some traits of the hybrid were observed to be inherited from the female parent, other from the male parent and a few intermediate inherited traits were observed.

These results suggest that

1) the hybrids of
O. niloticus females x *O. aureus* males.
Red tilapia females x *O. niloticus* males.
O. aureus females x *O. niloticus* males.
Red tilapia females x *O. aureus* males.

Thre were judged to be of high potential commercial value in fish farming;

2) Although the red tilapia hybrid show a bright potential for aquaculture, yet the inheritance of the red pigmentation need further work
3) Much further work is required to investigate the nutritional requirements of all these hybrids.

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Table (1): The ingredients and chemical analysis of the broodfish and fry diets.

Ingredients	Brood fish diet (%)	Fry diet (%)
Soybean meal	35	35
Fish meal	24	35
Wheat bran	30	25
Starch	10	4
Vitamin and minerals mixture	1	1
Chemical analysis:		
Dry matter	91.75	92.34
Crude protein	32.58	49.47
Crude fat	5.28	3.92
Crude fiber	10.27	4.17
Nitrogen free extract	41.00	33.16
Ash	10.87	9.28

Table (2): Brood fish means for growth parameters and survival percentage of tilapias.*

Item **	Group A: ♀ N x ♂R	Group B: ♀ R x ♂N	Group C: ♀R x ♂A	Group D: ♀ A x ♂R	Group E: ♀ N x ♂A	Group F: ♀ A x ♂N
♦ Brood fish /m2	4	4	4	4	4	4
♦ ♀: ♂ ratio	3:1	3:1	3:1	3:1	3:1	3:1
♦ Average initial weight of females (g)	55.0±4.10	36.7±2.91	34.7±1.43	50.0±3.92	50.0±1.98	30.5±2.36
♦ Average initial length of females (cm)	13.8±0.50	12.2±0.36	12.3±0.44	13.6±0.56	13.6±0.33	11.5±0.31
♦ Average initial weight of males (g)	57.5±1.50	59.0±2.80	48.0±1.12	56.0±2.08	40.0±0.95	52.5±3.60
♦ Average initial length of males (cm)	14.3±0.30	15.5±0.22	13.50±0.20	13.7±0.29	12.7±0.28	13.5±0.30
♦ Average final weight of females (g)	70.7±8.50	86.1±9.01	50.9±6.02	64.8±7.11	73.1±7.24	48.8±5.06
♦ Average final length of females (cm)	16.4±0.80	16.7±0.73	14.9±0.51	15.7±0.52	17.1±0.35	14.5±0.55
♦ Average final weight of males (g)	145.2 ±45.20	152.6±33.10	72.5± 7.25	83.1±8.11	74.8±3.10	124.1±26.11
♦ Average final length of males (cm)	20.0± 2.00	22.2± 1.67	16.5±0.54	17.0±0.55	16.7±0.63	19.6±10.9
♦ Gain in female weight (g)	15.7	49.4	16.2	14.8	23.1	18.3
♦ Gain in male weight (g)	87.7	93.6	24.5	27.1	34.8	71.6
♦ Gain in female length (cm)	2.6	4.5	2.6	2.1	3.5	3.0
♦ Gain in male length (cm)	5.7	6.7	3.0	3.3	4.0	6.1
♦ Survival rate (%)	100	100	100	100	100	100

* N: *O. niloticus* R: Red tilapia A: *O. aureus*

** Duration : 25 days

Table (3): Fry production (per female) in different tilapia crossess and the parental species.*

Parent species ♀ X ♂	Average fry production per female	Swim-up stage	
		Average body weight (g)	Average body length (cm)
N x R	1251	0.03	0.8
R x N	989	0.05	0.8
R x A	985	0.05	0.8
A x R	981	0.03	0.9
N x A	1256	0.03	0.9
A x N	1073	0.03	0.9
R x R	985	0.05	0.9
N x N	1290	0.04	0.8
A x A	986	0.04	0.7

* N: *O. niloticus* R: Red tilapia

A: *O. aureus*

Table (4): Growth performance of different tilapia hybrids and their parents during nursing period (90 days).*

Parent species ♀ X ♂	Mean weight gain per fish (g)	Mean daily wt.gain per fish (mg)	Specific growth rate (% /day)	Mean length gain per fish (cm)	Mean daily length gain per fish (mm)
N x R	1.20	13.33	3.70	2.95	0.33
R x N	2.27	25.22	4.06	3.83	0.42
R x A	1.85	20.55	3.86	3.65	0.40
A x R	1.10	12.22	3.92	2.72	0.30
N x A	4.43	49.22	4.88	4.29	0.48
A x N	1.85	20.55	3.92	3.43	0.38
R x R	2.07	23.00	3.73	3.82	0.45
N x N	1.91	21.22	3.98	3.70	0.30
A x A	1.60	17.78	3.82	2.74	0.30

* N: *O. niloticus* R: Red tilapia

A: *O. aureus*

Table (5): Condition factor , survival percentage and feed conversion ratio of different tilapia hybrids and their parents during nursing period (90 days). *

Parent species ♀ X ♂	Condition factor (k)	Survival (%)	Feed conversion ratio
N x R	1.88	85.00	1.02
R x N	2.00	83.00	1.09
R x A	2.00	85.00	1.36
A x R	1.82	100.00	1.14
N x A	2.01	91.00	1.13
A x N	1.83	96.00	1.56
R x R	1.85	74.40	1.90
N x N	2.10	78.30	1.80
A x A	2.07	76.10	1.91

* N: *O. niloticus* R: Red tilapia

A: *O. aureus*

Table (6): The heterosis values (%) of growth parameters and survival percentage of different tilapia hybrids, during nursing period (90 days).*

Parent species ♀ X ♂	Mean weight gain per fish (%)	Mean length gain per fish (%)	Specific growth rate (%)	Survival (%)
N x R	-39.70	-24.46	-3.90	11.33
R x N	14.07	1.86	5.45	8.71
R x A	1.09	7.99	2.39	12.96
A X R	-39.89	-19.53	-3.98	32.89
N x A	153.14	33.23	26.42	17.87
A x N	5.71	21.74	1.55	24.35

* N: *O. niloticus* R: Red tilapia

A: *O. aureus*

تجارب تهجين لبعض الأنواع الهامه من عائله أسماك البلطى وتقييم الهجين الناتج

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استهدفت هذه الدراسه تقييم ستة نواتج من هجين ثلاث أنواع سمكيه تنتمى إلى عائلـــه أسمـــاك البلطـــى Family Cichildae وهى :

١. البلطى النيلى *Oreochromis niloticus* السريع النمو.

٢. البلطى الحسانى *O. aureus* المقاوم لدرجات حرارة الماء المنخفضه.

٣. البلطى الأحمر *Oreochromis sp.* المعتدل النمو والحساس لدرجات حراره الماء المنخفضه.

حيث تم التقييم بأستخدام معايير اقتصاديه هامه فى الإستزراع السمكى كمعايير النمو والمعيشه ومعامل الحاله ومعـــدل التحويل الغذائى بالأضافه الى تقدير قوة الهجين لهذة المعايير الإقتصاديه فى مرحله خروجه من حياة الأسماك وهى مرحلـــة الحضانه للبرقات. ولقد أظهرت الدراسه نتائج اقتصاديه هامه نوجزها فى النقاط الآتيه:

(١) تحسنت معدلات نمو امهات كل من البلطى الحسانى والبلطى الأحمر كثيراً عند تعايشها مع ذكور البلطى النيلـــى (الآباء) لوجود توافق وراثى بينها.

(٢) أنتاج اليرقات فى حاله أناث البلطى النيلى تفوق على نظيره فى البلطى الحسانى والبلطى الأحمر بمقـــدار ١,٢، ١,٣ مره على التوالى.

(٣) هجين البلطى الأحمر ورث صفه مقاومه درجات حرارة الماء المنخفضه عند اجراء تهجين عكسى لإنـــاث البلطـــى الحسانى.

(٤) أظهر الهجين:

أناث	ذكور
البلطى النيلى	البلطى الحسانى
البلطى الأحمر	البلطى النيلى
البلطى الحسانى	البلطى النيلى
البلطى الأحمر	البلطى الحسانى

قوه هجين موجبه لمعايير النمو والمعيشه. كما ان هذا الهجين استخدم كمات اقل من الغـــذاء لأنتاج وحده وزن متفوقه فى ذلك على الأنواع الأبويه. لهذا فإننا نوصى بأستخدام هـــذا الهجـــين فى المزارع السمكيه بعد اجراء دراسات على احتياجاتها الغذائيه.

(٥) أظهر الهجين الناتج من تزاوج اناث البلطى النيلى أو الحسانى مع ذكور البلطى الأحمر قوة هجـــين سالبه لمعايير النمو وقوة هجين موجبه لمعدلات المعيشه فقط.