Bull. Nat. Inst. of Oceanogr. & Fish., A.R.E. 1996. Vol. (22); 255 - 266

EFFECT OF THE GENETIC STATUS OF OREOCHROMIS NILOTICUS AND O. AUREUS FROM THREE LOCALITIES ON THE MALE RATIO OF THEIR HYBRIDS

BY

E. A. BADAWY AND E. H. RIZKALLA*

***National Institute of Oceanography & Fisheries, Genetic lab; Barrage station; Dakahlia; Egypt.**

Key Words: Genetics, Fish genetics, Cichlid fish.

ABSTRACT

*Comparative electrophoretical studies for muscle proteines of two groups of female **Oreochromis niloticus** from Abbasa hatchery (T) and from ponds (F), a male group of **O. aureus** from a drain in the area and their hybrids were carried out. The study showed that females of **O. niloticus** from Abbassa hatchery are more genetically pure than those from the farm ponds, but the males of **O. aureus** from the drain were the lowest ones. This was clearly reflected in the male ratios of their produced hybrids. The male ratio was 84.2% for the 1st hybrid H1 (**O. niloticus** ♀T X **O. aureus** ♂D) and 79.8% for the and hybrid H2 (**O. niloticus** ♀F X **O. aureus** ♂D).*

INTRODUCTION

The early sexual maturity of Tilapia in addition to their prolific spawning cause overpopulation in rearing ponds (Hickling, 1963; Fryer & Iles, 1972; Wohlfarth & Hulata, 1981). Crossing between ***Oreochromis niloticus*** and ***O. aureus*** for producing hybrid progenies containing high ratio of males is one of the solutions to solve this problem (Pruginin et al, 1975; Rothbard & Pruginin, 1975 and Badawy, 1993). The male ratio of produced hybrids is greatly affected by the genetic purity of parents (***O. niloticus*** ♀ X ***O. aureus*** ♂). (Lovshin, 1982; Badawy, 1993).

Studying skeletal muscle proteins (myogens) of fish using polyacrylamide electrophoretic technique is one of the biochemical methods used to differentiate genetically between fish species (Herzberg & Pasteur, 1975, Basaglia, 1992, El-Saied, 1993).

This work aimed to declare the effect of the relative genetic purity of two female groups of ***O. niloticus*** and a male group of ***O. aureus*** from different areas on the male ratio of their produced hybrids.

MATERIALS AND METHODS

This study was carried out in 1994 at a private fish farm in Soaud area, Al-Husynia Center, Sharkia Governorate. This farm is irrigated by agricultural draining water mixed with sewage water of Bahy El-Bagar drain.

In this experiment, two female groups ***of Oreochromis niloticus*** were selected from two areas. The 1st group (♀NT) was brought to this farm from Abbasa hatchery(T) and the 2nd group (♀NF) was from the self spawning of ***O. niloticus*** in the pond farm (F). This in addition to the third group (♂AD), ***Oreochromis aureus*** males), which was selected from an adjacent small drain (I). All fish groups averaged 95-100 g/fish.

At the 1st of April 1994, females of the 1st and 2nd group (♀NT & ♀NF) were stocked with males of the 3rd group (♂AD) in two separate ponds (1000m2 each) at the rate of 1 fish/m2 (sex ratio 30:10). Using 0.5 meshed net, the produced hybrid fry were seined at weekly intervals starting from May 1st till end of October. A sample of fingerlings collected from each spawning pond was restocked in two ponds (2000m^2 each) at the rate of 2 fish/ m^2. Fish were fed wheat bran at the rate of 2% body weight. At the end of November 1994; sex ratio was determined by testing 1000 fish/pond.

For skeletal muscle electrophoresis, all fish samples were iced and sent to the Barrage Research Station. From each fish, 1.0 gram of the flesh was taken from the left side below the dorsal fin, mixed with 3 ml of cooled distilled water, homogenized in tissue homogenizer and centrifuged at 5000 rpm for 15 minutes. The supernatants were applied in disc electrophoresis of 7.5% acrylamide and tris-buffer system. (Herzberg & Pasteur, 1975). After running

process, gels were stained by amidoblack 10B for 30-60 minutes, destained and then stored in 7% acetic acid. The muscle protein fractions were scanned by densitometer.

Polymorphism of muscle protein (which of genetic character) of these two species was designed according to the frequency of appearance of each fraction in the samples of each species used (Payne at al., 1971; Wilkins, 1971). The fraction that appeared in 100% of individuals was given the symbol (a), (C, constant) was given to fractions that appeared in 90-99.9% of individuals from any locality, while (p) for those appeared in less than 90% of individuals and (d) for the fraction that disappeared completely in all individuals.

On the other hand, the similarity between mobilities of fractions of the electrophoretic pattern of the females of ***O. niloticus*** from the hatchery (♀NT) and the farm (♀NF) and males ***O. aureus*** from drain (♂AD) and their hybrids (H1 &H2) was assessed by simple matching coefficient of similarity (Ferguson, 1980). Lowest Similarity coefficient (S.C.) between members of the same species from different areas means that the species or at least one of them have low genetic purity and vice versa. (Ferguson, 1980, El-Saied, 1993).

RESULTS

Table (1) shows the results of the small hybrid fingerlings produced from the crossing of both female groups of ***O. niloticus*** that brought to the Farm from the Abbassa Hatchery (♀NT) and those selected from the farm (♀NF) with males of ***O. aureus*** that were selected from an adjacent drain (♂AD). The weekly sein in the two crossings obtained no fingerlings in April. The maximum numbers of fingerlings gained from the two crossings (♀NTX♂AD & ♀NFX♂AD) were found in July and then in September, being 18000 & 17422 and 13993 & 14087 fingerling/month, respectively. It was also found that the smallest number/month was in October in the two crosses, being 400 & 350 for the 1st and 2nd cross, respectively. On the other hand, the samples tested for the sex ratio from the two crosses declared that the male ratio was 84.2 & 79.8 for the 1st cross (♀NTX♂AD) and the 2nd cross (♀NFX♂AD), respectively.

Table (1): Number of small hybrid fingerlings per month collected from the two spawning ponds (2000m^2 each) that were stocked with the broods (average 95-100 g/fish) ♀NT X ♂AD and ♀NF X ♂AD at the rate of 1fish/m^2 (sex ratio 3♀:1♂)

	♀NT X ♂AD (H1)	***♀NF X ♂AD (H2)***
April, 1994	---	---
May	2890	3098
June	7825	7983
July	18000	17422
August	6977	6015
September	13993	14087
October	400	350
Total/pond	50085	48955
Male % (O %)	84.2	79.8

NT: ***O. niloticus*** from Hatchery
NF: ***O. niloticus*** from the farm
AD: ***O. aureus*** from the drain
H1 : first hybrid
H2 : second hybrid

Table (2,3 & 4) show the electrophoretic results of the muscle protein for the three experimental groups of ASD Oreochromis species (♀NT, ♀NF & ♂AD) and their hybrids (H1 & H2). Tables (2 & 3) show that the fractions of (♀NT) were absolute in 8 (No. 1,2,3,7,9,10,11 & 14), polymorphic in 4 fractions (No. 5,6,12 & 13) and those number 4 & 8 disappeared of females ***O. niloticus*** selected from the farm (ONF) were absolute in 8 bands or fractions (No. 1,3,5,7,10,11,12&14) and those number 2,4,6,8,9 & 13 showed polymorphism. However, the fractions of the sarcoplasmic proteinogram of ***O. aureus*** males (cought from the the adjacent drain) were absolute in 5 fractions (No. 1,3,10,11 & 1), polymorphic in 4 ones (5,8,9 & 12) and 5 fractions (No. 2,4,6,7&13) were not present. On the other hand, the sarcoplasmic electrophoretic patterns of the males of the first hybrid H1 {(***O. niloticus*** ♀(NT) X ***O. aureus*** ♂(AD)} were absolute in 6 fractions (No. 1,7,10,11,12& 14), polymorphic in 6 ones (2,5,6,8,9 & 13) and fraction number 3 disappeared.

Table (2): Comparative identification map, showing the frequency of appearance of individual sarcoplasmic protein fraction for different experimental groups of ***Oreochromis*** species and their hybrids.

Group Locality	**Sex**	**No. of Sample**	***Number of Fractions***												
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Abbasa Hatchery ***O.niloticus***	F.	(♀ONT) 5	a	a	a	d	p(20.0)	P(80.0)	a	d	a	a	a	p(80.0)	p(80.0)	a
Private ***O.niloticus***	F.	(♀ONF) 10	a	P(16.7)	a	p(16.7)	a	p(16.7)	a	p(33.3)	p(83.3)	a	a	a	p(50.0)	a
Adjacent ***O.aureus***	A.	(♂AD) 8	a	d	a	d	p(62.5)	d	a	p(50.0)	p(62.5)	a	a	p(75.0)	d	a
First Hybrid	M.	(H1) 15	a	p(40.0)	c	d	p(60.0)	p(50.0)	a	p(40.0)	p(50.0)	a	a	a	p(50.0)	a
♀ONT X ♂AD	F	6	a	p(28.6)	p(85.7)	p(28.6)	p(57.1)	p(57.1)	a	p(57.1)	p(42.9)	a	a	a	a	a
Second Hybrid	M.	(H2) 15	a	d	a	d	p(66.7)	p(66.7)	a	p(33.3)	a	a	a	p(66.7)	p(50.0)	a
♀ONF X ♂AD	F.	6	a	d	p(83.3)	d	p(50.0)	p(66.7)	a	p(33.3)	a	a	d	p(50.0)	p(50.0)	a

Table (3): Mean relative mobility of sarcoplasmic protein fraction of three groups of ***Oreochromis*** species from different areas and their hybrids.

		Hatchery	Farm	Drain	H1		H2	
		O. nilo (♀NT)	***O. nilo*** (♀NF)	***O. nilo*** (♂AD)				
	Fraction	female	female	male	male	female	male	female
1	X\	7.70	15.77	10.60	15.46	13.16	4.09	3.92
	± S.D.	4.00	4.42	4.54	3.62	4.16	1.03	2.21
2	X\	15.53	24.91	---	21.08	19.04	---	---
	± S.D.	5.22	---	---	3.51	5.38	---	---
3	X\	22.19	28.31	51.12	25.64	20.34	11.05	9.00
	± S.D.	4.60	7.43	5.00	6.53	4.32	2.65	3.18
4	X\	---	3.456	---	---	26.63	---	---
	± S.D.	---	---	---	---	5.24	---	---
5	X\	23.36	32.10	51.89	26.44	27.64	15.43	12.94
	± S.D.	---	7.14	2.84	6.63	3.82	4.38	5.98
6	X\	30.33	36.70	---	27.12	28.07	18.42	17.00
	± S.D.	5.35	---	---	4.34	2.01	3.20	3.00
7	X\	34.61	38.98	---	35.95	34.35	30.97	28.33
	± S.D.	5.16	7.78	---	3.98	5.03	4.45	4.21
8	X\	---	39.67	55.97	36.86	36.20	36.82	31.37
	± S.D.	---	1.88	1.66	4.93	6.26	2.68	6.37
9	X\	37.41	45.27	60.03	41.10	37.28	43.84	42.08
	± S.D.	4.77	6.55	1.92	2.96	6.78	4.16	3.15
10	X\	51.20	57.41	67.80	48.91	45.39	61.20	61.68
	± S.D.	4.64	7.99	2.03	3.20	5.16	7.99	4.67
11	X\	58.85	65.88	72.53	60.11	52.58	---	---
	± S.D.	7.82	6.16	3.32	3.93	5.30	---	---
12	X\	65.64	72.36	78.11	73.06	64.98	65.35	69.38
	± S.D.	5.97	5.18	4.45	4.08	7.06	3.99	0.07
13	X\	67.67	78.01	---	75.26	72.81	68.86	74.01
	± S.D.	6.19	3.75	---	2.76	6.28	3.74	4.56
14	X\	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	± S.D.	0.00	0.00	0.00	0.00	0.00	0.00	0.00

X\ : Mean value of mobility
S.D.: Standard deviation.

But those of their females were obvious in 7 fractions (No. 1,7,10,11,12,13 & 14) and individuals (No. 2,3,4,5,6,8 & 9) showed polymorphism. However, muscle electrophoretic patterns of the males of the second hybrid H2 {(***O. niloticus*** ♀(NF) X ***O. aureus*** ♂(AD)} were absolute in 6 fractions (1,2,7,9,10 & 14), polymorphic in 5 (5,6,8,12 & 13) and the fraction number 2,4 & 11 disappeared. On the other hand those of their females were absolute in 5 fractions (No. 1,7,9,10 & 14), polymorphic in 6 (No. 3,5,6,8,12 & 13) and three fractions (No. 2,4 & 11) disappeared.

From the relative mobility point of view, the similarity coefficient patterns of females ***O. niloticus*** from the hatchery (♀NT) and from the farm production (♀NF) was 0.86 (Table 4). However, on comparing the relative mobility of male ***O. aureus*** from the drain (♂AD) with ONT and with ONF the SC was 0.57 for the both (Table 3 & 4).

Table (4): Similarity coefficient (genetic similarity) of the mobility of electrophoretical sarcoplasmic protein fractions for two female groups of Oreochromis niloticus (♀NT & ♀NF) and the male group of ***O. aureus*** (♂AD) and their hybrids (H1 & H2)

	♀NT	***♀NF***	***♂AD***
♀NF	0.86	***	***
♂AD	0.57	0.57	***
♂H1	0.79	888	0.45
♀H1	0.93	888	0.50
♂H2	***	0.64	0.50
♀H2	***	0.64	0.50

NT: ***O. niloticus*** from Hatchery
NF: ***O. niloticus*** from the farm
AD: ***O. aureus*** from the drain
H1 : first hybrid
H2 : second hybrid

On the other hand, the genetic similarities or similarity coefficients(SC) between the males and also the females of the 1st hybrid (♂H1 & ♀H1) and their female parent (♀NT) were 0.79 & 0.93, respectively. While they were 0.45 & 50 for both ♂H1 & ♀H2 and their male parent ♂AD, respectively (Table 4). Furthermore, the SC was the same (0.64) between both males and females of the 2nd hybrid (♂H2 & ♀H2) and their female parent (♀NF). Also, the Sc was 0.50 between the two sexes of H2 (♂H2 & ♀H2) and their male parent (♂AD) (Table 4).

DISCUSSION

Producing all-male Tilapia is a method for decreasing over population in ponds (Mires, 1982 & Badawy, 1993). ***Oreochromis niloticus***, in addition to their high growth rate, play an important role in this way. Crossing female of ***Oreochromis niloticus*** with males of different Oreochromis species produces 9 hybrids containing high ratios of males that may reach 100% in some hybrids (Wohlfarth & Hulata, 1981). The male ratio in hybrids, especially produced by crossing of ***Oreochromis niloticus*** and ***Oreochromis aureus***, depends mainly on the degree of genetic purity of the parents (Lovshin, 1982; Mires, 1982 & Badawy, 1993).

Results of cross spawning showed that the net number of fingerlings per season (from April to October) was low, also the interspawning stops earlier than the intraspawning (Badawy, 1993 and Siliem & Badawy, 1995). This is due to many reasons; (1) - Incompatibility in interspawning where females do not have a strong urge to spawn with males of different species that may be often be aggressive at the time where there is a difference in color display, courting behavior and form of the nest, (2) - Inadequate spawning technique and high fry mortality where big fish cannibalize the fry and mud kills fry on seining, (3) - Lack of genetic purity where there is a phenotypical individual variability in fecundity of various tilapias. If more genotypes can be selected for culture strains; then higher yields of fry can be expected from both inter and intra-specific crosses (Mires, 1982).

The proteins of skeletal muscle are polymorphic in fish and species specific and many authors used them for studying the genetics of many species through electrophometric analysis (Tsuyuki, 1966; Grag & McKenzie, 1970 and Utter &

Hodgins, 1971). Chen & Tsuyuki (1970) reported that sarcoplasmic electrophorogram for mouth brooder species of Tilapia (Tilapia hornorum and T. mossambica) are very similar but differ from that of substratum spawner (Tilapia zillii and T. melanopleura). The same authors also recorded that the muscle myogen pattern of Fi hybrid (Tilapia mossambica ♀ X T. hornorum ♂ and reciprocal cross) have the characteristics of their parents but they could not differentiate the genotype of the hybrid. Heines & Yashouv (1970) found insignificant variations in some muscle proteins between T. aurea collected from different locations, although morphological variations were exist between these locations. Kirpichnikov (1981) mentioned that in certain fish species the variation in one or two zones (fractions) of muscle proteinogram could be shown and the number of alleles controlling the muscle protein polymorphism rarely exceeds two.

The sarcoplasmic eletrophoretic patterns showed polymorphism in 4 and in 6 fractions in case of two female groups of ***Oreochromis niloticus*** selected from hatchery (♀NT) and from the private farm (♀NF), respectively, and their similarity coefficient (SC) between their mobilities was 0.86. Also, 2 fractions of the last group (♀NF) disappeared completely. However, the 3rd group ***O. aureus*** (♂AD) that were selected from a small adjacent drain showed polymorphism in a fractions and 5 ones disappeared. In addition, the t-test for fraction mobility between this male group (♂AD) and each of the other 2 female 2 female groups (♀NT & ♀NF) showed significance nearly in the same fraction numbers, hence their similarity coefficient (SC) was the same (0.57) (Table 3 & 4).

On the other hand, the mobility of fractions between the 1st group (♀NT, the female parent of H1) and each of the males and females of 1st hybrid H1 (♂H1 & ♀H1) showed SC of 0.79 and 0.93, respectively. While it was 0.45 and 0.50 between the 3rd group male (♂AD, the male parent of H1) and each of ♂H1 and ♀H1, respectively. However, females of the 2nd group (ONF, female parent of H2) and each of ♂H2 and ♀H2 have the same SC. of 0.64. Also, the same SC of 0.50 was found between the 3rd group male (♂AD, male parent of H2) and each of ♂H2 and ♀H2 was observed. These results, in addition to the effect of locality, indicate that the 1st Oreochromis female group (♀NT) is relatively more genetically pure than the 2nd female group (♀NF). While the 3rd male group (♂AD) was the lowest one (Brody, et al., 1976 & Badawy, 1993). These

results agreed with the results obtained from the field where the male ratios were 84.2 & 79.8% for the two crosses ♀NT X ♂AD and ♀NF X ♂AD, respectively. Badawy (1993) obtained (among different crosses) the male ratio of 89.5 & 85.5% for two crosses between two female groups of ***O. niloticus*** (originally brought from the Nile and Serow Fish Farm) and a male group of ***O. aureus*** (originally from the Farm), respectively where each of the three groups was firstly spawned intraspecifically in separate ponds for two generations before crossing them. Thus, it can be concluded that, obtaining high male ratio for any crossing between ***O. niloticus*** and ***O. aureus*** requires pure genetic lines of females and of males, for the two species, respectively. This could be achieved by continuous selection and selfspawning for each species in completely separate ponds before crossing them.

REFERENCES

Badawy, E.A., 1993. Biological studies on Tilapia species as a major component of the Egyptian farming system. Ph. D. Thesis, Fac. Sci., Zagazig Univ., pp: 222.

Basaglia, F., 1992. Comparative examination of soluble red muscle proteins of fifteen Sparidae species. J. Fish. Biol., 40: 557-566.

Brody, T.; Moav, R.; Abamson, Z.V.; Hulata, G. and Wohlfarth, G., 1976. Application of electrophoretical genetic markers to fish breeding. II-Genetic variation within maternal half-sibs in carp. Aquaculture, 9: 351-365.

Chen Y.F. and Tsuyuki, H., 1970. Zone electrophoretic studies on proteins of Tilapia mossambica and Tilapia hornorum and their hybrids, Tilapia zillii and Tilapia melanopleura. J. Fish. Res. Bd. Can., 27: 2167-2177.

El-Saied, H.E., 1993. Genetical studies on family Mugilidae in two different habitats of Egyptian waters. M. Sc. Thesis, Fac. Sci., Zagazig Univ. 157 pp.

Ferguson, A., 1980. Biochemical systematic and evolution. Glasgow. Blackie.

Fryer, G. and Iles, T.D., 1972. The cichlid fishes of great lakes of Africa-their biology and evolution. T.F.H. publications, Neptune, N.J., 641 pp.

Grag, R.W. and McKenzie, J.A., 1970. Muscle protein electrophoresis in the genus Salmo of eastern Canada> J. Fish. Res. Bd Can., 27 (11): 2109-2112.

Heines, R. and Yashouv, A., 1970. Preliminary studies on muscle protein polymorphism occurring within the Tilapia. Bamidgeh, 22: 69.

Herzberg, A. and Pasteur, R., 1975. The identification of grey mullet species by disc electrophoresis. Aquaculture, 5: 99-106.

Hickling, C.F., 1963. The cultivation of tilapia. Sci. Am., 208 (5): 143-152.

Kirpichnikov, R.S., 1981. The genetic bases of fish selection. Springer, Berling-Heidelberg - New York.

Lovshin, L.L., 1982. Tilapia hybridization. The biology and culture of tilapias. ICLARM Conf. Proc. 7, 432P. Manilla, Philippines.

Mires, D., 1982. Study of the problem of mass production of hybrid tilapia fry. P. 317-329. The biology and culture of Tilapias. ICLARM Conference Proceedings 7, 432 p.

Payne, R.H.; Child, A.R. and Forrest, A., 1971. Geographical variation in the Atlantic salmon. Nature, 231: 250-252.

Proginin, Y.; Rothbard, S.; Wohlfarth, G.; Halevy, A.; Moav, R. and Hulata, G., 1975. All-male broods of *Tilapia nilotica* X *Tilapia aurea*. Aquaculture, 6: 11-21.

Rothbard, S. and Progini, Y., 1975. Induced spawning and artificial incubation of tilapia. Aquaculture, 5: 315-321.

Siliem, T.A.E. and Badawy, E.A., 1995. The prospective supply of ***Oreochromis niloticus*** seds for the Egyptian farming systrem from the sewage-fertilized ponds. Bull. Facu. Sci. Zagazig Univ., 17 (1): zoology 181:192.

Tsuyuki, H., 1966. Comparative electrophorogram of hemoglobins and other fish proteins. Proceed. 11th, Pacif. Sci. Congr., 7:8.

Utter, F.N. and Hodgins, H.O., 1971. Biochemikcal polymorphgisms on the Pacific hak (Merluccis products). Rapp. Proc. Ver. Renu., 161: 87-89.

Wilkins, N.P., 1971. Biochemical and serological studies on Atlantic salmon (Salmon salar). Rapp. Proc. Verb. Reun., 161: 91-95.

Wohlfarth, G.W. and Hulata, G., 1981. The applied genetics of Tilapia. ICLARM Studies and Review. 6, 26 p. Manila, Philippines.