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COMPARATIVE STUDIES OF SOLUBLE PROTEIN IN TESTIS AND PITUITARY GLAND OF MALE MUGIL CEPHALUS DURING SEXUAL MATURITY IN DIFFERENT HABITATS

BY

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Key words : Isoelectric focusing – testis - pituitary gland - *Mugil cephalus*.

ABSTRACT

*A comparative study of the variation which occur in soluble protein in the testis and pituitary gland of male **Mugil cephalus** in both natural habitat (El-Bardawill) and captivity habitat (El-Serw) during different stages of sexual maturity, by using isoelectric focusing (IEF) method. The electrophoretic patterns of testis and pituitary gland proteins showed differences in terms of maturation stages. The investigated organ of each habitat has a characteristic stage – specific and isoelectric focusing patterns.*

INTRODUCTION

Mugil cephalus is considered as one of the most important commercial fish. It is a euryhaline species, therefore it play an important role within Egyptian fish culture. Naturally the breeding season takes place in Autumn months. Although spermatogenesis was noticed to be completed in males in both natural and captivity habitat under studies. Yet the fish do not spawn in such environments, since the pre-spawning females was migrated from EL-Bardawill lagoon (natural habitat) to the sea for spawning. At the same time, the fish reared at EL Serw fish farm (captivity habitat) can not, of course, reach the sea to spawn, beside the ovarian oocytes stages in captive females was not completed (Eckstein, 1975 and Mousa, 1994).

Many environmental and internal factors are thought to act as toles for the initiation of complex behavioral, physiological, biochemical and neuroendocrine changes controlling the reproduction of teleosts. Among these factors, photoperiod, temperature and salinity are the most important ones to initiate pituitary activity in fish in temperate and sub-temperate regions. However, the relative importance of each factor varies with different species of teleosts (Zaki ***et al***, 1995).

The adaptation of a population to certain, environmental conditions usually occurs by the way of natural selection, which finally results in a genetic differentiation in consequence of some genes falling out of the gene pool (Starmach, 1976). The number and rate of protein band migration in the electric field depend on their structure differentiated according to the genetic information coded in the DNA; for this reason the electrophoretic separation of proteins reflecting the protein structure included in the biochemical systematics (Baron, 1975 and Starmach, 1977).

The efficiency of the breeding programs often depends on gonads and pituitary gland of parental fish and their maturation stages. Protein analysis is an important method that demonstrates the proper identification of internal picture reflecting the protein structure, which included in tissue and distinguished according to the maturation stages. Numerous studies in Egypt accurred in some species; (EL-Halfawy. 1993) identified Nemipterus species in Suez-Gulf using isoelectric focusing.

EL-Gharabawy ***et al***., (1995) determined changes in soluble proteins in different maturation stages of gonads and plasma in both female and male ***Mugil seheli***. Abdo, (1996) studied the comparative analysis of proteins in gonad, plasma and pituitary gland of female and male ***Dicentrarchus labrax*** during the sexual maturation stages.

Assem, (1998) studied the changes in protein patterns of gonad and pituitary gland during maturation stages of ***Solea aegyptiaca*** in the Egyptian Mediterranean waters.

The present study aims to determine the variation which occur in the soluble proteins of testis and pituitary gland in male ***Mugil cephalus*** during sexual maturation in both marine and fresh water habitats.

MATERIAL AND METHODS

Fish samples:

The fish ***Mugil cephalus*** were collected monthly from El-Bardawill lagoon (natural marine habitat) and El-Serw fish farm (fresh water habitat) during the period from April 1997 to May 1998. The maturity stages of testis were identified through studies of their morphological and histological characters. The gonadosomatic indices of males GSI = gonad weight/gutted weight x 100) calculated at different testicular stages.

Sample preparation:

"0.1gm" of testes from each stage of maturity were homogenized with 1ml of cold tris Hcl (PH. 8.0) wheras a pool of five pituitaries were homogenized with 0.5ml of tris Hcl (PH. 8.0). The homogenates were centrifuged at 6000 rpm for 10 minutes. The clear supernatant was pipetted into vials and stored at (-20°C) until use.

Silver staining technique:

It was derived from the method of Heukshoven and Dernik (1985).

Sample application and isoelectric point measurements:

Technical procedure for sample application and isoelectric points (PI's) of protein were performed as described by pharmacia LKB, (1987). Gels were dried and scanned using Hoefer GS 300 catalogue number 10663/5-14-92.

All treatments were replicated three times and the mean values were calculated.

RESULTS

Maturity stages: On the basis of seasonal changes of testis based on histomorphology and gonadosomatic index. Pattern of testicular activity of fresh water fish of ***M. cephalus*** followed more or less the same course as that in saline water fish. Their testicular cycle can be classified into five stages according to Nikolisky (1963) and El-Gharabawy and Zaki (1990) with some modification. The scale of maturity used was as follows:

Stage I: Immature

Testis are thin ribbon shaped and contain small size lobules, within it was primary germ cell and spermatogonia. Their gonadosomatic index (GSI) are 0.049 ± 0.019 in marine water fish and 0.039 ± 0.013 in fresh water fish.

Stage II: Maturing

Testis are pinkish white and occupy one-third of the body cavity of fish. The active spermatogenesis began and the spermatocytes are dominant in the testicular components. At this stage the GSI was 0.085 ± 0.042 in marine water fish and 0.06 ± 0.015 in fresh water fish.

Stage III: Nearly Ripe

The testis enlarged in size to occupy two-thirds of the body cavity and are reddish white in colour. The spermatogenic activity reach its peak, this stage is characterized by predominance of spermatids and spermatozoa. With GSI values of 0.58 ± 0.22 and 0.305 ± 0.14 for marine and fresh water fish respectively.

Stage IV: Ripe

Testis had almost filled the whole body cavity of fish and are milky-white in colour. The testicular lobules appear fully packed with mature spermatozoa. The GSI was about 3.9 ± 1.40 for marine males and 1.66 ± 0.84 for fresh water males.

Stage V: Spent and Resorption

Spent testis of marine fish (natural habitat) are empty and dirty-white in colour. Their lobules were distored vacuolated and contain spermatogonia and residual sperm; their GSI's are 0.50 ± 0.39. The resorbed testis of captive males in fresh water habitat are much reduced in size and pale white in colour. The sperm was resorbed, and the spermatogonia cells were detected in testicular tissue. The GSI was 0.069 ± 0.035.

A- Testis protein

The differences in electrophoretic pattern of testis protein in male ***M. cephalus*** of two habitat fish reflected the changes in sexual activity with maturation as shown in tables (1& 2) and plates (1, 2).

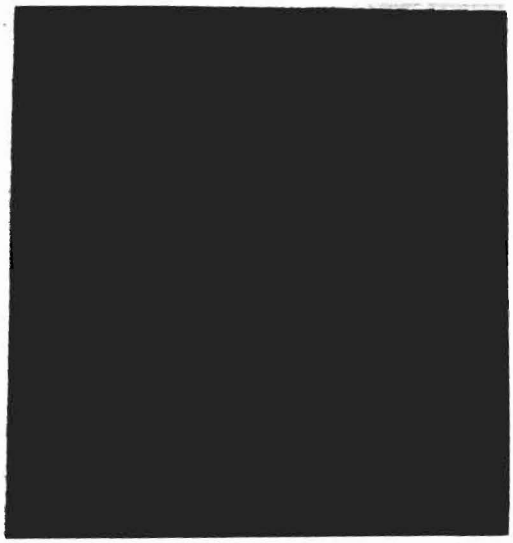

Plate (1): Isoelectric focusing of the testis protein of marine water fish at all stages of maturity in male of *M. cephalus* on polyacrylamide gel IEF (3-9): a – stages I; b – stage II; c & g standard protein calibration; – stage III; d – e – stage IV; f – stage V.

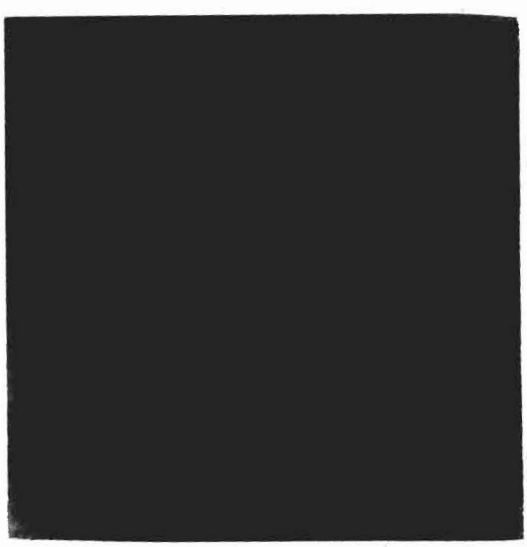

Plate (2): Isoelectric focusing of the testis protein of fresh water fish at all stages of maturity in male of *M. cephalus* on polyacrylamide gel IEF (3-9). The sequences are the same as in figure 1.

Table (1): The isoelectric point (PI's) values and their percentage of quantitative values of scanning protein pattern in testis of *Mugil cephalus* reared in marine water.

isoelectric point (PI's) values	percentage of concentration for different stages of maturity				
	I	II	III	IV	V
3.40	4.3 *	–	–	–	–
3.50	–	–	4.1 *	–	–
3.55	–	–	–	3.1	3.6
3.60	–	–	1.7	2.4	1.7
3.65	–	–	5.8 *	–	–
3.70	–	–	3.7 *	–	–
3.75	# 2.6	3.5	1.7	4.9	2.2
3.80	–	–	–	–	1.4 *
3.85	–	2.7	–	2.6	–
3.90	–	–	5.6 *	–	–
3.95	–	–	–	1.0 *	–
4.05	# 4.5	5.2	18.4	2.2	1.4
4.15	–	–	6.9 *	–	–
4.20	2.4	–	3.0	–	–
4.25	4.5	1.8	–	6.2	1.0
4.30	–	5.4 *	–	–	–
4.40	–	–	2.2 *	–	–
4.50	–	18.8 *	–	–	–
4.55	9.1 *	–	–	–	–
4.60	–	5.1 *	–	–	–
4.75	–	–	–	–	1.3 *
4.80	–	4.7	2.6	–	5.3
4.85	8.0	3.7	–	7.8	21.6
5.00	–	–	6.9	–	4.8
5.05	–	–	4.2	10.2	–
5.30	–	–	–	4.9	3.2
5.55	–	–	–	3.2	2.9
5.70	–	–	8.5	3.5	–
5.80	–	3.7	–	–	2.6
5.90	–	–	3.9 *	–	–
6.00	# 2.6	5.1	3.6	2.8	4.7
6.20	–	4.7	–	–	4.4
6.25	–	–	–	2.2 *	–
6.30	5.2 *	–	–	–	–
6.35	–	–	–	–	3.3 *
6.40	–	2.8	–	2.8	–
6.65	–	–	–	2.4 *	–
6.80	–	–	–	3.2 *	–
6.90	–	8.1 *	–	–	–
7.00	23.4 *	–	–	–	–
7.05	1.9	5.6	–	2.6	1.6
7.10	–	6.2 *	–	–	–
7.20	5.4	–	–	2.4	7.0
7.30	–	–	2.4	10.0	–
7.40	4.2	6.2	–	2.9	4.5
7.45	–	–	–	3.3 *	–
7.50	–	–	7.3 *	–	–
7.60	5.9 *	–	–	–	–
7.70	–	–	–	–	4.0 *
7.80	# 4.1	2.2	2.4	2.7	9.4
7.85	5.9 *	–	–	–	–
7.90	–	–	–	4.4	3.0
7.95	–	1.9	4.0	2.2	–
9.10	# 6.0	2.5	1.2	4.1	5.0

Common band
* Specific band

Table (2): The isoelectric point (PI's) values and their percentage of quantitative values of scanning protein pattern in testis of *Mugil cephalus* reared in fresh water.

isoelectric point (PI's) values	percentage of concentration for different stages of maturity				
	I	II	III	IV	V
3.40	1.6 *	–	–	–	–
3.50	3.2	2.8	–	–	–
3.55	–	–	1.3	2.5	
3.60	# 6.8	0.9	2.2	1.5	* 1.3
3.65	# 3.9	1.2	0.5	1.6	2.2
3.70	–	2.6 *	–	–	–
3.75	# 10.5	6.0	1.2	1.3	0.7
3.85	# 1.8	7.0	1.8	1.7	1.2
3.95	1.8	2.8	–	–	–
4.00	–	–	4.0	–	1.1
4.05	–	2.8 *	–	–	–
4.10	–	–	7.2	0.4	* –
4.15	1.8	–	–	–	0.6
4.30	–	–	7.8 *		–
4.35	–	–	1.7	4.4	–
4.40	–	–	–	–	0.5 *
4.50	–	–	–	5.1 *	–
4.55	–	–	2.4 *	–	–
4.60	–	–	4.4	–	0.8
4.70	–	–	–	15.7	1.2
4.80	–	–	2.2	–	11.0
4.85	–	–	–	5.6 *	–
4.90	–	–	1.9	–	15.3
4.95	–	–	–	–	–
5.00	–	3.5 *	–	–	–
5.05	–	2.4 *	–	–	–
5.10	–	–	15.6 *	–	–
5.15	1.7 *	–	–	–	–
5.20	–	2.6	4.0	–	–
5.30	2.5	16.5	10.2	–	–
5.40	1.6	16.7	6.5	–	3.1
5.45	–	–	6.4 *	–	–
5.55	–	–	4.6	1.9	3.0
5.60	20.6	8.1	–	–	3.5
5.70	–	–	–	2.7 *	–
5.80	# 8.7	5.5	6.5	1.7	3.0
5.90	5.1 *	–	–	–	–
6.00	–	–	–	–	2.1 *
6.05	–	7.8	–	–	5.0
6.25	–	2.8	–	–	5.2
6.35	–	–	1.8 *	–	–
6.40	8.8	3.7	–	7.8	3.8
6.45	–	–	–	9.7 *	
6.50	–	–	–	–	2.9 *
6.60	–	–	1.8 *	–	–
6.85		0.6 *	–	–	–
6.90	9.1 *	–	–	–	–
7.00	–	–	–	7.1 *	–
7.05	–	–	–		10.2 *
7.20	–	–	–	10.2 *	–
7.25	–	–	–	–	4.5 *
7.30	–	–	–	–	5.1 *
7.40	–	–	–	4.9 *	–
7.45	–	–	–	3.2 *	–
7.60	–	–	–	–	3.0 *
7.70	# 5.9	1.2	2.0	8.3	6.1
7.80	–	–	–	–	1.2 *
7.85	# 2.0	1.8	2.0	2.5	0.6
9.10	2.6	1.0	–	–	1.6

Common band
* Specific band

i- Marine water fish:

In stage I, seventeen protein bands were separated. Such a testis were characterized by six specific fractions at PI values of 3.40, 4.55, 6.3, 7.0, 7.6 and 7.85.

In stage II and stage III of maturity twenty and twenty-one protein fractions were separated from testis respectively. The stage II was identified by five specific bands separated at PI values of 4.3, 4.5, 4.6, 6.9 and 7.10. The stage III was identified by eight specific fractions separated at PI values of 3.5, 3.65, 3.7, 3.9, 4.15, 4.40, 5.90 and 7.50.

Stage VI had twenty-six protein bands. Five specific fractions were identified in this stage at PI values of 3.95, 6.25, 6.65, 6.80 and 7.45. In spent male (stage V) twenty-three protein fractions were separated and were characterized by four specific bands separated at PI values of 3.8, 4.75, 6.35 and 7.70.

In marine water fish, the testes were characterized by five common bands separated at PI values of 3.75, 4.05, 6.0, 7.8 and 9.10 with differences in their relative quantitative values according to maturity stages. The total number of protein fractions of exhibited a significant correlation with maturation ($R = 0.85$). The specific band number showed insignificant correlation with such total number ($R = -0.353$).

ii- Fresh water fish:

Nineteen protein fractions were separated from testis of fresh water male at stage I. The characteristic bands were identified at PI's values of 3.40, 5.15, 5.9 and 6.9. In stage II, twenty-two protein bands were separated from testis and were characterized by five bands separated at PI values of 3.7, 4.05, 5.0, 5.05 and 6.85.

Stage III was represented by twenty-four protein bands. The characteristic bands were identified at PI values of 4.30, 4.55, 5.10, 5.45, 6.35 and 6.60. Twenty-one protein bands were separated from testis of ripe male (IV). Eight specific bands were identified at PI values of 4.50, 4.85, 5.70, 6.45, 7.0, 7.20, 7.40 and 7.45.

In stage V (resorbed testes), twenty-eight bands were separated and could be identified by eight specific bands at PI values of 4.40, 6.00, 6.50, 7.05, 7.25, 7.30, 7.60 and 7.80.

In fresh water fish the electrophoretic patterns of protein in testis had seven common bands. These bands were present in all stages of maturity, but in different quantities. These bands were separated at PI's (3.60, 3.65, 3.70, 3.85, 5.80, 7.75 and 7.85. There were significant correlation between the total number of protein fractions and stage of maturity (R = 0.785). The number of specific bands exhibited same correlation trend with such total number (R = 0.62).

The total number of protein fractions of marine and fresh water testes exhibited no correlation with such total number in different stages of maturity (R = 0.378). The specific protein number of band in testes of two habitat exhibited insignificant correlation with stage of maturity (R =-0.424). One marked band only characterized the testes of two habitat was separated at PI value of 3.75.

B- Pituitary gland protein

For all stages of maturity the protein patterns of pituitary glands of male for both marine and fresh water fish *of **M. cephalus*** were given in tables (3 & 4) and plates (3 & 4).

i- Marine water fish:

In the immature male (stage I), eighteen bands were separated from the pituitary glands. Such a gland was characterized by four specific protein fractions at PI values of 4.40, 4.70, 5.30 and 6.45.

Twenty and twenty-one bands were separated from the pituitary glands of stage II and stage III of maturity. The pituitary gland at stage II was identified by five specific protein fractions at PI values of 4.05, 6.20, 6.50, 6.75 and 7.00, where as that at stage III was identified by six specific bands at PI of 3.70, 3.95, 4.90, 5.00, 5.65 and 6.80. In stage IV and stage V twenty eight and twenty protein bands were separated respectively. The pituitary at these stages were characterized by seven and five specific protein fractions at PI values of 4.00, 4.60, 5.40, 6.65, 7.05, 7.25, 7.85; and 3.55, 4.15, 4.40, 4.95 7.15 respectively (table 3)

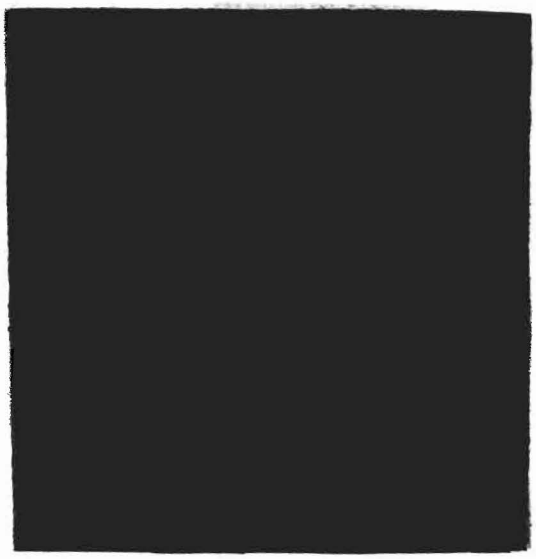

Plate (3): Isoelectric focusing of the pituitary gland protein in marine water fish at all stages of maturity in male of *M. cephalus* on polyacrylamide gel IEF (3-9). The seqtuences are the same as in figure 1.

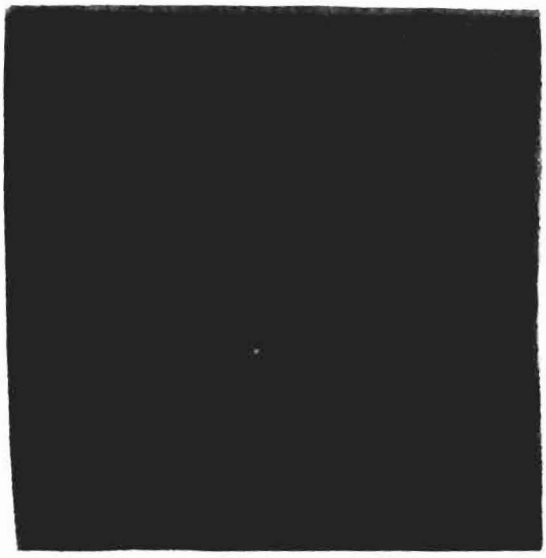

Plate (4): Isoelectric focusing of the pituitary gland protein in fresh water fish at all stages of maturity in male of *M. cephalus* on polyacrylamide gel IEF (3-9). The sequences are the same as in figure 1.

Table (3): The isoelectric point (PI's) values and their percentage of quantitative values of scanning protein pattern in pituitary gland of male *Mugil cephalus* reared in marine water.

isoelectric point (PI's) vlaues	percentage of concentration for different stages of maturity				
	I	II	III	IV	V
3.55	–	–	–	–	1.0 *
3.60	# 1.4	1.2	0.8	3.7	5.2
3.65	–	5.4	–	1.1	–
3.70	–	–	2.7 *	–	–
3.75	5.7	3.0	–	3.7	–
3.80	–	1.4	–	1.6	2.9
3.85	–	–	3.5	1.5	1.5
3.90	3.7	2.0	–	–	–
3.95	–	–	2.4 *	–	–
4.00	–	–	–	2.7 *	–
4.05	–	2.0 *	–	–	–
4.15	–	–	–	–	2.8 *
4.25	–	6.1	5.5	5.1	–
4.30	–	–	–	–	10.9 *
4.40	1.6 *	–	–	–	–
4.50	–	–	9.3		17.0
4.60	–	–	–	5.6 *	–
4.70	2.9 *	–	–	–	–
4.75	–	–	11.9	15.6	10.2
4.90	–	–	3.2 *	–	–
4.95	–	–	–	–	3.1 *
5.00	–	–	6.9 *	–	
5.15	2.3	–	2.4	–	2.6
5.30	3.6 *	–	–	–	–
5.40	–	–	–	3.7 *	–
5.50	–	13.2	–	–	3.6
5.55	12.4	–	–	2.7	–
5.65	–	–	3.8 *	–	–
5.70	–	–	–	2.3	2.5
5.80	8.2	2.4	–	–	–
5.90	6.9	4.5	5.9	2.2	–
6.10	6.1	–	–	2.0	–
6.20	–	4.5 *	–	–	–
6.25	2.3	2.9	7.7	2.5	–
6.40	26.2	8.9	8.2	2.6	–
6.45	6.3 *	–	–	–	–
6.50	–	6.4 *	–	–	–
6.65	–	–	–	1.3 *	–
6.75	–	18.8 *	–	–	–
6.80	–	–	3.1 *	–	–
6.90	–	–	–	8.9	3.6
7.00	–	11.4 *	–	–	–
7.05	–	–	–	4.4 *	–
7.10	5.1	–	13.1	–	–
7.15	–	–	–	–	5.4 *
7.20	–	–	2.7	5.8	–
7.25	–	–	–	3.6 *	–
7.40	–	–	–	3.1	4.6
7.60	–	1.4	–	2.7	3.5
7.65	–	–	–	1.5	3.7
7.75	# 3.5	1.0	1.9	4.8	6.1
7.80	0.6	–	3.0	–	4.8
7.85	–	–	–	1.1 *	–
7.95	–	2.3	1.0	1.5	–
9.10	# 1.3	1.2	0.8	2.9	5.1

Common band
* Specific band

Table (4): The isoelectric point (PI's) values and their percentage of quantitative values of scanning protein pattern in pituitary gland of male *Mugil cephalus* reared in fresh water.

isoelectric point (PI's) values	percentage of concentration for different stages of maturity				
	I	II	III	IV	V
3.60	–	–	–	–	1.7 *
3.70	# 3.3	1.4	0.5	2.8	1.9
3.75	3.8	–	3.8	1.3	2.5
3.80	–	–	–	2.1 *	–
3.85	7.1	–	3.3	1.9	1.3
3.90	–	–	3.9 *	–	–
3.95	–	–	–	1.0	10.7
4.00	17.8	–	–	3.2	3.7
4.05	–	–	8.9 *	–	–
4.15	–	3.7	–	1.1	18.1
4.20	–	–	22.6	2.6	–
4.25	–	–	–	–	11.8 *
4.30	–	–	10.9 *	–	–
4.50	6.8 *	–		–	–
4.60	–	–	3.5 *	–	–
4.65	–	1.8 *	–	–	–
4.90	–	–	–	13.0 *	–
5.30	–	–	–	–	3.5 *
5.40	3.9	3.3	–	–	–
5.45	–	–	5.0	–	9.9
5.50	3.4 *	–	–	–	–
5.55	–	2.5	9.4	–	2.0
5.60	4.8	2.6	–	–	–
5.80	# 8.6	1.6	11.8	9.9	5.6
5.90	12.4	–	–	11.3	–
6.00	–	4.5 *	–	–	–
6.10	8.0 *	–	–	–	–
6.20	–	22.7	–	3.4	–
6.35	–	5.5 *	–	–	–
6.40	–	3.7	–	8.5	8.1
6.45	2.8	3.4	–	–	–
6.60	5.9 *	–	–	–	–
6.75	–	–	–	9.2	2.4
6.80	–	7.9	–	11.3	5.2
6.90	1.4 *	–	–	–	–
7.00	–	3.1 *	–	–	–
7.05	2.9		–	4.4	–
7.15	–	11.1 *	–	–	–
7.20	–	2.8	–	5.3	–
7.30	–	–	–	1.5	3.4
7.40	–	7.9	4.2	2.3	–
7.45	–	1.7 *	–	–	–
7.55	3.1	–	–	2.7	–
7.60	2.9	1.4	–	–	–
7.65	–	4.3	4.3	–	–
7.75	# 1.1	2.9	2.0	1.1	5.4
7.85	–	–	4.7	–	0.8
9.10	–	–	1.3	–	1.7

Common band
* Specific band

At all maturity stages, the pituitary gland protein of marine male was characterized by three common bands at PI values of 3.60, 7.75 and 9.10 with different percentage of concentration for each stage. However, the total number of protein fractions of the pituitary gland exhibited insignficant correlation with maturation (R = 0.493). While the number of specific bands exhibited high significant with such total number (R = 0.92).

ii- Fresh water fish:

Eighteen protein bands were separated from the pituitary gland of immature male (stage I); five specific bands were identified at PI values of 4.50, 5.50, 6.10, 6.60 and 6.90. In stage II of maturity, twenty-one protein fractions were separated from pituitary gland. Six specific fractions were identified at PI values of 4.65, 6.00, 6.35, 7.00, 7.15 and 7.45.

In stage III and IV, sixteen and twenty-one protein fractions were separated respectively. The pituitary gland at stage III was identified by four specific protein fractions at PI value of 3.90, 4.05, 4.30 and 5.45. The characteristic bands of stage IV were identified at PI values of 3.80 and 4.90. At stage V nineteen protein bands were separated from the pituitary gland. Such a stage was identified by three specific bands at PI values of 3.60, 4.25 and 5.30.

The pituitary gland protein patterns of male of fresh water were identified by three common bands separated at PI values of 3.70, 5.80 and 7.75 with different quantitative values for each stage. The total number of protein bands of pituitary gland of male in this habitat showed no correlation with maturation (R = 0.15). The number of specific bands showed also no correlation with such total number (R = 0.074).

The total number of protein bands in pituitary gland of marine and fresh water male showed a weak correlation with such total number in different stages of maturity (R=0.46). Opposite correlation exhibited between the specific protein number of bands in pituitary glands of the two habitat through different stages of maturity (R=-0.69).

DISCUSSION

The Pattern of testicular activity of fresh water fish of *M. cephalus* followed more or less the same course as that in marine water fish. But the appearance of different stages month- wise was delayed considerably in fresh water and with relatively low GSI's. However, because of the impossibility of spawning in the two habitats, active males do not occur. Similar findings were reported by Mousa, 1994 for the same species of ***M. cephalus***. The GSI is an important parameter that can be used to reflect the effect of environmental factors on the gonadal activity of the fish (Zaki, ***et al***. 1995).

The electrophoretic separation of soluble protein by isoelectric focusing gave various patterns distinguishing the investigated tissue and their stages of maturation in each habitat in which fish live. These patterns were based upen the migration of a given protein to a fixed point within a stable PH gradient under the influence of an electric field. The fixed point where protein migration ceases, the isoelectric point (PI), corresponds to the PH at which the protein has neutral changes.

In the present results, the electrophoretic patterns of testis and pituitary glands of male in ***M. cephalus*** showed great differences reflecting the activity in maturities and the role of the environmental habitat in the process of maturation. Each stage in each habitat exhibited a distinct protein pattern and could be clearly differentiated by number of specific bands. Thus the present investigation supported the work of EL-Gharabawy and Zaki (1990) in ***Mugil capito***; El-Gharabawy ***et al***. (1995) in ***Mugil seheli;*** Abdo (1996) in ***Dicentrarchus labrax***; and Assem (1998) for ***Solea aegyptiaca***. They revealed that gonad and pituitary protein of male and female showed great differences corresponding to maturation stages and was characterized by a number of specific bands.

In the present work testis protein patterns of marine and fresh water males were characterized by five and seven common fractions respectively, each fraction exhibiting specific PI values. One marked band only characterized the testis of the two habitats was separated at PI value of 3.75. In the present study the protein patterns of testis showed a gradual increase in the number of bands reaching its maximum in the ripe testis of marine fish (R = 0.85) and in resorbed testis of fresh water fish (R = 0.785).

The great differences between the number of bands at each stage of maturity in marine and fresh water testes of the two habitats may reflect an intimate relationship between the maturation of germinal cells and nuclear protein composition. In agreament with our results Ando, ***et al.*** (1973) and Olivares ***et al.***, 1990 who suggested that spermatogenesis offers an excellent model to investigate the relationship between changes in nuclear protein composition and structural and functional translations that chromatin undergoes during the differentiation of the germinal cell line.

EL-Halfawy, (1993) determined the variation which occur in the gonad proteins during maturation stages. He also found differences between species in number and concentration of bands.

Environmental factors such as photoperiod, temperature and salinity were elucidated to influence reproductive activities in both male and female fish (Lee and Weber, 1986 and Micale, ***et al.***, 1987). It seems likely that such factors affect spermatogenesis and spawning in ***M. cephalus*** (Mousa 1994).

In the present results no obvious trend was recorded between number of bands of testis protein in marine and fresh water fish habitat (R = 0.38) for total number and (R = 0.42) for specific number.

Results of pituitary gland protein fraction of ***M. cephalus*** showed a wide variations in the electrophoretic patterns. These variations indicated a certain specificity of band for each stage of maturity in each habitat in which fish lives. The pituitary gland is believed to play an important role in the maturation of the gonads.

Assem (1992) in ***Oblada melanura*** observed that the pattern of pituitary gland protein showed a marked intraspecific differences for male and female and revealed characteristic bands for each sex. She also found that significant variations were found which could be attributed to degree of maturity stage.

The electrophoretic pattern of pituitary protein of marine and fresh water habitat characterized by the same number of common bands (three bands). Each one exhibited a specific PI values and quantitative values which were used for the identification of their stage and habitat. One marker band characterized the pituitary protein of the two habitats isolated at PI value of 7.75. the differences in the number of pituitary protein bands between marine and fresh water males of ***M. cephalus*** showed a weak correlation (R=0.46) with total number and

opposite correlation (R = - 0.69) with the specific number.

The characteristic band of proteins in both testis and pituitary glands of marine habitat was separated at PI value of 9.10 and in fresh water habitat at PI value of (5.80).

The present variation in testis or pituitary protein patterns linked to the environmental habitat in the present study may be discussed by Starmach (1976) who found the variability in the structure of proteins and thus the occurrence of a system which has an influence on the physiological adaptation of individual population to the condition of environment in which they live.

From the previous results, it could be concluded that the electrophoretic patterns of testis and pituitary gland proteins of ***M. cephalus*** showed great differences between them in terms of maturation stages. For each habitat the investigated organ has a characteristic stage specific and isoelectric focusing patterns. The patterns obtained in marine fish (testis and pituitary gland) are more clearly differentiated with maturity stages than those obtained in fresh water habitat.

REFERENCES

Abdo, M. A., 1996. Reproductive biology and induced spawning of ***Dicentrarchus labrax***. ph. D. Thesis, Faculty of Science, Alexandria University.

Ando, I., Yamaskaki, M. and Suzuki, K., 1973. Protamines: Isolation, characterization and function. Springer-verlag, New York.

Assem, S. S., 1992. Reproductive biology and physiology of one species of family sparidae in Mediterranean Sea. M.S.c. Thesis. Fac. of Science, Alexandria University.

Assem, S. S., 1998. Changes in protein patterns of gonad and pituitary gland during maturation stages of ***Solea aegyptiaca*** of the Egyptian Mediterranean waters. Bull. Fac. Sci, Assiut Univ., 27 (1-E), 81 – 96.

Baron, J. C., 1975. Note sur le serum de 4 especes de tilapia: ***Tilapia mossambica*** Peters,1852, ***T. nilotica*** (Linnaeus 1758), ***T. zillii*** (Gorvais 1848) et *T. macrochir* Boulenger 1912. Cahiess O.R.S.T.O.M Ser Hydrobiol. 9: 19 – 24.

Eckstein, B., 1975. Possible reasons for the infertility of grey mullets confined to freshwater. Aquaculture, 5: 9 – 17.

El-Gharabawy, M. M. and Zaki, M. I., 1990. Comparative study of protein pattern in gonads and pituitary gland of ***Mugil capito*** in relation to maturation stage using phast-system isoelectric focusing., Assiut, J. Agric. Sci. 21 (3): 283 – 299.

El-Gharabawy, M. M.; Zaki, M. I., Salem, S. B.; El-Shorbagy, I. K. and El-Boray, K. F., 1995. Changes in electrophoretic pattern of gonads and plasma protein during maturation stages in ***Mugil seheli*** in Suez Bay, Bull. Assiut. J. Agric. Sci. Vol. 21 (3): 301 – 311.

El-Halfawy, M. M., 1993. Reproductive studies of ***Nemipterus japonicus*** in Gulf of Suez. Unpublished M. Sc. Thesis, Faculty of Science, Suez Canal University.

Heukeshoven, J. and Dernick, R., 1985. Simplified method for silver staining of proteins in polyacrylamide gels and the mechanism of silver staining. Electrophoresis, 6: 103 – 112.

Lee, G. S. and Weber, G. M., 1986. Effects of salinity and photoperiod on 17 - methyltestestorone – induced spermatogenesis in the grey mullet (***Mugil cephalus*** L.) Aquaculture, 56: 53 – 62.

Micale, V.; Perdichizzi, F. and Santangelo, G., 1987. The gonadal cycle of captive white bream, ***Diplodus sargus*** (L.). J. Fish. Biol., 31: 435 – 440.

Mousa, M. A., 1994. Biological studies on the reproduction of mullet (***Mugil cephalus*** L.) in Egypt. ph. D. Thesis. Faculty of Science, Ain Shams University.

Nikolisky, C. R. , 1963. The Ecology of Fishes. Academic Press: London.

Olivares, C., Valdivia, R., Lafuente, N., Kulak, D and Kasinsky, H. E., 1990. Electrophoretic analysis of sperm basic proteins in ***Schroederychthys chilensis*** and comparison with other cartilaginous fish, Rev. Biol. Mar., Valparaiso, 25 (1): 99 – 108.

Pharmacia, L.K.B., 1987. Phast-system separation technique S – 75812 Uppsala, Sweden.

Starmach, J., 1976. Electrof rtyczny rozdzial bialek surowicy krwi i esteroz kielbi (***Gobio gobio*** L.) Zyjacych w roznych rzekach – Electrphoretic separation of proteins of blood serum and esterases in gudgeon (***Gobio gobio*** L.) living in various rivers. Acta Hydrobiol. 18 (2): 167 – 173.

Starmach, J., 1977. Electroforetyczny rozdzial na Żelu poliakryloamidowym bialek surowicy krwi siedmiu rodzin karpi - Electrophoretic separation of blood serum proteins on polyacryloamide gel in seven carp families. Acta. Hydrobiol. 19 (2): 163 – 167.

Zaki. M. I.; El-Gharabawy, M. M., El-Greisy, Z. A. and Baghdadi, H. H., 1995. Effect of some environmental and physiological factors on some reproductive parameters of ***Mugil capito*** during the breeding season. Bull. Nat. Inst. Of Oceanogr. & Fish., A.R.E. Vol. (21) No 2: 585 – 604.