



**BIOLOGICAL STUDIES ON THE
REPRODUCTION OF MULLET
(*Mugil cephalus* L.) IN EGYPT**

A THESIS SUBMITTED

BY

MOSTAFA ABD EL-WAHHAB EL-BAYOMY MOUSA

ASSISTANT LECTURER OF FISH BIOLOGY
NATIONAL INSTITUTE OF OCEANOGRAPHY AND FISHERIES

TO

THE FACULTY OF SCIENCE
AIN SHAMS UNIVERSITY

FOR

THE AWARD OF THE Ph. D. Degree
IN
ZOOLOGY - FISH BIOLOGY

SUPERVISED

BY


Dr. MAHMOUD A. EL-BANHAWY

PROF. OF EXPERIMENTAL ZOOLOGY
(Cell Biology and Histochemistry)
FACULTY OF SCIENCE - AIN SHAMS UNIVERSITY

Dr. MAGDA I. ZAKI

PROF. OF FISH REPRODUCTION
AND HEAD OF AQUACULTURE DIVISION
NATIONAL INSTITUTE OF OCEANOGRAPHY AND FISHERIES

Dr. SAID A. KAMEL

ASSOCIATE PROF. OF FISH REPRODUCTION
NATIONAL INSTITUTE OF OCEANOGRAPHY AND FISHERIES

1994

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿ وجعلنا من الماء كل شيء حي ﴾
صدق الله العظيم



To: My Parents

To: My Family

ACKNOWLEDGMENT

Firstly , I would like to offer my humble thanks to my GOD who has granted me the ability to accomplish this work .

It also gives me great pleasure to express my sincere gratitude and appreciation to Prof.Dr.Mahmoud A.El-Banhawy, Professor of Experimental Zoology (Cell Biology and Histochemistry), Department of Zoology , Faculty of Science ,Ain Shams University, for his continuous supervision, fruitful guidance, constructive criticism and critical reading of the manuscript .

Particular appreciation is also due to Prof. Dr.Magda I. Zaki, Professor of Fish Reproduction and Head of Aquaculture Division , National Institute of Oceanography and Fisheries , for planning this research, keen supervision and for the facilities she has kindly supplied to me.

My cordial thanks also go to Dr. Said A.Kamel, Associate Professor of Fish Reproduction, National Institute of Oceanography and Fisheries, for his constructive share in supervision, his continuous encouragement and valuable advice during the study .

Thanks are also due to my colleagues in the Laboratory of Fish Reproduction for their undeniable role in this work .

The assistance, support and encouragement offered to me from the Department of Zoology,Faculty of Science ,Ain Shams University are highly acknowledged.

Last and not least,I feel much grateful to my family for the continuous help and faithful encouragement extended to me during the progress of this work .

CONTENTS

	Page
LIST OF TABLES	i
LIST OF FIGURES	ii
CHAPTER :I	
INTRODUCTION	1
AIM OF THE WORK	4
REVIEW OF LITERATURE	5
.Identification of fishes by muscle protein electrophoresis .	5
.Histological structure and seasonal changes of the gonads and pituitary gland .	6
.Gonadotropin (s) and sex steroids during both reproductive cycle and induction of oocyte growth and maturation.	12
CHAPTER :II	
MATERIAL AND METHOD	16
.Environmental Factors	16
.Fish Collection .	18
.Boold Sampling .	18
.Experimental Design	19
- Experiment I:Effects of exogenous hormone treatment on oocyte growth .	19
- Experiment II : Effects of exogenous hormone treatment on oocyte maturation and changeover <i>M.cephalus</i> female to the spawning condition.	20
PhastSystem Isoelectric focusing (IEF) Method .	23
Preparation of extracts for isoelectric focusing .	23
Sliver staining technique .	24
Sample application and isoelelctric fousing (IEF)	24
Isoelectric point measurement .	24
Histological and Histochemical Preparations .	25
Quantitative Measurements	27

	Page
Radioimmunoassay (RIA)	27
- Gonadotropin (GTH) Radioimmunoassay .	27
- Steroid Radioimmunoassay.	28
Statistical Analysis	28
CHAPTER :III	
RESULTS AND OBSERVATIONS	29
I. Identification of <i>Mugil cephalus</i> by muscle protein isoelectric focusing .	29
II. Structure of the gonads and pituitary glands	33
The Gonads	33
The Testis	33
Morphology	33
Histology	33
The spermatogenic stages	36
Testicular cycle in saline water	41
Testicular cycle in fresh water	60
The ovary	64
Morphology	64
Histology	64
Oogenesis	66
Cytochemistry of oogenesis	66
Oil droplets	66
Yolk globules	67
Cortical granules	73
Ovarian cycle	88
A) Ovarian cycle in saline water	88
B) Ovarian cycle in fresh water	101
Degeneration of the oocytes	103
Follicular atresia	103
The Pituitary Gland	106
Morphology	106
Histology	106

	Page
Cyclic changes in the pituitary gland	126
A) In male fish	128
B) In female fish	134
III. Cyclic changes in gonadosomatic index and serum levels of gonadotropin and sex steroids .	145
A) Males :	145
1- The gonadosomatic index (GSI) .	145
2- Gonadotropin hormone (GTH) .	146
3- Progesterone	149
4- Testosterone	152
5- Estradiol- 17 β	155
B) Females :	158
1- The gonadosomatic index (GSI) .	158
2- Gonadotropin hormone (GTH) .	159
3- Progesterone	162
4- Testosterone	165
5- Estradiol- 17 β	168
IV. Effects of exogenous hormone treatment on growth and maturation of the oocytes of <i>M.cephalus</i> in captivity .	172
V. Effects of exogenous hormone treatment on gonadosomatic index and serum levels of gonadotropin and sex steroids during inducing growth and maturation of oocytes of <i>M.cephalus</i> in captivity :	182
1- The gonadosomatic index (GSI)	182
2- Gonadotropin hormone (GTH)	185
3- Progesterone	190
4- Testosterone	193
5- Estradiol- 17 β .	196
CHAPTER :IV	
DISCUSSION	200
.Identification of <i>M.cephalus</i> by muscle protein isoelectric focusing	200

	Page
.Structure of the gonads and pituitary glands .	201
.Cyclic changes in gonadotropin and sex steroids .	220
.Effects of exogenous hormone treatment on the levels of gonadotropin hormone (GTH) and sex steroids during inducing growth and maturation of oocytes of <i>M.cephalus</i> in captivity .	231
CHAPTER :V	
SUMMARY AND CONCLUSIONS	235
CHAPTER :VI	
REFERENECES	245
CHAPTER :VII	
ARABIC SUMMARY	

LIST OF TABLES

Table No.	Page
1. The experimental design and the protocol of hormone injection	22
2. The isoelectric point (PI's) values and % of concentration of scanning protein fractions of muscle.	32
3. Gonadosomatic index of males <i>M.cephalus</i> .	50
4. Monthly variations in the frequency (%) of testicular stages.	61
5. Monthly variations in the frequency (%) of ovarian stages.	90
6. Gonadosomatic index of females <i>M.cephalus</i> .	92
7. Oocytes diameter of females <i>M.cephalus</i> .	94
8. Serum gonadotropin hormone levels of males.	147
9. Serum progesterone hormone levels of males.	150
10. Serum testosterone hormone levels of males.	153
11. Serum estradiol-17 β hormone levels of males.	156
12. Serum gonadotropin hormone levels of females.	160
13. Serum progesterone hormone levels of females.	163
14. Serum testosterone hormone levels of females.	166
15. Serum estradiol-17 β hormone levels of females.	169
16. Effect of human chroric gonadotropin treatment on the frequency of ovarian stages.	174
17. Changes occurring in the ovary during the induction of oocytes maturation.	178 - 180
18. Gonadosomatic index of hormones injected females.	183
19. Oocytes diameter of hormones injected females.	186
20. Serum gonadotropin hormone levels of injected females.	188
21. Serum progesterone hormone levels of injected females.	191
22. Serum testosterone hormone levels of injected females.	194
23. Serum estradiol-17 β hormone levels of injected females.	197

LIST OF FIGURES

Figure No.	Page
1. Map showing the location of the Badawill lagoon and El-Serw Fish Farm.	17
2. Isoelectric focusing of muscle protein	30
3. The electrophoretograms and densitograms of muscle proteins	31
4. Section of the testis showing its shape.	34
5. Section of the testis showing its structure.	35
6. Section of the testis showing the spermatogenesis	37
7. Section of the testis showing the interstitial and Sertoli cells.	37
8. Section of the testis showing parachute shape of spermatozoa.	39
9. Section of the testis showing parachute shape of spermatozoa and the interstitial cells.	40
10. Testis section of immature male from saline water	42
11. Testis section of <i>M. cephalus</i> male during stimulating spermatogenesis from saline water.	43
12. Testis section of <i>M. cephalus</i> male during rapid spermatogenesis from saline water.	44
13. Section of ripe testis from saline water	45
14. Testis section of immature male from fresh water	46
15. Testis section of <i>M. cephalus</i> male during stimulating spermatogenesis from fresh water.	47
16. Testis section of <i>M. cephalus</i> male during rapid spermatogenesis from fresh water.	48
17. Section of ripe testis from fresh water.	49
18. Gonadosomatic index of male <i>M. cephalus</i>	51
19. Ovarian and testicular cycles of <i>M. cephalus</i> in relation to annual fluctuation in day length (photoperiod) and water temperatures.	52

Figure No.	Page
20. Section of immature testis	54
21. Testis section of <i>M. cephalus</i> during rapid spermatogenesis	56
22. Section of ripe testis	58
23. Section of spent testis	59
24. A magnified portion of the above section	59
25. Monthly variations in the frequency (%) of testicular stages.	62
26. Section of the ovary showing its shape.	65
27. Section showing the ovarian wall.	65
28. The distribution of general lipid in the vesicles oocyte and in the primary yolk oocyte.	68
29. The distribution of general lipid in the secondary yolk oocyte.	68
30. The distribution of general lipid in the tertiary yolk oocyte.	69
31. Coalescence of oil droplets in the ripe oocyte.	69
32. The proteid yolk in the vesicles oocyte and primary yolk oocyte.	70
33. The accumulation of proteid yolk globules in the secondary yolk oocyte.	70
34. The accumulation of proteid yolk globules in the tertiary yolk oocyte.	71
35. The accumulation of proteid yolk globules in the ripe oocyte.	72
36. The proteolysis and homogenization of proteid yolk in the ripe oocyte.	72
37 The cortical alveoli of the primary yolk oocytes.	74
38. The cortical alveoli of the secondary yolk oocytes	74
39. The cortical alveoli of the tertiary yolk oocytes	75
40. The cortical alveoli of the ripe oocyte.	76
41. A magnified portion of the above section.	76
42. Ovary section of <i>M. cephalus</i> during the period of previtellogenesis.	78

Figure No.	Page
43. Ovary section of <i>M. cephalus</i> during the period of early vitellogenesis.	80
44. The beginning of yolk deposition as yolk granules.	80
45. The proteid yolk of vesicles oocyte and the primary yolk oocyte.	81
46. The sudanophilic inner layer of oil droplets of the vesicles oocyte.	81
47. Ovary section of <i>M. cephalus</i> during the period of mid-vitellogenesis.	82
48. The distribution of oil droplets in the primary yolk oocytes.	84
49. The wall of the primary yolk oocyte.	84
50. Ovary section of <i>M. cephalus</i> during the period of late-vitellogenesis.	85
51. The wall of the secondary yolk oocyte.	85
52. Ovary section of <i>M. cephalus</i> during the prespawning period.	87
53. The wall of the tertiary yolk oocyte.	87
54. Section of ripe ovary.	89
55. The homogenized yolk in the ripe oocyte.	89
56. Monthly variations in the frequency (%) of ovarian stages.	91
57. Gonadosomatic index of female <i>M. cephalus</i> .	93
58. Oocytes diameter of females <i>M. cephalus</i> .	95
59. Section of the spent ovary.	100
60. Section of the ovary of <i>M. cephalus</i> from fresh water showing the atretic oocytes.	102
61. Ovary section of <i>M. cephalus</i> from fresh water at resorption stage.	104
62. Ovary section of <i>M. cephalus</i> from fresh water showing the follicular atresia.	104

Figure No.	Page
63. Ovary section of <i>M. cephalus</i> from fresh water at resorption stage showing the degeneration of zona radiata.	105
64. Ovary section of <i>M. cephalus</i> from fresh water at resorption stage showing the phagocytosis of proteid yolk.	105
65. Section of degenerating ovary showing the disturbance of cortical alveoli arrangement.	107
66. Section of degenerating ovary showing the move of oil droplets out of the atretic oocyte.	107
67. Section of degenerating ovary showing the invade of phagocytic follicular cells for the yolk material.	108
68. Midsagittal section of the pituitary gland of <i>M. cephalus</i> .	109
69. Section showing the prolactin cells of saline water fish.	110
70. Section showing the prolactin cells of fresh-water fish.	110
71. Section of the pituitary gland showing the orangeophilous prolactin cells.	112
72. Section of the pituitary gland showing the adrenocorticotrophic hormone cells, prolactin cells and thyrotrophs.	113
73. A magnified portion of the above section.	113
74. Section of the pituitary gland showing the pbH ⁺ adrenocorticotrophic hormone cells.	114
75. Section of the pituitary gland showing the somatotrophs and two types of gonadotrophs.	116
76. Section of the pituitary gland stained with AB-PAS-OG.	116
77. Section of the pituitary gland stained with Heidenhain's Azan	118
78. Section of the pituitary gland stained with Mallory's stain.	118
79. Section of the pituitary gland stained with PAS-pbH-OG.	119
80. Section of the pituitary gland stained with aldehyde fuchsin.	119
81. Section of the pituitary gland showing two types of gonadotrophs.	120

Figure No.	Page
82. Section of the pituitary gland showing the thyrotrophs.	121
83. A magnified portion of the above section.	121
84. Section of the pituitary gland showing the thyrotrophs, gonadotrophs and somatotrophs.	122
85. A magnified portion of the above section.	122
86. Section of the pituitary gland showing the cell types in the pars intermedia.	124
87. Section of the pituitary gland showing the PAS ⁺ cells and pbH ⁺ cells in the pars intermedia.	124
88. Section of the pituitary gland stained with Heidenhain's Azan, showing the pars intermedia.	125
89. Section of the pituitary gland showing pituicytes and neurohypophyseal fibres in the pars intermedia.	127
90. Section of the pituitary gland showing the neurosecretory material.	127
91. Section of the pituitary gland of <i>M. cephalus</i> immature male.	129
92. Section of the pituitary gland of <i>M. cephalus</i> male obtained during the period of stimulating spermatogenesis.	131
93. Section of the pituitary gland of <i>M. cephalus</i> male obtained during the period of rapid spermatogenesis.	131
94. Section of the pituitary gland of ripe male.	133
95. A magnified portion of the above section.	133
96. Section of the pituitary gland of <i>M. cephalus</i> spent male.	135
97. A magnified portion of the above section.	135
98. Section of the pituitary gland of <i>M. cephalus</i> female obtained during the period of previtellogenesis.	137
99. Section of the pituitary gland of <i>M. cephalus</i> female obtained during the period of early-vitellogenesis.	137