

EFFECTS OF ENVIRONMENTAL POLLUTANTS ON REPRODUCTIVE PERFORMANCE OF FARMED FISH

By

KAREEM MOHAMED AHMED AHMED

B.Sc. Agric. Sc. (Fish Production), Ain Shams University, 2002

M.Sc. Agric. Sc. (Animal physiology), Ain Shams University, 2009

A thesis submitted in partial fulfillment

of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

**Agricultural Sciences
(Animal Physiology)**

Department of Animal Production

Faculty of Agriculture

Ain Sham University

2015

EFFECTS OF ENVIRONMENTAL POLLUTANTS ON REPRODUCTIVE PERFORMANCE OF FARMED FISH

By

KAREEM MOHAMED AHMED AHMED

B.Sc. Agric. Sc. (Fish Production), Ain Shams University, 2002

M.Sc. Agric. Sc. (Animal Physiology), Ain Shams University, 2009

Under the supervision of:

Dr. Amin Abd El-Mouaty El-Gamal

Prof. Emeritus of fish Breeding, Department of Animal Production,
Faculty of Agriculture, Ain Shams University. (Principal supervisor)

Dr. Mohamed Fathy Osman

Prof. Emeritus of Fish Nutrition, Department of Animal Production,
Faculty of Agriculture, Ain Shams University

Dr. Mohamed Abdel Baky Amer

Associate Prof. of Fish Physiology, Department of Animal
Production, Faculty of Agriculture, Ain Shams University

ABSTRACT

Kareem Mohamed Ahmed Ahmed: Effects of Environmental Pollutants on Reproductive Performance of Farmed Fish. Unpublished Ph.D. Thesis, Department of Animal Production, Faculty of Agriculture, Ain Shams University, 2015.

The testis-ova or ovo-testis is the presence of female germ cells, or oocytes, within male gonads, it has been used as biomarker for detecting xenoestrogens exposure.

Two screening experiments in 2011 and 2012 were performed to detect signs of estrogenic compounds on male Nile tilapia *O. niloticus* fish inhabit Lake Manzala. Experimental samples were collected from five drainage endpoints areas 1) El-Bashtir, 2) El-Gamil, 3) El-Inaniya, 4) El-Sirw, and 5) El-Temsah where water was contaminated with different mixture of industrial, domestic and agriculture pollutants. In addition, Nile tilapia males were exposed to three different concentrations of estrogen like compound nonylphenol ethoxylate as a laboratory experiment. The contamination dosages of nonylphenol ethoxylate (NPE) were 25, 50 and 100 µg/l administrated for 126 days in rearing tanks, to be comparable with the data obtained from the above-mentioned locations. Eighteen to twenty wild male samples were dissected; both gonads and liver were taken the GSI and HSI was calculated and processed for histological examination.

The presence of oocytes within the testicular tissue was explicit; hence, severity index (0-4) was developed based on the number of oocytes per testicular tissue section. The number of histological section examined to clearly detect intersex in tilapia was 4-6 section per fish during the two sampling seasons; likewise, were done for the lab experiment. From each histological section, three microscopic fields were examined to determine the developmental stage in each fish; and 100% of the three sections were examined for the presence of tests-ova.

In the survey part, staging males gonads showed stages II Early spermatogenesis to stage IV Late spermatogenesis of normal histological development of the testes. Testes-ova (To) were found in all collection sites and most collected samples in both seasons with different occurrence percentage and severity. Although, females showed seasonal differences in the first season; stage (VI) spent of normal histological development of the ovaries appeared to be the common stage with almost 100% of the collected samples. Stages (III) cortical alveoli formation to stage (V) Ripe, where detected in the second season with almost all cases. A few atretic oocytes were found in both seasons due to seasonal differences and the multi-cyclic reproductive pattern of tilapia. In lab experiment, the percentages of testis-ova calculated from individuals of the experiment were 10, 28 and 50% for the treated groups 25, 50 and 100 $\mu\text{g l}^{-1}$, respectively. Furthermore, severity on all examined samples in the lab experiment were minimal to mild (1-3 testis-ova/section). Where, overall severity was mild for 25 $\mu\text{g l}^{-1}$ group and minimal for 50 and 100 $\mu\text{g l}^{-1}$ groups.

Key words: Xenoestrogens, Lake Manzala, Testis-ova, Endocrine disrupting chemicals, *O. niloticus*, Nonylphenol ethoxylate.

ACKNOWLEDGMENT

Firstly, all praise to Allah; the Magnificent, the merciful, without whose bless and guidance this work would never have been started nor completed.

All thanks and utmost respect and gratitude to my supervisors, **Prof. Dr. Amin Abd El-Mouaty El-Gamal, Prof. Dr. Mohamed Fathy Osman and Prof. Dr. Mohamed Abdel Baky Amer**, Fish Production Branch, Animal Production Department, Faculty of Agriculture, Ain Shams University for their guidance, technical and incorporeal support, encouragement, instructive discussion and constructive criticism throughout this study.

I would like to express my thanks to the **Academy of Scientific Research and Technology** for providing financial support to this study.

I would like to express my sincere gratitude to **Dr. Ahmed Abdo Hal**, Researcher, National Institute of Oceanography and Fisheries, for his help during sample collection from Lake Manzala.

I also wish to express my sincere thanks to all my colleagues and workers of Fish Production Branch and Animal Production Department for each help they offered throughout this study. Special thanks and sincere gratitude to my family for their patience and enthusiastic support throughout my journey to achieve this work.

LIST OF CONTENTS

No.		Page
I.	INTRODUCTION.....	1
II.	REVIEW OF LITERATURE.....	4
2.1.	Lake Manzala.....	4
2. 1. 2.	Characteristics of Lake Manzala.....	4
2.1.3.	Lake Manzala Fish production.....	6
2.1.4.	Contaminants monitored in Lake Mazala.....	7
2.2.	Tilapia Reproduction.....	10
2.3.	Hormonal Disruptors.....	10
2.3.1.	Hormonal Disruptors Mode of action.....	11
2.4.	Nonylphenol ethoxylate (NP9) physical and chemical properties.....	12
2.4.1.	NPEs world production and Usage.....	13
2.4.2.	Environmental occurrence and Environmental fate.....	16
2.4.3.	Metabolism and excretion.....	17
2.4.4.	Estrogenic effects of NP and NPE.....	18
2.5.	Vitellogenin.....	19
2.6.	Reproductive and developmental effects.....	20
III.	MATERIALS AND METHODS.....	24
3. 1.	Survey location Part I.....	24
3.1.1.	Sampling locations description.....	24
3.1.2.	Fish Samples.....	24
3.1.3.	Water Parameters.....	24
3.2.	Part II Laboratory Experiment.....	27
3.2.1.	Environmental factors.....	27
3.2.2.	Chemicals.....	27
3.2.3.	Experimental fish and management.....	28
3.2.4	Experimental Design.....	29
3.2.5	Nutritional parameters and feeding.....	29
3.2.5.1.	Fish samples and measurements.....	30
3.2.6.	Physiological and Morphological parameters.....	30

3.3.	Histology.....	31
3.3.1.	Males.....	31
3.3.2.	Females.....	32
3.4.	Statistical analysis.....	32
IV	RESULTS AND DISCUSSION.....	34
	Part I Survey.....	34
4.1.	Morphological parameters.....	34
4.2.	Gonadosomatic index (GSI).....	35
4.3.	Hepatosomatic Index (HSI).....	35
4.4.	Gonadal staging.....	36
	Part II Laboratory Experiment.....	47
4.5.	Anatomical and morphological parameters.....	47
4.6.	Survival rate.....	47
4.7.	Liver weight and Hepatosomatic index (HSI).....	49
4.8.	Gonads weight and GSI.....	51
4.8.1.	Testicular histology.....	52
V	CONCLUSION.....	58
VI	SUMMERY.....	59
VII	REFERENCES.....	63
VIII	APPENDICES.....	77
VIII	ARABIC SUMMERY.....	

LIST OF TABLES

No.		Page
1	Comparison of hormones and endocrine disruptors action....	15
2	Nonylphenol ethoxylate NPE9 general formula.....	16
3	Water Parameters.....	26
4	Summary of The experimental treatments.....	29
5	Daily formulated feed portion.....	30
6	Fish sex ratio in different investigated areas for season1 and 2 Samples.....	34
7	Males anatomical data in the first and Second Sample expressed as average $\pm$ SE.....	37
8	Females anatomical data in the first and Second Samples expressed as average $\pm$ SE.....	38
9	Lab experiment fortnightly average total tank weight $\pm$ SE during the experimental period for NP9 treated groups.....	48

LIST OF FIGURES

No.		Page
1	Sampling sites at Lake Manzala.....	25
2	Photograph of 5μ testicular transvers section of <i>O. niloticus</i> stained with H&E, El-Serw area during Season 1 (post-spawning season)	39
3	Photograph of 5μ testicular transvers section of <i>O. niloticus</i> stained with H&E, Bashteer area samples during post spawning season.....	39
4A	Photograph of 5μ testicular transvers section of <i>O. niloticus</i> stained with H&E, showing mature testes sampled during Season 2 (spawning season) 10x.....	40
4B	Photograph of 5μ testicular transvers section of <i>O. niloticus</i> stained with H&E, showing mature testes sampled during Season 2 (spawning season) 20x.....	40
5A	Photograph of 5μ ovarian transvers section of <i>O. niloticus</i> stained with H&E Showing different developmental stages of oocytes during first season 4x.....	43
5B	<i>O. niloticus</i> ovary during spawning season showing maturation phase 4x.....	43
5C	Photograph of 5μ ovarian transvers section of <i>O. niloticus</i> stained with H&E Showing different developmental stages of oocytes 4x.....	44
5D	<i>O. niloticus</i> ovary during post- spawning season 4x.....	44
6	Fortnightly Average body weight of <i>O. niloticus</i> exposed to different doses of NP9.....	48
7	Fortnightly average survival rate of <i>O. niloticus</i> exposed to different doses of NP9 during experimental period.....	49
8	Average liver weight of <i>O. niloticus</i> exposed to different doses of NP9 at the end of experimental period.....	50
9	GSI and HSI of <i>O. niloticus</i> exposed to different doses of NP9 at the end of experimental period.....	51

10A	Photograph of 5 μ testicular transvers section of <i>O. niloticus</i> stained with H&E, control group normal testis 10x.....	53
10B	Showing spermatogenesis stage IV in control group spermatozoa (SZ) are present and no presence of testis-ova 20x....	53
10C	General view of spermatogenesis stage II 4x.....	54
10D	Photograph of 5 μ testicular transvers section of <i>O. niloticus</i> stained with H&E, General view of spermatogenesis early stage III in NP9 treated groups 10x.....	54
10E	General view of degenerated testis 20x.....	55

LIST OF ABBREVIATIONS

Symbol	Meaning
&	And
°	Degree
/	Per
μ	Micron
μg	Microgram
-1	Per
am	ante meridiem
Avg	Average
C	Celsius
d	Day
DO	Dissolved oxygen
EDCs	Endocrine-disrupting chemicals
EEAA	Egyptian Environmental Affairs Agency
EPA	Environmental Protection Agency
EPO	Early perinucleolar oocyte
Fig.	Figure
FSH	Follicle stimulating hormone
g	Gram
GAFRD	General Authority for fish resources development
GnRH	Gonadotrophic releasing hormone
GSI	Gonadosomatic index
GTH	Gonadotropin
GTHI	FSH
GTHII	LH
H&E	Hematoxylin and eosin
Hp	Horse power
HSI	Hepatosomatic index
Kg	Kilogram(s)

l	Liter (s)
L	Length
LH	Luteinizing hormone
PO	perinucleolar oocyte
M	Month
m	Meter
m ²	Squared meter
m ³	Cubic meter
mg	Milligram
mm ²	Square millimeter
NH ₃	Unionized ammonia
NH ₄	Ionized ammonia
NO ₃	Nitrate-Nitrogen
NPE or NP ₉	Nonylphenoethoxylate
OAG	Office of the Auditor General of Canada
OCPs	Organochlorine pesticides
OSPAR	The Convention for the Protection of the marine Environment of the North-East Atlantic
PAHs	Polyaromatic compounds
PCBs	Polychlorinated biphenyls
pH	Hydrogen ions concentration
Pm	Post meridiem.
PPb	Part per billion
Spp.	Species
TO	Testes-ova
VTG	Vitellogenin
WHO	World health organization
WWF	World Wildlife Fund
YVO	Yolk vesicle oocyte
ZRP	Zona radiate protein