



Effect of Increased Water Temperatures on the Expression of Gonadotropin-Releasing Hormone (GnRH), Kisspeptin and their Receptors in the Regulation of Hypothalamus-Pituitary-Gonadal (HPG) Axis in Nile Tilapia (*Oreochromis niloticus*)

Azmaien Naziat

Roll No. 0122/04

Registration No. 1092

Session: 2021-22

**A thesis submitted in the partial fulfillment of the requirements for the degree of
Master of Science in Fish Biology and Biotechnology**

**Department of Fish Biology and Biotechnology
Faculty of Fisheries
Chattogram Veterinary and Animal Sciences University
Chattogram-4225, Bangladesh**

JUNE 2023

Authorization

I hereby declare that I am the sole author of the thesis. I also authorize the Chattogram Veterinary and Animal Sciences University (CVASU) to lend this thesis to other institutions or individuals for the purpose of scholarly research. I further authorize the CVASU to reproduce the thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

I, the undersigned, and author of this work, declare that the **electronic copy** of this thesis provided to the CVASU Library, is an accurate copy of the print thesis submitted, within the limits of the technology available.

Azmaien Naziat

June 2023

Effect Of Increased Water Temperatures on the Expression of Gonadotropin-Releasing Hormone (GnRH), Kisspeptin and their Receptors in the Regulation of Hypothalamus-Pituitary-Gonadal (HPG) Axis in Nile Tilapia (*Oreochromis niloticus*)

Roll No. 0122/04

Registration No. 1092

Session: 2021-2022

This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects and that all revisions required by the thesis examination committee have been made.

Dr. Md. Mahiuddin Zahangir
Supervisor

Md. Main Uddin Mamun
Co-supervisor

Dr. Md. Mahiuddin Zahangir
Chairman of the Examination Committee

Department of Fish Biology and Biotechnology
Faculty of Fisheries
Chattogram Veterinary and Animal Sciences University
Chattogram-4225, Bangladesh

JUNE 2023

ACKNOWLEDGEMENTS

All thanks belong to Allah, who has blessed me with the strength, ability, and patience to continue postgraduate education and complete the thesis for the Masters of Science (MS) degree in Fish Biology and Biotechnology.

My heartfelt gratitude goes to **Prof. Dr. M. Nurul Absar Khan**, Dean, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University (CVASU), who initiated the Master of Sciences program in the Faculty of Fisheries and furnished laboratories with modern instruments and facilities to conduct good quality research.

With great delight, I would really like to express my sincere gratitude, tremendous respect, and enormous indebtedness to my honorable teacher and research supervisor, **Dr. Md. Mahiuddin Zahangir**, Associate Professor and Head, Department of Fish Biology and Biotechnology, CVASU, for providing me with the opportunity to conduct research and for providing invaluable guidance and continuous support throughout this research. His passion, vision, integrity, and motivation have left an indelible impression on me. Working and studying under his direction was a wonderful honor and privilege. I owe him a huge debt of gratitude, as well as admiration for his cordial collaboration, sensitivity, and amazing sense of humor.

I also sincerely express my gratitude to my co-supervisor, **Md. Main Uddin Mamun**, Assistant Professor, Department of Fish Biology and Biotechnology, Chattogram Veterinary and Animal Sciences University, Chattogram for his valuable guidance to teach me to be more confident person.

I express my thanks to **Md. Moudud Islam**, Associate Professor and **Fatema Akhter**, Assistant Professor, Department of Fish Biology and Biotechnology, Chattogram Veterinary and Animal Sciences University, Chattogram for their valuable and constructive suggestions during the research work.

I would like to convey my heartfelt gratitude to my supportive friends and lab assistants of Department of Fish Biology and Biotechnology for their tireless efforts throughout the research time. I also want to convey my genuine gratitude, real appreciation to everyone who has helped me finish the research work. Finally, my sincere gratitude and respect to my loving parents for their unwavering support, inspiration, blessings, forbearance, and unending love in helping me finish my study.

The Author

June 2023

Table of Content

SI. NO.	TITLE	PAGE NO.
	Authorization Page	ii
	Signature Page	Iii
	Acknowledgements	iv
	Table of Content	v-vi
	List of Figures	vii
	List of Tables	vii
	List of Plates	viii
	Abstract	Ix
CHAPTER 1	INTRODUCTION	1-4
	Objectives	4
CHAPTER 2	REVIEW OF LITERATURE	5-12
	2.1. Climate change and reproduction	6-7
	2.2. Endocrine control and HPG axis	7
	2.3. Gonadotropin releasing hormone (GnRH)	7-10
	2.4. Kisspeptin	10-11
	2.5. Nile tilapia (<i>Oreochromis niloticus</i>)	11-12
CHAPTER 3	MATERIALS AND METHOD	13-21
	3.1. Experimental fish	14
	3.2. Experimental design	14-15
	3.3. Sample collection	15
	3.4. RNA extraction	16-17
	3.5. cDNA synthesis	17-18
	3.6. Real time PCR assays for <i>kiss2</i> , <i>gpr54</i> <i>gnrh1</i> and <i>gnrh1r</i>	18-19
	3.7. Calculation of gonadosomatic index (GSI) and gonad histology	19-20
	3.8. Water quality parameters	20-21
	3.9. Statistical analysis	21

CHAPTER 4	RESULTS	22-30
	4.1. Effect of higher acclimation temperatures on the expression of <i>kiss2</i> in the brain of <i>O. niloticus</i>	23
	4.2. Effect of higher acclimation temperatures on the expression of <i>gpr54</i> in the brain of <i>O. niloticus</i>	24
	4.3. Effect of higher acclimation temperatures on the expression of <i>gnrh1</i> in the brain of <i>O. niloticus</i>	25
	4.4. Effect of higher acclimation temperatures on the expression of <i>gnrh1r</i> in the brain of <i>O. niloticus</i>	26
	4.5. Effect of acclimation temperatures on the gonadal development in <i>O. niloticus</i>	27-28
	4.6. Effect of higher acclimation temperatures on GSI of <i>O. niloticus</i>	28-29
	4.7. Effect of temperature on water quality parameters of rearing tanks	30
CHAPTER 5	DISCUSSION	31-35
CHAPTER 6	CONCLUSION	36-37
CHAPTER 7	RECOMMENDATIONS AND FUTURE PROSPECTS	38-39
	REFERENCES	40-58

List of Figures

Figure no.	Title	Page no.
1.	Changes in mRNA levels of <i>kiss2</i> in the brain of <i>O. niloticus</i> at different acclimation temperature (31°C, 34°C, 37°C) in (A) male and (B) female	23
2.	Changes in mRNA levels of <i>gpr54</i> in the brain of <i>O. niloticus</i> at different acclimation temperatures (31°C, 34°C, 37°C) in (A) male and (B) Female	24
3.	Changes in mRNA levels of <i>gnrh1</i> in the brain of <i>O. niloticus</i> at different acclimation temperatures (31°C, 34°C, 37°C) in (A) males and (B) females.	25
4.	Changes in mRNA levels of <i>gnrh1r</i> in the brain of <i>O. niloticus</i> at different acclimation temperatures (31°C, 34°C, 37°C) in (A) male and (B) female	26
5.	Changes in gonadosomatic index (GSI) of <i>O. niloticus</i> at different acclimation temperatures (31°C 34°C, 37°C) in (A) males and (B) females.	29

List of Tables

Table No.	Title	Page No.
1.	Equipment and reagents required for RNA isolation	16
2.	List of primers used in the present study	18
3.	Water quality parameters recorded during the experiment	30

List of Plates

Plate No.	Title	Page No.
1.	Experimental Fish (Nile tilapia, <i>Oreochromis niloticus</i>)	14
2.	Experimental tank setup	15
3.	Final Sampling	15
4.	Isolation and Quantification of RNA	17
5.	RT-PCR analysis	19
6.	Gonad histology	20
7.	Measuring water quality parameters	21
8.	Changes in gonadal developmental stages at different acclimation temperatures (31°C, 34°C, 37°C) in male <i>O. niloticus</i> .	27
9.	Changes in the developmental stages at different acclimation temperatures (31°C, 34°C, 37°C) in female <i>O. niloticus</i>	28

ABSTRACT

Temperature is a preeminent factor in the regulation of fish reproduction and can hinder gonadal development and maturation beyond a specific threshold. To understand the mechanism that controls reproduction under different acclimation temperatures, genes encoding for kisspeptin (*kiss2*), gonadotropin-releasing hormones (*gnrh1*) and their receptors (*gpr54* and *gnrh1r*) in the brain along with gonadal maturation and gonadosomatic index (GSI) was studied in juvenile Nile tilapia (*Oreochromis niloticus*). The fish were subjected to three distinct acclimation temperatures including 31°C, 34°C and 37°C for a duration of 14 days. The findings showed that mRNA levels of *kiss2*, *gpr54*, *gnrh1* and *gnrh1r* were relatively decreased at 37°C compared to 31°C and 34°C in the brain of *O. niloticus*. Histologically, the gonads of both sex had normal growth of gametes at control temperature (31°C) compared to the other groups. At 37°C acclimation temperatures, the spermatogenesis and oocyte maturation were slowed down and atretic oocytes were found in the ovary. The GSI value was concomitant with other results, with a significant decrease at 37°C for both sexes. Taken together, the results imply that elevated temperatures beyond a specific threshold had a negative impact on reproduction by suppressing Kiss/GnRH system and eventually restraining normal growth and maturation of gametes.

Keywords: reproduction, kisspeptin, gonadotropin-releasing hormone, acclimation temperature, gonadal maturation, *Oreochromis niloticus*