

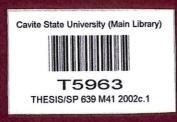
Salinity Tolerance of Oreochromis niloticus and Oreochromis niloticus ond Oreochromis niloticus and Their Successive Backcross

DENNIS ALMAZAN MATEO

Institute of Aquaculture

College of Fisheries and Ocean Sciences

v. p. in the Visavas





Salinity Tolerance of *Oreochromis niloticus* and O. *mossambicus* F₁ Hybrids and Their Successive Backcross

DENNIS ALMAZAN MATEO



Salinity tolerance of Oreochromis niloticus and O. mossambicus F1 hybrids 639 M41 2002 7.5963

A Thesis Submitted to the Graduate Faculty of the College of Fisheries and Ocean Sciences, University of the Philippines in the Visayas in Partial Fulfillment of the Requirements for the Degree of Master of Science in Fisheries (Aquaculture)

ABSTRACT

The effect of backcrossing on the salinity tolerance of the offspring of the hybrid O. mossambicus (Mo) x O. niloticus (Ni) was determined using median lethal salinity (MLS) and optimum salinity tolerance (OST) as indices of tolerance. The reciprocal Hybrids 1 (H1)(Mo x Ni and Ni x Mo), the first hybridization, were backcrossed with parental O. mossambicus in reciprocal crosses (i.e. with O. mossambicus as mother or father) to produce the reciprocal Hybrids 2 (H2) (H1 x Mo and Mo x H1), the backcross 1 and reciprocal Hybrids 3 (H3) (H2 x Mo and Mo x H2) and the backcross 2. These reciprocal hybrids and pure parental species were exposed to progressive changes in salinity at 6 ppt intervals until total mortality. Standard length (SL) was measured to correlate the size to salinity tolerance. Clinical signs and social behavior were observed during the test.

Parental O. mossambicus showed the highest salinity tolerance, followed by reciprocal H3, H2 and H1, while parental O. niloticus had the lowest. There was an increase of salinity tolerance in the offspring as they were backcrossed to the saline-tolerant parent O. mossambicus.

Both parents (O. mossambicus) contributed to the salinity tolerance of the offspring. Maternal inheritance was observed in reciprocal H1 and reciprocal H2, while paternal inheritance was observed in reciprocal H3. H1 had the highest heterosis. Both H2 and H3 stocks had negative and slight positive heterosis, indicating that they are good candidates as base population for selection.

Size and salinity tolerance were significantly related in H2 and H3 compared to the other group of fishes. Bigger fish survives longer in elevated salinities compared to smaller one. In the selection phase for growth, salinity tolerance might be indirectly selected, because larger fish survives longer regardless of age.

Social behavior during acclimation to salinity was observed. Generally, there was a decrease in the dominance of dominant individuals with the increase in salinity. O. niloticus and H1 stocks were increasingly aggressive at 30-36 ppt. H1 still had a residual dominant individuals until 96 ppt. Dominance disappeared in the other groups (H2 and H3 and pure O. mossambicus) at 48 ppt.

Loss of dominance among dominant individuals, cessation of feeding/minimal food intake, sunken eyes and abdomen, color changes and sluggish movement were observed before fish mortality.

Multicellular chloride cells increased its size and number in tilapias, including the degeneration of the size of their glomerulus as they were transferred to higher salinities. Specifically, O, niloticus has a smaller chloride cells and lesser degeneration as compared to O. mossambicus and hybrids 1, 2 and 3.

Transmission of salinity tolerance gene may be a multigene-factor inheritance. It is not controlled by a single gene, but by several genes summed together in the magnitude of salinity tolerance (i.e. adaptation of chloride cells and glumerulus, and adaptation of other osmoregulating organs, enzymes and hormones). Additive inheritance was observed due to their nearly zero heterosis. Maternal/paternal inheritance was observed due to the difference of salinity tolerance between the reciprocal breeds.

Hybrids 1 will not fit as base population for selection due to their high heterosis and aggressive behavior, that might be transmitted in the next generation. Hybrids 3 will not fit as base population for selection due to the high inbreeding values. Hybrids 2 best fit as base population for selection due to their low heterosis (additive inheritance), low aggressiveness and zero inbreeding. However H1 can contribute in the base population for selection, because heterosis not only governed by dominance-recessive effect but also additive. H3 can contribute to the base population, due to their high salinity tolerance.

TABLE OF CONTENTS

	Page		
Title page	i		
Approval Sheet	ii		
Dedication	iv		
Acknowledgement	v		
Vita	vii		
Table of Contents	ix		
List of Figures List of Tables List of Appendix Tables	xii xiii xiv		
		List of Appendix Figures	XV
		Abstract	1
Introduction	2		
Background of Study	2		
Significance of the Study	3		
Objectives of the Study	5		
Expected Outputs	6		
Duration and Place of Study	6		
Scope and Limitations of the Study	6		
Review of Related Literature	7		
Environmental Tolerances	7		
Dissolved Oxygen Tolerance	7		
Temperature Tolerance	8		
Toxic Gases Tolerance	8		
Salinity Tolerance			
Physiological Adaptation of Tilapia to Saline Environment	9		
Comparative Salinity Tolerance	11		
Factors Affecting Salinity Tolerance	11		
Age and Size	11		
Early Exposure to Salinity	12		
Nutritional Status	12		
Temperature	13		
Reproductive Performance at Various Salinity Levels	13		
Growth at Various Salinity Levels	14		
Seawater and Brakishwater Farming of Tilapia	15		

Constraint to Tilapia Saltwater Culture	16
Parasites and diseases in seawater/brackishwater	16
farming of tilapia	
Environmental concerns in seawater/brackishwater	16
farming of tilapia	
Techniques in Brakishwater Farming	17
Effects of testosterone in the salinity tolerance of tilapia	17
Tilapia polyculture to other species in brackishwater	17
Hybridization and crossbreeding of tilapia	17
Creation of a Synthetic Salinity Tolerant Tilapia Strain	18
Transmission of Genes	19
Transmission of Growth Rate Gene	20
Transmission of Salinity Tolerance Gene	21
•	
Materials and Methods	22
Rationale of Molobicus Project	22
Hybridization and Selection Protocol	22
Rotational Backcrossing Procedure	24
Selection of Breeders	26
Selection of Broads	
Methodology	27
Experimental Fish	27
Oreochromis mossamhicus	27
Reciprocal F ₁ Hybrids1 (1 st Hybridization)	27
Reciprocal F ₁ Hybrids2 (Backcross 1)	27
Reciprocal F ₁ Hybrids3 (Backcross 2)	27
Oreochromis niloticus	28
	20
Salinity Tolerance Test	28
Acclimation, Environmental Simulation and Water Quality	28
Salinity Levels	30
Experimental Units and Experimental Fish	30
Salinity Tolerance Indices	34
Mean Salinity Tolerance (MST)	34
Median Lethal Salinity (MLS)	34
Optimum Salinity Tolerance (OST)	34
-	2.5
Progressive Salinity Increase Tolerance Test	35
Experimental Design for Experiment 1	35
Experimental Design for Experiment 2	36
Data Collection	38
Analysis of Data	38

Results and Discussions	39
Salinity Tolerance Indices	39
Mean Salinity Tolerance (MST)	39
Median Lethal Salinity (MLS)	40
Optimum Salinity Tolerance (OST)	41
Size and Salinity Tolerance Correlation	43
Heterotic Effect	47
Maternal and Paternal Inheritance	48
Effects of Backcrossing and the Effects of Alternate Use of Sexes During Backcrossing	50
Computed Inbreeding Values of the Different Hybrids	51
Transmission if Salinity Tolerance Gene	53
Social Behavior of Tilapia During Salinity Increase	53
Fish Behavior Before Mortality in the Progressive Salinity Tolerance test	54
Comparative Morphology of Chloride Cells and Glomerulus of the Different Hybrids at Freshwater and Seawater	55
Conclusions	66
Recommendations	69
Literature Cited	71
Literature Cheu	0.5
Appendices	82

LIST OF FIGURES

Figures 1. Schematic diagram of Molobicus Project	Page 23
2. Rotational backcrossing scheme to develop a saline-tolerant tilapia	25
3. Production set up of experimental fish	29
4. Experimental set up	31
5. Stocking of experimental fish	32
6. Water management	33
7. Experimental set up on the effects of size to salinity tolerance	37
8. Graph of mean with standard deviation (MST, MLS and OST)	42
9. Linear regression graph of the reciprocal hybrids 1, pure O. mossambicus and pure O. niloticus	44
9a. Linear regression graph of the reciprocal hybrids 2 and reciprocal hybrids 3.	45
9b. Slope comparison between regression of Hybrid '3 and Hybrid '2	46
10. Path diagram of the different hybrids	52
11. Gills of O. niloticus and O. mossambicus in freshwater and elevated salinity levels	58
12. Gills of reciprocal hybrids 1 in freshwater and elevated salinity levels	59
13. Gills of reciprocal hybrids 2 in freshwater and elevated salinity levels	60
14. Gills of reciprocal hybrids 3 in freshwater and elevated salinity levels	61
15. Kidneys of O. niloticus and O. mossambicus in freshwater and elevated salinity levels	62
16. Kidneys of reciprocal hybrids 1 in freshwater and elevated salinity levels	63
17. Kidneys of reciprocal hybrids 1 in freshwater and elevated salinity levels	64
18. Kidneys of reciprocal hybrids 1 in freshwater and elevated salinity levels	65

LIST OF TABLES

Tables	Page
1. Six ppt salinity change per day in the experimental units	35
2. Experimental design for study 1	36
3. Experimental design for study 2	36
4. Mean Salinity Tolerance (MST) of the hybrids	
5. Median Lethal Salinity (MLS) of the hybrids	40
6. Optimum Salinity Tolerance (OST) of the hybrids	41
7. Relationship of size to salinity tolerance in the different hybrids and pure O. mossambicus and pure O. niloticus	43
8. Heterosis and the salinity tolerance difference in the reciprocal hybrids	47
9. Average salinity tolerance of offspring and their parents	50

LIST OF APPENDIX TABLES

Appendix Tables	Pages
1-1. Analysis of Variance (ANOVA) in the MST of hybrids	83
1-2. Duncan's Multiple Range Test (DMRT) in MST	83
2-1. Analysis of Variance (ANOVA) in the MLS of hybrids	83
2-2. Duncan's Multiple Range Test (DMRT) in MLS	83
3-1. Analysis of Variance (ANOVA) in the OST of hybrids	84
3-2. Duncan's Multiple Range Test (DMRT) in OST	84
4. Regression analysis of size and salinity tolerance in O. mossambicus	85
5. Regression analysis of size and salinity tolerance in Hybrid 1	85
6. Regression analysis of size and salinity tolerance in Hybrid '1	86
7. Regression analysis of size and salinity tolerance in Hybrid 2	86
8. Regression analysis of size and salinity tolerance in Hybrid '2	87
9. Regression analysis of size and salinity tolerance in Hybrid 3	87
10. Regression analysis of size and salinity tolerance in Hybrid '3	88
11. Regression analysis of size and salinity tolerance in O. niloticus	88

LIST OF APPENDIX FIGURES

Appendix Figures	Page
1. OST and MLS of O. mossambicus	90
2. OST and MLS of Hybrid 1	91
3. OST and MLS of Hybrid '1	92
4. OST and MLS of Hybrid 2	93
5. OST and MLS of Hybrid '2	94
6. OST and MLS of Hybrid 3	95
7. OST and MLS of Hybrid '3	96
8. OST and MLS of O. niloticus	97

Introduction

Background of Study

Tilapias are naturally distributed in Africa, and the Jordan Valley (Wohlfarth and Hulata, 1981; Pullin and McConnell, 1982; Trewavas, 1982) and were introduced to other places for aquaculture purposes.

Tilapias are popular culture species because of their high environmental tolerant ranges. They can withstand adverse conditions and are very tolerant of low dissolved oxygen concentrations, even below 1 mg/l (Colman and Edwards, 1987). During culture, tilapias are able to survive in cages and tanks with very low dissolved oxygen and toxic gases. They can withstand water conditions with carbon monoxide concentrations of 50 - 72.6 ppm. They tolerate very high concentrations of ammonia at 2.4 ppm of unionized ammonia (Daud *et al*, 1989). They tolerate high turbidities and are resistant to high levels of pollution and toxic substances, whether organic or inorganic, natural or artificial. Tilapias are cultured in wastewater in India (Colman and Edwards, 1987).

Almost tilapias are euryhaline, and are able to live and reproduce in salinity higher than 30 ppt. This salinity occurs in the coast, coastal lagoons and estuaries in Africa, and brackishwater ponds and river mouths in the Philippines. Some tilapias naturally breed in the canal of Suez in the Red Sea. These species which include *Tilapia guineensis*, *Sarotherodon melanotheron*, *Tilapia zilli and Oreochromis mossambicus* can tolerate salinities of 0 to 120 ppt (Trewavas, 1982).

Tilapias possess various characteristics which make them desirable species to culture in brackishwater farms. There are species of tilapias with potential for brackishwater farming. These include *Oreochromis niloticus*, *O. mossambicus*, *Oreochromis aureus*, *Oreochromis spilurus*, *Oreochromis hornorum*, *S. melanotheron* and the hybrid red tilapia.