STUDIES ON THE PREDATORY POTENTIAL OF SOME FISH SPECIES ON MOSQUITO LARVAE IN SOME PARTS OF NORTHERN NIGERIA

By

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DECLARATION

I declare that the work in this dissertation entitled 'Studies on the Predatory Potential of Some Fish Species on Mosquito Larvae in Some Parts of Northern Nigeria' has been carried out by me in the Department of Biological Sciences under the supervision of Professors I. H. Nock, J. Auta and T. O. L. Aken'Ova. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

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CERTIFICATION

This dissertation entitled ‘‘STUDIES ON THE PREDATORY POTENTIAL OF SOME FISH SPECIES ON MOSQUITO LARVAE IN SOME PARTS OF NORTHERN NIGERIA’’ by Bitrus AUTA meets the regulations governing the award of the degree of Doctor of Philosophy in Zoology of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my mother Saratu Auta, my wife Esther Bitrus and Children, Charles Auta and Nom Bitrus.

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ABSTRACT

This study was designed to evaluate the larvivorous potential of some indigenous fishes for the control of mosquito larvae and by extension, the adult mosquitoes. Twelve (12) fish species were collected from streams, rivers, ponds, swamps, ditches, reservoirs, lakes and dams in parts of northern Nigeria using hand nets, cast nets, drag nets and malian traps. These fishes were evaluated in the laboratory against the number of *Culex quinquefasciatus* larvae that each can consume in 24 hours, from which their predatory indices (PI) were computed. Similarly, the ability of nine of the fish species to prey on mosquito larvae in outdoor concrete ponds was evaluated for 12 months. Fish hardiness was measured by the ability to survive without being fed for five months (20 weeks), ability to survive under extreme pH, and ability to survive in different water volumes for 13 weeks. The fish species were bred under static and flow-through systems. Furthermore, *Clarias galmaensis* hatched in the laboratory using ovaprim hormone was tested for larvivorous efficacy at 5, 7 and 9 months. All the fish species evaluated preyed on mosquito larvae, but the consumption rate varied with species, the differences in the consumption rates among the 12 fish species were significant at $P<0.05$. The PI/ the mean number of mosquito larvae consumed per day by each of the fish species were as follows: *Poecilia reticulata* 265.00/53; *Clarias gariepinus* 101.36/223; *Clarias galmaensis* 49.40/988; *Epiplatys spilargyreius* 45.00/18; *Hemichromis bimaculatus* 35.96/187; *Oreochromis niloticus* 35.12/144; *Leptocypris niloticus* 29.33/88; *Schilbe mystus* 26.60/125; *Synodontis ouemeensis* 24.67/74; *Brienomyrus niger* 17.00/51; *Astatotilapia burtoni* 7.80/39 and *Brycinus nurse* 3.29/28. The number of larvae consumed by each of these fish species was lower when commercial feed was added but t-test showed that the differences were not significant ($P>0.05$). Except for ponds that contained *Brienomyrus niger, Brycinus nurse*

and *Synodontis ouemeensis*, all other ponds were devoid of larvae throughout the test period of 12 months. The lowest pH tolerated by the fish was 3.16, by *Astatotilapia burtoni* and the highest was 11.85, by *Brycinus nurse*. No fish species had mortality of more than 50 percent when starved in the laboratory for five months. Mortalities recorded among the different fish species kept in 10, 20 and 30 litres of water for 13 weeks were generally low; in six of the 12 fish species no mortality was recorded; in all, only 15 (5.21%) out of 288 fish died. *Poecilia reticulata* bred in the static and flow-through system and *Epiplatys spilargyreius* in the laboratory all year round. *Poecilia reticulata, Clarias gariepinus, Clarias galmaensis, Epiplatys spilargyreius, Hemichromis bimaculatus, Leptocypris niloticus, Oreochromis niloticus* and *Schilbe mystus* showed promise as good predatory agents for mosquito larvae with the potential that could be harnessed for the control of mosquitoes and consequently the diseases they transmit.

TABLE OF CONTENTS

	Title Page……	i
	Declaration……	ii
	Certification……	iii
	Dedication……	iv
	Acknowledgements……..……	v
	Abstract……...…….……	vi
	Table of Contents……	viii
	List of Tables……	xiv
	List of Figures……	xv
	List of Plates……	xvi
	List of Appendices……	xvii
	Abbreviations, Symbols and Definitions……. …….	xix
	CHAPTER ONE	
1.0	**INTRODUCTION**……	1
1.1	**Background of the Study**……	1
1.2	**Statement of the Research Problem**……	2
1.3	**Justification**……	4
1.4	**Aim and Objectives** ……	4
1.5	**Research Hypotheses**……..……	5

CHAPTER TWO

2.0 LITERATURE REVIEW…… 6

2.1 Mosquitoes …… 6

2.1.1 *Anopheles* mosquitoes……..…… 7

2.1.2 *Anopheles gambiae*…… 9

2.1.3 *Culex* mosquitoes …… 10

2.1.4 *Aedes* mosquitoes …… 11

2.2 Mosquito Borne Diseases…… 12

2.2.1 Malaria…… 12

2.2.2 Yellow fever…… 13

2.2.3 Dengue and dengue hemorrhagic fever…… 14

2.2.4 Elephantiasis/Filariasis…… 15

2.2.5 Chikungunya…… 16

2.2.6 Encephalitis…… 17

2.2.7 Animal diseases…… 19

2.3 Fish…… 19

2.3.1 Factors that affect fish…….. 20

2.3.2 Water quality…… 21

2.3.3 Temperature…… 22

2.3.4 pH……...…… 23

2.3.5 Total dissolved solids…… 24

2.3.6 Electrical conductivity…… 25

2.3.7 Feeding…… 26

2.3.8 Stress……..… 28

2.3.9 Quarantine…… 29

2.3.10 Reproduction…… 30

2.4 **Mosquito Control**…… 31

2.4.1 Environmental management…… 31

2.4.2 Physical methods of control…….. 31

2.4.3 Chemical methods of control…… 33

2.4.4 Biological methods of control **...** …… 35

2.4.5 Mosquito larvae control using fish……. . 39

CHAPTER THREE

3.0 **MATERIALS AND METHODS**…… 48

3.1 **Area of Study**…… 48

3.2 **Fish Collection** ……..……. 49

3.3 **Transportation and Handling of Fish** …… 50

3.4 **Research Site** …….. 52

3.5 **Acclimatization of Fish**.…… ……..**.....** 52

3.6 **Fish Identification**…… 52

3.6.1 *Brienomyrus niger* (Günther, 1866) …… 54

3.6.2 *Brycinus nurse* (Rüppell, 1832)…… 54

3.6.3 *Clarias galmaensis* Aken'Ova, 2007…… 54

3.6.4 *Clarias gariepinus* (Burchell, 1822) …… 55

3.6.5 *Epiplatys spilargyreius* (Duméril, 1861) ……. 56

3.6.6 *Astatotilapia burtoni* (Günther, 1894)…… 56

3.6.7 *Hemichromis bimaculatus* (Gill, 1862) …… 56

3.6.8 *Leptocypris niloticus* (Joannis, 1835) …… 57

3.6.9 *Oreochromis niloticus* (Linnaeus, 1758) …… 57

3.6.10 *Poecilia reticulata* (Peters, 1859) …… 58

3.6.11 *Schilbe mystus* (Linnaeus, 1758) …… 58

3.6.12 *Synodontis ouemeensis* Musschoot & Lalèyè, 2008…… 59

3.7 **Morphometric Parameters of Fish**…… 59

3.8 **Sourcing and Rearing Mosquito Larvae**…… 59

3.9 **Water Physicochemical Parameters** …… 60

3.10 **Test Aquarium**…… 60

3.11 **Studies on the Predatory Potential of Fish** …… 60

3.11.1 Indoor study on the predatory potential of fish on mosquito larvae ……. 60

3.11.2 Indoor study on the predatory potential of fish on mosquito larvae in the presence of commercial fish feed…… 62

3.11.3 Indoor study on the predatory potential of *Clarias galmaensis* bred in the laboratory …… 62

3.11.4 Outdoor study on the predatory potential of fish on mosquito larvae …… 63

3.12 **Test of Fish Hardiness**…… …… 64

3.12.1 Fish tolerance to pH changes…… 64

3.12.2 Fish survival in the absence of food…… 65

3.12.3 Fish tolerance to changes in water level…… 65

3.13 **Reproductive Potential of Fish in Captivity** …… 67

3.13.1 Fish breeding using static system…… 67

3.13.2 Fish breeding using the flow-through system…… 68

3.13.3 Breeding *Clarias galmaensis* using hormone…… 68

CHAPTER FOUR

4.0 **RESULTS**…… 71

4.1 **Sourcing Mosquito Larvae**…… 71

4.2 **Morphometric Indices of the Fish**…… 71

4.3 **Indoor Study on the Predatory Potential of Fish on Mosquito Larvae**…… 71

4.4 **Comparison of Larvae Consumed by Fish in the Absence and Presence of Fish Feed** …… 73

4.5 **Predatory Index** …… 75

4.6 **Indoor Study on the Predatory Potential of *Clarias galmaensis* Bred in the Laboratory** …… 75

4.7 **Relationship of Fish Morphometric and Mosquito Larvae Consumption**..... 75

4.8 **Outdoor Study on the Predatory Potential of Fish on Mosquito Larvae** …..... 81

4.9 **Physicochemical Parameters of Water in Experimental Ponds and Fish Morphometry**…… 81

4.9.1 Physicochemical parameters of water in experimental ponds …… 81

4.9.2 Morphometrics of fish in experimental ponds…… 83

4.10 **Tolerance of Fish to pH Changes** …… 83

4.11 **Effect of Keeping Fish without Feeding** …… 85

4.11.1 The physicochemical parameters of the aquaria of the fish not fed for five months …… 85

4.11.2 Mortality of fish kept for five months without feeding..... 87

4.12 **Effect of Keeping Fish in Different Water Volumes** …… 87

4.12.1 Morphometrics of fish used in three different volumes of water…… 87

4.12.2 Physicochemical parameter of aquarium with fish in relation to water volume…… 89

4.12.3 The effect of varying volumes of water on fish species…… 89

4.13 **The Reproductive Potential of the Fish Species**…… 90

4.13.1 The reproductive potential of fish species in static and flow-through systems…… 90

4.13.2 The physicochemical parameters of the water in the flow-through system…….. 90

4.13.3 Morphometrics of the fish in the flow-through system……..…….. 93

CHAPTER FIVE

5.0. **DISCUSSION**…… 95

CHAPTER SIX

6.0. **CONCLUSIONS AND RECOMMENDATIONS**…… 107

6.1. **Conclusions** …… 107

6.2. **Recommendations**…… …… 108

REFERENCES…… 110

APPENDICES…… 131

LIST OF TABLES

Table 3.1:	Fish species and the site of capture …….……	53
Table 4.1:	Some morphometric parameters of fish used for larvivorous test……	72
Table 4.2:	Changes in mean morphometrics of fishes kept in the outdoor Ponds for a period of 12 months……...	84
Table 4.3:	Morphometric parameters, lowest and highest pH tolerated by each fish species……	86
Table 4.4:	Reproductive potential of *Epiplatys spilargyreius* and *Poecilia reticulata* in the laboratory..…….....	92
Table 4.5:	Morphometrics of fishes used in flow-through system for 12 months……...	94

LIST OF FIGURES

Figure 1: A map of Nigeria showing the towns in northern Nigeria from which the fish species used in the study were obtained…….. 51

Figure 2: Mean Mosquito larvae consumption rate of fish fed on mosquito larvae only and those on mosquito larvae and coppens® …… 74

Figure 3: Predatory indices showing the comparison between the fish species fed on mosquito larvae only and those fed along with commercial feed (coppens®)..... 76

Figure 4: Mean mosquito larvae consumed compared with predatory indices of fish species fed on larvae only…….. 77

Figure 5: Changes in morphometric, mean mosquito larvae consumption and predatory indices of *Clarias galmaensis* at 5, 7 and 9 months…… 78

Figure 6: Mosquito larvae consumed and fish weight relationship……... 79

Figure 7: Mosquito larvae consumed and fish length relationship……... 80

Figure 8: Population of mosquito larvae in outdoor ponds with fish species …….. 82

Figure 9: Total mortality in fish species kept without feeding for five months…… 88

Figure 10: Total mortality of fish species resulting from the effect of different water volumes…… ……... 91

LIST OF PLATES

Plate I a:	Dead fish used as food source for mosquito larvae ……...	61
Plate I b:	Harvested third and fourth instars mosquito of larvae ……	61
Plate II:	Ponds used for the outdoor predatory potential experiment ……..	66
Plate III:	Experimental set-up of flow-through system for the breeding of nine species of fish ……	70

List of Appendices

Appendix I:	Photographs of the fish species used in this study ……	131
Appendix II a:	Sorting fish at Akwa village, Godo-Godo, Jama'a Local Government Area, Kaduna State……..	135
Appendix II b:	Catching fish at New Bussa, Niger State…….	135
Appendix II c:	Collection of *Epiplatys spilargyreius* from a swamp at Munnai, New Bussa, Niger State…….	136
Appendix III:	Fish morphometric and mean number of mosquito larvae consumed with standard deviation and standard error……...	137
Appendix IV:	Fish morphometric and mean number of mosquito larvae consumed in the presence of coppens® with standard deviation and standard error……	138
Appendix V:	Predatory Indices of the fish species fed on mosquito larvae with or without coppens®……..	139
Appendix VI:	Morphometrics, mean mosquito larvae consumed and Predatory Indices of *Clarias galmaensis* at 5, 7 and 9 months …….	140
Appendix VII:	Monthly mean temperature (^{o}C) recorded in each pond for 12 months…….. ……..	141
Appendix VIII:	Monthly mean pH for each pond for 12 months……...	142
Appendix IX:	Monthly mean total dissolved solids (mg/L) for each pond for 12 months…….. …….....	143
Appendix X:	Monthly mean electrical conductivity (µSiemens/cm) for each pond for 12 months…….	144
Appendix XI:	Monthly and total mosquito larvae from each respective pond for 12 months period ……	145
Appendix XII:	Morphometric of the fish species starved for five months…….	146
Appendix XIII:	Monthly mean temperature (^{o}C) record of fish species starved for five months ……. ……..	147
Appendix XIV:	Monthly mean pH record of the fish species starved for five months……	148

Appendix XV: Monthly mean total dissolved solids (mg/L) record of the fish species starved for five months…… 149

Appendix XVI: Monthly mean electrical conductivity (µSiemens/cm) record of the fish species starved for five months…….. 150

Appendix XVII: Monthly and total mortality record of the different fish species starved for five months ……. 151

Appendix XVIII: Morphometrics of the experimental fish species kept for 13 weeks in three different water volumes……... 152

Appendix XIX: Weekly temperature (^{o}C) record of the fish species in three different volumes of water ……..... 153

Appendix XX: Weekly pH record of the fish species in three different volumes of water …… 154

Appendix XXI: Weekly total dissolved solids (mg/L) record of the fish species in three different volumes of water…… 155

Appendix XXII: Weekly electrical conductivity (µSiemens/cm) record of the fish species in three different volumes of water……... 156

Appendix XXIII: Weekly mortality records of the fish species in different volumes of water ……..... 157

Appendix XXIV: Monthly means temperature (^{o}C) changes in flow-through system .. 158

Appendix XXV: Monthly means pH changes in flow-through system …… 159

Appendix XXVI: Monthly means total dissolved solids (mg/L) changes in flow-through system …… 160

Appendix XXVII: Monthly means electrical conductivity (µSiemens/cm) changes in flow-through system ……... 161

Appendix XXVIII: Flow-through chart…… 162

Appendix XXIX Details of Artificial breeding of *Clarias galmaensis*……. 163

Appendix XXX Photographs of *Clarias galmaensis* breeding…… 165

Abbreviations, Symbols and Definition of terms

Abbreviations

Ae.	*Aedes*
AES	Aventis Environmental Science
AMCA	American Mosquito Control Association
An.	*Anopheles*
Anopheles m and s forms	*Anopheles* mopti and savanna forms
Anopheles gambiae s.l.	*Anopheles gambiae sensu lato*
Anopheles gambiae s.s.	*Anopheles gambiae sensu stricto*
ASM	African Summit on Roll Back Malaria
Bs	*Bacillus sphaericus*
Bti	*Bacillus thuringiensis* var. *israelensis*
C.	*Culex*
CDC	Centres for Disease Control and Prevention
CDMP	Communicable Disease Management Protocol
CHP	Centre for Health Protection
CWT	Clean Water Team
DDT	Dichloro-diphenyl-trichloroethane
DHF	Dengue Hemorrhagic Fever
EC	Electrical Conductivity
EEE	Eastern Equine Encephalitis
FAF	Freshwater Aquarium Fish
FCT	Federal Capital Territory

GAELF	Global Alliance to Eliminate Lymphatic Filariasis
GMVC	Guidelines for Malaria Vector Control in Ethiopia
GPELF	The Global Programme to Eliminate Lymphatic Filariasis
HCH	Hexachlorocyclohexane
IDPH	Illinois Department of Public Health
IDRC	International Development Research Centre
ITMs	Insecticide Treated Materials
ITNs	Insecticide Treated mosquito Nets
JE	Japanese Encephalitis
LAC	LaCrosse Encephalitis
LF	Lymphatic Filariasis
NBTE	National Board for Technical Education
NEA	National Environment Agency
NIFFR	National Institute for Freshwater Fisheries Research
NPS	National Park Service
PCR	Polymerase Chain Reaction
RFA	Rural Freshwater Aquaculture Manual
SL	Standard Length
SLE	St. Louis Encephalitis
TDS	Total dissolved Solids
TL	Total Length
TMB	Tropical Medical Bureau
USA	United States of America
USAID	United States Agency for International Development

WEE	Western Equine Encephalitis
WHO	World Health Organisation
WNE	West Nile Encephalitis
WNV	West Nile Virus

Symbols

µS/cm:	microsiemens per centimeter
µSiemens/cm:	microsiemens per centimeter
µmhos/cm:	micromhos per centimeter
S/m:	Siemens per meter

Definitions

sensu lato	In the wide or broad sense
sensu stricto	In the strict sense

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Mosquitoes are ubiquitous insects found in nearly every climatic region of the world (AES, 2000). They are vectors of malaria, yellow fever, dengue and dengue hemorrhagic fever, filariasis/elephantiasis, chikungunya and encephalitis. Malaria is endemic in more than 100 countries and forty-one percent of the world's population lives in areas where the disease is transmitted (CDC, 2007; USAID, 2007). There are approximately 200,000 cases of yellow fever with 30,000 deaths every year world-wide (Sumodan, 2001; AMCA, 2005). Yellow fever occurs mainly in western sub-Saharan region and occasionally in some eastern countries; about 90% of all cases are found in Africa (TMB, 2004). Dengue is the most important tropical disease, after malaria, in 1998; more than 2.5 billion persons lived at risk (Gubler, 1998). It is estimated that there are 50 to 100 million cases of dengue fever and 500,000 of dengue hemorrhagic fever each year (WHO, 2006; McCormack, 2007). More than one billion people are threatened by lymphatic filariasis, the estimated at risk population is 390 million, which accounts for approximately one-third of the global burden, thirty-eight percent of the at-risk population are children under 15 years (GAELF, 2008). Encephalitis is a viral disease that causes inflammation of the brain and spinal cord; there are different types of encephalitis. Not only can mosquitoes carry diseases that afflict humans, they also transmit several diseases and parasites that dogs, sheep and horses are very susceptible to (AMCA, 2005). Mosquito-borne diseases have plagued civilization for thousands of years and throughout history; no insect has contributed more to human discomfort, disease and death than the mosquito (IDPH, 2007).

1.2 Statement of the Research Problem

A lot of human activities are geared towards improving human health and a peaceful environment. Among the challenges facing human beings particularly in the developing countries is the menace of mosquito and mosquito–borne diseases which are on the increase despite of steps being taken to address the problem. Methods used for mosquitoes and mosquito-borne diseases control in Nigeria include, chemotherapy, removal and proper disposal of water retaining containers in the environment, draining marshes, removal of aquatic weeds and debris which shelter and protect mosquito larvae, chemicals, the use of mosquito repellents, use of mosquito nets, the use of mosquito screens on windows and doors in houses. However, some of these methods of mosquito control are often associated with problems. When the insecticidal properties of Dichloro-diphenyl-trichloroethane (DDT) were discovered in the 1940s, it became the principal method for *Aedes aegypti* eradication programmes in the Americas (WHO, 1997). The use of chemicals such as DDT and Hexachlorocyclohexane (HCH) are nowadays discouraged due to their residual effects, widespread resistance, soaring price and other operational difficulties (Goddard, 2003; Ghosh *et al.,* 2005a). Proper disposal of water-holding containers has not been very successful. The use of repellants in the form of coils, creams, lotions, or in pressurized cans is expensive and not accepted by some people. The use of mosquito screens on windows and doors in houses is also expensive. Increase in irrigation schemes, dams for generating hydroelectric power, car washing activities, excavation projects, construction activities, road construction, damaged roads, wells, and floods all contribute to increase in potential mosquito breeding sites. Other factors that increase the number of mosquitoes or diseases associated with them include resistance of mosquitoes to some common previously effective insecticides, human drug resistance, increasing cost of treatment, non-availability

of cheap and effective drugs, poor drainage, lack of trained and dedicated scientist and specialists who work on mosquitoes, lack of vaccine breakthrough, poverty which hinders many people from accessing treatment, purchasing nets and insecticides. Dragonflies, birds, bats, dragonfly nymphs and diving beetles which are natural predators of adult mosquitoes and larvae are inadequate as their predatory effect is minimal in nature. The bacterium, *Bacillus thuringiensis* var. *israelensis* (Bti), which is known to kill mosquito larvae, is expensive to purchase and apply, it does not kill the pupae, it is effective only on standing and not moving water; must be reapplied often, and not all mosquito species respond to it, *Bacillus sphaericus* (Bs) is highly effective on *Culex* but not on *Anopheles* larvae (Gouge *et al.*, 2001; Goddard, 2003). Therefore, in spite of efforts and money invested in combating mosquitoes, their number and diseases associated with them seems to be on the increase. Thus, mosquito control is far from being successful in Nigeria as there is hardly any method that is perfect in eliminating them. However, some fish species are known to eat mosquito larvae if they have access to them and Global experience has shown that in many situations fish are effective in controlling mosquitoes. Fish have been used as early as 1903 in some countries. The World Health Organization (WHO) recognizes the roles of larvivorous fish either alone or in an integrated approach for control of mosquito-borne diseases (WHO, 2003). In Nigeria biological control of mosquitoes using fish has remained unexploited because information on fish species with mosquito larvivorous potential is lacking, thus the mosquito control value of its vast fish population has remained unknown, undetermined and unutilised. Therefore, the essence of this study is to search for potential larvivorous indigenous fish species that can be used to control mosquito larvae towards the integration of biological control (using fish) into the overall mosquito control programme in the country.

1.3 Justification

The use of larvivorous fish can greatly reduce mosquito population in the environment; this will reduce mosquito nuisance and bites thus leading to a decrease in mosquito-transmitted diseases. The use of fish to control mosquito larvae will improve the health status of many Nigerians. The use of fish to control mosquito is more economical than chemical control methods (WHO, 1982; GMVC, 2002; WHO, 2003). Fish are environmentally friendly, acceptable and have no deleterious ecological consequences (GMVC, 2002; NVBDCP, 2007). Fish need no special equipment for introduction and maintenance (GMVC, 2002).

1.4 Aim and Objectives

This study is aimed at evaluating the predatory potential of some fish species on mosquito larvae from parts of northern Nigeria.

The objectives of this research are to determine:

i. The predatory potential of some fish species from parts of northern Nigeria on mosquito larvae.

ii. The rate of consumption of mosquito larvae by selected fish species in the absence and presence of commercial fish feed.

iii. The ability of the selected fish species to survive in different water pH levels.

iv. The ability of the selected fish species to survive in different water volumes.

v. The ability of the selected fish species to survive without being fed for five months.

vi. The reproductive potentials of the selected fish species in captivity.

1.5 Research Hypotheses

i. Fish species indigenous to northern Nigeria are not good mosquito larvivorous agents.

ii. There is no significant difference in the rate of mosquito larvae consumption by the selected fish species in the absence or presence of commercial fish feed.

iii. Changes in pH do not affect the survival of the selected fish species.

iv. Change in water volume does not affect the survival of the selected fish species.

v. The ability of the selected fish species to survive is not affected by withdrawal of feeding.

vi. The reproductive potential of the selected fish species is not affected by captivity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mosquitoes

There are over 3,000 mosquito species; they are vectors of malaria, yellow fever, dengue and dengue hemorrhagic fever, filariasis/elephantiasis, chikungunya fever and encephalitis. These diseases are negatively affecting the development of large areas of the tropics, especially Africa (Gubler, 1998). In addition to these diseases, mosquito bites cause skin irritation which often leads to secondary infections (AMCA, 2005; NPS, 2010). Mosquito noise is also a nuisance both indoors and outdoors and if proper nets or screens are not used, this may prevent sleep at night. However, in all species that require blood, it is only the female mosquitoes that bite; some species prefer humans while others target other mammals, birds or even reptiles (Geary *et al.*, 2000). A female mosquito usually detects a host through body odour, carbon dioxide increase, air pressure, heat and moisture (IDPH, 2007).

Mosquitoes breed in water, different species exhibit considerable plasticity in their selection of breeding places, some breed in polluted water and others in clean water and while some breed in small amount of water others prefer large collections ((Hopkins, 1952). In nature, mosquitoes are found in a variety of habitats, e.g. small pools, large marshes, rock pools, tree holes, plant axils, flower bracts, fallen leaves, fruit husks, empty mollusk shells, and a variety of man-made containers (Service, 1976). Culverts, storm drains and roadside ditches usually become mosquito-breeding areas and the presence of lakes and the increase in dams in several areas to boost hydro-electric generation and

agriculture has helped increase mosquito population in such places (Goddard, 2003). When rainfall is abundant, many species lay eggs continuously and under ideal conditions with high temperatures, development can be completed in less than a week, resulting in large populations of mosquitoes (NPS, 2010). However, wave action in large bodies of water and rapid current of swiftly flowing streams often deter mosquito breeding (Hopkins, 1952).

2.1.1 *Anopheles* mosquitoes

Anopheles species are distributed world-wide, they bite both indoors and outdoors (Sumodan, 2001; Oyewole and Awolola, 2006; Oyewole *et al*., 2007). Every species has its preferred water body for oviposition, depending on climate, physical geography and human activities, breeding sites can be natural or man-made of various sizes, located in running or stagnant waters, shaded or sunny, permanent or temporary (Machault *et al.*, 2009). They are likely to be found in clean and unpolluted or non-turbid breeding sites with a size less than one meter (Service, 1980; Sattler *et al.*, 2005). In a potential habitat survey in Dar es Salaam (Tanzania) by Vanek *et al.* (2006), they found almost a third of the habitats occupied by *Anopheles* larvae. Mutuku *et al.* (2006) in a survey in western Kenya also reported that 35 % of the habitats had anopheline larvae only. Bigoga *et al*. (2007) in Cameroon reported the following percentages of *Anopheles* among other mosquitoes present in the area: *Anopheles gambiae* (57%), *An. funestus* (10%), *An. nili* (5%) and *An. rufipes* (9%), but analysis of the females indicated that 79% were *An. gambiae*, 14% *An. funestus* and 7% *An. nili*.

Different *Anopheles* species have been found breeding in animal shells, tree holes, leaves stalk, ground and rock pools in Nigeria (Okogun *et al*., 2005; Anosike *et al*., 2007; Adebote

et al., 2008). In a study on mosquito breeding in Edo State, Nigeria, Okogun *et al*. (2003) found that 37% were *Anopheles* spp. which bred in plastic containers, metal cans, earthenware pots and bamboo stumps. Okogun *et al*. (2005) found the most preferred breeding site to be clay pots and the least plastic ware.

Onyabe and Conn (2001) found that in terms of distribution the two major malaria vectors, *Anopheles gambiae* and *An. arabiensis* occurred in each of the 5 ecological zones in Nigeria. They reported that one of the two species was usually more prevalent than the other in at least one locality of the five ecological zones, and that the two species coexisted in several localities but disproportionately. In Lagos area, *Anopheles gambiae* s.s., *An. arabiensis, An. funestus* s.s. and *An. rivulorum* were found (Oyewole and Awolola, 2006; Okwa *et al.,* 2007). Even though Oyewole and Awolola (2006) encountered *Anopheles arabiensis* and *An. moucheti* throughout the year in Lagos area, but they were predominant in the dry season. Okwa *et al*. (2009) found five *Anopheles* species in Nigeria, *An. gambiae* s.s. made up approximately 29.2-36.6% of the population in each zone of the country and species distribution in four areas suggests that members of *An. gambiae*, *An. moucheti nigeriensis* and *An. funestus* complexes can be found in sympathy, they found considerable numbers of *Anopheles arabiensis* in the rainforest zone of the country and wherever *An. arabiensis* occurred in the rainforest, it was often associated with a history of extensive land clearance, they reported that *Anopheles arabiensis* appears to be a good vector of malaria, especially in the savannah-forest, *An. melas* occurred only in the coastal rainforest and mangrove forest while *An. moucheti nigeriensis* was identified only in the southern zones and was more abundant in the rainforest. A study in Ugah, Nasarawa State by Ayanda (2009) revealed that *Anopheles gambiae* s.l., *An. funestus* and *An. ardensis* were

abundant in that area. In a study in Ogun State, Ajayi *et al.* (2010) reported that out of a total of 1,308 mosquitoes examined 18.85 % were *Anopheles.* Adeleke *et al*. (2008) in Abeokuta and Oringanje *et al*. (2011) in Calabar reported *Anopheles gambiae* s.l. and *An. funestus* among other mosquito species to be abundant in these areas. Molecular characterization of the *Anopheles gambiae* s.l.using PCR assays revealed two sibling species: *An. gambiae* s.s. and *An. arabiensis* (Oringanje *et al*., 2011).

2.1.2 *Anopheles gambiae*

Sattler *et al.* (2005) in Dar es Salaam (Tanzania) found *Anopheles gambiae* s.l. larvae in highly organically polluted habitats, polluted by rotting vegetation, human faeces and oil. *Anopheles gambiae* complex larvae often prefer water with abundant aquatic plants that provide protection from fish and other predators (NPS, 2010). Clearing forest improves growing conditions of *Anopheles gambiae* complex (Minakawa *et al*., 2005). Robert *et al.* (2002) in Madagascar found *Anopheles gambiae* s.l. larvae in rice fields which did not contain emergent vegetation and were exposed to the sun. Most *Anopheles gambiae* collected in East Africa (Charlwood *et al.*, 2000), Cameroon (Bigoga *et al*., 2007) and Nigeria (Oyewole and Awolola, 2006; Oringanje *et al.,* 2011) coincided with periods of rain fall.

Onyabe *et al*. (2003) in the study of the distribution of M and S molecular forms of *Anopheles gambiae* in Nigeria found that, both S and M forms occurred across Nigeria with no apparent relationship to the ecological transition from savannah to forest zone. In each area one form was always much more common than the other, the S form alone was found in Giwa (Kaduna State) and Lafiya (Nasarawa State), whereas only the M form occurred in

Lagos. However, Oyewole and Awolola (2006) reported the existence of both the molecular M and S forms of the *Anopheles gambiae* s.s. in Lagos.

2.1.3 *Culex* mosquitoes

They are found in paddy canals, pit habitat, rain and stream pools (Fakoorziba and Vijayan, 2006; Muturi *et al*., 2007). *Culex* species prefer breeding in polluted standing water with a large amount of organic material (CHP, 2004; Sattler *et al.,* 2005). They breed in both small containers and large pools (Hopkins, 1952; NPS, 2010); rock pools (Adebote *et al*., 2008); tree holes (Anosike *et al*., 2007); plastic containers, metal cans, earthenware pots and bamboo stumps (Okogun *et al*., 2003, 2005) and tyre (Qualls and Mullen, 2006).
Muturi *et al*. (2007) in rice agro-system in Kenya found culicines larvae made of 43% of the total mosquito larvae in one area and 66.8% in another, *Culex duttoni* and *Culex quinquefasciatus* accounted for 73.1% of the total mosquitoes they identified in the area. *Culex* spp. also formed 92% of the mosquitoes collected in Accra, Ghana by Klinkenberg *et al*. (2008).

Culex mosquitoes are very abundant in Nigeria; 34% of the mosquitoes in midwestern (Edo State) Nigeria (Okogun *et al*., 2003) and 49.6% of mosquitoes collected in Lagos (Okwa *et al.,* 2007) were found to be *Culex*. Adeleke *et al.* (2008) in Abeokuta found *Culex quinquefasciatus* in 4 out of the 5 habitats surveyed. In a study in Ogun State, it was reported that out of a total of 1308 mosquitoes 62.7% were *Culex*, out of a total of 675 mosquitoes caught in Calabar, 64.1% were culicines, these high proportion of the culicines are thought to be a result of environmental practices which had subjected most available breeding sites to pollution, hence, encouraging the proliferation of more culicines and

reducing the suitability of these sites for anopheline and Aedes proliferation (Ajayi *et al.*, 2010; Oringanje *et al.*, 2011).

2.1.4 *Aedes* mosquitoes

Aedes species breed in plastic containers, metal cans, earthenware pots and bamboo stumps (Service, 1980; Okogun *et al*., 2003, 2005). Qualls and Mullen (2006) found that 70.4% of the mosquitoes that bred in tyres in Alabama USA were *Aedes albopictus*. *Aedes albopictus* breeds easily in water-filled containers, so breeding sites are commonly available (NPS, 2010). *Aedes aegypti* prefer containers and water tanks as breeding sites in Asia and the Americas (WHO, 1997; Sumodan, 2001; WHO, 2006; Tipathi, 2008). Muturi *et al.* (2007) in rice fields in Kenya found that *Aedes* spp. were also represented in different habitat types except ditches and tree holes. Ground and rock pools are preferred breeding sites for *Aedes vittatus* (Okogun *et al*., 2005; Adebote *et al*., 2008). In a study in Edo State, Nigeria, *Aedes* were reported to be 23-29% of all the mosquitoes that bred in the area (Okogun *et al*., 2003). *Aedes albopictus*, *Ae. palpalis*, *Ae. africanus* and *Ae. luteocephalus* breed in plant and animal shells, tree holes and on leaf stalks (Okogun *et al*., 2005). Anosike *et al*. (2007) found *Aedes aegypti*, *Ae. africanus*, *Ae. simpsoni*, *Ae. albopictus*, *Ae. stokesi*, *Ae. taylori* and *Ae. apicoargenteus* among the mosquitoes breeding in tree holes in Imo State. *Aedes aegypti*, *Ae. albopictus* and *Ae. vittatus* were found in Abeokuta (Adeleke *et al*., 2008). In a study in Ogun State Ajayi *et al.* (2010) found that out of a total of 1308 mosquitoes only 18.5% were *Aedes.*

2.2 Mosquito Borne Diseases

Mosquitoes transmit many debilitating and fatal diseases, which are a major problem, especially in tropical developing countries (Chandra *et al.*, 2008a; NPS, 2010). Millions of people are killed and debilitated by malaria, yellow fever, dengue and dengue hemorrhagic fever, filariasis, chikungunya and encephalitis.

2.2.1 Malaria

Ronald Ross, a British physician in the Indian medical service in 1897 proved that mosquitoes were responsible for transmitting malaria (Sumodan, 2001). Nobel prizes have been awarded for work associated with malaria to Sir Ronald Ross (1902), Charles Louis Alphonse Leveran (1907), Julius Wagner-Jauregg (1927) and Hermann Muller (1948) (CDC, 2007). Each year, 350-500 million cases occur worldwide and over one million people die, most of them young children in sub-Saharan Africa (GMVC, 2002). The disease is a major cause of prenatal mortality, low birth weight, and maternal anaemia; some children who survive may suffer learning impairment or brain damage and in some countries, the disease may account for as much as 40 % of public health expenditure, 30–50 % of inpatient admissions, and up to 50% of outpatient visits (USAID, 2007). In Africa, more than 74% of the people live in endemic areas, 18% live in epidemic prone areas and only 7% live in free areas (GMVC, 2002). An estimated 990,000 people died of the disease in the region in 1995, in 2002; it was the fourth cause of death in children in developing countries (CDC, 2007). Nine out of ten cases worldwide occur in Africa south of the Sahara, those who suffer most are some of the continent's most impoverished, and the disease keeps them poor, a poor family living in affected areas may spend up to 25% or more of its annual income on prevention and treatment, the disease has slowed economic

growth in African countries by about 1.3% per year; the result of this over 35 years is the fall in GDP for African countries by 32%, the disease costs Africa more than US$ 12 billion annually (ASM, 2000). The World Health Organisation donated US$ 9 million to the African region for accelerated implementation of malaria control programmes in 1996 (GMVC, 2002). The disease however, seems to be on the increase as a result of drug resistance (Ogungbamigbe *et al*., 2008; Leach, 2009).

2.2.2 Yellow fever

The yellow fever virus originated in Africa as a monkey viral disease, transmitted by *Aedes africanus* in African forests (Sumodan, 2001). Yellow fever was carried to the western hemisphere during slave trade (Staples and Monath, 2008) and the first recorded outbreak was in Mexico in 1648 (Sumodan, 2001). To date, the disease has not appeared in Asia or the Pacific region (CDMP, 2001). About 90% of all cases are found in Africa, the disease occurs mainly in western sub-Saharan region and occasionally in some eastern countries, reports are regularly received from Nigeria, Ghana, Sierra Leone, Senegal, Mali, Guinea, Burkina Faso and Sudan (TMB, 2004). Nigeria has reported far more cases than any other country during the past 15 years; between 1984 and 1993 there were 20,000 cases with more than 4,000 deaths, in 1986 and 1987, the population at risk in Benue State was 200,000 with 9,800 cases and 5,600 deaths and in Oyo State there were about 116, 000 cases and 24,000 deaths, in 1994 an outbreak in Imo State spread to neighbouring districts of Cameroon (WHO, 1998).

The rainfall pattern and the vegetational zones determine the abundance of mosquitoes which also determine the disease distribution in Africa (WHO, 1998). The disease is highly communicable where many susceptible people and abundant vector mosquitoes coexist, the

overall case fatality rate among indigenous population in endemic region is less than 5%, recovery is followed by lasting immunity and transient passive immunity in infants born to immune mothers may persist for up to six months (CDMP, 2001). Yellow fever vaccine was one of the earliest viral vaccines developed (Staples and Monath, 2008). In 1881 Carlos Finly of Cuba suggested that *Culex cubensis* (now *Aedes aegypti*) was responsible for the disease; Walter Reed proved that *Aedes aegypti* was actually responsible for its spread (Staples and Monath, 2008). The main vectors in Africa are mosquitoes of the genus *Aedes*, subgenera *Stegomyia* and *Diceromyia*. Seven species are thought to play an important role: *Aedes (Stegomyia) aegypti, Ae. (Stegomyia) africanus, Ae. (Stegomyia) opok, Ae. (Stegomyia) luteocephalus, Ae. (Stegomyia) simpsoni group, Ae. (Diceromyia) furcifer, Ae. (Diceromyia) taylori* and *Ae. bromeliae, o*nce infected, mosquitoes remain infective for life, which in some instances exceed three months (WHO, 1998; CDMP, 2001).

2.2.3 Dengue and dengue hemorrhagic fever

Dengue is the most important tropical disease after malaria (Gubler, 1998). It is the most important human viral disease transmitted by arthropod vectors in the tropics and subtropics and the disease is rapidly increasing in public health importance (WHO, 1997; NEA, 2002; WHO, 2006). It was an uncommon illness until it became widespread in Southeast Asia in the 1950s; it is estimated that there are 50 to 100 million cases of dengue fever and 500,000 of dengue hemorrhagic fever (DHF) each year, almost half of the world's population is estimated to be at risk, the annual mortality is estimated at 24,000 (Gubler, 1998; Sumodan, 2001; WHO, 2006; McCormack, 2007). Since 1967, dengue virus has been reported in Angola, Burkina Faso, Comoros, Côte d'Ivoire, Democratic Republic

of Congo, Djibouti, Ethiopia, Ghana, Guinea, Kenya, Madagascar, Mauritius, Mozambique, Nigeria, Pakistan, Saudi Arabia, Senegal, Seychelles, Sierra Leone, Somalia, Sudan and the United Republic of Tanzania (WHO, 1997; WHO, 2006). Treatment is simply supportive i.e. drinking plenty of fluid to prevent dehydration, lowering the temperature and reducing the pain with paracetamol, Without specialised treatment, 20% of severe dengue patients die, but with suitable treatment the mortality is usually less than one-per-1,000, there is currently no specific clinically useful diagnostic test, no drugs and no vaccine, the development of a vaccine is complicated by the need to incorporate all four virus serotypes into a single preparation (WHO, 2006; McCormack, 2007).

Dengue viruses are transmitted to humans through the bite of infected *Aedes* mosquitoes, principally *Ae. aegypti* (WHO, 1997; Sumodan, 2001; McCormack, 2007) and the Asian tiger mosquito, *Ae. albopictus* (Goddard, 2003; Wan-Norafikah *et al*., 2009). Dengue outbreaks have also been attributed to *Aedes polynesiensis* and several species of the *Ae. scutellaris* complex, each of these species has its own particular geographical distribution; however, they are less efficient epidemic vectors than *Aedes aegypti* and an infected mosquito remains so for life, transmitting the virus to susceptible individuals during probing and feeding (WHO, 1997).

2.2.4 Elephantiasis/Filariasis

The disease is prevalent in humid and hot areas of Africa, the Americas, Asia and numerous islands in the Pacific Ocean (Sumodan, 2001). Thirty-nine out of 46 countries in the African region are endemic, the estimated at-risk population is 390 million, which accounts for approximately one-third of the global burden, thirty-eight percent of the at-risk

population are children under 15 years, the disease has both economic and psycho-social consequences since it prevents afflicted individuals from experiencing a normal working and social life, thus furthering the cycle of poverty (GAELF, 2008). The Global Programme to Eliminate Lymphatic Filariasis (GPELF) was established in 1998 under the leadership of WHO to eliminate LF as a public health problem by 2020; in 1998, SmithKlineBeecham (now GlaxoSmithKline-GSK) and Merck & Co. Inc. each announced their commitment to donate the drugs Albendazole and Mectizan® (the largest drug donations in history) valued at more than US$1 billion, the Bill & Melinda Gates Foundation provided a US$ 20 million grant to strengthen the Global Alliance to Eliminate Lymphatic Filariasis (2002-2006) (GAELF, 2008).

Culex quinquefasciatus and some *Anopheles* species are vectors of filariasis (Service, 1980 Sumodan, 2001). In a study in Plateau and Nasarawa States, in Central Nigeria, *Anopheles gambiae* s.s. and *An. arabiensis* were both found to carry the developing stages (L1-L3) of *W. bancrofti* (Lenhart *et al.*, 2007).

2.2.5 Chikungunya

Chikungunya is a relatively rare form of viral fever caused by an alphavirus that is spread by bites from *Aedes aegypti*. It was originally described in the early 1950s after an outbreak in a Swahili village on the Makonde Plateau that lies between Tanzania and Mozambique, since its discovery, the disease has occurred occasionally in Africa, South Asia, and Southeast Asia, but recent outbreaks have spread it over a wider area, between the1960s and 1980s the virus was isolated repeatedly from numerous countries in central and southern Africa as well as in Senegal and Nigeria, the symptoms are similar to those of dengue fever, the disease is almost always self-limiting and rarely fatal, it confers life-long

immunity and there is no vaccine or specific antiviral treatment (Srivastava, 2008; Tipathi, 2008).

Chikungunya virus is transmitted in the Savannah and forests of tropical Africa by *Aedes* species that belong to the subgenera Stegomyvia-*Aedes africanus, Ae. luteocephalus, Ae. opok* and Diceromyia-*Ae. furcifer, Ae. taylori, Ae. cordellieri; Ae. aegypti* is the primary vector that transmits the disease to humans, *Aedes aegypti* and *Ae. albopictus* (the Asian tiger mosquito) transmit the disease to human in Asia, Africa and Europe (Srivastava, 2008; Tipathi, 2008; Sumodan, 2010).

2.2.6 Encephalitis

Encephalitis is a viral disease that causes inflammation of the brain and spinal cord, there are different types, Eastern Equine Encephalitis (EEE**)** occurs in North, Central and South America, and the Caribbean; it affects only a small number of people but it is often fatal, the disease is spread to horses and humans by infected mosquitoes; a vaccine is available for horses, but not humans (AMCA, 2005; NPS, 2010).

West Nile Virus (WNV) emerged in 1937 from Africa (Uganda) into Europe, the Middle East, West and Central Asia and associated islands (Sumodan, 2001; AMCA, 2005; Hotta, 2005). Heavy rains followed by floods, irrigation, higher than usual temperature or formation of suitable breeding sites for mosquitoes increase the incidence of West Nile fever (Hubálek and Halouzka, 1999). Not all people infected with the virus develop clinical symptoms (Goddard, 2003). About 0.7% of all infected individuals develop central nervous system symptoms called West Nile encephalitis (Hotta, 2005). Fatality rates range from 2-20% with most deaths occurring in people 60 years of age or older (Goddard, 2003). The

virus is maintained between avian hosts and mosquitoes, infected mosquitoes are known to hibernate over the winter and may serve as the source of outbreaks the following year, and humans are the final host (Goddard, 2003; Hotta, 2005). According to Hotta (2005), infections may occur through blood transfusion, organ transplant, breast-feeding or in the foetus within the uterus; the only therapy available is symptomatic treatment and research into the development of vaccines is ongoing. The Bill Clinton administration gave US$ 7.7 million in 2000 to fight West Nile encephalitis in the USA (Sumodan, 2001).

Japanese encephalitis (JE) occurs not only in Japan but also in India, China, Malaysia, Korea and other areas of South-east Asia (Service, 1980). It has an annual mortality of 10,000 and an annual morbidity of 30,000-40,000 (Sumodan, 2001). In Asia about 50,000 cases of JE are reported annually, LaCrosse encephalitis is much less common and occurs in children younger than 16 years; there is no vaccine, outbreaks of St. Louis encephalitis (SLE) usually occurs in midsummer to early fall, the elderly and young people are more susceptible than those between 20 and 50 (Goddard, 2003; AMCA, 2005). West equine encephalitis (WEE**)** was first recognized in 1930 in a horse in California and since 1964 fewer than 1,000 cases have been reported; a vaccine is available for horses against WEE but not for humans, Humans can become infected only if bitten by an infected mosquito (Goddard, 2003; AMCA, 2005).

West Nile encephalitis (WNE) mosquito vectors in many places include *Culex tarsalis, C. pipiens pallens, C. pipiens molestus, C. tritaeniorhynchus, C. quinquefasciatus, C. salinarius* and *C. restuans* (Hubálek and Halouzka, 1999; Gouge *et al.*, 2001). *Culex vishnui* which prefers to breed in paddy fields is the vector of Japanese encephalitis (Sumodan, 2001). *Culex tarsalis* is the primary vector of Eastern Equine Encephalitis

(EEE) and Western Equine Encephalitis (WEE) while the vector of LaCrosse encephalitis (LAC**)** is a type of woodland mosquito *Aedes triseriatus* called the tree-hole mosquito (AMCA, 2005). *Aedes albopictus* is a vector of West Nile virus (WNV) (Goddard, 2003; Hotta, 2005).

2.2.7 Animal diseases

Not only can mosquitoes carry diseases that afflict humans, they also transmit several diseases and parasites that dogs, sheep and horses are very susceptible to. These diseases include dog heartworm, Rift valley fever and encephalitis. Dog heartworm (*Dirofilaria immitis*) can be a life-threatening disease for canines (AMCA, 2005). It is a filarial parasitic disease transmitted by a number of different mosquitoes to dogs and rarely humans; the disease can be fatal to dogs if left untreated (NPS, 2010). Rift valley fever is a viral disease spread by mosquitoes; it is prevalent in Africa and found mostly in sheep (Sumodan, 2001). The three equine encephalitis viruses (EEE, WEE and WNE) are very virulent in horses (Service, 1980). Western equine encephalitis (WEE) was first recognized in 1930 in a horse in California and over 200 horses were confirmed with EEE in 50 counties in USA in 2003 (AMCA, 2005). For WNV horses, beside humans, also contract infection via mosquito bites, but again they are final hosts for this virus (Hotta, 2005).

2.3 Fish

There are about 25,000-28,000 fish species; they constitute slightly more than one-half of the total number of approximately 54,711 recognized living vertebrate species, they belong to 57 orders, 200 to 300 species are discovered and named per year, they exhibit enormous diversity in their morphology, habitats and their biology, they live in almost every

conceivable type of aquatic habitat; their lifespan vary from a little over 1 year to about 120 years and they ranged from 7-10 mm *Schindleria brevipinguis* to the giant 12-16 metre whale shark (Coleman, 2006; Nelson, 2006; Orr, 2007; Pandey and Shukia, 2007).

Freshwater lakes and rivers comprise less than 0.001% of the volume of water on earth, but harbour 40% of fish species (Orr, 2007). In Nigeria over 34 well-known freshwater bodies (rivers, lakes, and reservoirs) which constitute about 12% of Nigeria's total surface area put at 94,185,000 hectare are known to support different fish species (Ita, 1993). Welman (1948) produced a list of 181 species of fish that could be found in Nigeria inland waters, the report revealed that Kaduna, Sokoto-Rima, Cross River, Ogun, and Osun Rivers have 28, 22, 39, 23, and 23 fish species respectively. Lévesque *et al.* (1992) estimated the freshwater fishes found in Nigeria to be about 268 species but Ita (1993) estimated 230 species. Odo *et al.* (2009) in Anambra River basin reported 52 fish species belonging to 17 families in the area. Achionye-Nzeh and Isimaikaiye (2010) in their study of a reservoir in Ilorin found a total of 12 species from 8 families. In Cross River, Offem *et al.* (2009) found 46 species belonging to 28 genera and 16 families. Allison and Okadi (2009) found 25 species belonging to 14 families in the lower Nun River Niger Delta. Araoye (2009) in Asa Lake, Ilorin found 21 species belonging to 11 families.

2.3.1 Factors that affect fish

Good water quality is essential to the health of fish at all stages of development, water quality requirements differ between species and between the different life stages as the fish develop, many of the water-quality parameters are interlinked and a change in one feature can have an effect on another (RFA, 2010). Therefore, it is important to understand the various parameters that affect fish.

2.3.2 Water quality

Understanding the physical and chemical qualities of water is important in keeping or working with fish (Rakocy, 1989; Huntley and Langton, 1994). No matter how clean the water used for fish, with time it will become polluted because fish are totally dependent upon the water to breathe, feed, excrete wastes, maintain salt balance and reproduce. Therefore not exceeding the carrying capacity of fish in ponds and tanks is very important (Rottmann *et. al.,* 1992; Swann, 1997). Nitrate, ammonia and other toxins often accumulate in an aquarium (DuHamel, 2009); therefore confinement of fish in tanks at high densities creates stressful conditions and increases the risk of disease outbreaks (Rakocy, 1989). Regular water change is therefore important in keeping fish healthy and in stimulating fish to breed (Huntley and Langton, 1994). Water should however, never be change all at once, as this will kill the beneficial bacteria which sustain aquarium's ecosystem and also put fish at risk of shock due to changes in the water chemistry. Changing 20-40% of the water once in two weeks is appropriate (Walker, 1978; Sommer, 1993). Fresh water replenishes trace minerals that are used up in the tanks by fish, plants and bacteria (DuHamel, 2009). It is important that the water to be introduced should be of similar temperature, pH etc. before being introduced (Huntley and Langton, 1994). Flow-through systems which involve the continual flow of a high quality water source through a tank or raceway is often used in some farms; this ensures quality and fish wastes are flushed, however, treatment of the fish wastes is often required before the water can be discharged into the environment (Terlizzi *et al*., 2004; DeTolla *et al*., 2008).

2.3.3 Temperature

Many aquatic organisms are sensitive to changes in water temperature (Bellingham, 2005). In keeping and handling fish, temperature is probably the most important water-quality variable, fish are not able to regulate their own body temperature and therefore have a body temperature similar to that of the water around them (RFA, 2010). All metabolic, physiological and life processes such as feeding, reproduction, growth, movement and distribution of fishes are greatly influenced by water temperature (Huntley and Langton, 1994; Swann, 1997; Bellingham, 2005; Pandey and Shukia, 2007). These metabolic processes are slowest at colder temperatures and speed up as external temperature increases (Huntley and Langton, 1994). All fish have a minimum and maximum lethal temperature limit, metabolic rates in fish double for each 18ºF rise in temperature (Swann, 1997). Rapid changes in temperature should definitely be avoided as few things kill fish faster than a rapid temperature change; tropical fish thrive in a very narrow temperature range, temperatures even slightly above or below this range will stress fish over time and could lead to disease or death (Mayland, 1976; FAF, 2004; PETCO, 2004; Pandey and Shukia, 2007). It is safer not to move fish into water more than 2ºC different from that which they came from, if fish are moved between ponds they should never be moved without checking that the two ponds are the same temperature; if they are different, it is important that they are allowed to get used to the new temperature by floating the container holding the fish in the new pond until the two temperatures are the same, it is safer to move fish to slightly cooler water rather than warmer water, as it contains more oxygen which will assist the fish in overcoming the stress of handling (RFA, 2010). Fish however tolerate gradual change in temperature (PETCO, 2004; Pandey and Shukia, 2007). The preferred temperature range

for optimum tilapia growth is 82º to 86ºF (27.8 to 30ºC) (Rakocy, 1989; Swann, 1997) and 85ºF (29.4ºC) for catfish (Swann, 1997). Tilapia, carp and catfish thrive in warm ponds of up to 33ºC. Tilapia are more adversely affected by too low temperatures, and usually die if the water goes below 12-13ºC for lengthy periods, carp and catfish are tolerant of a wide range in temperature (RFA, 2010).

2.3.4 pH

Some water molecules (H_2O) often disassociate into hydrogen ions (H^+) and hydroxide ions (OH^-), other compounds entering the water may react with these, leaving an imbalance in the numbers of hydrogen and hydroxide ions, this leads to the water becoming basic or acidic (Behar, 1997). pH is a measure of the number of hydrogen ions and therefore a measure of acidity. Most aquatic animals and plants have adapted to life in water with a specific pH and may suffer from even a slight change (Mesner and Geiger, 2005). There is no normal pH that applies to all fish species because fish come from sources with different pH levels, however, almost all freshwater fish can inhabit waters with a pH from 5.0-9.0 (Butler, 2006). The acceptable range for fish production is normally between pH 6.5-9.0 (Swann, 1997). The desirable pH range is 6.5-9.5 and acceptable range is 5.5-10.0 according to Stone and Thomforde (1977). A pH below 4 or above 10 will kill most fish (Mesner and Geiger, 2005), rapid changes can cause stress and even death (PETCO, 2004). When transferring fish from one environment to another, the pH of the water in both environments is measured and fish are only transfered when the pH of the receiving water is very near, or below, that of the transport tank water (Tucker and D'Abramo, 2008). Fish are more stressed and susceptible to disease when exposed to a change in pH greater than 0.3 in a 24 hour period (PETCO, 2004), they are prone to attack of parasites and diseases in

acidic waters (Pandey and Shukia, 2007). Even moderately acidic waters may reduce the hatching success of fish eggs and irritate fish (Mesner and Geiger, 2005).

Pond water pH fluctuates throughout the day due to photosynthesis, this may be stressful and even damaging to fish health even though may still be within the preferred range (FishDoc, 2004). Typically, pH is highest at dusk and lowest at dawn, because night time respiration increases carbon dioxide concentrations that interact with water producing carbonic acid and lowering pH, which can limit the ability of fish blood to carry oxygen (Stevens, 2009).

2.3.5 Total dissolved solids

Total dissolved solids (TDS) are the amount of inorganic salts, organic matter and other dissolved materials in water (Weber-Scannell and Duffy 2007; Abdalla and Drohan, 2009). TDS is expressed in units of mg per unit volume of water (mg/L), also refers to parts per million (ppm), it can be naturally present in water or can be the result of mining, oil and gas drilling, or some industrial or municipal treatment of water, it can be toxic to aquatic life through increases in salinity or changes in the composition of the water, or it may include substances that are toxic to people or aquatic life, some constituents such as arsenic, lead, and nitrate, can have health effects if they exceed drinking water standards (Abdalla and Drohan, 2009). Increase of salinity in the external environmental leads to excess water loss in fish overcoming the fish ability to osmoregulate thus leading to dehydration and death (Weber-Scannell and Duffy 2007).

TDS of fresh water typically ranges between 100 and 500 mg/L, maximum recommended level is 500 mg/L, when it exceed 1,000 mg/L it is considered unfit for human

consumption, ocean water typically has a TDS of about 35,000 mg/L (Abdalla and Drohan, 2009).

2.3.6 Electrical conductivity

Electrical conductivity (EC) is the ability of water to conduct electric current (Stone and Thomforde, 1977; CWT, 2004). This is an indicator of the concentration of dissolved electrolyte ions in the water, distilled water has a conductivity level of zero, as it has been stripped of all its salts and minerals during the distilling process, significant increases in conductivity may be an indicator that polluting discharges have entered the water (Behar, 1997). The EC of aqueous solution will thus depend on the presence of charged ions (Moore *et al*., 2008). The source of EC may be an abundance of dissolved salts due to poor irrigation management, minerals from rain water runoff or other discharges (Bellingham, 2005). Electrical conductivity indicates the total amount of ions in the water but does not indicate whether they are Mg, Ca or Fe (Butler, 2006; Behar, 1997). EC is mostly influenced by dissolved salts such as sodium chloride and potassium chloride (Bellingham, 2005). Because the mobility of a charged ion depends on ionic size and charge, the overall EC of a fluid will depend on which chemical are present and not just their concentration (Moore *et al*. 2008). Salts that dissolve in water break into positively and negatively charged ions, the major positively charged ions are sodium (Na^{+}), calcium (Ca^{2+}), potassium (K^{+}) and magnesium (Mg^{2+}); the major negatively charged ions are chloride (Cl^{-}), sulfate ($SO4^{-2}$), carbonate ($CO3^{-2}$), and bicarbonate ($HCO3^{-}$); Nitrates ($NO3^{-2}$) and phosphates ($PO4^{-3}$) are minor contributors to conductivity, although they are very important biologically (CWT, 2004). Some minimum salt content is desirable to help fish maintain

their osmotic balance, fresh water fish generally thrive over a wide range of EC, and the upper range of EC varies with fish (Stone and Thomforde, 1977).

The common unit for electrical conductivity is Siemens per meter (S/m) (Bellingham, 2005). The basic unit of measurement is micromhos per centimeter (µmhos/cm) or microsiemens per centimeter (µS/cm); either can be used, they are the same (Behar, 1997). The desirable range of EC is 100-2,000 µSiemens/cm while the acceptable range is 30-5,000 µSiemens/cm (Stone and Thomforde, 1977). Distilled water has 0.5-3.0, melted snow 2-42, potable water in USA 30-1,500 and freshwater streams 50-2,000 (umhos/cm) (Behar, 1997; CWT, 2004). Freshwater streams ideally should have conductivity between 150 to 500 µS/cm to support diverse aquatic life (Behar, 1997). EC also can be used to give a rough estimate of TDS in water, typically, the TDS value in mg/L is about half of the EC (µSiemens/cm) (Stone and Thomforde, 1977).

2.3.7 Feeding

Fish, like other animals, require the common components of foods i.e. carbohydrates, proteins, fats, minerals, vitamins and water; most fish feed daily (Bond, 1979). Food is the most important and vital factor in determining the population levels, rate of growth and condition of fish (Devi and Jauhari, 2011). Natural foods are the best foods for fish and include algae (phytoplankton), zooplankton, detritus, snails, worms, insects and insect larvae, small plants such as duckweeds and various other weeds and grasses that are found in a fish pond (RFA, 2010). Each fish species is unique in its nutritional requirements and

feeding behaviour (Pandey and Shukia, 2007; Francis-Floyd, 2009; RFA, 2010). Although some fish will only accept a narrow range of foods, most will take different types of foods if they are introduced to them slowly and allowed to get used to them (Huntley and Langton, 1994). Fish generally should be fed with a high quality diet that meets their nutritional requirements (Rottmann *et. al.,* 1992). Supplementing diets with fresh vegetables and live food is a good way to provide a balanced diet for fish, whose nutritional requirements are poorly understood (Francis-Floyd, 2009). Fish have limited access to natural foods when grown in tanks; they must therefore be fed on a complete diet containing vitamins and minerals (Rakocy, 1989). Fish grown in ponds have an advantage over fish raised indoors, because of their exposure to a variety of natural foods (Francis-Floyd, 2009). An important consideration in selecting a species to grow is the availability of commercially formulated diet and many species that appear attractive because of their high value may prove difficult and uneconomical to produce if nutritional requirements are poorly understood (Terlizzi *et al*., 2004).

The quantity of food given to a fish depends on many factors including species, age, current physical condition and type of food (Huntley and Langton, 1994). Active predators with their high metabolic rates require more food energy than do sluggish fishes, the predator feeding on the rich bodies of other animals such as insects, crustaceans, squids and other fishes, is ingesting high protein, high calorie food that provide nutritional requirements without much bulk, on the other hand herbivores ingest large quantities of food (Bond,

1979). Correct food should be provided at all times in order to maximize growth, carnivorous fish require a high protein diet while herbivorous species require lower protein content and can be fed on a higher proportion of plant meal (RFA, 2010). The eyes, the auditory organs, the lateral system, or the electric sense serve to orient the fish toward the food source (Bond, 1979). Feeding is better accomplished at least two or three times per day (Walker, 1978; Wainwright, 1996). It is always advisable to feed fishes at the same time of day and at the same part of the tank, young fishes normally need to be fed several times a day receiving only a small portion each time (Mayland, 1976). Fish kept in the laboratory should be given food that they can consume within fifteen minutes (Mayland, 1976; Rakocy, 1989; Sommer, 1993; Wainwright, 1996). Over-feeding or starving should be avoided (Rottmann *et. al.,* 1992). Uneaten food and fish waste contribute to poor water quality (DuHamel, 2009). The remains of food left in a tank will decompose rapidly and lead to an accumulation of ammonia (which is poisonous) and of nitrates (Mayland, 1976). Fish feed should always be stored in a cool dry place to prevent deterioration (Rottmann *et.al.,* 1992).

2.3.8 Stress

Stress is a condition in which an animal is unable to maintain a normal physiological, biochemical and behavioral state because of various factors affecting its well–being; it is caused by placing a fish in a situation which is beyond its normal level of tolerance (Bartelme 2006; Francis-Floyd, 2009). Stress can cause fish to exhibit behaviours, cease behaviours or modify the degrees to which these behaviours are exhibited (Bartelme 2006). Some stressors that may induce behavioral changes are: overcrowding, low dissolved oxygen and high carbon dioxide levels, photo-stress, thermal stress, pathogens, heavy

metals, toxins, poor water quality, pollution, metabolic wastes, dissolved gasses, handling, electric charges, aggression by other fish, nitrogenous waste, excessive water velocity, noise or vibration, low/high pH, transport, nutritional deficiency and injury (Bond, 1979; Bartelme 2006; Francis-Floyd, 2009). Signs of an unhealthy or stressed fish include wobbly swimming, severe undulations, problems with maintaining buoyancy, paleness and folded fins (DeTolla *et al.*, 2008). Fish are initially able to adapt to stress for a period of time during which they may look and act normal. However, energy reserves are eventually depleted and hormone imbalance occurs, suppressing their immune system and increasing their susceptibility to disease (Rottmann *et al*., 1992; Francis-Floyd, 2009). Stressful conditions can make fish more susceptible to infections, leading to decreased production and death (Terlizzi *et al*., 2004). If a fish is unable to adapt to a stress, decreased reproductive capacity and growth may occur, this might be associated with stress-mediated energy-repartitioning that diverts energy substrate away from vital life processes, such as reproduction and growth, in order to cope with the enhanced energy demand associated with stress (Iwama *et al.* 2004).

2.3.9 Quarantine

Fish brought into the laboratory for experiment or into ponds for rearing should be quarantine; this is to monitor the fish in isolation for any sign of disease which can be treated before the fish are allowed to come into contact with the existing stock (Mayland, 1976; Sommer, 1993; Wainwright, 1996). Quarantine is extremely important when acquiring stocks of fish, regardless of their source, however, it can only reduce the chances of spreading disease as some chronic infections and internal parasites may not become apparent during the period (Huntley and Langton, 1994).

2.3.10 Reproduction

Males of many fish species are more brightly coloured than the females, and may have larger fins and bolder markings; fish are well known for their high potential fecundity, with most species releasing thousands to millions of eggs annually (Bond, 1979). Some environmental factors that have been shown to play a significant role in the reproductive cycle are: photoperiod, time of day, water temperature, rainfall, water quality, change in electrical conductivity (change in salt content of the water), pH, flooding, water current, tides, cycles of the moon, weather cycles, spawning substrate, nutrition, disease, parasites and presence of other fish (Mayland, 1976; Rottmann *et al*., 1991; Sommer, 1993; Stevens, 2009). A variety of sensory receptors detect these cues, including the eye, pineal gland (an organ in the dorsal part of the forebrain that is sensitive to light), olfactory organs, taste buds, and thermoreceptors (Mittelmark and Kapuscinski, 2008).

In order to guarantee the survival of a particular species 'nature' provides a large number of young or establishes a particular type of behaviour, such as brood care, which assures minimal losses due to predation or adverse environmental influences, females of livebearers that have been fertilized just once can produce up to 10 lots of young without further copulation (Sommer, 1993). Some fish may require hiding places and some may require substrate to attach their eggs, aquatic plants must be provided when oviparous species are to be bred (Wainwright, 1996). Proper type and quantity of food ensure the best physical conditions for breeding as ova in certain species will only mature when a specific type of food is given (Sommer, 1993; Huntley and Langton, 1994). Brood fish should be fed on high quality feed at a rate of 2% of their body weight per day (Rakocy, 1989). Some species e.g. the African Catfish will not readily breed in captivity since the environmental

factors such as the rise in water level and inundation of shallow areas do not occur on the fish farms to complete the reproductive process, hormone induce spawning is the only reliable method to induce reproduction in these fishes (Rottmann *et al*., 1991; De Graaf and Janssen, 1996; Mittelmark and Kapuscinski, 2008).

2.4 Mosquito Control.

2.4.1 Environmental management

Environmental management involves the modification of the environment to make it unfavourable for the mosquitoes to breed. These include draining or filling up of ponds, intermittent draining of irrigated areas and maintenance of irrigation channels, construction of drainage channels (GMVC, 2002; USAID, 2007). Increase sunlight on water surface as a result of trimming overhanging vegetation prevents breeding of mosquitoes which prefer shaded habitats; the removal of rooted and floating vegetation reduces breeding of mosquitoes such as the *Mansonia* species which require plants to obtain their oxygen supplies (CHP, 2004). Using the synchronized cropping method for rice e.g. rice paddies are left dry for 2 months each year, this periodic wet and dry rice agricultural practice has led to a significant reduction of adult mosquito populations in Indonesia, alternatively fields can be flooded for several days and then left to dry (USAID, 2007).

2.4.2 Physical methods of control

Traps are used to capture adult mosquitoes; several types of light traps are commonly used. Only selected species of mosquitoes are attracted by light traps and mosquito catches are increased by hanging a container of dry ice or an octenol lure near the light trap (Goddard, 2003). The attractant used could also be carbon dioxide released by breaking down propane

into water and carbon dioxide, some mosquito traps have a fan to suck the insects flying nearby into some collection chamber or bag (CHP, 2004). New Jersey light traps and CDC light traps are the traps most commonly used; different models of traps vary in the numbers, species, and the proportion of males to females they catch (NPS, 2010). The CDC light trap, developed by the Centers for Disease Control was used by Amusan *et al*. (2005) to determine mosquito species composition and abundance in rice field and plantation in Ogun State. Adult mosquitoes were collected by CDC light traps in three villages in a survey to determine the mosquito species diversity and abundance in relation to land use in the Mwea rice scheme, Kenya (Muturi *et al*., 2006). Hoel *et al*. (2009) compared six adult mosquito traps for effectiveness in collecting *Aedes albopictus*, trap selection included two commercial propane traps (Mosquito MagnetTM Professional trap and Mosquito Magnet Liberty trap), two *Aedes*-specific traps (Fay-Prince Omnidirectional trap and Wilton trap), one experimental trap (Mosquito Magnet-X trap) and a standard surveillance CDC light trap that served as a control. Significantly more *Aedes albopictus* were collected from the experimental and commercial traps than from the CDC light trap and *Aedes*-specific traps.

Long and thick garments reduce areas exposed by human to mosquito bites, mosquito nets have been used for a long time as a protection against mosquitoes (GMVC, 2002). Use of Insecticide treated mosquito nets (ITNs) and insecticide treated materials (ITMs) has increased since 2000 (USAID, 2007). Insecticide treated mosquito nets have had significant impact in reducing morbidity and mortality particularly among children under five years old and pregnant women in Nigeria (WHO, 2006). Pyrethroid-impregnated bednets or curtains appear to be effective and save against mosquitoes that feed at night and house

screening/ proofing of doors, windows and other openings using netting materials is also effective in mosquito control (WHO, 1997).

2.4.3 Chemical methods of control

During World War II (1939–1945) Dichloro-diphenyl-trichloroethane (DDT) was used in malaria control (USAID, 2007). The use of the DDT and other chlorinated hydrocarbons as chemical agents for control of insects and other pests which gained momentum around the mid twentieth century lost its appeal by the seventies because of their ill effects on human health (Pandey and Shukia, 2007). As mosquito resistance to DDT emerged, costs of the campaigns increased, and efforts to expand campaigns to endemic tropical areas failed, the pursuit of worldwide malaria eradication was abandoned (USAID, 2007). However, as resistance to DDT emerged in the early 1960s, organophosphate insecticides, including fenthion, malathion, fenitrothion permethrin, propoxur and temephos, were used for *Aedes aegypti* control (WHO, 1997). But some mosquito species have developed resistance to some of these commonly used insecticides (Goddard, 2003).

Insect growth regulators (IGRs) are pesticides that disrupt the normal activity of the endocrine or hormone system of insects, the affect the development, reproduction or the metamorphosis of targeted insects. Examples of insect growth regulators are hydroprene, hexaflumuron, lufenuron, diflubenzuron, kinoprene, pyriproxifan and methoprene. Methoprene interferes with an insect's life cycle and prevent it from reaching maturity or reproducing; it has been used in some countries for controlling the breeding of mosquito larvae, this prevent the development of the larvae rather than killing them, it is sold under a number of trade names including Altosid®, Precor®, Kabat®, Pharorid®, Dianex®,

Apex®, Fleatrol®, Ovitrol®, Extinguish® and Diacon®. Methoprene is only effective when applied to the larval stage; any pupae that are present in the water during application will not die and will successfully emerge as adults; it is target specific and does not harm mammals, waterfowl or predatory insects, it was first registered by the Environmental Protection Agency of America in 1975 (Goddard, 2003; CHP, 2004; Tunaz, 2004; USAID, 2007; NPS, 2010).

Monomolecular films are low-toxicity pesticides that spread a thin film on the surface of water, making it difficult for mosquito larvae, pupae and emerging adults to attach to the water's surface thus causing them to drown, films typically remain active for 10–14 days on standing water (USAID, 2007). Agnique is a biodegradable oil emulsion sprayed as a larvicide, it acts by suffocating mosquito larvae as they float at the surface to breathe, it is also safe to use in fish habitats and in the presence of biological predators (Goddard, 2003).

Repellents are normally applied directly to skin or clothing, the most commonly used insecticide is Deet (Diethyl-toluamide), they are recommended for people staying outdoors at night and those working in plantations. Some plants such as basil, lemon grass, marigold, rosemary, garlic and burned leaves and barks of Neem (Azadirachta indica) and Eucalyptus (Eucalyptus globulus) keep mosquitoes away; different types of coils for mosquito control are sold commercially, they are often used in rooms; they often contain allethrin, pyrethrum or citronella in a vapouriser base, when these coils are heated, they release the insecticide in form of vapour or smoke (GMVC, 2002; Goddard, 2003).

Pyrethrins are natural insecticidal chemicals extracted from the flowers of Chrysanthemum, the natural mixture of the six pyrethrins is known as pyrethrum. Synthetic pyrethroids have

been in use for over 30 years and account for 25% of the world insecticide market; almost all the Raid, Mortein, PeaBeu, Budget and Ultrapel aerosols or coils sold in supermarkets and shops contain synthetic pyrethroids, such as: tetramethrin, permethrin, allethrin, cypermethrin and bioresmethrin, the names of the pyrethroids used as insecticides usually end with "-thrin" (McCormack, 2007). Synthetic pyrethroids are used in indoor residual sprays, space sprays and impregnated bed nets; they are also used in aircrafts (Tiwari *et al*., 2010). Ndams *et al.* (2006) in Zaria reported that *Aedes aegypti* and *Culex quinquefasciatus* are susceptible to permethrin, whereas *Anopheles gambiae* was resistant to the insecticide in the area. In Benin, Nigeria, *Anopheles gambiae* s.s. was found to be resistant to pyrethroids (Djouaka *et al*, 2008).

2.4.4 Biological methods of control

Following the resurgence of malaria in the early 1970s in some countries, reports of the development of insecticide resistance in malaria vectors and increased fear of environmental pollution with insecticide led to renewal of interest in developing environment-friendly methods such as the use of biological control agents (WHO, 2003). Since biological control does not cause chemical pollution, it is considered as a better method for mosquito control by many people (CHP, 2004).

Microcrustacean copepods are found to prey on mosquito larvae (Hintz 1951, Ray and O'Connell, 2004). The following species of copepods have been found to control mosquito larvae in Australia, Oceania, Brazil, Honduras and Vietnam: *Mesocyclops longisetus*, *Mesocyclops thermocyclopoides*, *Metacyclops mendocinus*, *Tropocyclops prasinus*, *Eucyclops serrulatus*, *Eucyclops solitarius*, *Eucyclops ensifer* and *Macrocyclops albidus*

(USAID, 2007). Copepods were observed to kill the following number of first instar *Anopheles quadrimaculatus* larvae in the laboratory: *Mesocyclops ruttneri* (36 larvae/day), *Macrocyclops albidus* (23 larvae/day), *Mesocyclops longisetus* (24 larvae/day), and *Acanthocyclops vernalis* (15 larvae/ day) (Marten *et al*., 1999). Larvae of the aquatic coleopterans (dytiscid beetle) *Acilius sulcatus* Linnaeus 1758 was found to also prey on mosquito larvae (Chandra *et al.,* 2008b). Larvae of hydrophilid beetle of the genus *Tropisternus* also prey on mosquito pupae (Hintz, 1951).

Certain species of *Turbellaria* flatworms prey on mosquito larvae, the nematode *Romanomermis iyengari* has been found to be an effective parasite of aquatic stages of mosquitoes in rice fields (USAID, 2007). Kar and Aditya (2003) in India reported that aquatic planarians (free-living helminthes) feed and can control *Culex* larvae.

Mosquitoes are infected by several viruses, the baculovirus group, which causes high infectivity and pathogenicity, offers the most promise for biological control potential; however, most virus agents are difficult to mass produce and store for long periods of time (USAID, 2007). There are many fungi that infect and kill mosquitoes at the larval and/or adult stage, and the following have been studied extensively: *Lagenidium*, *Coelomomyces* and *Culicinomyces* (Scholte *et al*., 2004). *Erynia aquatica* is known to infect the immature aquatic stages of mosquitoes and the aquatic fungus *Coelomomyces indicus* found naturally in rice fields infects anophelines and culicines (USAID, 2007).

Many species of water-going predaceous insects, such as dragonfly larvae and water bugs, eat mosquito larvae and pupae; for instance, adults and nymphs of *Anisops bouveri* feed on mosquito larvae (USAID, 2007). *Cyrinus natator*, *Nepa cinerea* and *Cybister tripunctatus*

are remarkably predaceous on mosquito larvae (Mohanray *et al*., 2012). Larvae of the mosquito genus *Toxorhynchites*, such as *T. lendens* attack and kill mosquito larvae; however, some species are very selective in their oviposition sites, limiting them to tree holes and containers, which greatly restrict their usefulness (USAID, 2007).

Many species of bats and birds are voracious feeders on mosquito adults; however, their feeding is not generally sufficient to fully control adult mosquito population (USAID, 2007). Most aquatic turtles are general predators and juvenile turtles have been found to have the capacity to eat more than 500 third and fourth instar mosquitoes per day (Marten, 2007). The idea of using frogs and tadpoles to control mosquitoes in India is receiving attention (Raghavendra *et al*., 2008).

Sterile-male release techniques are being studied in some countries for getting a high proportion of infertile insemination. Mosquitoes that are refractory to infection with human diseases such as malaria are released for replacing the natural susceptible ones (CHP, 2004).

Plants cover and different plant parts have been tried and used for mosquito larvae control in different countries. In Dhaka city (Bangladesh), the vegetation cover in breeding grounds influenced mosquito larval population; 25% cover with water hyacinth and duckweeds showed 62.7% less larvae in the former than the latter (Ameen *et al*., 1999). Experiments with duckweeds showed a 40-100% reduction in oviposition due to duckweed cover, a decline in hatching, survival of immature stages, and adult emergence (16-52%) compared to the controls (57-69%) was observed; in case of full cover no larvae survived beyond second instar (Ameen *et al*., 1999). Two indigenous aquatic weeds in Pakistan *Lemna*

minor (duckweed) and *Spirodella* spp. (large duckweed) were also used for the biological control of mosquitoes with local guppy fish also called Rainbow fish *Poecilia reticulata* in stagnant and polluted dirty water; the plants and fish supported each other in symbiotic condition (Tariq *et al*., 2009). Chansang *et al*. (2005) in Thailand, bioassayed aqueous extracts of nine medicinal plants against larvae of *Culex quinquefasciatus* and *Aedes aegypti*; among these plants, the long pepper, *Piper retrofractum* showed the highest level of activity against mosquito larvae. Mohan and Ramaswamy (2007) found leaf extract of *Ageratina adenophora* to be toxic to both *Aedes aegypti* and *Culex quinquefasciatus* and could be used for the control of mosquito larvae. In a study in Kaduna, plants extracts of *Peristrophe bicalyculata* leaves and *Zingiber officinale* (ginger) rhizome were found to be effective in controlling mosquito fourth instar larvae, while *Allium sativum* (Garlic) bulbs were found to be less effective (Yohanna *et al*., 2011). Aina *et al*. (2009a) found that both the ethanolic and aqueous extracts of the fruits of *Physalis angulata* and *Xylopia aethiopica* and seeds of *Piper guineense* and *Jatropha curcas* were effective in eliminating the second instar larvae of *Anopheles gambiae*. Aina *et al*. (2009b) also found that extracts of *Tetrapleura tetraptera* (*Aridan*), *Delonix regia* (Flame of the forest) and *Raphia vinifera* (Raffia palm) were toxic to the larvae of *Anopheles gambiae*. Ohaga *et al*. (2007) showed that *Piper guineense* and *Spilanthes mauritiana* crude-powder were larvicidal against *Anopheles gambiae* and *Culex quinquefasciatus* in Kilifi District, Kenya.

Bacillus sphaericus (Bs) and *Bacillus thuringiensis* var *israelensis* (Bti) are bacteria used as larvicides, they are effective only on mosquito larvae that are still feeding and not effective on pupae or larvae that have stopped feeding (NPS, 2010). Duration of effectiveness depends primarily on the mosquito species, the environmental conditions, the formulation

of the product, and water quality (USAID, 2007). These bacteria are nonhazardous to humans, other animals, fish, and predacious insects (WHO, 1997; CHP, 2004). Bti was first registered by USA Environmental Protection Agency (EPA) as an insecticide in 1983 while *B. sphaericus* was initially registered by the EPA in 1991 (USAID, 2007). Treatment with Bti in wild rice fields in USA significantly reduced mosquito larvae population and in Kenya the bacterial isolates showed broad spectrum larvicidal potency against *Anopheles, Culex* and *Aedes* mosquito larvae (Kramer *et al*. 1988). While Bti is effective against only anopheles larvae, Bs is more effective against culex larvae (CHP, 2004), but the formulations require frequent reapplications and because the toxin is photolabile it could be destroyed by sunlight (WHO, 1997). *Bacillus cereus, Bacillus thuringiensis pakistani* H-13 and *Bacillus thuringiensis tohokuensis* H-17 have biocontrol potential against *Culex* larvae in Zaria environment (Umaru, 2000).

2.4.5 Mosquito larvae control using fish

Fish with larvivorous potentials should: be surface feeders, as mosquito larvae are found on the water surface. They should have terminal or superior mouth and swim above the surface stratum of aquatic habitats; they must be hardy enough to survive handling, transport and temperature variations. They should be carnivorous, small in size so as to reach grassy pond edges, show preference for mosquito larvae over other types of food, be swift and agile to escape predators, live in turbid and polluted water, and should be insignificant as food for other predators. They should have high fecundity and ability to breed throughout the year under natural conditions and in confined waters; and they should be locally available (GMVC, 2002; WHO, 2003; CHP, 2004; NVBDCP, 2007; USAID, 2007).

Most fish fry are potential predators of mosquito larvae (GMVC, 2002). More than 100 fish species are known to eat mosquito larvae (Jianguo and Dashu, 1995). Therefore mosquitoes are rarely a problem in a body of water that contains fish (NPS, 2010). Small insectivorous fish are a valuable tool in controlling mosquitoes in ponds, patio tanks, abandoned swimming pools and pools no longer maintained by their owners (Kern, 2004). Since 1937, different types of fish had been used to control mosquito larvae (Chandra *et al*., 2008a). Global experiences has shown that in many situations fish are effective in controlling mosquito-borne disease either alone or as part of an integrated control approach; the most promising larvivorous fish belong to the families: Umbridae (Mudminnow), Poeciliidae (Live bearer or Guppy), Centrarchidae (Sunfish), Cyprininidae (Killifish) and Cichlidae (WHO, 2003; Kern, 2004). Fish can be used cost-effectively, especially when there is also community participation, such as the maintenance of fish stocks and hatcheries, removal of weed from water bodies, distribution of fish, replenishment of fish in mosquito breeding habitats and the use of larvivorous fish with food value; indigenous fish are generally preferred over the exotic species since they are well adapted to the local environment (WHO, 2003; Chandra *et al*., 2008a).

Gambusia affinis

Gambusia affinis is a small surface feeder and can penetrate into shallow vegetated areas, it can grow and reproduce rapidly and tolerate a wide range of temperature and physical conditions (CHP, 2004). The World Health Organisation has used this fish for decades to fight malaria in many parts of the world (Goddard, 2003). The fish was purposely introduced from its native Texas (southern USA) to Hawai in 1905; in 1921, it was introduced in Spain, then from there in Italy during 1920s and later to 60 other countries, in

Egypt imported *Gambusia affinis* from Italy and Georgia for mosquito control has been used as far back as 1920 (Gerberich, 1985; WHO, 2003). A study of this fish in Gezira irrigation canals by El- Safi *et al.* (1985) showed that the fish could be useful for mosquito control in the area. The fish was able to reduce anopheline larvae and thus contributed to reducing malaria in Iran (Tabibzadeh *et al.,* 1971). In Tunis the fish was introduced in 1929 to control mosquito larvae under laboratory condition and the good efficacy of the fish was confirmed in the area recently by Ghrab and Bouattour (1999). Bellini *et al.* (1994) in northern Italy showed that the fish can be used to control mosquitoes in rice fields. In America, studies showed that the fish controlled mosquito larvae (Cech and Linden, 1987; Kramer *et al*. 1987, 1988; Linden and Cech, 1990; Nelson and Keenan, 1992; Duryea *et al*., 1996; Offill and Walton, 1999; Billman *et al.,* 2007). Emergent vegetation is the greatest problem associated with the inability of *Gambusia* to control *Anopheles* spp. larvae (Duryea *et al*., 1996). In India, the mosquito control potential of the fish was evaluated in the Shahjahanpur district of Uttar Pradesh; it was found that the larval and pupal densities of the mosquitoes in experimental field dropped (Das and Prasad, 1991; Haq *et al.,* 1992; Ambrose *et al*., 1993; Prasad *et al.,* 1993). Culture of *Gambusia* along with edible fish in village ponds is recommended to get the dual benefit of fish production and control of mosquito proliferation in village ponds (Haq *et al.,* 1991). Some mosquito researchers have expressed concern about the environmental impact of placing the fish in habitats to which it is not native (Rupp, 1996). However, in India, Haq *et al.* (1992) found that predatory fishes and birds played a major role in eliminating *Gambusia,* therefore it was unable to eliminate the local fauna to become a dominant species and is therefore unlikely to pose any ecological hazard in vector-control programmes in that area.

Poecilia reticulata

Beginning in 1908 *Poecilia reticulata* (guppy), a native of South America, was introduced for malaria control into British India and many other countries (Gerberich, 1985; WHO, 2003). The fish has been used in vector control programs around the world for the past 50 years (USAID, 2007). In Dhaka, Elias *et al*. (1995) studied the predatory potential of the fish, on the third instar larvae of *Culex quinquefasciatus* under laboratory conditions and suggested that the fish could be used to control filariasis and other mosquito borne diseases. Nalim and Tribuwono (1987) used this fish to control *Anopheles aconitus* in rice fields in central Java; this led to a reduction in malaria cases in the area. In Cuba, it was found that the fish was able to control the population of *Culex quinquefasciatus* larvae in ponds and ditches (Koldenkova *et al.,* 1989; Garcia *et al*., 1991). In Indonesia, studies on the predatory capability of the fish for the control of *Aedes aegypti* in the laboratory was carried out and found to be effective (Kamah, 1995). Studies on indigenous fish species in water storage tanks in Southern Mexico showed that the fish was able to control *Aedes aegypti* (Martinez-Ibarra *et al.*, 2002). In the Comoros, Sabatinelli *et al.* (1991) used the fish to control the malaria vector *Anopheles gambiae* in the field. In a community-based study of the fish in water storage containers for dengue control in Cambodia, there was a reduction in *Aedes* infestation in the intervention community (Seng *et al*., 2008). In Sri Lanka, Ekanayake *et al.* (2007) also found the fish effective in controlling mosquito larvae. In India, effectiveness of the fish in controlling well breeding *Culex quinquefasciatus* was found to be successful (Joshi *et al.,* 1989). *Poecilia reticulata* and *Gambusia affinis* are being mass-produced by fish farmers as part of an environmental management malaria control program in India (USAID, 2007).

Cichlids

In Kenya, Asimeng and Mutinga (1993) reported that *Oreochromis niloticus* and *Tilapia zilli* demonstrated a strong predation for mosquito larvae. In the western Kenyan highlands the fish was use to control both *Anopheles gambiae* s.l. and *An. funestus* in abandoned fishponds (Howard *et al.* 2007). Fletcher *et al.* (1993) in a search of indigenous fish with larvivorous potentials in Ethiopia identified *Oreochromis niloticus* among others as efficient predator of *Anopheles gambiae* s.l. and *Culex andersoni* under laboratory conditions. In Egypt a study of the fish in Gezira irrigation canals by El-Safi *et al.* (1985) showed that the fish smaller than 150 mm were marked carnivorous and could be useful for mosquito control. In India predation experiments in the laboratory and in the field on the fish among others indicated that the fish had potential as biocontrol agents for *Anopheles stephensi* mosquito larvae and pupae (Ghosh *et al*., 2005a).

Mohamed (2003) used *Oreochromis spilurus spilurus* to control mosquito larvae in reservoirs in Somalia. Fletcher *et al.* (1993) in Ethiopia found *Oreochromis spilurus spilurus* among seven indigenous fish species tested in the laboratory to be efficient predators of *Anopheles gambiae* s.l. and *Culex andersoni.* In Sri Lanka, Ekanayake *et al.* (2007) experimented on the larvivorous potential of eight common freshwater fish species on *Aedes aegypti* and *Ae. albopictus*, they found that *Oreochromis mossambicus* with six others were effective in controlling mosquito larvae. In India, a pilot study in the laboratory and field using *Oreochromis mossambicus* was carried out to evaluate its potentiality to control *Aedes* and *Anopheles* larvae, in the laboratory studies, the fish showed a greater feeding affinity for *Aedes* than either *Anopheles* or artificial fish diet; in the field trial, the

introduction of the fish into water storage tanks proved to be effective in eliminating *Aedes* larvae within three days (Surendran *et al.*, 2008).

Aphanius dispar, Clarias gariepinus, Panchax panchax, Grass carp and *Barbus pobeguini*

Natural occurring *Aphanius dispar* in shallow channels was able to control mosquito larvae in Saudi Arabia (Ataur-Rahim, 1981). Louis *et al.* (1988) reported that the fish was able to suppress the breeding of *Anopheles arabiensis* and *An. gambiae* breeding in wells, cisterns, barrels and containers in Djibouti. In Ethiopia, the fish was use to control *Anopheles culicifacies adanensis, An. gambiae* s.l. and *Culex andersoni* (Fletcher *et al*., 1992, 1993). In Israel, Goren (1995) found that the fish met some conditions required by fish for mosquito larvae control. Predation experiments in the field and laboratory on *Clarias gariepinus* by Ghosh *et al*. (2005a) in India indicated that the fish had potential as biocontrol agents for *Anopheles stephensi* mosquito larvae and pupae. In India Hora and Nair (1938) found that *Panchax panchax* preyed on mosquito larvae in both field and laboratory experiments. Grass carp has been used purposely in pond for removing aquatic vegetation that provides shelter for mosquito larvae and pupae (CHP, 2004). Liao *et al.* (1991) in their study in China found that Grass carps which were bred in rice field were able to control *Anopheles sinensis* and *Culex tritaeniorhynchus.* Jianguo and Dashu (1995) also found grass carp to be more effective in the laboratory and in eliminating weeds and mosquito vectors in ricefield than Silver crucian carp and the Nile tilapia. *Barbus pobeguini* was able to control the breeding of *Anopheles gambiae* s.l. and *Culex quinquefasciatus* in Ouagadougou (Burkina Faso), the fish is indigenous and thus of advantage in the area (Mancini and Romi, 1988).

Other fishes tried for mosquito larvae control in some countries

Okorie and Abiodun (2010) found that *Aphyosemion gularis* have mosquito biocontrol potentials in Nigeria. Fletcher *et al.* (1993) in Ethiopia identified *Aplocheilichthys antinorii, Micralestes acutidens, Alestes affinis* and *A. macrolepidotus* as efficient predators of mosquito larvae. In Sri Lanka, Kusumawathie *et al*. (2006) and Ekanayake *et al.* (2007) found the following fish species with larvivorous potentials*: Danio malabaracus, Oreochromis mossambicus, Oreochromis niloticus, Poecilia reticulata, Aplocheilus dayi, Puntius bimaculatus, Puntius filamentosus, Belontia signata* and *Rasbora daniconius.* In Pakistan a study was carried out on a total of 426 fish specimens belonging to 12 different species of local fishes and mosquito larvae were found in the gut of the following carnivores: *Barilus vagra, Channa punctatus,* and *Mystus bleekeri* (Rasool and Suleman, 1999). In India a lot of work has been done on the larvivorous potential of different fish species (Joshi *et al.*, 1989; Yadav *et al.*, 1992; Jayasree and Panicker, 1992; Ambrose *et al.*, 1993; Haq *et al.*, 1993; Sharma, 1994; Victor *et al.*, 1994; Sumodan and Kumar, 1998; Ghosh *et al*., 2004, 2005a; Gupta and Banerjee, 2009; Devi and Jauhari, 2011). *Esomus danrica* and *Trichogaster fasciatus* were found to control mosquito larvae (Joshi *et al.*, 1989). Yadav *et al.*, (1992) on laboratory evaluation selected these six potential larvivorous fishes: *Aplocheilus panchax, Oryzias melastigma, Oreochromis mossambicus, Gambusia Affinis, Danio (B.) rerio* and *Esomus dandricus*. Jayasree and Panicker (1992) showed that *Gambusia affinis, Ophiocephalus striatus* and *Macropodus cupanus* were able to consume mansonioides larvae. Ambrose *et al.* (1993) found that *Gerris (A) sponolae* and *Laccotrephes griseus* are capable of preying on culicine larvae. Haq *et al.* (1993) found that six fish species (*Chela bacaila, Puntius stigma, Rasbora daniconis, Esomus danricus,*

Colisa fasciatus and *Danio* sp.) had good larvivorous potentials among others. Sharma (1994) found *Puntius ticto, Colisa fasciata, Aplocheilus panchax, Rasbora daniconius, Chanda nama* and *Esomus danricus* to possessed good feeding potential on mosquito larvae. Composite culture of 11 edible fishes by Victor *et al.* (1994) resulted in reduction of immature mosquito population; the study showed that common carp (*Cryprinus carpio*) and silver carp (*Hypopthalmithys molitrix*) are effective larvivores. Sumodan and Kumar (1998) studied fifteen fish species and based on their findings they recommended *Aplocheilus blockii* and *Rasbora daniconius* for use in malaria control operations. Predation experiments on *Betta splendens, Pseudotropheus tropheops, Osphronemus gorami, Pterophyllum scalare, Cyprinus carpio* and *Ctenopharyngodon idella* indicated that all these fishes have potential as biocontrol agents for mosquito larvae and pupae (Ghosh *et al*., 2004, 2005a). *Aplocheilus panchax* fed well on mosquito larvae (Devi and Jauhari, 2011). Gold fish (*Carassius auratus)* was also observed to control mosquito larvae (Gupta and Banerjee, 2009). In Indonesia, studies on the predatory capability of *Aplocheilus panchax* and *Colisa lalia* on mosquito larvae was found to be successful (Kamah, 1995). In America, Nelson and Keenan (1992) showed that native killfish can control mosquito larvae. *Gambusia affinis* controlled mosquito larvae in experimental paddies but Sacremento blackfish (*Orthodon microlepidotus*) did not (Cech and Linden, 1987). The inland silverside *Menidia beryllina* did not survive well in rice field system and were not able to control mosquito larvae (Kramer *et al*., 1987). In Columbia, Torrente *et al*. (1993) made a survey of 16 fish species from 11 families and gut analyses showed that 11 fish species included mosquito larvae in their diets while the other five did not. Hurst *et al*. (2004) reported the predation efficacy of some Australian fresh water fishes: *Melanotaenia duboulayi, Retropinna semoni, Pseudomugil signifier, Craterocephalus stercusmuscarum,*

Hyposeleotris galli, Hypseleotris compressa and *Ambassis marianus* on mosquito larvae in the laboratory, all these with the exception of *Ambassis marianus* performed well. Bellini *et al.* (1994) in northern Italy showed that *Carassius auratus* and *C. carpio* can be used to control mosquitoes in rice fields. Studies in southern Mexico showed that *Lepisosteus tripicus, Astyanax fasciatus, Brycon guatamalensis* and *Ictalurus meridionalis* were able to control *Aedes aegypti* (Martinez-Ibarra *et al.*, 2002). Marti *et al*. (2006) reported that *Cnesterodom decemmaculatus* and *Jenynsia multidentata* were able to feed and control *Culex pipiens* in human made ditches in Argentina. In China, since 1988 fish have been employed for controlling *Aedes* larvae in water containers in Liuchiu, Pingtung (Hwang and Chow, 1990). Liao *et al.* (1991) found that grass carp controlled mosquito larvae. Wu *et al*. (1991) found that the common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*) and *Tilapia* spp. were also able to control mosquito larvae. Kim *et al.* (1994) demonstrated biological control of mosquito larvae by *Moroco oxycephalus* and *Misgurnus anguillicandatus* in Korea. The ability of two freshwater fishes, *Melanoteania splendida splendida* and *Craterocephalus stercusmuscarum stercusmuscarum*, native to North Queensland, Australia, to prey on mosquito larvae was studied by Russell *et al.* (2001), *Melanoteania splendida splendida* was found to be very promising, and have the advantages that it could easily be handled, transported and kept in captivity for extended period with negligible mortality.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Area of Study

Fish were collected from parts of Kaduna, Katsina, Kano and Niger States in northern Nigeria. All the collection sites fall within the Guinea and Sudan savanna ecological zones of Nigeria. Variations in the weather are governed by the interaction of moist southwest monsoon and dry northeast winds. Mean maximum temperatures are 33-35ºC in the north and humidity is high from June to September, low humidity coincides with the dry season. The Guinea savanna is the broadest vegetational belt in the country occupying almost half the area of the country and located in the middle; it covers the area which has 100 to 150 cm of rainfall with a wet season which lasts 6 to 8 months. Beyond the Guinea savanna lies the drier Sudan savanna, a region of shorter grasses and more scattered, drought-resistant trees such as the baobab, tamarind, and acacia; it covers an area over a quarter of the whole country with an annual rainfall between 65 and 100 cm, the dry season is about 6 to 8 months.

The River Niger and River Benue are the two principal rivers in northern Nigeria. River Niger, the largest in West Africa; flows for a distance of 4,000 km from Guinea through Mali, Niger, Benin and Nigeria before emptying into the Gulf of Guinea. The River Benue flows for 1,400 km from Cameroon into Nigeria, where it empties into the River Niger. Kainji Lake, created in the late 1960s by the construction of the Kainji Dam on the Niger River, is Nigeria's only large lake in addition to Lake Chad. Northern Nigeria's rivers and

lakes are now getting attention as means of irrigation and power generation. (Iloeje, 1981; Microsoft ® Encarta ® 2007; Library of Congress, 2008).

3.2 Fish Collection

The search for the larvivorous fishes was mainly concentrated on small-size fish species as recommended by World Health Organisation (WHO, 2003). The fishes were collected from swamps, ditches, streams, rivers, reservoirs, lakes and ponds using hand nets, cast nets, drag nets and malian traps. Hand paddling and motorized canoes were used when sampling from large water bodies. During collection, the fish were collected with enough water to cover them; they were then kept under tree shade for one hour to adjust. They were then transferred into transporting water (a mixture of dechlorinated tap water and water from the sampling site) for immediate transportation to the research laboratory. The fish were collected from parts of Kaduna, Katsina, Kano and Niger States in northern Nigeria. In Kaduna, fish were obtained from River Kaduna by Ahmadu Bello Stadium 10^{o}29’10.05’’N, 7 ^{o}26’06.73’’E and an unnamed stream that runs behind the National Board for Technical Education (N.B.T.E.) 10^{o}31’10.95’’N, 7^{o}26’32.55’’E. Fish were also sampled from Kangimi Dam at Mararaban Jos (Katabu) 10^{o}39’28.29’’N, 7^{o}31’15.88’’E. In Zaria, fish were collected from Kubanni (Ahmadu Bello University) Reservoir 11^{o}08’06.36’’N, 7^{o}39’27.58’’E; Bomo Lake 11^{o}11’17.68’’N, 7^{o}37’26.53’’E; Zaria Dam (Reservoir), 11^{o}08’08.23’’N, 7^{o}46’09.56’’E; River Galma 11^{o}04’54.33’’N, 7^{o}44’20.45’’E and Unguwar Sarkin Kogi 11^{o}02’35.81’’N, 7 ^{o}45’07.28’’E. Fish were also collected from Hunkuyi (Kudan Local Government Area) 11^{o}13’08.10’’N, 7 ^{o}38’12.96’’E. In the southern part of Kaduna State, fish were collected from Akwa village and Godo-Godo 9^{o}24’30.91’’N, 8^{o}21’50.77’’E (Jama’a Local Government Area). In other States, fish were collected from

streams in Funtua (Katsina State) 11°29'N, 7 °17'E; Bagauda and Kano 11°56'N, 8°28'E (Kano State) and New Bussa (Niger State) 9°19'26.49''N, 3°49'09.77''E. Figure 1 shows some towns where fish were obtained. Appendix II is pictures of some of the fish collection sites.

3.3 Transportation and Handling of Fish

The fish samples were transported from the different sampling locations to the Department of Biological Sciences, Ahmadu Bello University, Zaria in the morning or evening hours in partially open 50 litre plastic containers half filled with water; the open containers allowed exchange of atmospheric oxygen. Overcrowding of the containers and jerky vehicular movement were avoided to reduce mortality. Young hatchlings were not collected because they cannot withstand the stress of transportation and were also not suitable for the experiments. Ice cubes were added to the water during transportation for cooling and to decrease the metabolic rate of fish and consequently the amount of ammonia excreted into the water. Furthermore, fish samples were not fed for 24 hours before the journey from sampling sites to the research laboratory in Zaria; to reduce their metabolic activities. The temperature of the water in which the fish samples were transported and of the water into which they were introduced in the laboratory were similar to avoid sudden changes in water temperature. The fish samples were not lifted out of water during transportation, handling and transfer (Rottmann *et al.,* 1992; Haq *et al.,* 2003; WHO, 2003; NVBDCP, 2007; DeTolla *et al*., 2008; RFA, 2010).

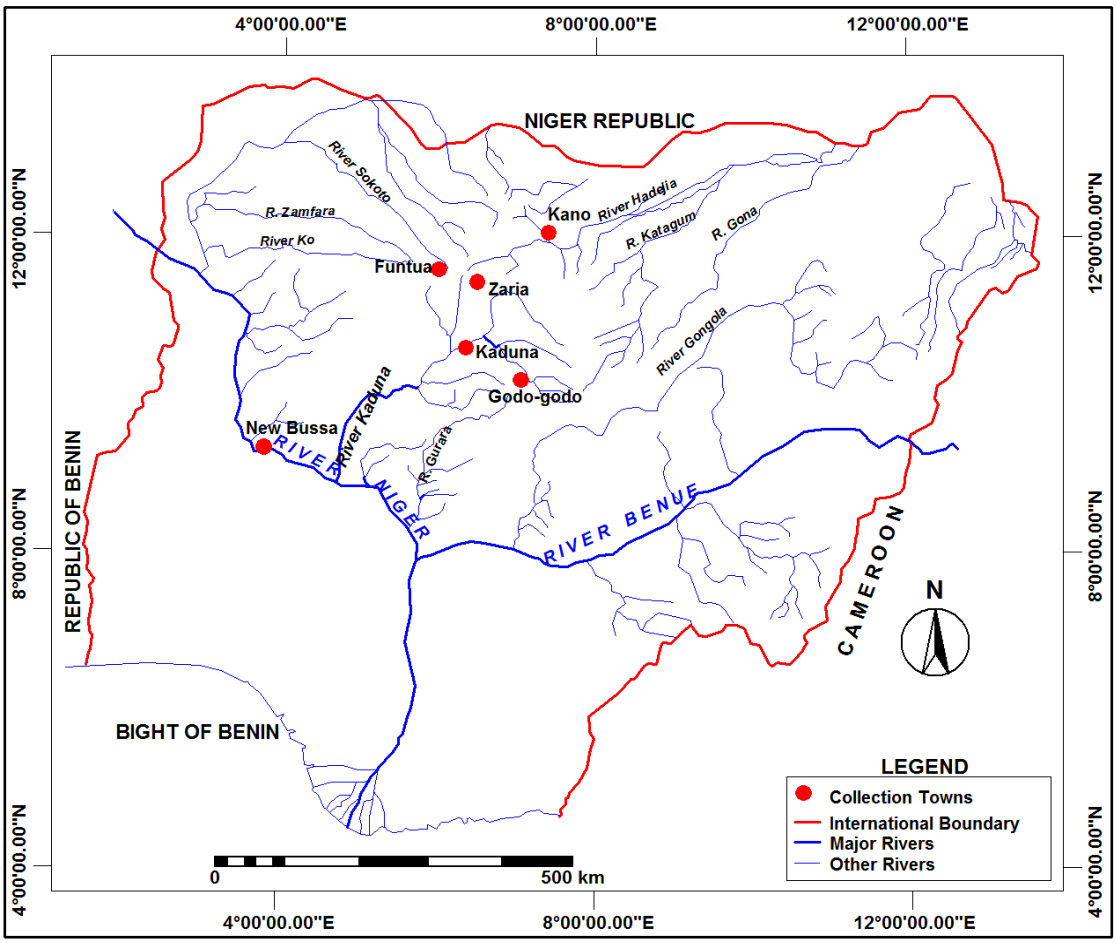


Figure 1: A map of Nigeria showing the towns in northern Ngeria from which the fish species used in the study were obtained

Source: Adapted and modified from Nigeria Drainage System

3.4 Research Station

The research work was carried out in the Fisheries laboratory and the departmental garden of the Department of Biological Sciences, Ahmadu Bello University, Zaria.

3.5 Acclimatization of Fish

All the fish samples brought from the different sampling sites were initially kept in a shaded area in the departmental garden for 10 days to acclimatize before the commencement of any experiment. During this period they were kept in dechlorinated tap water in plastic containers measuring 45×30×30 cm and fed twice daily with commercial feed (coppens®). Different species were kept in separate containers and overcrowding was avoided. During the acclimatizing period, sick and dead fish were removed to prevent spread of diseases (Rottmann *et. al.,* 1992; DeTolla *et al*., 2008).

3.6 Fish Identification

Twelve fish species were collected and identified with the aid of Reed (1967), Holden and Reed (1972), Teugels (1986), Olaosebikan and Raji (1998), Idodo-Umeh (2003), Aken'Ova (2007) and Froese and Pauly (2012). The identity of the fish species and where they were obtained are shown in Table 3.1. The fish species are described below and their photographs are presented in Appendix I.

Table 3.1: Fish species and the site of capture

S/n	Scientific name	Family	Common name	Source
1.	*Brienomyrus niger* (Günther, 1866)	Mormyridae	-	Bagauda, Kano, Zaria
2.	*Brycinus nurse* (Rüppell, 1832)	Alestidae	-	New Bussa, Zaria
3.	*Clarias galmaensis* Aken'Ova, 2007	Clariidae	-	Mararaban Jos, Zaria
4.	*Clarias gariepinus* (Burchell, 1822)	Clariidae	-	Buruku, Funtua
5.	*Epiplatys spilargyreius* (Duméril, 1861)	Nothobranchiidae	-	New Bussa
6.	*Astatotilapia burtoni* (Günther, 1894)	Cichlidae	Burton's Nigerian mouthbrooder	Zaria
7.	*Hemichromis bimaculatus* Gill, 1862	Cichlidae	Jewel fish	Kaduna, New Bussa, Zaria
8.	*Leptocypris niloticus* (Joannis, 1835)	Cyprinidae	-	Zaria
9.	*Oreochromis niloticus* (Linnaeus, 1758)	Cichlidae	Nile mouthbrooder, Nile Tilapia	Bagauda, Godo-Godo, Hunkuyi, Kaduna, Zaria
10.	*Poecilia reticulata* Peters, 1859*	Poeciliidae	Guppy, million fish, rainbow fish	Kaduna
11.	*Schilbe mystus* (Linnaeus, 1758)	Schilbeidae	African butter catfish	Godo-Godo, Kano, Zaria
12.	*Synodontis ouemeensis* Musschoot & Lalèyè, 2008	Mochokidae	-	Mararaban Jos, Kano, Zaria

*Not indigenous

3.6.1 *Brienomyrus niger* (Günther, 1866)

The fish has a dark steel grey colour, darker on the back and lighter on the head, the fins are light grey and there are dark spots on the head. The mouth is terminal. The species is very common and grows to a length of about 13 cm (Olaosebikan and Raji, 2004; Froese and Pauly, 2012). It is a bottom feeder and feeds on insect larvae, detritus and mud; the fish inhabits fringing vegetation and migrates onto the floodplain to spawn (Idodo-Umeh, 2003; Froese and Pauly, 2012).

3.6.2 *Brycinus nurse* (Rüppell, 1832)

It has a rather strongly compressed body, slightly projecting snout when the mouth is closed. The colour is generally silvery on the sides and olive bronze on the back. Dorsal, ventral, anal and adipose fins are marked red. There is a dark patch near the end of the caudal peduncle and a less distinct, reddish mark above the eye. The fish is widely distributed; maximum size is 25 cm and weight 127-200 g (Reed, 1967; Idodo-Umeh, 2003; Olaosebikan and Raji, 2004; Froese and Pauly, 2012). The fish inhabit rivers, lakes, irrigation canals and fringing vegetation and it feed on zooplankton, *Caridina*, insects, snails and vegetation (Froese and Pauly, 2012).

3.6.3 *Clarias gariepinus* (Burchell, 1822)

Clarias are also called mudfish. The head is somewhat between rectangular and pointed in dorsal outline; the snout is broadly rounded. The eyes have a supero-lateral position and are relatively small. The fish have a maximum size of one metre, it has a maximum published weight of 60.0 kg and a maximum reported age of 8 years (Olaosebikan and Raji, 2004;

Froese and Pauly, 2012). They are generally classified as omnivores or predators, are bottom feeders which occasionally feed at the surface (De Graaf and Janssen, 1996; Froese and Pauly, 2012). They have accessory breathing organs attached to the gills and can live out of water for several hours or in damp muddy places for months, adults occur mainly in quiet waters, lakes and pools and prefer rather shallow and swampy areas with a soft muddy substrate; they may also occur in fast flowing rivers and rapids, migrate to rivers and temporary streams to spawn (Reed, 1967; Froese and Pauly, 2012). The fish has a high growth rate, and it is very resistant to handling and stress. The African Catfish does not spawn spontaneously in captivity since the environmental factors such as the rise in water level and inundation of shallow areas do not occur on the fish farms (De Graaf and Janssen, 1996).

3.6.4 *Clarias galmaensis* Aken'Ova, 2007

Clarias galmaensis resembles other species of *Clarias* except that the adult is naturally smaller than other species known from water bodies in northern Nigeria. The species is described as small size, oval head in dorsal outline and olive to brownish in colour with many tiny cream to dirty-white spots, in live specimens and was named *Clarias galmaensis* after its locality, River Galma, Zaria, Kaduna State, Nigeria (Aken'Ova, 2007). Aken'Ova (2007) found this fish to have a maximum weight of 75 g and maximum standard length of 20.8 cm. A holotype of the species with reference No. BMNH 1981.9.10.1 was deposited by Prof. T. O. L. Aken'Ova in the Natural History Museum, London, in 1981 (Aken'Ova, 2007, 2011).

3.6.5 *Epiplatys spilargyreius* (Duméril, 1861)

Their back is grey and belly white with 10-14 bold oblique dark stripes on the flanks which may continue onto the caudal fin. A grey line runs across the body. The caudal fin may either be pointed or broadly rounded with a small point. The mouth faces upwards, males are coloured and smaller than females. They mostly come from areas along the coasts of West Africa where they live in standing slow-flowing waters, lay eggs attached to plants which hatch 7-28 days after they are laid (Mayland, 1976). Have a maximum total length of 4.20-6 cm and weight 1.8 g, they are omnivores feeding mainly on dipteran larvae, other insects, other benthos, algae, zooplankton and some detritus (Froese and Pauly, 2012).

3.6.6 *Astatotilapia burtoni* (Günther, 1894)

The anal fin of the male is marked with egg dummies. After hatching, the young spend a short period using up the contents of their yolk sac, and then become free-swimming, but they still remain under the protection of the female (Mayland, 1976). The fish inhabits slow streams, river mouths and shallow parts of lake near rivers; common length is 7 cm but may grow to 12-15 cm (Froese and Pauly, 2012).

3.6.7 *Hemichromis bimaculatus* Gill, 1862

The general colouration is usually ochre or tan, with a faint iridescent spangle on each scale and more spangles on the dorsal caudal fins. The body is elongated and moderately compressed, the snout pointed, and the mouth rather small. It has a conspicuous black spot, bordered in gold, at the upper rear corner of the operculum; a second large spot on the middle of the side; and a third, often inconspicuous spot on the caudal peduncle. This is a small species; adult may reach 10-13.6 cm in total length and weight 10-12 g (Reed, 1967;

Idodo-Umeh, 2003; Froese and Pauly, 2012). The fish is carnivorous, widely distributed, found in both dry and rainy season, it seems to prefer streams and swamps, both parents give parental care to the young (Reed, 1967; Idodo-Umeh, 2003; Olaosebikan and Raji, 2004).

3.6.8 *Leptocypris niloticus* (Joannis, 1835)

It has a silver body, fins without colour. It is widely distributed, has a maximum total length of about 8- 9.5 cm and a weight of 7-10 g (Idodo-Umeh, 2003; Olaosebikan and Raji, 2004; Froese and Pauly, 2012).

3.6.9 *Oreochromis niloticus* (Linnaeus, 1758)

The dorsal surface and sides are silvery-dark in colour while the belly is white, the body elongate and usually shows a number of rather narrow bands on the back, fins also have regular vertical black stripes, have a terminal mouth. This is one of the largest cichlids, it may reach a maximum size of 60.0 cm, maximum published weight is 4.3 kg and maximum reported age is 9 years (Reed, 1967; Olaosebikan and Raji, 2004; Froese and Pauly, 2012). The fish is mainly diurnal and feed mainly on phytoplankton or benthic algae (Froese and Pauly, 2012). The fish species is oviparous, there may be 2,000 eggs in a clutch, a typical mouth brooder, in which the female takes up the eggs and later takes sole charge of guarding and rearing the fry (Reed, 1967; Froese and Pauly, 2012). The fish is widely distributed, occur in a wide variety of freshwater habitats like rivers, lakes, sewage canals and irrigation channels (Olaosebikan and Raji, 2004; Froese and Pauly, 2012). Is currently the most widely cultivated fish in Africa (De Graaf and Janssen, 1996).

3.6.10 *Poecilia reticulata* Peters, 1859

The males are coloured and the females are bigger than the males. No parental care is exercised; parents even prey on their young. Males mature at two months and females at three months of age, females may reach 5 cm standard length (Froese and Pauly, 2012). Occurs in a range of fresh and brackish warm water habitats; slow-flowing or still water typically associated with well-vegetated margins of ponds/streams, diet consists of zooplankton, small aquatic insects and larvae, detritus (Froese and Pauly, 2012). This fish had been introduced to many regions of the world, including Africa, the Middle East, and southwest Asia probably due to fish farm or aquarium releases and in some areas as a result of mosquito control (Nico and Neilson, 2011). In terms of availability guppies are found in the Federal Capital Territory, Benue, Plateau, Kaduna and Lagos States, the suitable climatic condition prevalent within these areas favours the breeding of this exotic fish species (NIFFR, 2002; Anogwih and Makanjuola, 2010).

3.6.11 *Schilbe mystus* (Linnaeus, 1758)

Have no adipose fin and the pectoral fins are equipped with spines, the colour of adult is silver on the sides. The fish should be handled with care because the spines penetrate easily, inflicting a painful wound (Holden and Reed, 1972). They are generally more active at night or in subdued light, feed from mid-water and surface waters on a wide variety of foods including fish, insects, shrimps, snails, plant seeds, and fruit (Froese and Pauly, 2012). Breed during the rainy season, they are oviparous and lay eggs on vegetation, eggs are usually unguarded and adult fish may weighed up to 250 g and adult may live up to 6-7 years (Froese and Pauly, 2012).

3.6.12 *Synodontis ouemeensis* Musschoot & Lalèyè, 2008

The fish is brownish black dotted all over with small black spots, except the belly, which is white. Has the anterior soft rays of the dorsal fin about uniformly elongated, giving a 'square-sail rig' shape to the dorsal fin, the dorsal spine is very strong. The fish reaches a maximum length of 15.7 cm (Froese and Pauly, 2012).

3.7 Morphometric Parameters of Fish

For all experiments involving fish in the laboratory and outdoor, the fish were weighed to the nearest 0.1 g using Sartorius electronic metre balance model CP 8201 (Germany) and their standard and total lengths were measured using a meter rule.

3.8 Sourcing and Rearing of Mosquito Larvae

Culex quinquefasciatus (the brown house mosquito) which usually breeds in organically polluted water near human communities was used. The mosquito is frequently found breeding in high number in unsealed septic tanks and can be present throughout the year (Whelan and Warchot, 2009). Blood engorged female *Culex quinquefasciatus* mosquitoes were caught on the walls of Akenzua and Sulaiman students' residential halls, Ahmadu Bello University, Zaria. They were placed in meshed cages with water containers for egg deposition. The oval rafts of deposited eggs were then transferred with a spoon into plastic basins (65 cm diameter and 30 cm height) containing dechlorinated tap water for hatching into larvae. The basins were kept in dark corners of the laboratory; in some basins, 20 g chicken droppings were added while to other basins dead fish (*Oreochromis niloticus* and/or *Synodontis ouemeensis*) were added to serve as food for larvae (Woodhouse, 2006). *Culex quinquefasciatus* larvae and adults were identified with the following guides (Russel,

1996; Hill and Connelly, 2009; Cutwa and O'meara, 2012). Plate IIa are mosquito larvae feeding on a dead fish while Plate IIb is harvested mosquito larvae for laboratory larvivorous test.

3.9 Water Physicochemical Parameters

The physicochemical parameters [temperature, pH, total dissolved solids (TDS) and electrical conductivity (EC)] of the water in the ponds, flow-through system and various aquaria for the various experiments were determined using Hannah portable Instruments meter (Model HI 98129). pH was also determined using Hanna Instruments pH 210 microprocessor in the laboratory.

3.10 Test Aquarium

All tests in the laboratory were carried out in transparent plastic aquaria measuring 45×30×30 cm, each aquarium was covered with perforated plastic lids to prevent the fish from jumping out (Kamah, 1995; Wainwright, 1996; Mohamed, 2003).

3.11 Studies on the Predatory Potential of Fish

The capability of the different fish species to consume mosquito larvae was tested both indoor, in the laboratory and in outdoor ponds.

3.11.1 Indoor study on the predatory potential of fish on mosquito larvae

The capability of each of the twelve (12) fish species to feed on mosquito larvae in the laboratory was tested as described (with modifications) by Fletcher *et al.* (1993), Jianguo and Dashu (1995), Mohamed (2003), and Ekanayake (2007)). Acclimatized fish were brought from the reservoir in the garden to the laboratory and allowed to adjust to the



Plate I a: Dead fish used as food source for mosquito larvae



Plate I b: Harvested third and fourth instars of mosquito larvae

laboratory environment for 3 days; aquaria with 8 litres dechlorinated tap water were used. For each fish tested, the average weight, standard and total lengths were measured and recorded before commencement of the experiments. For each fish species, three replicates each with a single fish in an aquarium were set up. At the beginning of the experiment, fifty (50) III and IV instars of mosquito larvae were added to each aquarium with the fish inside. The number of larvae consumed by each fish was checked at 2-hours interval, when the numbers were observed going down, more were added (number added each time in each aquarium was recorded). The mean number of mosquito larvae consumed by each fish species per day (24 hours) was determined for 4 days. The predatory index, which is the mean number of mosquito larvae consumed by a fish in a day divided by the weight of the fish in gramme (Jayasree and Panicker, 1992), was also determined.

3.11.2 Indoor study on the predatory potential of fish on mosquito larvae in the presence of commercial fish feed

This test was done to ascertain if the presence of commercial fish feed affect mosquito larvae consumption of the experimental fish species. The set up in the experiment is similar to the one described in 3.11.1 above, however, the fish were fed twice daily with 2.5 % of their body weight commercial fish feed (coppens®) in addition to the mosquito larvae.

3.11.3 Indoor study on the predatory potential of *Clarias galmaensis* bred in the laboratory

The preliminary test result of *Clarias galmaensis* in consuming mosquito larvae in the laboratory coupled with the fact that it is an indigenous fish recently identified in River Galma, Zaria motivated more studies on it. The fish was hatched by artificial induction using ovaprim hormone (Appendices XXIX and XXX). The frys that hatched were reared

to juveniles; at 5, 7 and 9 months, were tested for their ability to prey on mosquito larvae as described in 3.11.1 and 3.11.2.

3.11.4 Outdoor study on the predatory potential of fish on mosquito larvae

In open water bodies, mosquitoes lay eggs which hatch into larvae, these larvae developed into pupae and later on adults if control measures are lacking. The number of mosquito larvae therefore observed or enumerated in a water body with a fish gives a clue to the effectiveness of the fish preying on the larvae, the more effective the fish is, the less the mosquito larvae that will be observed or captured with a dipper in the water body. The methods of Mohamed (2003), Ghosh *et al.* (2005a) and Howard *et al*. (2007) were adopted with slight modifications to determine the ability of the experimental fish to control mosquito larvae in an open body of water. Nine (9) of the fish species (*Brienomyrus niger, Brycinus nurse, Clarias galmaensis, Clarias gariepinus*, *Hemichromis bimaculatus, Oreochromis niloticus*, *Poecilia reticulata, Schilbe mystus* and *Synodontis ouemeensis*) were used for this experiment. Ten concrete ponds, each measuring 140×85×25 cm (Plate II), were constructed and water level in each was maintained at 20 cm depth. The water in each pond was allowed to stand for 2 weeks to weakened or destroy the effect of cement which was used for the construction, mosquito larvae were observed in all the ponds before the fish were introduced. The fish were introduced into nine (9) of the ponds, each pond had eight (8) fish of the same species. The mean weights, standard and total lengths of each of the fish species were determined and recorded before and after the experiments. One (1) pond without fish served as control. Dead fish were removed and replaced during the period of the experiment. Wire nets (pore size of 2.5 cm) were used to cover the ponds in order to prevent contamination, predators and the fish from jumping out. The pore size of

the wire nets however, allowed mosquitoes to have access and lay their eggs in the ponds. The surroundings of the ponds were swept while leaves and objects that fell into the ponds were removed twice a week. The temperature, pH, total dissolved solids (TDS) and electric conductivity (EC) of the ponds water were recorded weekly. A 400 ml-capacity dipper was used for the weekly mosquito larvae sampling. The ponds were approached cautiously as shadow casted on water and heavy footfalls disturbed larvae and caused them to dive to the bottom. Every week, each of the ten ponds was sampled for mosquito larvae, during sampling four dips, one from each of the four corners of each pond was done and the number of the mosquito larvae was obtained by counting (O'Malley, 1989). The experiment lasted for twelve (12) months.

3.12 Test of Fish Hardiness

The hardiness of each of the 12 experimental fish species was tested by their ability to tolerate a wide range of pH, survive five months without feeding and survive in different water volumes.

3.12.1 Fish tolerance to pH changes

Each fish species has an optimum pH in which it thrives, it also has a range, which it may tolerate, pH below or above this range is detrimental and will kill the fish. This test was to determine the minimum and maximum pH range which each fish species can tolerate. Eight (8) fish from each of the 12 experimental fish species in an aquarium with 10 litres dechlorinated tap water was used to determine the minimum pH and another 8 was similarly used to determine the maximum. Starting with the pH of water around 7.0, the pH of each fish aquarium was altered by adding drops of diluted Hydrochloric acid (HCl).

Acidity levels were adjusted from 7.0, to 6.9, to 6.8, to 6.7, this adjustment was continued until a pH that resulted in permanent signs of stress or death of 50% of the tested fish was reached, this was considered the lowest pH tolerated by that fish species. Tolerance test for alkalinity was carried out in a similar manner by altering the pH from 7.0, to 7.1, to 7.2 by addition of solution of dissolved Sodium hydroxide (NaOH) pellets, this adjustment was continued until a pH that resulted in permanent signs of stress or death of 50% of the tested fish was reached; this was considered the highest pH tolerated for that fish species.

3.12.2 Fish survival in the absence of food

This experiment was set up to determine the ability of each of the 12 fish species to survive for five months (20 weeks) in an aquarium without supplementary feed. Eight (8) fish from each of the experimental fish species were separately placed in an aquarium with 30 litres dechlorinated tap water. The fish in each aquarium was kept for 20 weeks in the laboratory without feeding. A record of mortality of each fish species was kept and the temperature, pH, total dissolved solids (TDS) and electrical conductivity (EC) in each aquarium was determined and recorded weekly.

3.12.3 Fish tolerance to changes in water level

This experiment tested the survival of each of the 12 fish species in three different water volumes in the laboratory. The average weight, standard and total lengths of each species were determined and recorded at the beginning of the experiment. Eight (8) fish from each species were introduced into each aquarium containing 10.0, 20.0 and 30.0 litres of dechlorinated tap water. The fish in each aquarium was fed with 2.5% of their body weight coppens® twice daily for 13 weeks (three months) experimental period. Temperature, pH,



Plate II: Ponds used for the outdoor predatory potential experiment

total dissolved solids (TDS), electrical conductivity (EC) and mortality of each aquarium was determined and recorded weekly.

3.13 Reproductive Potential of Fish in Captivity

The static and flow-through systems were used

3.13.1 Fish breeding using static system

The breeding ability of nine (9) fish species (*Brienomyrus niger, Brycinus nurse, Clarias galmaensis, Clarias gariepinus, Hemichromis bimaculatus, Oreochromis niloticus, Poecilia reticulata, Schilbe mystus* and *Synodontis ouemeensis*) used for the outdoor larvivorous studies was studied. Each fish species comprising four males and four females placed in a pond. The breeding potential of each species was monitored for twelve (12) months. Temperature, pH, TDS and EC of the ponds were recorded weekly during the study period.

Based on its high predatory index and its reproductive ability, the number of offspring delivered by *Poecilia reticulata* was determined. Five gravid females were randomly picked at different times and each placed inside a plastic container diameter 13 cm and height 15 cm filled to ¾ with water. The fish were monitored and offspring delivered counted.

The breeding ability of *Epiplatys spilargyreius, Leptocypris niloticus* and *Astatotilapia burtoni* was not studied under the static and flow-through systems because they were not available when the tests were set up. Their breeding ability was however, studied in the laboratory later. Eight (four males and four females) of each of these fish species were

maintained in separate aquaria. *Azola africana* and *Ceratophylum submersum* were added as in the flow-through system. The fish were fed twice daily with 2.5% of their body weight coppens® and the experiment was for 12 months.

3.13.2 Fish breeding using the flow-through system

The ability of nine fish species (*Brienomyrus niger, Brycinus nurse, Clarias galmaensis, Clarias gariepinus, Hemichromis bimaculatus, Oreochromis niloticus, Poecilia reticulata, Schilbe mystus* and *Synodontis ouemeensis*) to breed in a flow-through system was tested. Small stones (diameter 3-5 cm), gravel (diameter 3-4 mm) and sand (diameter 1-1.5 mm) washed in potassium permanganate (to kill all attached organisms) were placed on the floor of transparent plastic containers. The flow-through arrangement allowed for constant flow of water by gradual trickling from a reservoir through an inlet pipe and excess removed through an outlet pipe; about 30 litres of water was always maintained. The water plants *Azola africana* and *Ceratophylum submersum* were added to the aquarium tanks to improve oxygen supply; their roots also served as egg-laying substrate and hideout for hatchlings. Eight (8) fish of the same species (four males and four females) were introduced into each tank and fed twice daily with coppens®. Water temperature, pH, TDS and EC of the water in the containers were monitored weekly. The experiment was allowed to run for twelve (12) months. The experimental set up is shown in Plate III and in a sketch in Appendix XXVIII.

3.13.3 Breeding *Clarias galmaensis* using hormone

Based on the good performance of *Clarias galmaensis* in the laboratory larvivorous studies and its inability to reproduce under the static and flow through systems, it was subjected to

artificial breeding using ovaprim hormone in the laboratory. Details of the breeding are presented in Appendices XXIX and XXX.



Plate III: Experimental set-up of flow-through system for the breeding of nine species of fish

CHAPTER FOUR

4.0 RESULTS

4.1 Sourcing Mosquito Larvae

Mosquito larvae were observed 5-7 days in the basins where hatching and rearing of the larvae took place. Enough larvae were raised and collected for the indoor predatory potential test of all the experimental fish species in the laboratory.

4.2 Morphometric Indices of the Fish

All the fish used for the indoor predatory potential study and other experiments where obtained from streams, swamps, ditches, rivers and ponds, except *Clarias gariepinus* which was obtained from Yula farm, Buruku (Kaduna State) and Dangiwa fish farm, Funtua (Katsina State). The largest fish used for the indoor larvivorous study was *Clarias galmaensis* (mean standard length 14.9 cm and mean weight 20 g) and the smallest was *Poecilia reticulata* (mean standard length 2.0 cm and mean weight 0.2 g). The morphometrics indices of the 12 fish species used for the test are given in Table 4.1.

4.3 Indoor Study on the Predatory Potential of Fish on Mosquito Larvae

All the 12 experimental fish species tested of their predatory potential on mosquito larvae in the laboratory preyed on the larvae, but their consumption rates were different. *Clarias galmaensis* consumed the highest number of mosquito larvae (988/day) while *Epiplatys spilargyreius* consumed the least (18/day). Analysis of variance (ANOVA) among the 12 fish species showed that the differences in the consumption rates of mosquito larvae was

Table 4.1: Some morphometric parameters of fish species used in indoor larvivorous test

S/No	Fish	Average weight (g)	Average standard length (cm)	Average total length (cm)
1	*Clarias galmaensis*	20	14.9	16.5
2	*Clarias gariepinus*	2.2	5.8	6.5
3	*Hemichromis bimaculatus*	5.2	5.6	7.0
4	*Oreochromis niloticus*	4.1	5.2	6.5
5	*Schilbe mystus*	4.7	7.0	8.0
6	*Leptocypris niloticus*	3.0	5.2	6.8
7	*Synodontis ouemeensis*	3.0	3.5	5.7
8	*Poecilia reticulata*	0.2	2.0	3.0
9	*Brienomyrus niger*	3.0	6.3	7.2
10	*Astatotilapia burtoni*	5.0	6.0	7.6
11	*Brycinus nurse*	8.5	7.2	9.0
12	*Epiplatys spilargyreius*	0.4	3.0	3.8

significant (P<0.05). The mean mosquito larvae consumed, standard error and standard deviation are presented in Appendix III.

For the indoor study on the predatory potential of the 12 fish species on mosquito larvae in the presence of fish feed, all the tested species also consumed the larvae but at different rates. In all the fish species the larvae consumption was lower as compared to when the fish were fed only on the mosquito larvae. *Clarias galmaensis* consumed the highest number of larvae (649/day) while *Epiplatys spilargyreius* consumed the least (12/day). Analysis of variance (ANOVA) among the 12 fish species showed that the differences in the consumption rates of mosquito larvae in the presence of coppens® was significant (P<0.05). Figure 2 gives the mean mosquito larvae consumed by each fish when fed on only mosquito larvae and the mean mosquito larvae consumed when fed on mosquito larvae along with coppens®. The mean mosquito larvae consumed when fed along with coppens®, standard error and standard deviation are presented in Appendix IV.

4.4 Comparison of Larvae Consumed by Fish in the Absence and Presence of Fish Feed

In all the tested fish species the mean mosquito larvae consumed by the fish in the laboratory per day in the presence of commercial fish feed (coppens®) were less as compared to when they were fed only on the mosquito larvae. Paired t-test however, showed that the larval consumption rate of each fish species when fed on mosquito larvae only and when fed on mosquito larvae together with coppens® was not significantly different (P>0.05).

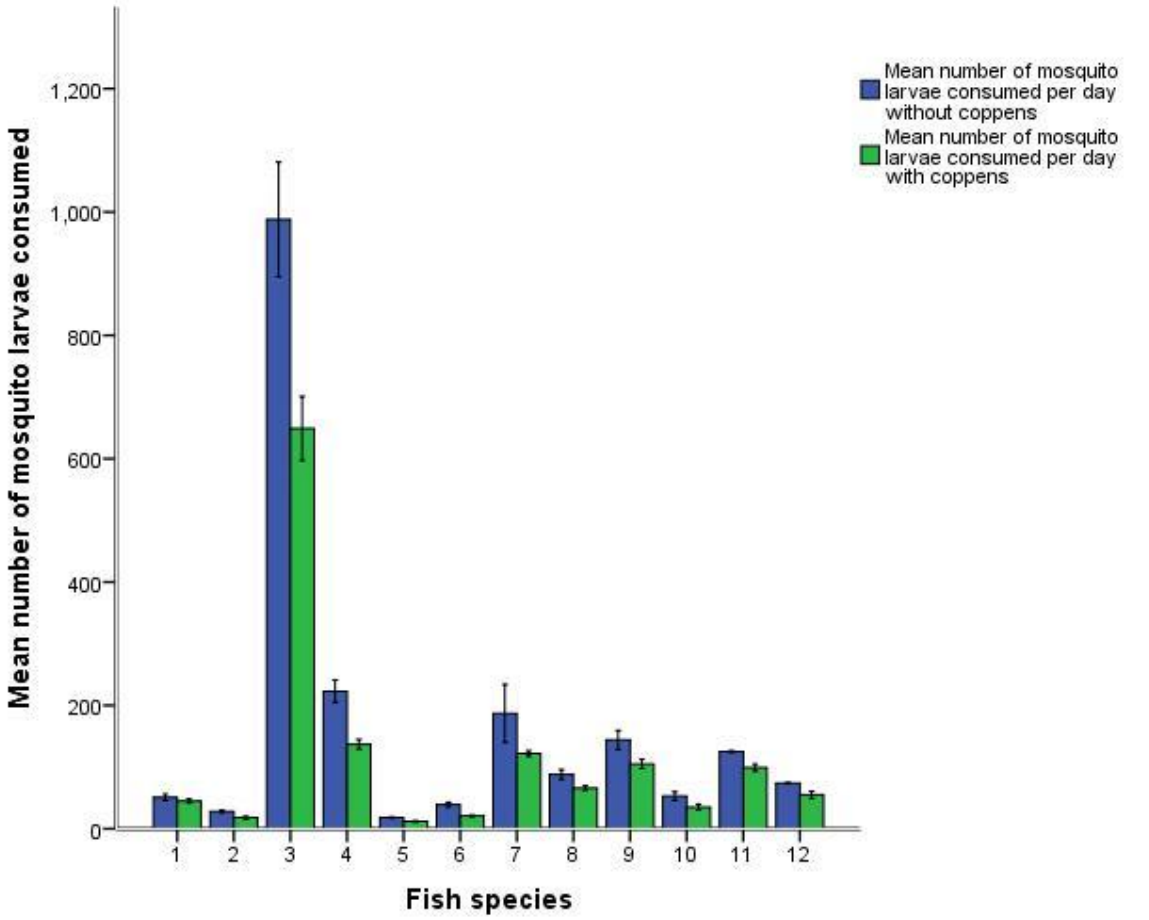


Figure 2: Mean mosquito larvae consumption rate of fish fed on mosquito larvae only and those on mosquito larvae and coppens®

Key: 1. *Brienomyrus niger*, 2. *Brycinus nurse*, 3. *Clarias galmaensis*, 4 .*Clarias gariepinus*, 5. *Epiplatys spilargyreius* , 6. *Astatotilapia burtoni*, 7. *Hemichromis bimaculatus*, 8. *Leptocypris niloticus*, 9. *Oreochromis niloticus*, 10. *Poecilia reticulata*, 11. *Schilbe mystus*, 12. *Synodontis ouemeensis*

4.5 Predatory Index

When the predatory index of each fish species fed on mosquito larvae only was computed, *Poecilia reticulata* had the highest index of 265.00 while *Brycinus nurse* had the lowest (3.29). When the predatory index of each fish fed on mosquito larvae together with coppens® was computed, *Poecilia reticulata* again had the highest index (175.00) and *Brycinus nurse* the lowest (2.12). The predatory indices of the fish fed on mosquito larvae in the absence and presence of fish feed are summarized in Figures 3 and 4 and Appendix V.

4.6 Indoor Study on the Predatory Potential of *Clarias galmaensis* Bred in the Laboratory

The ability of *Clarias galmaensis* hatched and reared in the laboratory to consume mosquito larvae at 5, 7, and 9 months was tested, the result showed that with increase in age there was an increase in mean weight, mean standard and total lengths. Mean number of mosquito larvae consumed with or without coppens® and predatory index with or without coppens® also increase with increase in age as shown in Figure 5 and Appendix VI.

4.7 Relationship of Fish Morphometric and Mosquito Larvae Consumption

There is a strong relationship between the fish weight and mosquito larvae consumed, and fish length and mosquito larvae consumed as the correlation coefficient (r) in each case is greater than 0.8 (Figures 6 and 7).

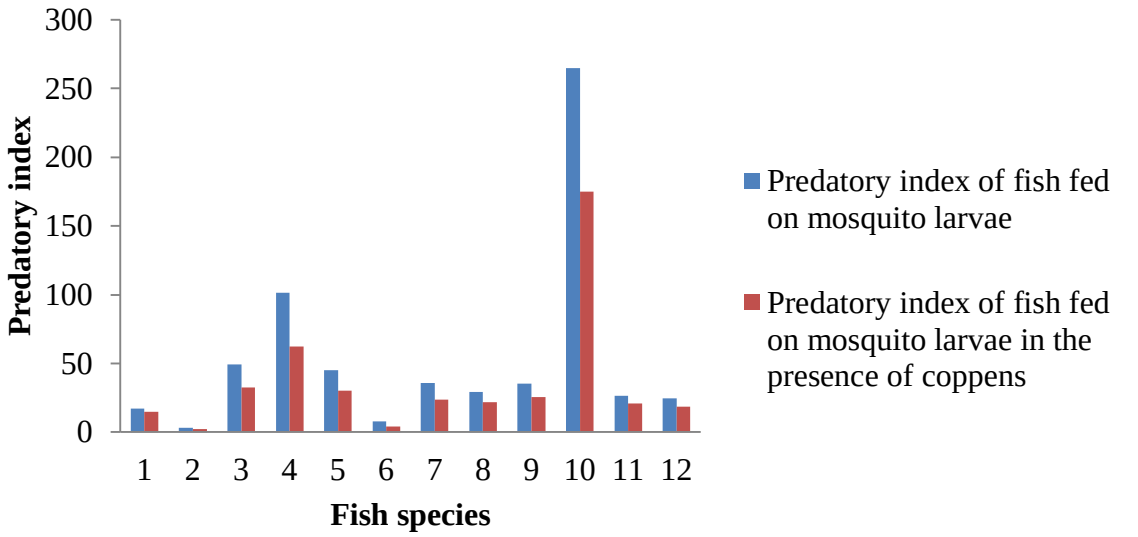


Figure 3: Predatory indices showing the comparison between the fish species fed on mosquito larvae only and those fed along with commercial feed (coppens®)

Key: 1. *Brienomyrus niger*, 2. *Brycinus nurse*, 3. *Clarias galmaensis*, 4 .*Clarias gariepinus*, 5. *Epiplatys spilargyreius* , 6. *Astatotilapia burtoni*, 7. *Hemichromis bimaculatus*, 8. *Leptocypris niloticus*, 9. *Oreochromis niloticus*, 10. *Poecilia reticulata*, 11. *Schilbe mystus*, 12. *Synodontis ouemeensis*

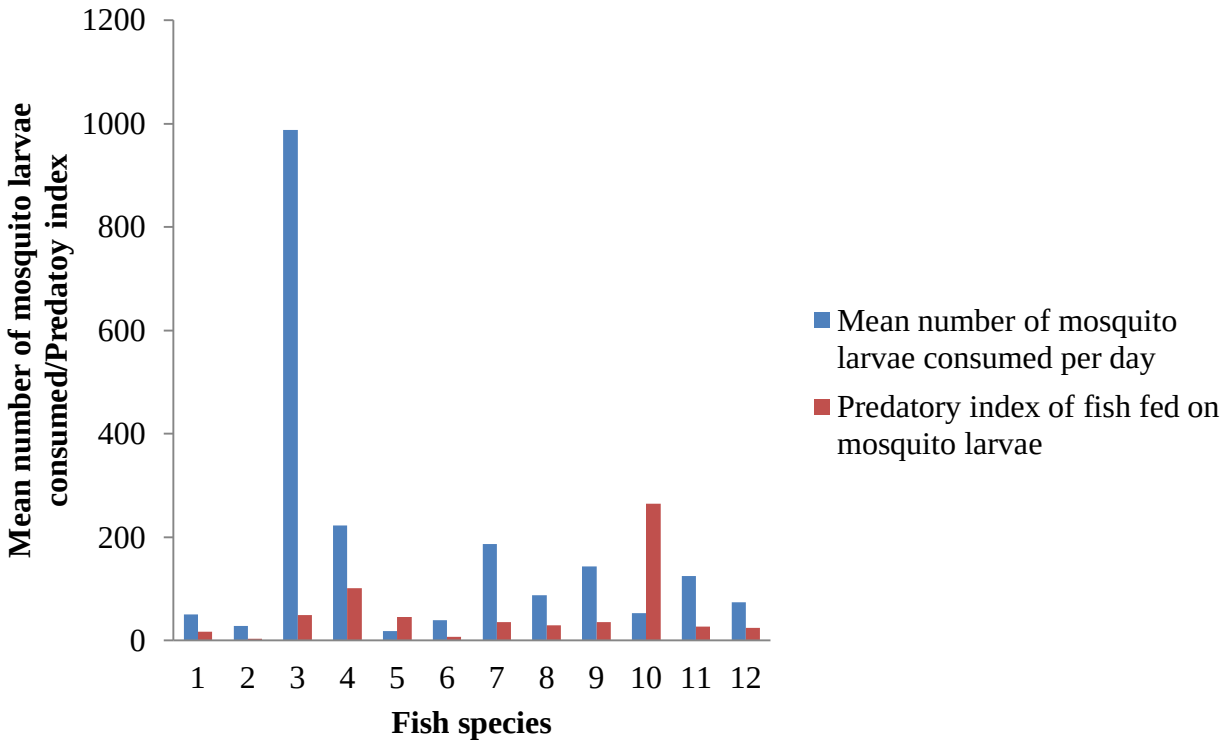


Figure 4: Mean mosquito larvae consumed compared with predatory indices of fish species fed on larvae only

Key: 1. *Brienomyrus niger*, 2. *Brycinus nurse*, 3. *Clarias galmaensis*, 4 .*Clarias gariepinus*, 5. *Epiplatys spilargyreius* , 6. *Astatotilapia burtoni*, 7. *Hemichromis bimaculatus*, 8. *Leptocypris niloticus*, 9. *Oreochromis niloticus*, 10. *Poecilia reticulata*, 11. *Schilbe mystus*, 12. *Synodontis ouemeensis*

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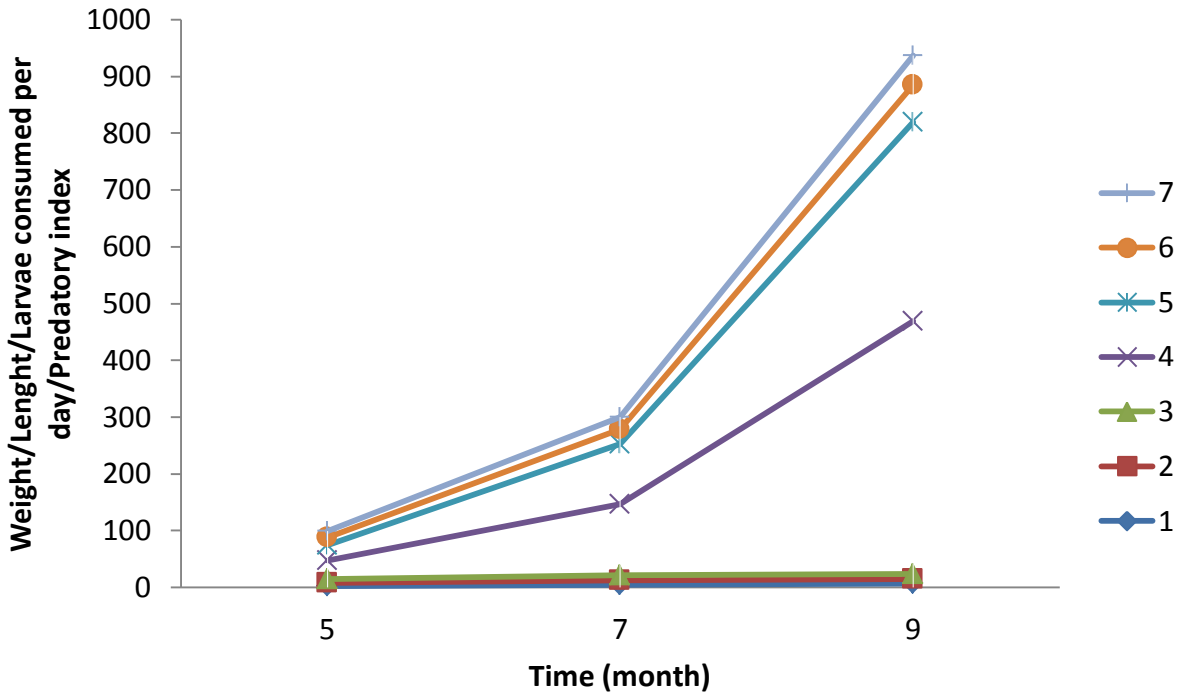


Figure 5: Changes in morphometric, mean mosquito larvae consumption and predatory indices of *Clarias galmaensis* at 5, 7 and 9 months

Key: 1. Mean weight (g); 2. Mean standard length (cm); 3. Mean total length (cm); 4. Mean number of mosquito larvae consumed per day when fed along coppens®; 5. Mean number of mosquito larvae consumed per day; 6. Predatory index with coppens®; 7. Predatory index without coppens®

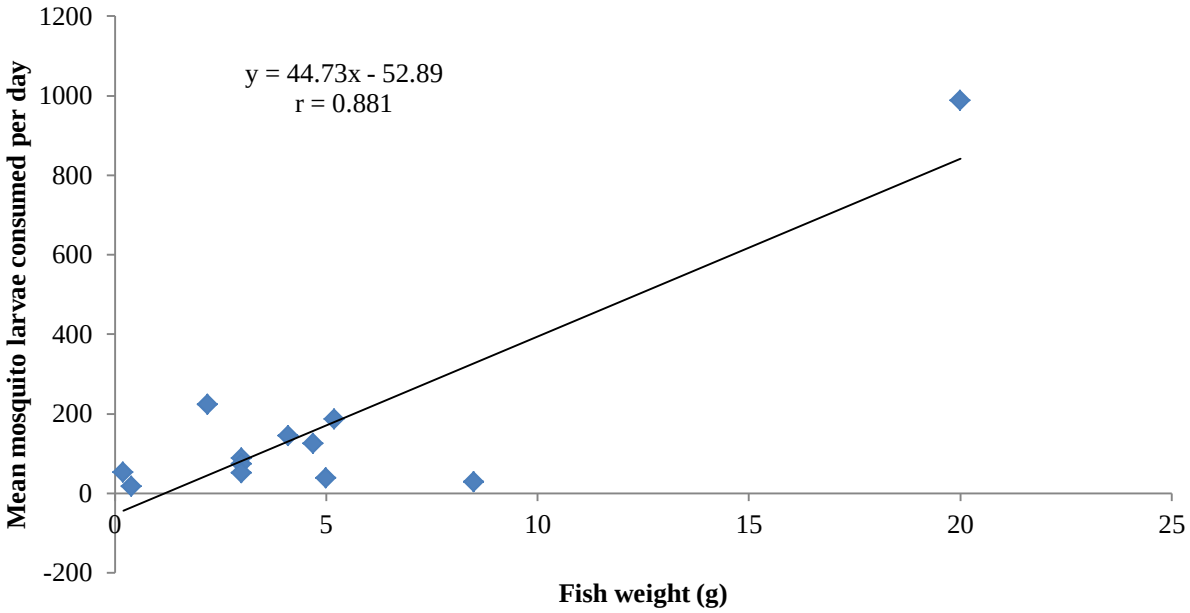


Figure 6: Mosquito larvae consumed and fish weight relationship

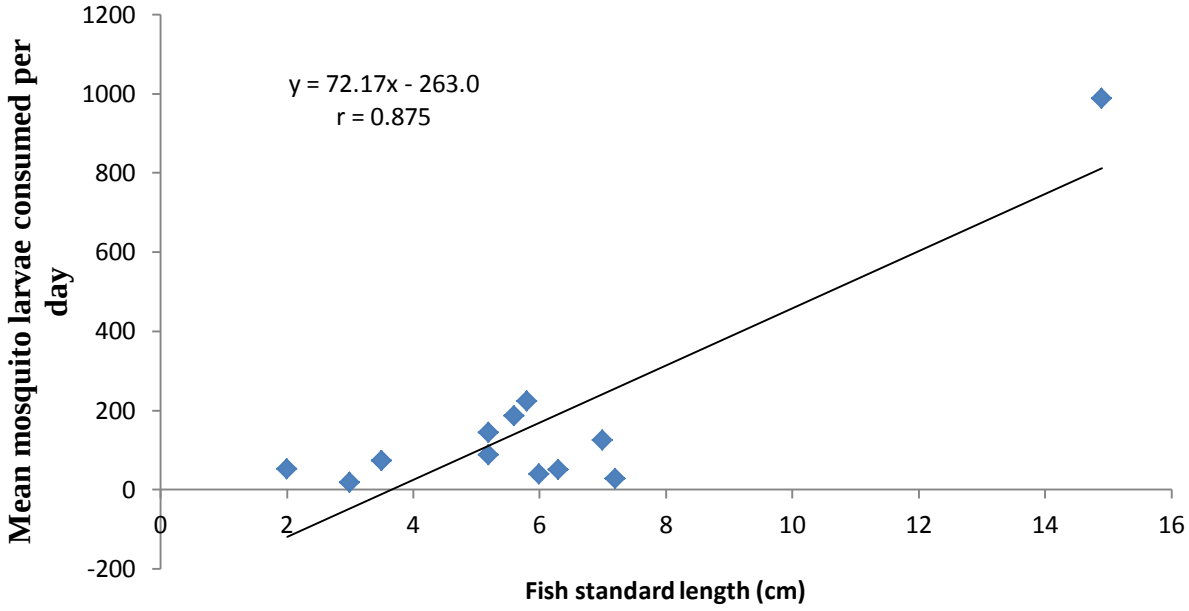


Figure 7: Mosquito larvae consumed and fish length relationship

4.8 Outdoor Study on the Predatory Potential of Fish on Mosquito Larvae

The control pond (which had no fish) gave the highest mosquito larvae count each week and month; it also gave the highest total mosquito larvae count for the year (2,630). In the ponds with *Brienomyrus niger, Brycinus nurse* and *Synodontis ouemeensis,* mosquito larvae were encountered in varied number each week and the total count for the year for them was 155, 165 and 625 respectively. However, in the ponds with *Clarias galmaensis, Clarias gariepinus, Hemichromis bimaculatus, Oreochromis niloticus, Poecilia reticulata* and *Schilbe mystus,* not a single mosquito larva was encountered throughout the test period. *Epiplatys spilargyreius, Astatotilapia burtoni* and *Leptocypris niloticus* were not available when this test was started and were therefore not tested with the others. Figure 8 below and Appendix XI gives the monthly and total mosquito larvae collected from each of the 10 ponds.

4.9 Physicochemical Parameters of Water in Experimental Ponds and Fish Morphometry

4.9.1 Physicochemical parameters of water in experimental ponds

During the 12 months test period, the lowest monthly mean temperature (17.95ºC) was obtained in the pond that had *Clarias galmaensis* in the fifth month (January 2010) and the highest was 27.36ºC also in the same pond that had *Clarias galmaensis* in the eighth month (April 2010). The lowest monthly mean pH (6.85) was obtained in the eleventh month (July 2010) in the pond that had *Oreochromis niloticus* while the highest (8.98) was obtained in the fifth month (January 2010) coincidentally also in the same pond. The lowest monthly mean (TDS) was 51.25 mg/L and the lowest monthly mean EC (98.75 µSiemens/cm) were obtained in the first month (September 2009) in the control pond which had no fish. The

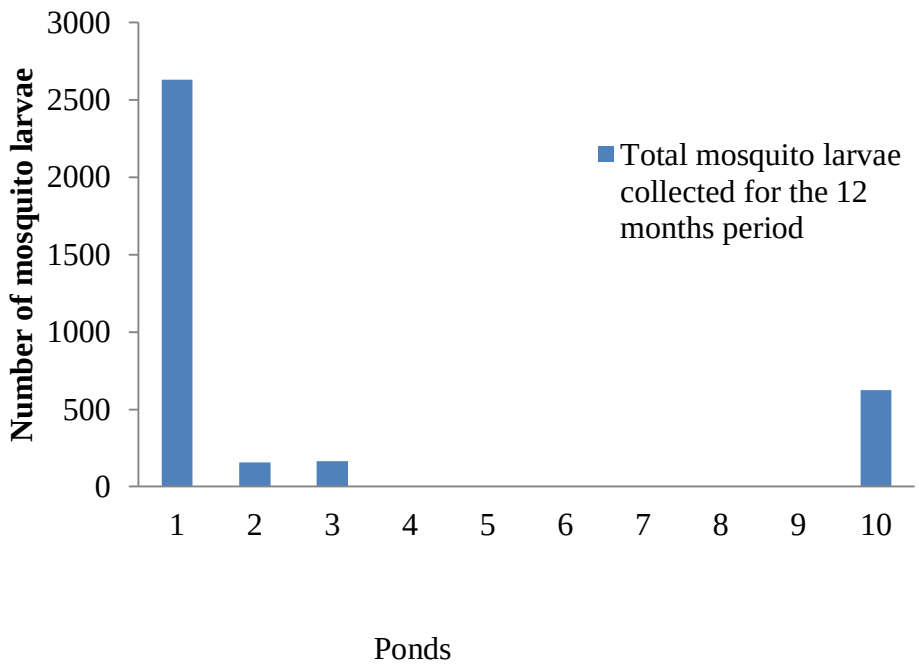


Figure 8: Population of mosquito larvae in outdoor ponds with fish species

Key: 1. Control pond, 2. *Brienomyrus niger*, 3. *Brycinus nurse*, 4. *Clarias galmaensis*, 5. *Clarias gariepinus*, 6. *Hemichromis bimaculatus*, 7. *Oreochromis niloticus*, 8. *Poecilia reticulata*, 9. *Schilbe mystus*, 10. *Synodontis ouemeensis*

highest monthly mean TDS (181.25 mg/L) and EC (349.25 µSiemens/cm) were all obtained in the fifth month (January 2010) in the pond that had *Brycinus nurse*. There were no significant differences ($P>0.05$) in the monthly means of these parameters in all the ten (10) ponds. The monthly mean temperature, pH, TDS and EC recorded in each respective pond for the 12 months period is presented in Appendices VII-X.

4.9.2 Morphometrics of fish in experimental ponds

Seven out of the 12 fish species (*Brienomyrus niger, Brycinus nurse, Clarias galmaensis, Clarias gariepinus, Hemichromis bimaculatus, Oreochromis niloticus* and *Synodontis ouemeensis*) did not show any appreciable increase in size at the end of the 12 months test period. *Oreochromis niloticus* and *Clarias gariepinus* increased in weight by 52 % and 300 % respectively. However, *Poecilia reticulata* grew both in size and number during the period. In the case of *Schilbe mystus* its mean weight and standard length showed a decrease at the end of the experiment. The changes in mean weights, standard and total lengths of each of the fish species are presented in Table 4.2.

4.10 Tolerance of Fish to pH Changes

Fish tolerance to changes in pH showed that the different fish species generally tolerated wide pH ranges. The lowest pH tolerated was 3.16 by *Astatotilapia burtoni* while the highest was 11.85 by *Brycinus nurse* as shown in Table 4.3.

Table 4.2: Changes in mean morphometrics of fishes kept in the outdoor ponds for a period of 12 months

Fish	Start of experiment			End of experiment			Differences calculated		
	Wt (g)	S/l (cm)	T/l (cm)	Wt (g)	S/l (cm)	T/l (cm)	Wt (g)	S/l (cm)	T/l (cm)
Brienomyrus niger	2.9	6.2	7.2	3.7	7.0	8.0	0.8	0.8	0.8
Brycinus nurse	3.0	4.9	6.0	4.1	6.0	7.2	1.1	1.1	1.2
Clarias galmaensis	5.1	8.5	9.4	5.8	9.0	10.4	0.7	0.5	1.0
*Clarias gariepinus**	0.9	4.3	5.0	3.7	7.2	8.4	2.8	2.9	3.4
Hemichromis bimaculatus	3.3	5.0	6.3	4.9	6.5	7.5	1.6	1.5	1.2
*Oreochromis niloticus**	2.7	4.5	5.8	4.1	6.0	7.2	1.4	1.5	1.4
*Poecilia reticulata***	0.2	2.1	3.1	0.6	3.4	4.2	0.4	1.3	1.1
Schilbe mystus	2.2	6.0	7.0	1.8	5.8	7.0	-0.4	-0.2	0.0
Synodontis ouemeensis	2.1	3.6	4.5	4.4	5.8	7.5	2.3	2.2	3.0

*There was significant increase in size

**There was a significant increase in size and number

Wt, S/l, T/l = mean

Key: Wt=weight; S/l=Standard length; T/l=Total length

4.11 Effect of Keeping Fish without Feeding

4.11.1 The physicochemical parameter of the aquaria of the fish not fed for five months

The lowest monthly mean temperature (20.98ºC) was obtained in the aquarium that contained *Synodontis ouemeensis* during the fourth month of the experiment, while the highest was 25.85ºC in the aquarium that had *Clarias galmaensis* in the third month of the experiment. The lowest monthly mean pH (6.25) was in the aquarium that had *Clarias galmaensis* during the second month of the experiment while the highest mean pH (8.69) was in the aquarium that contained *Astatotilapia burtoni* in the third month of the experiment. The lowest monthly means TDS and EC were 42.50 mg/L and 81.50 µSiemens/cm respectively they were obtained in the aquarium that had *Oreochromis niloticus* in the third month of the experiment while the highest were 102.25 mg/L and 197.25 µSiemens/cm obtained in the aquarium that had *Clarias galmaensis* in the first month of the experiment. There was no significant difference ($P>0.05$) in temperature of water harbouring the different fish species. The monthly means of pH, total dissolved solids (TDS) and electrical conductivity (EC) of the water in the different aquaria harbouring the different fish species were significantly different ($P<0.05$). The morphometrics of the fish and the monthly means of the physicochemical parameters are presented in Appendices XII-XVI.

Table 4.3: Morphometric parameters, lowest and highest pH tolerated by each fish species

Fish species	Mean weight (g)	Mean length (cm)		pH range		Differences
		Standard	Total	Lowest	Highest	
Brienomyrus niger	6.6	8.6	10.4	4.85	10.52	5.67
Brycinus nurse	8.7	7.3	9.1	4.54	11.85	7.31
Clarias galmaensis	4.6	7.6	8.8	3.84	11.28	7.44
Clarias gariepinus	8.5	9.7	10.2	3.68	11.03	7.35
Epiplatys spilargyreius	0.4	3.0	3.7	3.97	11.06	7.09
Astatotilapia burtoni	5.5	6.1	7.7	3.16	10.64	7.48
Hemichromis bimaculatus	5.1	7.0	5.5	4.74	10.82	6.08
Leptocypris niloticus	3.2	5.3	6.9	4.44	10.78	6.34
Oreochromis niloticus	5.1	5.4	6.7	4.99	11.32	6.33
Poecilia reticulata	0.2	2.0	3.0	4.26	10.86	6.60
Schilbe mystus	15.5	11.2	13.1	4.26	11.08	6.82
Synodontis ouemeensis	11.4	8.1	10.1	3.96	11.16	7.20

4.11.2 Mortality of fish kept for five months without feeding

The mortality of fish starved for five months is presented in figure 9. There was a significant difference ($P<0.05$) in the mortality of the different fish species. Six (6) of the fish species, *Clarias galmaensis, Clarias gariepinus, Epiplatys spilargyreius, Astatotilapia burtoni, Hemichromis bimaculatus* and *Poecilia reticulata* all survived (zero mortality). Aquaria containing *Leptocypris niloticus* and *Oreochromis niloticus* had one mortality each. Aquaria containing *Brycinus nurse* and *Schilbe mystus* had 3 (37.50%) deaths each while aquaria containing *Brienomyrus niger* and *Synodontis ouemeensis*) had 4 (50%) deaths each, which were the highest. The monthly details of the mortality are given in Appendix XVII.

4.12 Effect of Keeping Fish in Different Water Volumes

4.12.1 Morphometrics of fish used in three different volumes of water

The morphometrics of the twelve fish species used for the 13 weeks test period showed that *Clarias galmaensis* had the highest morphometric value (mean weight 15.5 g, mean standard length 12.8 cm and mean total length 14.1 cm) while *Poecilia reticulata* had the least (mean weight 0.2 g, mean standard length 2.0 cm and mean total length 2.7 cm). The weight of the fishes used including their standard and total lengths are presented in Appendix XVIII.

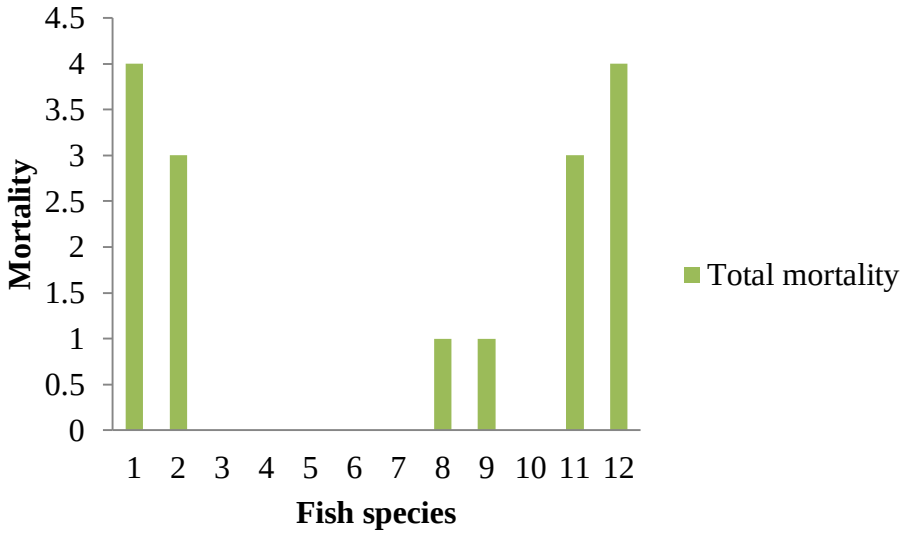


Figure 9: Total mortality of each fish species kept without feeding for five months

Key: 1. *Brienomyrus niger*, 2. *Brycinus nurse*, 3. *Clarias galmaensis*, 4 .*Clarias gariepinus*, 5. *Epiplatys spilargyreius* , 6. *Astatotilapia burtoni*, 7. *Hemichromis bimaculatus*, 8. *Leptocypris niloticus*, 9. *Oreochromis niloticus*, 10. *Poecilia reticulata*, 11. *Schilbe mystus*, 12. *Synodontis ouemeensis*

4.12.2 Physicochemical parameter of aquarium with fish in relation to water volume

The minimum temperature (20.9ºC) was in the aquarium that had *Clarias gariepinus* in 10 litres of water in the thirteenth week of the experiment and the maximum temperature (30.8ºC) was obtained in the first week of the test in the aquarium that had 20 litres of water containing *Epiplatys spilargyreius*. The minimum pH (5.91) was obtained in the aquarium with 10 litres of water containing *Clarias galmaensis* in the seventh week of the experiment, and the maximum pH (8.99) was in the aquarium that had 20 litres of water and *Epiplatys spilargyreius,* in the eleventh week of the test. The minimum TDS (64 mg/L) was recorded in the aquarium that had 30 litres of water and *Oreochromis niloticus* in the first week of the experiment. The minimum EC (125 µSiemens/cm) was obtained twice in the aquarium that had 30 litres of water containing *Oreochromis niloticus* and also in the aquarium that had 30 litres of water containing *Epiplatys spilargyreius,* both in the first week of the experiment. The maximum TDS (964 mg/L) and EC (1850 µSiemens/cm) were obtained in the aquarium containing 10 litres of water and *Clarias galmaensis* in the thirteenth week of the test. Appendices XIX-XXII gives the weekly water physicochemical parameters for the period.

4.12.3 The effect of varying volumes of water on fish species

Mortalities among the different fish species kept in 10, 20 and 30 litres of water for 13 weeks were generally low. At the end of the test period, only 15 out of 288 fish (5.21%) died. There was no aquarium in which more than 2 fish (25%) died. Of six of the fish species (*Clarias galmaensis, Clarias gariepinus*, *Epiplatys spilargyreius, Poecilia reticulata, Schilbe mystus* and *Synodontis ouemeensis*), no death was recorded in any of the

three different volumes of water. In aquaria containing *Astatotilapia burtoni*, *Leptocypris niloticus* and *Oreochromis niloticus*, there was only single (1) mortality in one out of the three different water volumes (4.2%). In the case of *Brienomyrus niger, Brycinus nurse* and *Hemichromis bimaculatus* there were four mortalities in the different water volumes (16.7%) in each case. Figure 10 and Appendix XXIII give the detail of the mortalities recorded.

4.13 The Reproductive Potential of the Fish Species

4.13.1 The reproductive potential of fish species in static and flow-through systems

Poecilia reticulata was the only fish that bred in the static pond and flow-through system. Breeding took place every months of the year, because young ones were observed and isolated throughout the year. When gravid females were isolated and studied in the laboratory, the mean number of offspring delivered was 38. *Epiplatys spilargyreius* bred all year round in the laboratory; the mean number of young observed was six. The number of young delivered by five females *Poecilia reticulata* and hatched by *Epiplatys spilargyreius* are presented in Table 4.4.

4.13. 2 The physicochemical parameters of the water in the flow-through system

During the 12 months test period, the lowest monthly mean temperature (21.85ºC) was in the aquarium that contained *Schilbe mystus* in the twelfth month (August 2010) and the highest (31.28ºC) was in aquarium that had *Oreochromis niloticus* in the eighth month (April 2010). The lowest monthly mean pH (6.67) was in the eleventh month (July 2010) in the aquarium that had *Hemichromis bimaculatus* while the highest was 9.48 in the fifth month (January 2010) in the aquarium that had *Clarias gariepinus*. The lowest monthly

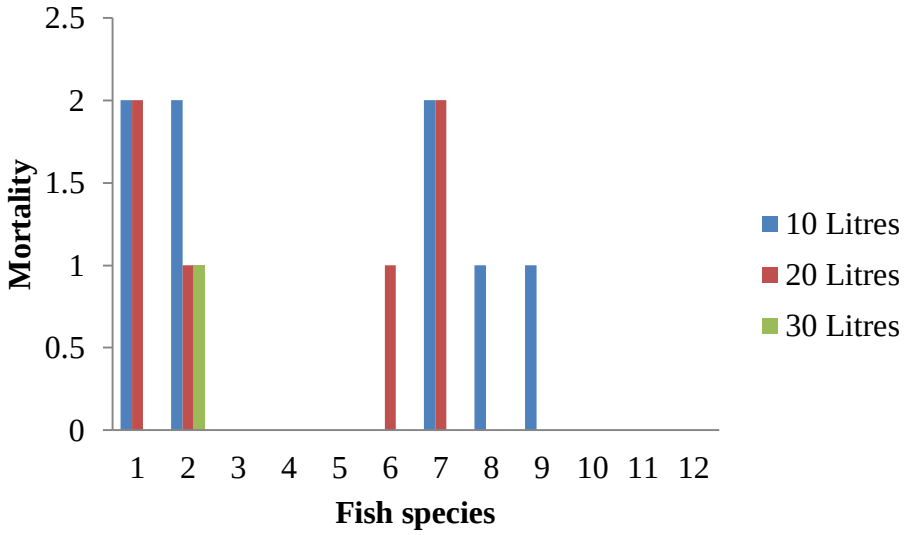


Figure 10: Total mortality of fish species resulting from the effect of different water volumes

Key: 1. *Brienomyrus niger*, 2. *Brycinus nurse*, 3. *Clarias galmaensis*, 4 .*Clarias gariepinus*, 5. *Epiplatys spilargyreius* , 6. *Astatotilapia burtoni*, 7. *Hemichromis bimaculatus*, 8. *Leptocypris niloticus*, 9. *Oreochromis niloticus*, 10. *Poecilia reticulata*, 11. *Schilbe mystus*, 12. *Synodontis ouemeensis*

Table 4.4: Reproductive potential of *Epiplatys spilargyreius* and *Poecilia reticulata* in the laboratory

	Number reproduced	
S/no.	*Epiplatys spilargyreius*	*Poecilia reticulata*
1	5	40
2	7	30
3	6	35
4	7	50
5	5	35
Mean ± standard deviation (standard error)	6 ± 1.00 (0.447)	38 ±7.58 (3.391)

mean TDS (39.50 mg/L) was in the aquarium that had *Poecilia reticulata* in the twelfth month (August 2010). The lowest monthly mean EC was 76.75 µSiemens/cm, obtained twice, in the twelfth month (August 2010) in the aquarium that had *Poecilia reticulata* and in the first month (September 2009) in the aquarium that had *Schilbe mystus*. The highest monthly mean TDS was 122.00 mg/L and that of EC was 234.50 µSiemens/cm, all obtained in the ninth month (May 2010) in the aquarium that had *Oreochromis niloticus*. There were no significant differences ($P>0.05$) in the monthly means of these water parameters in all the nine (9) aquaria. The mean monthly water parameters (temperature, pH, total dissolved solids and electrical conductivity) of the flow-through system are presented in Appendices XXIV-XXVII.

4.13.3 Morphometrics of the fish in the flow-through system

In the flow through system, all the experimental fish species showed appreciable increase in size at the end of the 12-month test period. For example *Clarias galmaensis, Clarias gariepinus* and *Oreochromis niloticus* had about 2,000%, 3,000% and 8,000% increase in weights respectively. The morphometrics of the fish species used are given in Table 4.5.

Table 4.5: Morphometrics of fishes used in flow-through system for 12 months

S/No.	Fish	Beginning of the experiment			End of the experiment			Differences calculated		
		Wt (g)	S/l (cm)	T/l (cm)	Wt (g)	S/l (cm)	T/l (cm)	Wt (g)	S/l (cm)	T/l (cm)
1	*Brienomyrus niger*	0.85	4.5	5.0	3.5	6.5	7.3	2.65	2.0	2.3
2	*Brycinus nurse*	8.3	7.0	8.9	9.5	7.4	9.5	1.2	0.4	0.6
3	*Clarias galmaensis*	2.1	3.7	4.6	45.1	16.2	18.1	43.0	12.5	13.5
4	*Clarias gariepinus*	41.1	16.0	18.3	1250	32.2	37.5	1208.9	16.2	19.2
5	*Hemichromis bimaculatus*	4.2	5.2	6.5	5.1	5.4	6.7	0.9	0.2	0.2
6	*Oreochromis niloticus*	2.7	4.5	5.8	220	18.0	22.5	217.3	13.5	16.7
7	*Poecilia reticulata*	0.2	2.1	3.1	0.7	3.4	4.2	0.5	1.3	1.1
8	*Schilbe mystus*	2.2	6.0	7.0	5.0	6.8	7.5	2.8	0.8	0.5
9	*Synodontis ouemeensis*	2.7	6.0	7.0	34.2	10.7	13.8	31.5	4.7	6.8

Key: Wt=weight; S/l=Standard length; T/l= Total length

CHAPTER FIVE

5.0 DISCUSSION

All the experimental fish species preyed on mosquito larvae in the laboratory but their consumption rates differed significantly ($P<0.05$). The availability and density of mosquito larvae affects the number consumed by a larvivorous fish, the more the larvae within a small area, the more susceptible they would be to predation because searching for them by the fish would be less tedious. Okorie and Abiodun (2010) and Ghosh *et al*. (2004, 2005a) remarked that the predatory potential of a fish was positively related to the density of mosquito larvae and inversely related to water volume. It is for this reason that mosquito larvae were supplied in abundance at all times during this study, so that the fish had enough to feed on, as some fed only when the surroundings were quiet, some at night, while others preferred mornings, afternoons or evenings. It was generally observed in this research, that the ability of fish to feed often reduced when the water temperature was low, when the aquarium water was changed, and whenever there was disturbance/movement around the aquarium. Therefore during the mosquito larvae feeding test, disturbance in the experimental area was minimal and tests were conducted at 27-30ºC.

In spite of the fact that all the experimental fish species fed on fewer mosquito larvae in the presence of commercial fish feed (coppens®), there was no significant difference between the mean number of mosquito larvae consumed by the fish either in the presence or absence of commercial fish feed. This finding of the fish feeding on less mosquito larvae in the presence of fish food concur with that of *Poecilia reticulata* which exhibited less preference for mosquito larvae in the presence of alternative prey as reported by Manna *et*

al. (2008). In India, Haq *et al*. (1993) also obtained similar results. Ekanayake (2007) in Sri Lanka found that in four fish the absence or presence of fish food did not appear to affect larval consumption by fish, but in four species there was reduction in larval consumption when commercial fish food was available. It can therefore be said that, if a fish feeds on other available nutrients then the amount of mosquito larvae it may consume is likely going to be less whenever such nutrients are available. Thus, the number of mosquito larvae each of the tested fish fed on per day in the absence of the commercial fish feed in the laboratory is likely to be less when these fish are in natural bodies of water where other foods may entice them. For a fish species to be used to control mosquito larvae, the fish should be able to consume mosquito larvae in the presence of other alternative foods, which may be present and even abundant in the environment.

The ability of fish to prey on mosquito larvae depended on the species under consideration; for example, the number of mosquito larvae consumed by *Clarias gariepinus* in a day differed from that consumed by *Oreochromis niloticus* of similar weight. This is probably because the two species have different feeding/nutritional requirements. A fish that is herbivorous may not feed on mosquito larvae and is therefore not likely to impact noticeably on mosquito larvae numbers. A fish species which is carnivorous at one stage and herbivorous at another could reduce mosquito larvae only at the carnivorous stage. *Oreochromis* species are known to be carnivorous only as juveniles (Fletcher *et al*., 1993), therefore, larger fish may not be effective for reduction in the numbers of mosquito larvae. *Clarias gariepinus* and *Clarias galmaensis* are omnivorous and may therefore prey on high numbers of mosquito larvae throughout their life.

In this study, the mosquito larvae consumed by *Clarias galmaensis* at 5, 7 and 9 months increased with increase in age/size/weight. Correlation analysis also showed that there was a strong positive relationship between mean mosquito larvae consumed by all the experimental fish and their weight and also between mean mosquito larvae consumed by the fish and their standard lengths. It can therefore be inferred that the number of larvae consumed by each species depends on age/size/weight. The mean number of larvae consumed by a 2.4 g *Clarias galmaensis* per day, differed from that consumed by one that was 4.9 g. Fletcher *et al*. (1993) also found that larval consumption in *Aplocheilichthys loati*, *Aphanius dispar* and *Micralestes* sp. in Ethiopia increased with increase in size. Okorie and Abiodun (2010) in Nigeria and Devi and Jauhari (2011) in India, made the same observation on *Aphyosemion gularis* and *Aplocheilus panchax*, respectively.

The mean number of mosquito larvae consumed by a fish is often expressed per 1, 2, 3, 6, 12 or 24 hours (Kamah, 1995; Kusumawathie *et al*., 2006; Ekanayake *et al*., 2007; Gupta and Banerjee, 2009; Okorie and Abiodun, 2010; Phukon and Biswas, 2011). In this study, the result is presented and discussed per 24 hours (day). The mean number of mosquito larvae consumed in the laboratory by a fish and its predatory index (PI) are used in explaining the predatory potential of each fish species.

The mean number of mosquito larvae consumed by *Poecilia reticulata* per day agreed with that of Menon and Rajagopalan (1978) in India, who found that the average predation of the fish per day was 53.1 and close to that of Elias *et al*. (1995) in Bangladesh, who found that the fish consumed 41.0 mosquito larvae per day. Chatterjee and Chandra (1997) also in India found that the adult and juveniles consumed 32 and 18 larvae respectively in 24 hours.

The mean number of larvae consumed by *P. reticulata* per day in this study is, however lower than the number reported by Ameen *et al*. (1999) in Bangladesh and Manna *et al*. (2008) in India. Ameen *et al*. (1999) reported that 0.6, 1.8 and 2.7 cm standard length *P. reticulata* consumed 10, 25 and 146 mosquito larvae respectively in 2 hours. Manna *et al*. (2008) also found the consumption of mosquito larvae by the fish ranging between 65 and 84 in a 3 hour feeding period. This difference could be due to the fact that Ameen *et al*. starved their experimental fish for 24 hours before utilizing them for the experiment and they used larger fish (2.5-3.5 cm versus 2.0 cm used in this study). Both Ameen *et al*. (1999) and Manna *et al*. (2008) reported that the consumption of mosquito larvae by *P. reticulata* increased with body size. Because of its good performance in reducing mosquito larvae, the fish have been used successfully to reduce mosquito larvae in some water bodies in Bangladesh, India and Grand Comore Island (Sharma and Ghosh, 1989; Sabatinelli *et al*., 1991; Gupta *et al*., 1992; Ameen *et .al*., 1999; Ghosh *et al*., 2005b, 2011). Based on the fish's predatory index and the result obtained from the pond experiment, this fish will be very effective for use in mosquito larvae control. Though *P. reticulata* is not indigenous, it is available in Nigeria, it is found in the Federal Capital Territory, Benue, Plateau, Kaduna and Lagos States (NIFFR, 2002; Anogwih and Makanjuola, 2010). The suitable climatic conditions prevalent within these areas favour the breeding of this exotic fish species (NIFFR, 2002).

Clarias gariepinus ranked second in terms of both predatory index and mean number of mosquito larvae consumed per day of all the fish species studied. Ghosh *et al*. (2005a) in India reported that *Clarias gariepinus* consumed between 1,325 and 2,550 mosquito larvae per day in the laboratory and Ameen *et al*. (1999) in a 1996 survey in Dhaka city,

Bangladesh found that a related species (*Clarias batrachus*) consumed 2,180 larvae in only 2 hours. This big difference in the numbers of mosquito larvae consumed may be due to the smaller size of *Clarias gariepinus* used in this study (2.2 g and 5.8 cm standard length) as compared to larger sizes used by Ghosh *et al*. (3.55 g) and Ameen *et al.* (16 cm).

Clarias galmaensis preyed on the highest number of mosquito larvae per day among all the tested fish species. *Clarias galmaensis* hatched, reared and experimented with, at five, seven and nine months in the laboratory revealed that as the age of the fish increased there was also an increase in its weight and lengths and a corresponding increase in the mean number of mosquito larvae consumed and the predatory index both in the presence and absence of coppens®. The predatory index of *Clarias galmaensis* at nine months (6.8 g) was 65.44 and older ones obtained from the field (20 g) was 49.40 in the absence of coppens®. This indicates that even though the older fish ate more mosquito larvae compared to the 9-month olds, more mosquito larvae were consumed by body weight at nine months (thus a higher predatory index). Based on the mean number of larvae consumed in the laboratory, its predatory index and the fact that it was able to control the larvae in the experimental pond; this fish will be a very good candidate for mosquito larvae control. In addition, the fish is indigenous and available in some rivers of northern Nigeria.

Both *Clarias gariepinus* and *Clarias galmaensis* are larvivorous and the great difference observed in the amount of mosquito larvae consumed between the two species may likely be attributed to the fact that the mean weight of the *Clarias galmaensis* used was 20 g while that of the *Clarias gariepinus* was 2.2 g, if the average weight of both the *Clarias gariepinus* and *Clarias galmaensis* were the same, the number of larvae consumed may also be similar. *Clarias gariepinus* used by Ghosh *et al*. (2005a) and *Clarias batrachus*

used by Ameen *et al*. (1999) demonstrated this assertion. In addition to consuming large numbers of mosquito larvae, *Clarias* species are known to withstand stress, survive even when out of water and handling them is easier as they do not have scales which when lost cause damage to skin (RFA, 2010).

Oreochromis niloticus had a predatory index of 35.12 and consumed an average of 144 mosquito larvae per day in this study but Ghosh *et al*. (2005a) found that the fish species (mean weight 2.1 and 2.95 cm total length) consumed between 192 and 407 mosquito larvae in the laboratory per day. A survey in Dhaka City, Bangladesh by Ameen *et al*. (1999) showed that the fish (standard length 14 cm) consumed 398 larvae in only 2 hours. There is a difference between the result in this study and that reported by Ameen *et al*. (1999) and Ghosh *et al*. (2005a). While the difference is difficult to explain in the case of Ghosh *et al*. (2005a) who used smaller size fish. On the other hand, the higher number obtained by Ameen *et al*. (1999) could be as a result of the large size of the fish used and the fact that they were starved for 24 hours before experimentation. Asimeng and Mutinga (1993), Ghosh *et al*. (2005a, 2006), Kusumawathie *et al*. (2006) and Howard *et al*. (2007) had all used this fish species to control mosquito larvae. Other species of *Oreochromis* had also been tried in other places with success in reducing the number of mosquito larvae; Mohamed (2003) found that *Oreochromis spilurus* greatly reduced the number of mosquito larvae in Kalabeydh village, northern Somalia. *Oreochromis mossambicus* in India proved to be effective in eliminating mosquito larvae (Sharma and Ghosh, 1989; Surendran *et al.*, 2008).

Even though there are no previous reports of mosquito larvae consumption by *Epiplatys spilargyreius, Hemichromis bimaculatus, Leptocypris niloticus* and *Schilbe mystus* for

comparison, the results obtained in this study show that they are good candidates for mosquito larvae control. There are also no reports from previous studies of mosquito larvae consumption by *Synodontis ouemeensis, Brienomyrus niger, Astatotilapia burtoni* and *Brycinus nurse* for comparison. However, owing to the low mean number of larvae they consumed per day as observed in this study, it can be inferred that they may not be good for use in the control of mosquito larvae.

In testing the ability of nine of the experimental fish to control mosquito larvae in ponds, the control pond which had no fish gave the highest total mosquito larvae count each month; it also gave the highest total mosquito larvae count for the year. Different numbers of mosquito larvae were counted in the ponds containing *Brienomyrus niger, Brycinus nurse* and *Synodontis ouemeensis*. However, in the ponds containing *Clarias galmaensis, Clarias gariepinus, Hemichromis bimaculatus, Oreochromis niloticus, Poecilia reticulata* and *Schilbe mystus,* not even a single mosquito larva was encountered throughout the 12-month test period; this implies that these six fish species were able to consume all the mosquito larvae in their ponds. In some countries where fish with larvivorous potentials were introduced into ponds or other water collections for mosquito larvae control, the result is encouraging because the numbers of mosquito larvae in such water bodies was observed to drop within a short period e.g. lakes and ponds stocked with various fish species had low average larval density compared to other water bodies without fish in Dhaka city, Bangladesh (Ameen *et al*., 1999). *Poecilia reticulata* successfully reduced mosquito larvae in some water bodies in India (Sharma and Ghosh, 1989; Gupta *et al*., 1992; Ghosh, 2005b, 2011), Bangladesh (Ameen *et al*., 1999) and in Grand Comore Island (Sabatinelli *et al*., 1991). In India, Ghosh *et al*. (2005a) observed a significant decrease in larval abundance

after introduction of *Cyprinus carpio*, *Ctenopharyngodon idella*, *Oreochromis niloticus* and *Clarias gariepinus* in field conditions. A study by Surendran *et al.* (2008) in India using *Oreochromis mossambicus* in water storage tanks proved to be effective in eliminating mosquito larvae within 3 days. Sharma and Ghosh (1989) also in India found that *Oreochromis mossambicus* was effective for controlling mosquitoes in cow dung pits.

In the open pond experiment, seven (7) of the experimental fish species (*Brienomyrus niger, Brycinus nurse, Clarias galmaensis, Clarias gariepinus, Hemichromis bimaculatus, Oreochromis niloticus* and *Synodontis ouemeensis*) showed no appreciable increase in size at the end of the 12-month test period. In the case of *Schilbe mystus* its average weight and standard length showed a decrease at the end of the experiment. It was only the *Poecilia reticulata* that increased in size and number during the period. The inability of the tested fish species to show appreciable weight gain in the open ponds is advantageous if they are used in controlling mosquito larvae in bodies of water as small in size makes them unattractive to the general public for nutritional and/or economic purposes; they can therefore be used for mosquito larvae control without fear that they will be harvested for food. It is even disadvantageous to use large *Oreochromis niloticus* as a study by El-Safi *et al.* (1985) in Gezira irrigation canals in Egypt showed that *Oreochromis niloticus* smaller than 15.0 cm were markedly carnivorous, but this decreased with age; therefore, only small fish of this species could be useful for biological control of mosquito larvae as fish larger than 15.0 cm showed a marked preference for higher aquatic macrophytes. For mosquito larvae control in large bodies of water, large size larvivorous fish could be a disadvantage because bigger fish may be restricted to deep water and may not come in contact with mosquito larvae which are often found at shallow edges. The movements of large fish may

also send signals to mosquito larvae to disperse. Therefore, for mosquito larvae control, smaller larvivorous fish species are preferred.

The absence of any significant differences in the mean monthly water parameters is not surprising since the ponds were more or less exposed to the same environmental conditions. Even when more water was added to replace the amount lost as a result of evaporation or seepage; it was done for all the ponds within the same period.

The fact the different fish species generally tolerated wide pH ranges is a testimony to their hardiness. The wider the pH range a fish is able to tolerate, the greater its chances of survival in the environment, if it is used for mosquito control in water bodies that may be accidentally contaminated with acidic or basic minerals.

In a natural environment such as the river, fish survive by depending on food that is available in the environment. Cultured fish need to be fed if they are to survive and grow. When the experimental fishes in this research were deprived of food for five months, there was no fish species that had more than 50% mortality. In six of the fish species no mortality was recorded during the test period, two species had one mortality each; the other four species had mortalities between 37.50 to 50.00%. The starved fish probably survived on phytoplankton, zooplankton or microorganisms that developed on the walls of the aquarium (Mayland, 1976). The ability of each of the fish species to survive for a long period without feeding may depend on several factors such as food components stored in the form of fat in the body of the fish, health status, age/size, sex and period of the year. The result showed that the tested fish species can survive for a long period without feeding, this indicates that the fish species are very hardy in terms of being able to survive in the absence

of food and can be recommended for use in mosquito larvae control since they would last long in water habitat at a time when mosquito larvae or other foods are scarce.

Mortalities recorded in different water volumes were also generally low; there was no aquarium in which more than two fish (25%) died. Where mortalities were recorded it was likely due to chance and not the amount of water in which the fish was kept as there was no significant difference in fish mortality among the different aquaria used for the test. The low mortalities recorded among all the fish species kept in different volumes of water suggest that all the fish species are likely to be hardy in terms of survival if introduced into small or large bodies of water. It also implies that if any of these fish is used for mosquito larvae control, it will tolerate flooding, drought and seepages, which are often experienced in our environment. If a fish species is not able to survive such changes then it may not be a good candidate for mosquito control as it has to be reintroduced any time such a phenomenon is experienced.

Poecilia reticulata was the only fish that bred in both the static and flow-through systems throughout the year as young ones were observed all year round. Although these fish were not fed in the pond/static system, natural food items including mosquito larvae in the pond were probably adequate to support their small size. The inability of the other fish species to breed could be attributed to several factors related to water temperature and volume, hardness, photoperiod, food, seasons etc. these factors were probably not suitable in the ponds and flow-through system. In captivity for example, *Clarias* does not spawn spontaneously since environmental factors such as the rise in water level and inundation of shallow areas do not occur in the laboratory (De Graaf and Janssen, 1996). Though fish were adequately fed with coppens® in the flow-through system, the fact that the

experimental fishes were mostly brought from the wild and their specific nutritional requirements were not known, coppens® alone might not have met their specific need. Some of these fish in their natural environment might depend on insects, insect larvae, phytoplankton, zooplankton, microorganisms and other dead or living organisms which were probably lacking in the flow-through system which could also have contributed to their inability to breed. Some of these fish species are used to large volumes of water in their natural environments, but in the static system, the water level in the ponds was maintained at just 20 cm and the aquarium size in the flow-through system was also probably not large enough to support the growth and reproductive activities of the fish. For example, even though the experimental *Oreochromis niloticus* did not reproduce, another was observed breeding in a close by concrete pond (130×90) cm with water maintained at 70 cm depth and in an earthen pond (11.0×7.0 metres) with water volume maintained at about one metre. Since ages of fish used in the study was not known (as they were collected from the wild), it is possible that most of them were not mature and could not have bred during the 12-month study period. The temperature, pH, TDS and EC recorded in both the static and flow-through systems were within optimum limits of fish requirement.

Pregnant *Poecilia reticulata* were isolated so as to count the number of offspring delivered, the numbers ranged from 30-50. The exact numbers were often difficult to ascertain as the mothers often prey on their young after delivery. Even though steps were taken in this work to separate the new born from their mother, it was difficult to prevent the mother from preying on some of her young, therefore these numbers may have been underestimated. Where steps were not taken to separate the young from the mother in some cases, the mother ate all the young. The numbers of *Poecilia* born in this study is comparable to that

of García *et al*. (2008) and Balon (1990) who obtained ranges of 45-64 and 20 to 40 newborns per female, respectively. The larger the aquarium or the container in which the expectant mother was kept, the greater the number of offspring recovered; this may be because some of the offspring were able to escape from being eaten by the mother. The presence of water plants and stones with spaces in between also gave the offspring places to hide from the mothers and therefore, large numbers of young ones were recovered from such environments. It was also observed that most offspring survived when adequate food was provided for the mother before and after delivery. The availability of adequate food may have contributed to the delivery of large offspring because in natural environment where food is lacking number delivered and the frequency of delivery may be lower as found by García *et al*. (2008).

In the twelve months that the breeding ability of *Epiplatys spilargyreius, Leptocypris niloticus* and *Astatotilapia burtoni* were monitored in the laboratory, only *Epiplatys spilargyreius* bred, the number of young observed were 5-7. This implies that the growing and breeding requirements of this fish were met. The inability of the other fish species to breed could be attributed to factors including unsuitable temperature, water quality, photoperiod, quantity of water, seasons, food and age.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

- All The twelve experimental fish species used in the study consume *Culex quinquefasciatus* larvae but at different rates.
- Based on predatory indices and mean number of mosquito larvae consumed, *Poecilia reticulata, Clarias gariepinus, Clarias galmaensis, Epiplatys spilargyreius, Hemichromis bimaculatus, Oreochromis niloticus, Leptocypris niloticus* and *Schilbe mystus* have better larvivorous potential than *Synodontis ouemeensis, Brienomyrus niger, Astatotilapia burtoni* and *Brycinus nurse*.
- The fingerlings of normally large-sized fish species such as *Clarias gariepinus* are more suitable than the adults for use in the control of mosquito larvae,
- Small size fish have the advantage of being able to move to the shallow edges of water bodies where mosquito larvae often reside. The movement of large fish often makes mosquito larvae disperse.
- All the tested fish species consume fewer mosquito larvae in the presence of alternative food.
- The tested fishes are able to tolerate wide pH ranges, showing that they are hardy and if used for mosquito larvae control, they will survive natural, accidental or deliberate exposure to wide pH changes in the environment.
- The fish species used in this study, particularly *Clarias galmaensis, Clarias gariepinus, Epiplatys spilargyreius, Astatotilapia burtoni, Hemichromis*

bimaculatus, Poecilia reticulata, *Leptocypris niloticus* and *Oreochromis niloticus* can survive for a long period without feeding, and would therefore be able to withstand long periods of food scarcity in the wild.

- All the tested fish species can survive varying volumes of water and would therefore be able to tolerate flooding and drought which are often experienced in our environment.
- *Poecilia reticulata* (viviparous) and *Epiplatys spilargyreius* (egg-laying) are capable of breeding in captivity.
- *Clarias galmaensis,* which ranked highest in the consumption of mosquito larvae can be hatched in captivity using ovaprim hormone.
- *Culex quinquefasciatus w*as used as a model with the understanding that the result obtained can be applied to other mosquito species.
- Nigeria has a number of potential fish species that can be incorporated into its mosquito/malaria/filariasis control programme to complement other efforts in combating these and related diseases.

Recommendations

From the result obtained, the following eight out of the 12 experimental fish species are recommended for mosquito larvae control *Poecilia reticulata, Clarias gariepinus, Clarias galmaensis, Epiplatys spilargyreius, Hemichromis bimaculatus, Oreochromis niloticus, Leptocypris niloticus* and *Schilbe mystus.* These eight species in addition to other fish species which may proof effective should be adopted for mosquito larvae control in the country.

It is crucial to identify native/indigenous fish species that thrive under the conditions prevalent in mosquito breeding sites in different parts of Nigeria for use in the control of mosquitoes. The Federal Government of Nigeria, through the Federal Ministry of Health or other agencies involved in disease control should set up a unit/department that will concern itself with the possibility of mosquito control using larvivorous fishes. The unit should identify, breed and supply the best larvivorous fish for each part of the country. The unit should have offices and officers that monitor and evaluate the success of the programme in all the States and Local Government Areas of the country.

Mosquito control using fish should be used in addition to other strategies already in place, such as environmental alteration, habitat manipulation, getting rid of stagnant water, proper disposal of water holding containers, removal of aquatic vegetation, use of mosquito nets and drugs.

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APPENDICES

Appendix I: Photographs of the fish species used in this study



Brienomyrus niger (Günther, 1866)



Brycinus nurse (Rüppell, 1832)



Clarias galmaensis Aken'Ova, 2007



Clarias gariepinus (Burchell, 1822)



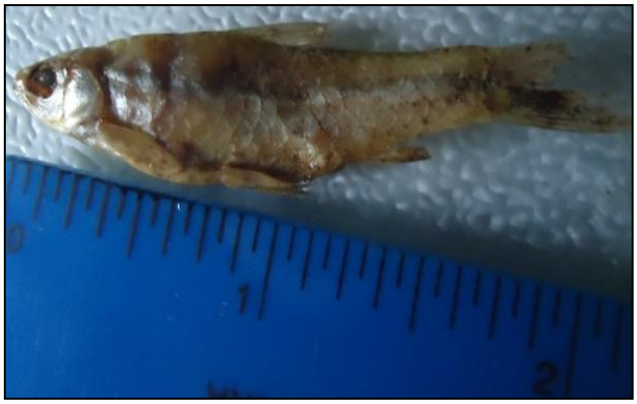
Epiplatys spilargyreius (Duméril, 1861)



Astatotilapia burtoni (Günther, 1894)



Hemichromis bimaculatus (Gill, 1862)



Leptocypris niloticus (Joannis, 1835)



Oreochromis niloticus (Linnaeus, 1758)



Poecilia reticulata Peters, 1859



Schilbe mystus (Linnaeus, 1758)



Synodontis ouemeensis Musschoot & Lalèyè, 2008

Appendix II a: Sorting fish at Akwa village, Godo-Godo, Jama'a Local Government Area, Kaduna State



Appendix II b: Catching fish at New Bussa, Niger State



Appendix IIc: Collection of *Epiplatys spilargyreius* from a swamp at Munnai, New Bussa, Niger State



Appendix III: Fish morphometric and mean number of mosquito larvae consumed with standard deviation and standard error

S/No	Fish	Weight (g)	Standard length (cm)	Mean Total length (cm)	Mean mosquito larvae consumed/day	Std. Deviation	Std. Error
1	*Clarias galmaensis*	20	14.9	16.5	988 [a]	161.11	93.02
2	*Clarias gariepinus*	2.2	5.8	6.5	223 [b]	31.19	18.01
3	*Hemichromis bimaculatus*	5.2	5.6	7.0	187 [b]	81.18	46.87
4	*Oreochromis niloticus*	4.1	5.2	6.5	144 [bc]	26.96	15.57
5	*Schilbe mystus*	4.7	7.0	8.0	125 [bcd]	2.65	1.53
6	*Leptocypris niloticus*	3.0	5.2	6.8	88 [cde]	14.11	8.15
7	*Synodontis ouemeensis*	3.0	3.5	5.7	74 [cde]	2.00	1.16
8	*Poecilia reticulata*	0.2	2.0	3.0	53 [cde]	12.17	7.02
9	*Brienomyrus niger*	3.0	6.3	7.2	51 [cde]	9.00	5.20
10	*Astatotilapia burtoni*	5.0	6.0	7.6	39 [de]	7.00	4.04
11	*Brycinus nurse*	8.5	7.2	9.0	28 [de]	3.61	2.08
12	*Epiplatys spilargyreius*	0.4	3.0	3.8	18 [e]	2.00	1.16

Means with different alphabet(s) are statistically different at $P< 0.05$

Appendix IV: Fish morphometric and mean number of mosquito larvae consumed in the presence of coppens® with standard and standard error

S/No	Fish	Weight (g)	Mean			Std. Deviation	Std. Error
			Standard length (cm)	Total length (cm)	Mosquito larvae consumed /day		
1	*Clarias galmaensis*	20	14.9	16.5	649 [a]	89.96	51.94
2	*Clarias gariepinus*	2.2	5.8	6.5	137 [b]	13.45	7.77
3	*Hemichromis bimaculatus*	5.2	5.6	7.0	122 b	7.94	4.58
4	*Oreochromis niloticus*	4.1	5.2	6.5	105 [bc]	13.08	7.55
5	*Schilbe mystus*	4.7	7.0	8.0	99 [bcd]	9.85	5.69
6	*Leptocypris niloticus*	3.0	5.2	6.8	66 [cde]	6.08	3.51
7	*Synodontis ouemeensis*	3.0	3.5	5.7	55 [def]	10.54	6.08
8	*Poecilia reticulata*	0.2	2.0	3.0	35 [ef]	8.19	4.73
9	*Brienomyrus niger*	3.0	6.3	7.2	45 [ef]	5.57	3.22
10	*Astatotilapia burtoni*	5.0	6.0	7.6	21 [ef]	2.00	1.16
11	*Brycinus nurse*	8.5	7.2	9.0	18 [ef]	3.61	2.08
12	*Epiplatys spilargyreius*	0.4	3.0	3.8	12 [f]	2.00	1.16

Means with different alphabet(s) are statistically different at $P< 0.05$

Appendix V: Predatory Indices of the fish species fed on mosquito larvae with or without coppens®*

S/No.	Fish species	Predatory Index	
		Without coppens®	**With coppens®**
1	*Poecilia reticulata*	265.00	175.00
2	*Clarias gariepinus*	101.36	62.27
3	*Clarias galmaensis*	49.40	32.45
4	*Epiplatys spilargyreius*	45.00	30.00
5	*Hemichromis bimaculatus*	35.96	23.46
6	*Oreochromis niloticus*	35.12	25.61
7	*Leptocypris niloticus*	29.33	22.00
8	*Schilbe mystus*	26.60	21.06
9	*Synodontis ouemeensis*	24.67	18.33
10	*Brienomyrus niger*	17.00	15.00
11	*Astatotilapia burtoni*	7.80	4.20
12	*Brycinus nurse*	3.29	2.12

* arranged by ranking

Appendix VI: Morphometrics, mean mosquito larvae consumed and Predatory indices of *Clarias galmaensis* at 5, 7 and 9 months

Age (Months)	5	7	9
Mean weight (g)	2.4	4.9	6.8
Mean standard length (cm)	6.1	7.8	8.5
Mean total length (cm)	6.4	8.9	9.0
Mean number of mosquito larvae consumed per day without coppens®	32.5	125	445
Mean number of mosquito larvae consumed per day with coppens®	27.5	106	350
Predatory Index without coppens®	13.54	25.5	65.44
Predatory Index with coppens®	11.46	21.6	51.47

Appendix VII: Monthly mean temperature (ºC) recorded in each pond for 12 months

Months	Control	*Brienomyrus niger*	*Brycinus nurse*	*Clarias galmaensis*	*Clarias gariepinus*	*Hemichromis bimaculatus*	*Oreochromis niloticus*	*Poecilia reticulata*	*Schilbe mystus*	*Synodontis ouemeensis*
Sep 2009	24.70	24.60	24.65	23.95	25.25	24.65	24.90	24.80	24.75	24.55
Oct 2009	23.66	23.68	23.60	23.66	24.16	23.68	24.96	24.12	23.90	23.78
Nov 2009	21.08	20.33	19.88	19.88	20.88	19.95	21.90	21.43	20.75	19.90
Dec 2009	19.18	19.24	18.18	18.36	19.50	18.72	20.18	20.16	19.84	18.46
Jan 2010	19.35	19.33	18.15	17.95	19.50	19.10	21.13	20.50	19.83	18.63
Feb 2010	21.35	22.23	20.33	20.30	21.60	20.75	22.75	22.30	21.75	20.55
Mar 2010	22.78	22.63	22.78	23.65	23.08	22.43	24.58	23.83	23.33	22.40
Apr 2010	25.06	24.92	26.70	27.36	25.28	24.02	25.92	25.36	25.22	25.10
May 2010	24.48	24.80	25.50	25.53	24.80	24.73	25.18	24.90	24.75	24.90
Jun 2010	23.05	23.10	23.78	23.93	23.10	23.18	23.23	23.18	23.15	23.28
Jul 2010	21.92	22.10	23.16	23.08	22.00	22.30	22.10	21.98	22.16	22.08
Aug 2010	23.33	21.63	22.35	22.30	21.30	21.68	21.65	21.63	21.78	21.68

No significant difference in the means of the ponds each month at $P>0.05$

Appendix VIII: Monthly mean pH for each pond for 12 months

Months	control	*Brienomyrus niger*	*Brycinus nurse*	*Clarias galmaensis*	*Clarias gariepinus*	*Hemichromis bimaculatus*	*Oreochromis niloticus*	*Poecilia reticulata*	*Schilbe mystus*	*Synodontis ouemeensis*
Sep 2009	8.30	8.17	7.99	7.99	8.28	8.24	7.89	7.80	8.09	8.22
Oct 2009	7.95	8.34	7.87	7.88	7.87	7.75	7.99	7.82	8.07	7.81
Nov 2009	7.95	8.25	8.51	8.20	8.13	8.64	8.30	8.35	8.05	8.72
Dec 2009	8.48	8.54	8.44	8.20	8.05	8.62	8.64	8.67	8.44	8.58
Jan 2010	8.40	8.55	8.31	8.47	8.47	8.57	8.98	8.79	8.68	8.33
Feb 2010	8.17	8.24	8.33	8.36	8.24	8.39	8.46	8.49	8.33	8.35
Mar 2010	8.08	8.13	8.19	8.26	8.09	8.16	8.51	8.37	8.13	8.14
Apr 2010	7.88	7.96	7.96	8.23	7.91	8.08	7.99	8.01	7.92	8.01
May 2010	7.43	7.55	7.23	7.66	7.56	7.58	7.53	7.37	7.47	7.60
Jun 2010	7.19	7.19	7.15	7.51	7.16	7.26	7.15	7.17	7.19	7.27
Jul 2010	6.94	7.05	7.18	7.29	6.87	7.21	6.85	6.90	6.92	7.18
Aug 2010	6.95	6.94	7.17	7.02	7.02	7.05	6.96	6.99	6.98	6.96

No significant difference in the means of the ponds each month at $P>0.05$

Appendix IX: Monthly mean total dissolved solids (mg/L) for each pond for 12 months

Months	control	*Brienomyrus niger*	*Brycinus nurse*	*Clarias galmaensis*	*Clarias gariepinus*	*Hemichromis bimaculatus*	*Oreochromis niloticus*	*Poecilia reticulata*	*Schilbe mystus*	*Synodontis ouemeensis*
Sep 2009	51.25	88.00	77.25	54.25	79.25	76.75	57.50	70.75	57.50	53.75
Oct 2009	71.60	88.80	101.20	79.40	94.80	87.60	79.80	89.40	84.40	70.80
Nov 2009	87.50	105.25	124.50	83.00	112.50	88.00	79.75	91.25	112.00	85.75
Dec 2009	99.40	120.80	159.80	86.80	126.20	88.40	95.00	104.40	110.80	110.40
Jan 2010	153.75	99.25	181.25	91.50	142.75	84.50	145.25	120.25	125.00	144.25
Feb 2010	179.25	92.00	123.25	114.25	149.00	124.25	162.50	122.50	138.75	125.00
Mar 2010	151.75	124.00	127.00	119.25	143.50	126.75	152.50	108.75	137.00	131.75
Apr 2010	163.60	153.80	135.60	136.20	159.00	113.40	153.60	121.60	139.60	158.00
May 2010	132.25	118.25	128.75	107.75	139.75	104.00	146.00	109.25	133.75	110.00
Jun 2010	79.25	99.75	96.00	87.25	89.00	78.25	91.75	78.25	91.00	99.50
Jul 2010	67.40	92.60	84.60	74.80	82.60	78.00	73.40	55.00	72.40	83.60
Aug 2010	65.25	91.75	80.75	71.00	72.25	73.25	67.75	61.75	62.75	75.00

No significant difference in the means of the ponds each month at $P>0.05$

Appendix X: Monthly mean electrical conductivity (µSiemens/cm) for each pond for 12 months

Months	control	*Brienomyrus niger*	*Brycinus nurse*	*Clarias galmaensis*	*Clarias gariepinus*	*Hemichromis bimaculatus*	*Oreochromis niloticus*	*Poecilia reticulata*	*Schilbe mystus*	*Synodontis ouemeensis*
Sep 2009	98.75	169.50	150.25	104.50	153.75	147.75	110.50	137.25	110.75	103.75
Oct 2009	138.00	168.20	193.40	152.00	184.00	166.60	154.40	162.40	162.40	137.00
Nov 2009	170.75	202.25	238.50	160.75	218.00	168.75	155.50	175.50	217.50	165.50
Dec 2009	192.60	232.40	305.40	168.80	236.00	171.00	184.60	194.20	214.60	210.80
Jan 2010	296.75	214.75	349.25	177.50	275.00	165.25	283.50	234.00	249.25	278.25
Feb 2010	341.25	180.75	237.75	223.75	288.00	239.50	316.00	237.50	267.25	241.00
Mar 2010	292.00	240.50	244.75	229.75	276.25	245.00	295.25	209.50	263.50	253.50
Apr 2010	294.80	296.40	260.60	261.20	306.00	219.40	294.80	235.40	269.40	263.80
May 2010	255.00	229.50	250.00	207.75	268.75	201.75	281.75	212.75	258.25	212.00
Jun 2010	154.00	190.50	182.75	166.50	171.50	152.00	177.50	151.25	176.00	190.50
Jul 2010	133.20	177.60	164.20	142.60	158.80	151.00	142.20	107.20	140.20	156.60
Aug 2010	126.25	176.50	156.25	137.75	139.25	142.25	131.25	120.50	121.00	144.75

No significant difference in the means of the ponds each month at $P>0.05$

Appendix XI: Monthly and total mosquito larvae from each respective pond for 12 months period

Months	Control	*Brienomyrus niger*	*Brycinus nurse*	*Clarias galmaensis*	*Clarias gariepinus*	*Hemichromis bimaculatus*	*Oreochromis niloticus*	*Poecilia reticulata*	*Schilbe mystus*	*Synodontis ouemeensis*
Sep 2009	206	10	11	0	0	0	0	0	0	40
Oct 2009	282	11	23	0	0	0	0	0	0	28
Nov 2009	189	4	27	0	0	0	0	0	0	37
Dec 2009	263	7	18	0	0	0	0	0	0	20
Jan 2010	194	5	6	0	0	0	0	0	0	29
Feb 2010	114	3	5	0	0	0	0	0	0	74
Mar 2010	238	9	8	0	0	0	0	0	0	108
Apr 2010	306	23	10	0	0	0	0	0	0	198
May 2010	247	19	21	0	0	0	0	0	0	36
Jun 2010	240	15	8	0	0	0	0	0	0	29
Jul 2010	224	29	21	0	0	0	0	0	0	19
Aug 2010	127	20	7	0	0	0	0	0	0	7
Total	**2630**	**155**	**165**	**0**	**0**	**0**	**0**	**0**	**0**	**625**

Differences within ponds were significant (P<0.05)

Appendix XII: Morphometric of the fish species starved for five months

Fish species	Mean		
	weight(g)	standard length (cm)	total length (cm)
Brienomyrus niger	2.1	6.0	7.1
Brycinus nurse	8.0	7.0	8.6
Clarias galmaensis	22.0	15.0	16.5
Clarias gariepinus	5.1	8.5	9.4
Epiplatys spilargyreius	1.2	3.8	4.8
Astatotilapia burtoni	2.8	4.0	5.3
Hemichromis bimaculatus	4.5	5.4	6.7
Leptocypris niloticus	3.4	5.8	7.3
Oreochromis niloticus	3.1	4.5	5.8
Poecilia reticulata	0.2	2.1	3.1
Schilbe mystus	4.1	6.7	8.0
Synodontis ouemeensis	2.0	3.5	4.4

Appendix XIII: Monthly mean temperature (ºC) record of fish species starved for five months

S/no	Fish species	First month	Second Month	Third month	Fourth month	Fifth month
1	*Brienomyrus niger*	24.93	22.33	21.93	22.50	22.88
2	*Brycinus nurse*	25.08	23.10	21.15	22.40	22.95
3	*Clarias galmaensis*	23.50	24.15	25.85	24.38	21.73
4	*Clarias gariepinus*	24.48	22.70	21.78	22.75	23.08
5	*Epiplatys spilargyreius*	24.15	22.38	21.78	22.43	22.63
6	*Astatotilapia burtoni*	24.78	22.95	21.19	22.50	22.95
7	*Hemichromis bimaculatus*	24.50	22.40	22.00	22.48	22.65
8	*Leptocypris niloticus*	25.75	23.83	25.13	24.08	22.08
9	*Oreochromis niloticus*	23.50	24.20	25.73	24.58	22.00
10	*Poecilia reticulata*	23.43	24.30	23.85	24.23	21.68
11	*Schilbe mystus*	25.18	23.05	21.48	22.05	23.20
12	*Synodontis ouemeensis*	25.38	23.25	21.78	20.98	23.68

Differences within months were not significant ($P>0.05$)

Appendix XIV: Monthly mean pH record of the fish species starved for five months

S/no	Fish species	First month	Second month	Third month	Fourth month	Fifth month
1	*Brienomyrus niger*	7.51	7.84	8.21	7.47	8.04
2	*Brycinus nurse*	7.28	7.41	7.62	7.55	7.08
3	*Clarias galmaensis*	7.62	6.25	7.33	6.73	7.69
4	*Clarias gariepinus*	7.05	8.08	8.12	7.86	8.19
5	*Epiplatys spilargyreius*	8.13	8.21	8.11	8.32	8.09
6	*Astatotilapia burtoni*	7.91	8.24	8.69	8.61	8.48
7	*Hemichromis bimaculatus*	7.58	7.92	7.60	7.42	8.35
8	*Leptocypris niloticus*	8.00	8.25	8.31	7.98	8.15
9	*Oreochromis niloticus*	7.42	6.39	7.06	6.61	7.67
10	*Poecilia reticulata*	8.05	6.72	7.48	7.78	7.19
11	*Schilbe mystus*	6.65	7.36	7.66	7.80	6.82
12	*Synodontis ouemeensis*	7.03	7.44	7.68	7.64	7.35

Differences within months were significant ($P<0.05$)

Appendix XV: Monthly mean total dissolved solids (mg/L) record of the fish species starved for five months

S/no	Fish species	First month	Second month	Third month	Fourth month	Fifth month
1	*Brienomyrus niger*	48.25	52.00	56.75	54.25	58.75
2	*Brycinus nurse*	54.00	51.25	54.00	55.50	53.25
3	*Clarias galmaensis*	102.25	85.75	62.75	62.75	68.75
4	*Clarias gariepinus*	48.50	51.00	55.25	55.00	54.25
5	*Epiplatys spilargyreius*	46.00	53.50	64.00	64.25	67.50
6	*Astatotilapia burtoni*	47.00	56.00	53.25	59.00	56.00
7	*Hemichromis bimaculatus*	52.75	51.75	56.25	59.00	62.00
8	*Leptocypris niloticus*	53.00	59.00	60.50	60.00	57.00
9	*Oreochromis niloticus*	80.00	61.00	42.50	45.75	55.00
10	*Poecilia reticulata*	70.25	58.50	65.50	73.25	66.25
11	*Schilbe mystus*	44.50	59.50	53.75	57.00	58.25
12	*Synodontis ouemeensis*	45.75	49.50	49.50	49.00	51.25

Differences within months were significant ($P<0.05$)

Appendix XVI: Monthly mean electrical conductivity (µSiemens/cm) record of the fish species starved for five months

S/no	Fish species	First month	Second month	Third month	Fourth month	Fifth month
1	*Brienomyrus niger*	95.00	100.75	109.25	103.75	112.50
2	*Brycinus nurse*	104.75	99.50	104.50	107.25	100.75
3	*Clarias galmaensis*	197.25	167.00	119.50	122.00	133.50
4	*Clarias gariepinus*	92.75	99.25	101.25	106.00	103.00
5	*Epiplatys spilargyreius*	89.75	104.25	125.00	126.75	132.50
6	*Astatotilapia burtoni*	90.00	106.25	100.75	113.50	107.50
7	*Hemichromis bimaculatus*	101.25	100.25	109.00	114.00	118.25
8	*Leptocypris niloticus*	102.75	116.00	118.00	116.50	109.75
9	*Oreochromis niloticus*	155.50	118.50	81.50	89.50	106.75
10	*Poecilia reticulata*	120.75	114.25	127.50	142.75	129.00
11	*Schilbe mystus*	87.25	114.50	104.25	109.50	113.00
12	*Synodontis ouemeensis*	88.50	96.50	97.50	93.25	99.50

Differences within months were significant ($P<0.05$)

Appendix XVII: Monthly and total mortality record of the different fish species starved for five months

s/no	Fish species	Mean monthly mortality					**Total (%)**
		First month	Second month	Third month	Fourth month	Fifth month	
1	*Brienomyrus niger*	1	1	1	0	1	**4 (50.00)**
2	*Brycinus nurse*	1	0	1	0	1	**3 (37.50)**
3	*Clarias galmaensis*	0	0	0	0	0	**0 (0.00)**
4	*Clarias gariepinus*	0	0	0	0	0	**0 (0.00)**
5	*Epiplatys spilargyreius*	0	0	0	0	0	**0 (0.00)**
6	*Astatotilapia burtoni*	0	0	0	0	0	**0 (0.00)**
7	*Hemichromis bimaculatus*	0	0	0	0	0	**0 (0.00)**
8	*Leptocypris niloticus*	1	0	0	0	0	**1 (12.50)**
9	*Oreochromis niloticus*	0	1	0	0	0	**1 (12.50)**
10	*Poecilia reticulata*	0	0	0	0	0	**0 (0.00)**
11	*Schilbe mystus*	0	0	2	0	1	**3 (37.50)**
12	*Synodontis ouemeensis*	0	2	2	0	0	**4 (50.00)**

Differences within months were significant ($P<0.05$)

Appendix XVIII: Morphometrics of the experimental fish species kept for 13 weeks in three different water volumes

Fish species	Mean		
	Weight (g)	standard length (cm)	total length (cm)
Brienomyrus niger	2.6	6.2	7.2
Brycinus nurse	5.6	8.5	10.0
Clarias galmaensis	15.5	12.8	14.1
Clarias gariepinus	2.7	6.2	7.0
Epiplatys spilargyreius	1.4	3.9	5.1
Astatotilapia burtoni	3.5	4.6	5.4
Hemichromis bimaculatus	4.8	3.7	6.7
Leptocypris niloticus	5.4	6.0	7.5
Oreochromis niloticus	3.3	4.4	5.5
Poecilia reticulata	0.2	2.0	2.7
Schilbe mystus	6.5	6.4	7.8
Synodontis ouemeensis	4.9	6.1	8.2

Appendix XIX: Weekly temperature (ºC) record of the fish species in 3 different volumes of water

Fish	Water volume	Weeks/ weekly Temperature recorded (ºC)												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Bri.	30 L	24.6	23.8	22.6	21.3	25.2	22.7	21.3	23.2	23.2	22.1	22.8	22.6	21.9
n.	20 L	24.8	24.2	22.8	21.8	25.2	22.9	21.5	22.8	23.2	22.2	22.9	22.6	21.9
	10 L	25.2	24.4	22.2	21.9	25.1	22.5	21.2	23.4	23.1	22.6	22.8	22.5	21.6
Bry.	30 L	25.2	24.4	22.1	22.4	25.2	22.4	21.2	23.6	23.2	22.1	23.2	22.3	22.4
n.	20 L	25.6	24.3	22.2	22.3	25.3	22.6	21.5	22.8	23.2	22.2	23.6	22.2	22.5
	10 L	25.7	24.2	22.2	22.2	25.3	22.6	21.8	22.9	23.1	22.1	23.6	22.2	22.6
Cl.	30 L	25.0	24.3	22.1	22.7	25.2	22.7	21.6	23.9	23.8	22.6	23.9	22.7	22.0
gal.	20 L	24.9	23.9	21.8	22.3	25.0	22.4	21.5	23.5	23.6	22.4	23.6	22.5	21.6
	10 L	24.6	23.5	21.5	21.7	24.8	22.4	21.1	22.9	23.7	22.0	22.9	22.3	21.5
Cl.	30 L	29.4	28.2	28.0	28.1	28.5	24.5	24.6	24.0	21.7	22.7	25.0	22.2	21.5
gar.	20 L	29.3	28.2	28.1	28.1	28.6	24.3	24.5	23.8	21.4	22.1	25.2	22.4	21.2
	10 L	29.4	28.1	28.1	28.1	28.5	24.0	24.1	23.4	21.1	21.3	24.8	21.9	20.9
Epi.	30 L	30.5	27.7	27.2	29.6	27.9	23.7	23.5	25.2	21.8	23.1	24.6	23.2	22.6
s.	20 L	30.8	29.7	28.4	28.4	28.7	22.5	24.8	24.4	21.8	21.4	25.7	22.7	21.7
	10 L	28.4	29.6	28.6	28.9	29.8	24.7	24.4	23.5	22.7	22.5	24.1	23.1	22.2
Ast.	30 L	28.9	28.6	27.4	28.8	28.1	26.7	25.6	24.9	23.3	23.5	25.5	23.6	22.8
b.	20 L	29.3	29.1	28.4	28.2	28.8	25.9	22.8	24.3	22.9	23.6	25.0	23.8	22.0
	10 L	30.1	28.6	28.6	28.9	28.4	24.5	24.9	22.8	22.8	22.1	24.0	22.4	22.8
Hem.	30 L	29.9	28.7	27.9	28.2	28.5	25.0	25.3	24.4	22.3	23.5	25.5	23.3	22.1
b.	20 L	30.3	29.0	28.3	28.2	28.8	24.9	25.5	24.6	22.5	23.2	25.4	23.1	21.9
	10 L	30.3	28.9	28.2	28.3	28.8	24.6	24.9	23.8	21.8	22.1	25.0	22.4	21.4
Lep.	30 L	25.6	23.4	21.6	22.2	24.4	22.5	21.7	22.2	22.9	23.1	22.8	22.5	22.9
n.	20 L	24.7	24.5	22.8	21.6	25.6	22.9	21.2	22.6	23.9	22.6	22.7	22.2	21.8
	10 L	25.5	23.4	22.7	21.7	24.1	22.5	22.8	23.0	23.1	22.4	22.6	21.5	21.9
Ore.	30 L	25.2	23.6	22.5	21.3	25.2	22.3	21.5	23.2	23.6	22.2	23.4	22.2	21.6
n.	20 L	25.2	23.8	22.4	21.3	25.1	21.3	21.6	23.1	23.7	22.3	23.7	22.4	21.7
	10 L	25.1	23.5	22.5	21.2	25.3	22.3	21.6	23.4	23.7	22.2	23.1	22.2	21.9
Poe.	30 L	30.2	28.7	28.2	28.6	28.9	22.4	24.6	24.1	21.7	22.1	25.7	23.0	21.8
r.	20 L	30.6	29.4	28.8	28.9	28.4	22.4	24.3	24.4	21.5	21.9	25.4	22.8	21.5
	10 L	29.4	29.8	28.9	28.8	29.1	24.2	24.3	23.8	21.2	22.2	25.1	22.2	21.2
Sch.	30 L	29.9	28.3	28.7	28.2	28.4	24.5	24.8	23.9	21.2	22.2	24.9	22.4	21.4
m.	20 L	30.1	28.3	28.5	28.3	28.6	24.4	24.5	23.7	21.5	22.3	25.0	22.4	21.4
	10 L	29.6	27.8	28.2	28.1	28.1	24.1	24.1	23.4	21.6	22.0	25.0	22.2	21.2
Syn.	30 L	29.2	28.6	28.2	28.3	24.7	25.0	25.2	24.2	21.1	23.0	25.4	23.1	21.8
o.	20 L	29.2	28.5	27.9	28.5	24.6	25.0	25.2	24.1	21.8	22.7	25.2	22.8	21.6
	10 L	29.7	28.7	28.3	28.8	24.4	24.6	25.2	23.8	21.5	21.9	25.0	22.3	21.2

KEY: *Bri. n.= Brienomyrus niger; Bry. n.= Brycinus nurse; Cl. gal.= Clarias galmaensis; Cl. gar.= Clarias gariepinus; Epi. s.= Epiplatys spilargyreius ; Ast. b.= Astatotilapia burtoni; Hem. b = Hemichromis bimaculatus; Ore. n.= Oreochromis niloticus; Poe. r.= Poecilia reticulata; Lep. n.= Leptocypris niloticus; Sch. m. = Schilbe mystus; Syn.* o. = *Synodontis ouemeensis;* L= Litres.

Appendix XX: Weekly pH record of the fish species in 3 different volumes of water

Fish	Water volume	Weeks/ Weekly pH Recorded												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Bri.	30 L	7.63	7.52	7.64	7.69	7.43	7.50	7.52	7.75	7.50	7.80	7.69	7.49	7.50
n.	20 L	7.26	7.40	7.52	7.49	7.52	7.62	7.44	7.20	7.35	7.46	7.50	7.52	7.36
	10 L	7.72	7.82	7.92	7.32	7.60	7.72	7.96	7.85	7.77	7.91	7.26	7.69	7.80
Bry.	30 L	7.26	7.42	7.45	7.50	7.55	7.60	7.69	7.59	7.50	7.55	7.53	7.57	7.61
n.	20 L	7.25	7.32	7.32	7.40	7.35	7.33	7.51	7.42	7.55	7.40	7.12	7.05	7.15
	10 L	7.13	7.21	7.21	7.42	7.23	7.21	7.35	7.41	7.38	7.25	7.22	7.24	7.28
Cl.	30 L	7.23	7.00	6.78	6.87	6.62	6.38	6.30	6.80	6.92	6.99	6.95	7.05	7.61
gal.	20 L	7.19	6.99	6.69	6.70	6.25	6.15	6.13	6.67	6,89	6.83	6.98	6.86	6.81
	10 L	7.16	7.03	7.09	6.68	6.15	5.96	5.91	6.72	6.91	6.30	7.04	6.80	6.99
Cl.	30 L	7.67	7.97	8.05	8.06	8.11	7.73	7.85	7.80	7.80	7.97	7.66	7.09	7.14
gar.	20 L	7.60	7.96	8.10	8.02	8.09	7.69	7.81	7.75	7.68	8.02	7.80	7.16	7.13
	10 L	7.72	7.99	8.11	8.03	7.86	7.58	7.30	7.62	7.49	7.73	7.34	6.87	6.83
Epi.	30 L	8.56	8.19	8.26	8.34	8.16	8.04	7.99	7.97	8.56	8.53	8.52	7.37	8.22
s.	20 L	8.76	8.10	8.23	8.69	7.99	7.86	8.05	7.90	8.13	8.37	8.99	8.27	8.28
	10 L	8.05	8.56	8.34	8.65	8.43	7.99	7.88	8.09	8.38	8.56	8.23	8.67	8.22
Ast.	30 L	8.74	8.65	8.71	8.82	8.00	8.37	8.25	8.21	8.68	8.48	8.58	8.76	8.69
b.	20 L	8.60	8.86	7.83	7.87	7.25	7.76	7.89	7.77	8.08	7.82	8.84	8.55	8.62
	10 L	8.64	8.78	8.82	8.78	8.77	8.43	7.67	7.87	7.92	8.67	8.68	7.58	7.62
Hem.	30 L	7.74	7.89	7.71	7.82	8.00	7.30	7.27	7.23	6.98	7.08	6.78	6.78	6.60
b.	20 L	7.66	7.84	7.81	7.80	7.20	7.25	7.26	7.19	6.85	7.12	6.84	6.57	6.67
	10 L	7.68	7.74	7.84	7.77	7.70	7.23	7.17	7.07	6.81	6.94	6.68	6.53	6.57
Lep.	30 L	8.54	8.57	8.55	8.78	8.49	8.66	8.57	8.79	8.45	8.45	8.69	8.47	8.52
n.	20 L	8.27	8.45	8.57	8.45	8.64	8.56	8.33	8.28	8.36	8.40	8.53	8.43	8.31
	10 L	8.72	8.56	8.43	8.32	8.23	8.54	8.56	8.85	8.56	8.75	8.26	8.68	8.89
Ore.	30 L	7.25	7.26	7.50	7.40	7.30	7.10	7.07	7.20	7.40	7.50	7.56	7.40	7.42
n.	20 L	7.15	7.14	7.20	7.35	7.32	7.15	7.20	7.25	7.16	7.17	7.23	7.19	7.25
	10 L	7.16	7.20	7.25	7.20	7.21	7.31	7.42	7.72	7.15	7.17	7.20	7.24	7.28
Poe.	30 L	7.98	8.08	8.27	8.20	8.06	7.78	7.92	7.97	8.82	8.52	8.49	7.32	7.29
r.	20 L	7.78	8.10	8.13	8.19	7.94	7.71	7.90	7.98	8.16	8.37	8.22	7.27	7.29
	10 L	8.05	8.45	7.94	8.05	8.15	7.75	7.88	7.89	7.98	8.23	8.00	7.22	7.22
Sch.	30 L	7.90	7.70	7.98	7.82	7.74	7.25	7.26	7.26	7.29	6.96	6.50	6.42	6.39
m.	20 L	7.85	7.49	7.70	7.80	7.62	7.22	7.23	7.12	7.17	6.85	6.47	6.32	6.26
	10 L	7.70	7.89	7.84	7.62	7.48	7.19	7.10	7.09	7.19	6,80	6.46	6.23	6.10
Syn.	30 L	7.67	7.82	7.93	8.34	7.58	7.67	7.65	7.65	7.38	7.69	7.22	6.87	6.92
o.	20 L	7.66	7.92	7.72	8.30	7.48	7.53	7.50	7.49	7.19	7.50	6.97	6.62	6.68
	10 L	7.62	7.81	7.90	8.08	7.30	7.28	7.35	7.27	6.90	7.17	6.70	6.30	6.43

KEY: *Bri. n.= Brienomyrus niger; Bry. n.= Brycinus nurse; Cl. gal.= Clarias galmaensis; Cl. gar.= Clarias gariepinus; Epi. s.= Epiplatys spilargyreius ; Ast. b.= Astatotilapia burtoni; Hem. b = Hemichromis bimaculatus; Ore. n.= Oreochromis niloticus; Poe. r.= Poecilia reticulata; Lep. n.= Leptocypris niloticus; Sch. m. = Schilbe mystus; Syn.* o. = *Synodontis ouemeensis;* L= Litres.

Appendix XXI: Weekly total dissolved solids (mg/L) record of the fish species in 3 different volumes of water

Fish	Water volume	Weeks/ Weekly Total dissolved solids recorded (mg/L)												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Bri. n.	30 L	72	73	83	87	92	97	103	112	113	119	124	128	133
	20 L	73	77	85	95	95	100	120	125	130	130	135	147	145
	10 L	95	99	102	111	111	115	139	140	150	157	160	160	164
Bry. n.	30 L	73	76	79	87	95	102	108	118	122	125	127	138	143
	20 L	72	77	83	95	99	104	121	127	133	133	139	152	147
	10 L	77	83	89	101	107	117	137	139	150	152	171	174	179
Cl. gal.	30 L	97	140	150	167	188	210	218	230	232	260	308	324	355
	20 L	102	164	186	221	299	368	382	427	440	466	520	548	619
	10 L	143	287	384	430	582	756	761	832	860	906	915	953	964
Cl. gar.	30 L	70	79	84	87	100	108	115	131	142	150	163	173	179
	20 L	71	83	87	94	101	110	118	134	142	152	165	180	185
	10 L	72	92	96	111	131	167	182	187	179	195	236	268	273
Epi. s.	30 L	65	70	77	77	80	82	81	84	85	87	92	95	99
	20 L	67	73	75	79	83	85	90	96	96	97	99	102	105
	10 L	71	79	85	87	88	93	94	98	102	105	120	123	135
Ast. b.	30 L	65	70	73	75	85	100	102	110	112	121	125	127	135
	20 L	70	74	80	83	95	111	115	122	149	152	155	160	174
	10 L	75	99	108	120	126	145	155	191	215	245	273	290	301
Hem. b.	30 L	65	69	73	77	83	96	102	111	112	112	120	127	130
	20 L	68	74	80	83	96	107	107	121	144	147	150	160	158
	10 L	70	93	108	111	126	139	157	189	217	240	470	281	280
Lep. n.	30 L	72	73	84	85	90	97	100	112	112	121	124	127	136
	20 L	73	77	85	96	97	100	123	125	133	134	140	147	159
	10 L	95	100	102	115	120	125	139	140	155	157	160	171	175
Ore. n.	30 L	64	67	70	75	82	90	92	95	102	118	110	120	122
	20 L	65	68	70	77	83	92	99	101	110	114	121	122	132
	10 L	67	72	76	83	99	113	115	120	127	139	140	152	159
Poe. r.	30 L	69	74	77	77	79	80	81	84	85	87	90	95	101
	20 L	69	73	76	81	83	87	92	94	94	94	95	102	105
	10 L	70	79	83	87	88	93	94	103	103	105	117	123	131
Sch. m.	30 L	73	84	91	92	110	120	128	143	151	163	177	180	191
	20 L	75	87	93	93	113	130	145	158	179	196	212	212	234
	10 L	75	104	124	140	200	201	216	229	254	265	288	307	343
Syn. o.	30 L	71	85	85	93	99	104	105	111	118	123	128	134	136
	20 L	72	88	93	96	104	108	111	121	133	140	157	170	176
	10 L	74	95	98	134	170	178	177	181	189	202	225	247	245

KEY: *Bri. n.= Brienomyrus niger; Bry. n.= Brycinus nurse; Cl. gal.= Clarias galmaensis; Cl. gar.= Clarias gariepinus; Epi. s.= Epiplatys spilargyreius ; Ast. b.= Astatotilapia burtoni; Hem. b = Hemichromis bimaculatus; Ore. n.= Oreochromis niloticus; Poe. r.= Poecilia reticulata; Lep. n.= Leptocypris niloticus; Sch.m. = Schilbe mystus; Syn.* o. *= Synodontis ouemeensis;* L= Litres

Appendix XXII: Weekly electrical conductivity (µSiemens/cm) record of the fish species in 3 different volumes of water

Fish	Water volume	Weeks/ Weekly Electrical conductivity Recorded (µSiemens/cm)												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Bri. n.	30 L	140	144	162	170	181	189	202	219	223	235	239	252	260
	20 L	142	150	166	185	185	195	234	244	250	255	261	284	293
	10 L	186	190	201	210	211	222	270	277	292	304	310	316	320
Bry. n.	30 L	142	150	155	170	185	201	211	232	240	247	250	272	281
	20 L	142	151	162	185	188	202	235	242	261	265	275	289	292
	10 L	151	163	175	199	210	225	270	277	294	300	335	342	351
Cl. gal.	30 L	186	271	290	324	364	405	422	435	450	490	592	622	673
	20 L	196	317	376	426	574	706	729	820	860	883	999	1052	1186
	10 L	274	551	736	824	1119	1450	1463	1650	1701	1745	1800	1829	1850
Cl. gar.	30 L	135	152	162	170	194	207	221	251	273	287	314	334	340
	20 L	136	162	169	181	194	211	227	259	275	293	315	347	350
	10 L	140	178	187	210	255	317	365	360	342	376	452	512	532
Epi. s.	30 L	125	133	149	150	154	159	160	164	163	170	180	183	185
	20 L	130	142	147	155	162	168	175	185	182	184	187	199	203
	10 L	140	154	165	166	170	182	183	197	199	204	232	237	262
Ast. b.	30 L	126	136	142	147	165	195	198	214	220	236	245	250	265
	20 L	137	146	155	162	185	219	225	234	296	301	305	315	352
	10 L	146	196	213	235	248	282	306	377	436	483	520	574	596
Hem.	30 L	126	133	142	150	162	186	198	213	219	220	234	245	248
b.	20 L	131	143	154	160	184	206	208	234	276	280	285	293	299
	10 L	135	179	207	210	244	268	301	365	412	460	520	541	551
Lep. n.	30 L	140	144	164	167	177	189	196	219	221	239	241	252	265
	20 L	142	151	166	185	185	197	242	244	262	265	275	286	319
	10 L	186	196	201	225	236	245	273	277	303	304	315	336	345
Ore. n.	30 L	125	130	137	145	159	175	180	186	201	213	215	234	239
	20 L	126	135	141	150	159	181	192	200	215	220	235	240	260
	10 L	130	140	150	161	191	220	225	235	251	270	275	299	320
Poe. r.	30 L	133	143	149	150	153	159	156	164	164	170	175	183	197
	20 L	134	142	148	155	160	169	178	181	182	183	185	199	202
	10 L	134	152	161	166	170	179	183	198	198	201	224	237	250
Sch. m.	30 L	142	163	175	180	213	232	247	273	292	317	345	351	370
	20 L	145	169	180	182	217	251	278	304	345	375	408	410	450
	10 L	152	197	239	270	375	386	412	439	489	508	548	591	648
***Syn.* o.**	30 L	137	165	166	179	191	203	206	215	228	237	247	263	264
	20 L	140	171	179	186	199	209	215	234	255	270	300	326	337
	10 L	144	183	190	259	325	393	340	346	362	386	432	473	481

KEY: *Bri. n.= Brienomyrus niger; Bry. n.= Brycinus nurse; Cl. gal.= Clarias galmaensis; Cl. gar.= Clarias gariepinus; Epi. s.= Epiplatys spilargyreius ; Ast. b.= Astatotilapia burtoni; Hem. b = Hemichromis bimaculatus; Ore. n.= Oreochromis niloticus; Poe. r.= Poecilia reticulata; Lep. n.= Leptocypris niloticus; Sch. m. = Schilbe mystus; Syn.* o. *= Synodontis ouemeensis;* L=Litres

Appendix XXIII: Weekly mortality records of the fish species in different volumes of water

Fish species	Water volume	Weeks/ Fish weekly mortality													**Total**
		1	2	3	4	5	6	7	8	9	10	11	12	13	
Brienomyrus niger	30 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	20 Litres	0	0	1	0	0	0	0	0	0	1	0	0	0	**2**
	10 Litres	0	0	0	0	0	0	0	2	0	0	0	0	0	**2**
Brycinus nurse	30 Litres	0	0	0	0	0	0	1	0	0	0	0	0	0	**1**
	20 Litres	0	0	0	0	0	0	0	0	0	0	0	1	0	**1**
	10 Litres	0	0	0	0	0	0	0	2	0	0	0	0	0	**2**
Clarias galmaensis	30 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	20 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	10 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
Clarias gariepinus	30 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	20 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	10 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
Epiplatys	30 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
spilargyreius	20 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	10 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
Astatotilapia	30 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
burtoni	20 Litres	0	0	0	0	0	0	0	0	0	0	1	0	0	**1**
	10 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
Hemichromis	30 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
bimaculatus	20 Litres	0	0	0	0	1	0	0	0	0	0	1	0	0	**2**
	10 Litres	0	0	0	0	2	0	0	0	0	0	0	0	0	**2**
Leptocypris	30 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
niloticus	20 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	10 Litres	0	0	0	0	0	0	0	0	0	1	0	0	0	**1**
Oreochromis	30 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
niloticus	20 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	10 Litres	0	0	0	0	0	0	0	0	0	1	0	0	0	**1**
Poecilia reticulata	30 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	20 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	10 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
Schilbe mystus	30 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	20 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	10 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
Synodontis	30 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
ouemeensis	20 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	10 Litres	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**

Appendix XXIV: Monthly means temperature (^{o}C) changes in flow-through system

Months	*Brienomyrus niger*	*Brycinus nurse*	*Clarias galmaensis*	*Clarias gariepinus*	*Hemichromis bimaculatus*	*Oreochromis niloticus*	*Poecilia reticulata*	*Schilbe mystus*	*Synodontis ouemeensis*
Sep 2009	27.75	27.65	27.55	27.88	28.00	28.25	27.78	27.10	28.63
Oct 2009	27.20	28.12	27.30	26.60	26.40	27.52	27.08	27.62	27.52
Nov 2009	25.25	26.00	26.13	25.30	24.88	26.33	28.23	24.78	26.08
Dec 2009	25.30	27.34	26.54	25.60	25.10	27.22	26.26	28.08	26.98
Jan 2010	24.48	27.03	25.90	25.00	24.78	27.18	25.98	27.85	26.38
Feb 2010	28.78	30.03	29.38	28.85	28.43	30.33	29.90	30.30	29.55
Mar 2010	29.25	30.30	29.73	29.53	28.95	30.75	30.18	30.48	30.00
Apr 2010	30.56	30.72	30.74	30.60	30.38	31.28	30.82	30.32	31.00
May 2010	27.63	28.15	27.75	27.63	27.63	27.93	27.80	27.70	28.08
Jun 2010	23.58	23.64	23.52	23.64	23.70	23.66	23.64	23.54	23.80
Jul 2010	22.63	22.73	22.55	22.55	22.53	22.43	22.50	22.20	22.58
Aug 2010	22.08	21.93	22.05	22.23	22.55	22.03	22.08	21.85	22.15

No significant difference in the means of the different aquaria each month (P>0.05)

Appendix XXV: Monthly means pH changes in flow-through system

Months	*Brienomyrus niger*	*Brycinus nurse*	*Clarias galmaensis*	*Clarias gariepinus*	*Hemichromis bimaculatus*	*Oreochromis niloticus*	*Poecilia reticulata*	*Schilbe mystus*	*Synodontis ouemeensis*
Sep 2009	8.25	7.78	8.54	8.52	8.52	7.40	7.16	8.23	8.45
Oct 2009	7.48	7.73	7.67	8.40	8.27	7.88	7.73	7.71	7.80
Nov 2009	8.69	8.53	8.55	8.44	8.34	8.69	8.49	8.47	8.56
Dec 2009	9.08	8.94	8.85	8.86	8.67	8.75	9.23	8.78	8.89
Jan 2010	9.18	9.32	9.23	9.48	9.20	9.50	9.34	9.30	9.35
Feb 2010	9.09	9.18	8.62	9.10	8.84	9.22	9.22	9.06	9.13
Mar 2010	9.04	8.94	8.79	8.88	8.77	9.45	9.13	9.15	9.03
Apr 2010	8.55	8.40	8.43	8.37	8.33	8.64	8.54	8.61	8.65
May 2010	7.68	7.52	7.68	7.60	7.47	7.62	7.67	7.55	7.42
Jun 2010	7.10	7.19	7.07	7.05	6.95	7.01	7.23	7.20	7.11
Jul 2010	6.76	6.84	6.87	6.84	6.67	6.74	6.84	6.90	6.82
Aug 2010	7.05	7.05	7.01	7.02	7.06	7.06	7.05	7.17	6.95

No significant difference in the means of the different aquaria each month (P>0.05)

Appendix XXVI: Monthly means total dissolved solids (mg/L) changes in flow-through system

Months	*Brienomyrus niger*	*Brycinus nurse*	*Clarias galmaensis*	*Clarias gariepinus*	*Hemichromis bimaculatus*	*Oreochromis niloticus*	*Poecilia reticulata*	*Schilbe mystus*	*Synodontis ouemeensis*
Sep 2009	44.75	40.00	42.25	41.50	43.50	47.00	39.75	40.75	42.50
Oct 2009	50.60	45.00	49.00	46.60	49.20	50.60	40.80	41.60	43.20
Nov 2009	49.25	51.00	50.50	47.50	49.75	52.75	45.00	54.25	51.00
Dec 2009	51.60	62.00	54.20	51.40	51.00	59.20	50.20	59.60	49.60
Jan 2010	56.25	59.00	55.75	56.00	55.75	77.75	60.75	55.25	57.50
Feb 2010	60.00	64.00	61.00	65.25	70.00	78.00	72.00	62.75	65.25
Mar 2010	64.25	73.50	73.75	81.50	80.25	98.25	90.75	66.25	72.50
Apr 2010	72.80	89.00	83.60	91.60	85.20	109.00	88.60	70.80	82.40
May 2010	86.75	90.75	94.25	98.50	103.25	122.00	75.50	83.25	89.25
Jun 2010	78.25	80.00	98.00	82.00	97.75	102.50	65.25	88.75	83.75
Jul 2010	49.60	46.40	72.60	73.40	58.60	82.60	42.60	69.60	64.20
Aug 2010	62.50	45.25	84.00	69.00	59.25	52.25	39.50	62.75	70.50

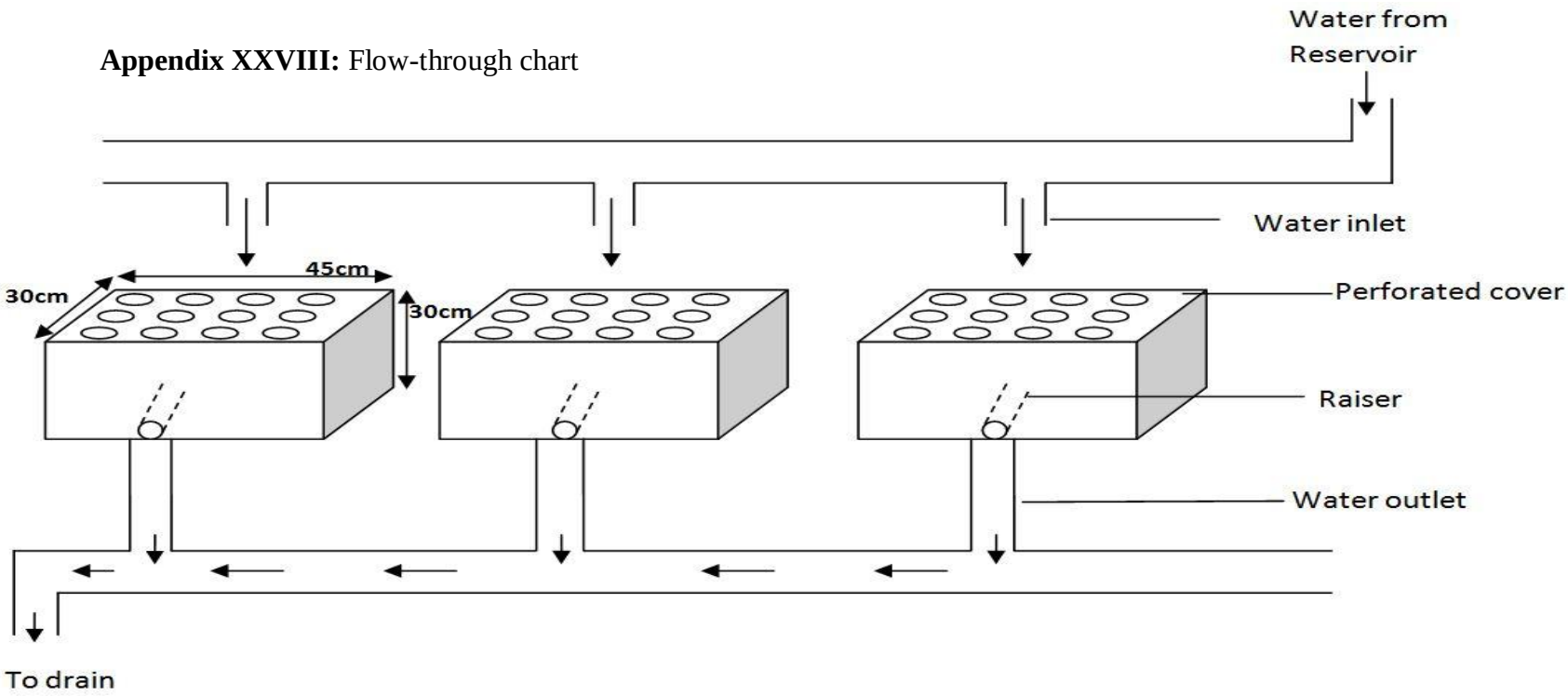
No significant difference in the means of the different aquaria each month ($P>0.05$)

Appendix XXVII: Monthly means electrical conductivity (µSiemens/cm) changes in flow-through system

Months	*Brienomyrus niger*	*Brycinus nurse*	*Clarias galmaensis*	*Clarias gariepinus*	*Hemichromis bimaculatus*	*Oreochromis niloticus*	*Poecilia reticulata*	*Schilbe mystus*	*Synodontis ouemeensis*
Sep 2009	86.75	79.00	80.75	79.75	84.50	91.50	77.50	76.75	80.75
Oct 2009	98.80	86.80	94.80	92.60	93.60	98.60	80.20	80.60	84.00
Nov 2009	95.75	102.75	98.00	93.50	97.25	102.25	87.25	105.00	98.25
Dec 2009	100.00	121.00	107.60	100.80	99.00	112.00	96.80	116.60	95.20
Jan 2010	108.75	113.75	109.00	108.75	108.50	147.75	118.00	104.75	110.00
Feb 2010	116.25	124.50	117.75	126.00	135.75	160.75	139.25	119.75	126.00
Mar 2010	125.50	142.00	144.00	161.25	155.25	185.25	177.50	136.50	140.50
Apr 2010	141.20	171.20	161.20	177.60	163.80	210.00	171.60	136.00	159.00
May 2010	168.25	174.50	181.75	190.75	199.75	234.50	147.00	167.50	171.25
Jun 2010	151.50	155.75	188.75	158.00	188.75	196.50	127.25	174.25	162.00
Jul 2010	96.00	91.40	141.00	142.00	111.20	158.40	83.80	136.60	123.60
Aug 2010	121.25	89.50	163.00	135.25	114.50	101.25	76.75	121.75	133.25

No significant difference in the means of the different aquaria each month ($P>0.05$)

Appendix XXVIII: Flow-through chart



Appendix XXIX: Details of Artificial breeding of *Clarias galmaensis*

Male and female brooders were weighed and measured (standard and total lengths) using a weighing balance and meter ruler respectively. The brooders were brought from the outside pond into the laboratory, they were kept in darkness for five hours to allow them calm down and adjust to laboratory environment; the females were injected intramuscularly above the lateral line with ovaprim hormone at an angle of 45 degrees (Mittelmark and Kapuscinski, 2008). 0.5 ml of the hormone was used per kilogram of fish as instructed by the manufacturer. The injected fish were returned to the water for 10-14 hours, after which they were removed and stripped by initially applying little pressure on the abdomen and allowing the eggs to flow into a plastic container (De Graaf and Janssen, 1996; Mittelmark and Kapuscinski, 2008). Sperm was obtained from 3 male fish, three males were used so as to increase genetic variability and reduce the risk of infertility of any of them (RFA, 2010). After killing the male fish, their testes were dissected using a new razor blade to avoid contamination and the milt was pressed out with a teaspoon; the milt was then mixed with the stripped eggs, an equal volume of distilled water was added to activate the sperm so as to fertilized the eggs; the combination was then mixed by gently shaking continuously for 60 seconds (De Graaf and Janssen, 1996; RFA, 2010). The fertilized eggs become sticky on contact; using a spoon the fertilized eggs were spread out on a kakaba (screen made from mosquito mesh), which was spread in a water bath with dechlorinated water running at a depth of 20 cm. The running water kept the fertilized eggs well-oxygenated (De Graaf and Janssen, 1996; RFA, 2010). The room was kept warm by continuously boiling water in 3 buckets using immersion water heaters. The fertilized eggs started hatching after about 20 hours at a water temperature of 24ºC and room temperature of 26ºC. After the eggs hatched

the free embryos fell to the bottom of the tank (RFA, 2010). The kakaba was then removed with all the unhatched eggs and shells of the fertilized eggs; dead eggs in the water bath were siphoned out in order to avoid fungal infection and mortality of hatchlings (De Graaf and Janssen, 1996). High mortalities were experienced at this stage because of handling and their fragile nature (RFA, 2010). The hatchlings were then transferred out of incubation facilities to an aquarium in the laboratory (De Graaf and Janssen, 1996). The hatchlings were kept in the dark without being fed for three days as they relied on their yolk sac (De Graaf and Janssen, 1996); thereafter they were fed on zooplankton and Artemia three times a day for 2 weeks, from then on they were fed on crushed 2 mm coppens®; as they grew into juveniles, they were regularly size-sorted to prevent cannibalism of the smaller ones by the 'shooters' which outgrew the rest (RFA, 2010). The fish were fed with coppens® and their larvivorous potential was tested at 5,7 and 9 months as described in 3.11.3 to determine when the species preys best on mosquito larvae.

Appendix XXX: Photographs of *Clarias galmaensis* breeding



Injecting a female *Clarias galmaensis* with ovaprim hormone



Stripping and collecting eggs from an ovaprim-injected *Clarias galmaensis* female