ARTIFICIAL HYBRIDIZATION OF *CLARIAS GARIEPINUS* AND *HETEROBRANCHUS LONGIFILIS*

BY

YISA, NDAGI SILAS (B.Sc. ABU)
M.Sc./Sc./38243/02-03
DEPARTMENT OF BIOLOGICAL SCIENCES
AHMADU BELLO UNIVERSITY, ZARIA

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA FOR THE AWARD OF MASTERS OF FISHERIES
(M. SC. FISHERIES) DEPARTMENT OF BIOLOGICAL SCIENCES, FACULTY OF SCIENCE,
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

FEBRUARY, 2011

DECLARATION

I declare that this work in the thesis entitled "ARTIFICIAL HYBRIDIZATION OF *CLARIAS GARIEPINUS* AND *HETEROBRANCHUS LONGIFILIS*" was conducted by me in the Department of Biological Science under the supervision of Prof. Jehu, Auta. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any University.

_____ _____

Yisa, N. Silas **Date**

CERTIFICATION

This thesis entitled "ARTIFICIAL HYBRIDIZATION OF *CLARIAS GARIEPINUS* AND *HETEROBRANCHUS LONGIFILIS"* by YISA, NDAGI SILAS meets the regulation governing the award of the degree of Masters of Fisheries of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

_____ _____

Prof. J. Auta Date
Chairman, Supervisory Committee

_____ _____

Dr. S. A. Abdullahi Date
Member

_____ _____

Prof. P. I. Bolorunduro Date
Member

_____ _____

Prof. J. Auta Date
Head of Department

_____ _____

Prof. A. A. Joshua Date
School of Postgraduate Studies

DEDICATION

This work is dedicated to God The Father, God The Son, and God The Holy Spirit who Purposed and Willed in me

and to

Dr (Mrs) Modupe Taiwo Odubela who is a mother to me.

ACKNOWLEDGEMENT

I thank CHRIST JESUS my LORD, who has enabled me, for that He counted me worthy of His Mercy and Exceeding Abundant Grace; Now unto The King Eternal, Immortal, Invisible, The Only Wise God, be Honour and Glory for ever and ever. Amen!

I thank God for my major Supervisor Prof. J. Auta who right from the conception of the idea for a higher degree, avail himself to be used of God to keep the fire burning and my zeal going. Be exceeding abundantly blessed. I also thank God profusely for my other Supervisors Pastor (Prof.) P.I. Bolorunduro and Dr. S.A. Abdullahi who have given their best, avail themselves and contributed in no small way. A rich reward awaits you all at the feet of the Lord.

I am also grateful to Dr. A. Raji the Executive Director of NIFFR, New Bussa, Dr. C.T. Madu, H.O.D. Aquaculture and Fish Biotechnology Division and Dr. S.O. Olufeagba, H.O.P. Fish Biotechnology Programme. My unfeigned gratitude goes to my brother, my friend, my lecturer, my Pastor, Uncle Moses Yisa who laboured tirelessly to ensure that I do this work and complete it, his wife Aunty Florence Yisa for her love who took care of me beyond measure and Dorathy their daughter for her love. You shall be planted by the Rivers and watered by the LORD. Worthy of mention are the staff in the Fish Biotechnology Programme of NIFFR, Bayo Aremu, Woru, Shuaibu (The Iroko man) and Lawrence. To you all I say thank you!

I am everlastingly indebted to my darling and loving wife Mrs. Rachel W. Yisa and the gifts of God to our lives Mordecai Ejinda and Sharon Emapa Be Soko who patiently tolerated me, endured my ever-long absence from home, prayed for me and ensure that I succeed. The Lord shall open the Fountain of Life for you and give you the Crowns of Life and Glory.

My deep and heart felt appreciation goes to my dear parents Baba Ezekiel Santali Yisa, JP. and Mama Rebecca Yisa and my elder brother Pastor Benjamin E. Yisa, Dr (Mrs) Modupe Taiwo Odubela all of who planted the seed of greatness in me. Heaven is for you.

Lastly, to everyone that has contributed in one way or the other to my realizing and fulfilling my dreams and all those that Love His appearing and that will be there at the marriage supper. Keep shinning unto His appearing. Shalom!

YISA, NDAGI SILAS 2011.

ABSTRACT

Intergeneric hybridization studies of *Heterobranchus longifilis* and *Clarias gariepinus* were carried out at the Fish Biotechnology Programme of National Institute for Freshwater Fisheries Research (NIFFR), New Bussa, Niger State, Nigeria with the aim of identifying the mating combination with the best performance in terms of hatchability, survival and growth. Four mating combinations were generated from *H. longifilis* and *C. gariepinus* and were successfully induced under controlled hatchery condition. The percentage hatchability showed no significant difference ($P>0.05$) between the mating combinations. However, the highest percentage hatchability of 56% was recorded in female *Clarias gariepinus* and male *Heterobranchus longifilis* and the least of 40% was recorded in parental *Clarias gariepinus*. The percentage indoor survival showed no significant difference ($P>0.05$). But the cross involving the intergeneric crosses gave the highest survival of 86% and parental of *H. longifilis* gave the least of 75%. However, the outdoor survival after 8 weeks rearing also showed no significant difference ($P>0.05$). However, female *Clarias gariepinus* and male *H. longifilis* gave the highest survival of 91.5% and the parental crosses gave the least of 71.5%. The 14 days growth indoors showed significant difference ($P<0.05$) in final pooled weight. Highest final pooled weight (9.0g) was obtained in parental *C. gariepinus* and the parental *H. longifilis* gave the least (4.57g). There was no significant difference in their length. The eight weeks outdoor growth performance also showed significant difference ($P<0.05$) between the crosses. Maximum final pooled weight (293.75g) was observed in *H. longifilis*, while female *H. longifilis* and male *C. gariepinus* gave the least final pooled weight (219.00g). There was no significant difference ($P>0.05$) in length between the crosses in the Outdoor rearing. In this study, the inter-generic cross between female *Clarias gariepinus* and Male

Heterobranchus longifilis gave the best growth performance while in the intra-specific cross *Heterobranchus longifilis* parental group gave the best growth performance.

TABLE OF CONTENTS

Title page

Declaration ii

Certification iii

Dedication iv

Acknowledgement v

Abstract vii

Table of Contents ix

List of tables xii

List of figures xiii

List of plates xiv

CHAPTER ONE

1.0 Introduction 1

1.1 Biology 3

1.2 Research Problem 4

1.3 Justification 5

1.4 Aim 6

1.5 Objectives 6

1.6 Hypothesis 6

CHAPTER TWO

2.0 Literature Review 8

2.1 Taxonomy 10

2.2 Distribution 11

2.3 Breeding prospects in fish 11

2.4 Potential Interest of Hybridization 12

2.4.1 Hybrids 12

2.4.1a viability of Hybrids 12

2.4.1b Growth Potential of Hybrids 13

2.4.1c Reproduction 13

2.4.1d Hybridization and Sex Ratio 13

2.4.2 Research Trends in Hybridization 14

2.4.2.1 Basic Research 14

2.4.2.1a Cytogenetics of Hybrids 14

2.4.2.1b Hybridization and classification 14

2.4.3 Applied Research 15

2.4.3.1 Production of New Hybrids 15

2.5 Genetics Approaches in Hybridization 16

2.6 Advantages of Hybridization 16

2.7 Disadvantages of Hybridization 17

2.8 Future Areas of research in Hybridization 18

CHAPTER THREE

3.0 Materials and method 19

3.1 Sources of Fish 19

3.2 Experimental Site 19

3.2.1 Record keeping 19

3.2.2 Tank Preparation 19

3.3 Selection of Brooders 20

3.3.1 Female brooder Selection 20

3.3.2 Male Brooder Selection 20

3.4 Induced breeding 20

3.5 Milt and Egg Collection 22

3.6 Fertilization 24

3.7 Incubation 25

3.8 Growth Performance 26

3.9 Water quality Parameters 27

3.10 Statistical Analysis 27

CHAPTER FOUR 28

4.0 Results 28

4.1 Percentage Hatchability 28

4.2 Percentage Survival Indoor 29

4.3 Percentage survival outdoor 30

4.4 Growth Performance Indoor 31

4.5 Growth Performance Outdoor 32

4.6 Description of the Hybrid Fish 34

4.7 Water quality parameters 36

CHAPTER FIVE

5.0 Discussion 36

CHAPTER SIX

6.0 Conclusion and recommendation 40

References 41

Appendix 49

LIST OF TABLES

Table 1:	Growth Performance of the fry of the various Mating combinations at 14 days indoor	31
Table 2:	Length parameters of the fry of the various mating combinations at 14 days indoor	32
Table 3:	Growth performance of the fingerlings of the various mating combinations at the end of 8 weeks of outdoor rearing.	33
Table 4:	Length parameters of the fingerlings of the various mating combinations at the end of the 8 weeks of outdoor rearing.	34

LIST OF FIGURES

Figure 1:	Percentage hatchability	28
Figure 2:	Percentage survival of fry indoor	29
Figure 3:	Percentage survival of juveniles	30

LIST OF PLATES

Plate I:	*Heterobranchus longifilis*	4
Plate II:	*Clarias gariepinus*	4
Plate III:	Weighing of fish	21
Plate IV:	Injection of fish with the spawning hormone	21
Plate V:	Removal of testis from the male fish	22
Plate VI a & b:	*Heterobranchus* testis, *Clarias* testis	23
Plate VII:	Stripping of the gravid female to collect the eggs	24
Plate VIII:	Dispensing the fertilized eggs on the kakaban in the incubating tanks.	25
Plate IX:	Hybrid of female *H. longifilis* and male *C. gariepinus*	35
Plate X:	Hybrid of female *C. gariepinus* and Male *H. longifilis*	35
Plate XI:	fingerlings of the mating combination produced.	36

LIST OF FIGURES

Figure 1:	Percentage hatchability	28
Figure 2:	Percentage survival of fry indoor	29
Figure 3:	Percentage survival of juveniles	30

CHAPTER ONE

1.0. INTRODUCTION

Aquaculture is the World's fastest-growing food-producing sector. The sub-sector's expansion began in the 1970s stimulated by advances in hatchery technology and pond husbandry. According to the FAO (2007), aquaculture continues to grow more rapidly than all other animal food-producing sectors, with an average global annual growth rate of 8.8% per year since 1970, compared to only 1.2% for captured fisheries. The contribution of aquaculture to global supplies of fish, crustaceans, mollusks and other aquatic animals, increased from 3.9% of total production by weight in 1970 to 27.1% in 2000 and 32.4% in 2004. This growth is however impeded by a lack of adequate attention to genetics and selective breeding, leading to stagnating yields (CGIAR, 2006).

With global population expansion, the demand for high-quality protein, especially from aquatic sources is rising dramatically. Increased aquaculture production is clearly needed to meet this demand in the third millennium, because capture fisheries are at capacity or showing precipitous declines due to overfishing and habitat destruction. Further increases in capture fisheries are not anticipated under the current global conditions (Dunham *et al*; 2000).

Since the end of the 19th century, a large number of studies has been devoted to hybridization in fish, with descriptions of "hybrid-like" individuals among natural populations or including results of artificial insemination of eggs from one species by sperm from another one (Chevassus, 1983). Intraspecific, interspecific and intergeneric crosses are methods employed in fish hybridization.

During the recent decades remarkable improvement of productivity has been achieved for all important species of livestock. In the growing aquaculture industry future

development will also largely depend on the potential of the stock used. It is surprising that up to now integrated breeding and selection programs rarely have been practiced even though in some regions fish farming has a long tradition. The main reasons for failure in aquaculture breeding strategies have been unsuccessful artificial reproduction of some farmed species, technical difficulties in applying sophisticated testing procedures, and also inflexible aquaculture tradition.

This contribution deals with the encouraging prospects for selective improvement of aquaculture candidate species and sets out principles for developing advanced breeding and selection strategies in fresh water fish. Hence, Chevassus, (1983) defined hybridization as the union of gametes from two different species, resulting on a new organism. Hybridization therefore, is one of the methods used to introduce new fish species into intensive fish production (Klaus, 1987). Simple hybridization may also help sometime to bring in some improvements in the hybrid progeny. Hybrids are the progeny of parents from different lines, strains (intraspecific hybrids), or species (interspecific hybrids). The mean phenotypic value of hybrids is often greater than the value of either parent line.

A good supply of fry is essential for successful aquacultural production. The process of hybridization helps the fish farmer to select for desirable characteristics such as fast growth, food conversion, resistance to diseases etc which can increase the profitability of the farmer.

The Clariid catfishes from wild and farmed sources are economically important food fish in Nigeria, like other parts of the world; the catfish species of the family Clariidae have gained much prominence as important food fish. The species are very popular with fish farmers and consumers alike and as such command a very good commercial value in the market (Venden Bossiche and Bernacsek, 1990).

Heterobranchus and *Clarias* species are leading freshwater fish candidates for aquaculture in Africa. The African catfish, *C. gariepinus* is farmed mainly in Africa and Europe and of Late in India, China, Brazil and some East European countries (Huisman and Richter, 1987).

1.1. Biology of *Heterobranchus longifilis* and *Clarias gariepinus*

Fish of the species *Heterobranchus* and *Clarias* belong to the catfish family clariidae, which is widely distributed in Africa and parts of Asia. There are about 13 genera in this family (Teugels, 1990). They contribute about 20% of the total yield of freshwater fishes captured in Africa (Clay, 1977). The clariids are scaleless fishes with slimy skin. They are darkly pigmented on the dorsal and lateral parts of the body. The pigment turns lighter in colour when exposed to light. During stress the fish develop a mosaic-like pattern of dark and white spots. *Heterobranchus longifilis* and *Clarias gariepinus* have eight barbels around the mouth, which serve as tentacles with strong olfactoric sense, enabling the fish to identify food and prey in the night and in area of low light penetration. Both species have well developed pectoral fins serving locomotory and protective functions. They are able to migrate long distances on moist soil/vegetation using the spines. They have functional gills for gas exchange in water and they also possess accessory respiratory organs, which enable air breathing to survive very adverse oxygen deficient conditions.

Generic differences between *Clarias gariepinus* and *Heterobranchus longifilis* are well marked. *Clarias gariepinus* has a single dorsal fin, with 65 to 80 rays, which extends from the head region to the caudal peduncle, whereas *H. longifilis* has two distinct dorsal fins, namely a rayed dorsal with 29 to 36 rays and a fleshy adipose fin

of about the same length . The barbels of *H. longifilis* are much longer than those of *C. gariepinus*, the longest almost reaching the pelvic fins (Bruton, *et al.,* 1982).



Plate I: *Heterobranchus longifilis*



Plate II: *Clarias gariepinus*

1.2. RESEARCH PROBLEMS

The purpose of hybridization is to combine useful, positive, or desirable traits from one species to another with the aim of producing offsprings of superior quality than the parent species. Intraspecific and intergeneric hybrids of clariid catfishes have been produced (Madu *et al* 1993; Salami *et al* 1993; Nwadukwe 1995; and Aluko *et al* 2003); but which have not been thoroughly elevated as to their suitability for aquaculture. *Clarias* species do not grow as large or as quickly as *Heterobranchus.*

Heterobranchus species do not have the same survival rate as *Clarias*. Furthermore, the problem of low fecundity of *Heterobrachus* compared to high fecundity of *Clarias* has not been solved.

The decline in production has been a consequence of scarcity and high cost of fingerlings. The fish also does not readily breed in captivity. To profer solution(s) to these problems and improve the production of these two fish species for commercial culture, artificial hybridization using induced spawning hormone is necessary. This can increase the production and supply of fingerlings and the technique when popular with fish farmers will ease the breeding problem under pond culture.

1.3. JUSTIFICATION

Fish are wide spread over the oceans and rivers of the world. They are a major source of food for humans providing a significant portion of the protein intake in the diets of a large proportion of the people, particularly in the developing countries. Fish are a cheap source of animal protein with little or no religious rejection of it which gives it an advantage over pork (Ligia, 2002). It has been reported to be superior to meat as a protein source because it has a greater number of free amino acids, need lighter cooking and is more digestible (Jones, 1974). Fishery as an industry is highly placed in the agricultural sector providing not only food but also employment for many.

The catfishes (family clariidae) are very popular for fish farming in Nigeria. The fish are hardy and can grow to a large size of over 10kg (Reed *et al.,* 1967; Olaosebikan and Raji 1999) which are attractive to the consumers. The fish are scaleless, tasty and therefore, excellent as food fish.

They are also able to survive in ponds at extremely low oxygen concentrations because they have accessory respiratory organs, which enable them to breathe atmospheric air, and can obtain oxygen by cutaneous exchange. It is expected, that hybrid species of *C. gariepinus* and *H. longifilis* will grow to solve the problem of stunted growth in ponds and also, the **hybrid** fish will grow faster than their parent stock.

1.4 AIM

To Cross *Clarias gariepinus* and *Heterobranchus longifilis* to produce the much better quality hybrid.

1.5. OBJECTIVES

1. To determine from the parent stock and intergeneric mating combinations the best performance in terms of Hatchability, larvae survival and utilization of natural fish feed (food utilization by the larvae).
2. To determine the growth potentials of the hybrid fish under culture compared with the parent stocks.

1.6 HYPOTHESIS

NULL (H_O):

There is significant difference between the parent stock and intergeneric mating combinations in terms of Hatchability, survival, utilization of natural fish feed (feed utilization by the fish).

ALTERNATE (H_1):

There is no significant difference between the parent stock and intergeneric mating combinations in terms of Hatchability, survival, utilization of natural fish feed (feed utilization by the fish).

HYPOTHESIS 2

NULL (H_O):

There is significant difference in growth potentials of the hybrid fish under culture compared with the parent stock.

ALTERNATE (H_1):

There is no significant difference in growth potentials of the hybrid fish under culture compared with the parent stock.

CHAPTER TWO

2.0. LITERATURE REVIEW

Aquaculture in Nigeria is in the developing stage, because it has not been able to meet the demand and supply of the ever-increasing population. It is acknowledged as the efficient means of providing food which is rich in protein source, income and employment opportunities for the populace. Interest in fish culture is growing very rapidly in Nigeria but the scarcity of fingerlings of widely acceptable species of catfish such as *Heterobranchus longifilis* and *Clarias species* tend to constitute a major constraint to the rapid development of fish farming in Nigeria (Ojutiku, 2008). Economically productive aquaculture like agriculture is heavily dependent on adequate supply of seeds of fertile eggs and juvenile fish with which to stock the pond enclosures and other culture systems.

Fish culture is hardly imaginable without artificial or semi-artificial mass propagation of fish seeds of culture fish species. Heterobranchus and Clarias happen to be among the more than 300 species of fin fishes that have been cultivated but not spawn in captivity which therefore implies that their seed have to be obtained from the wild. The supply of fingerlings from the wild is most unreliable, unstable and inadequate, since the breeding habit of most culturable species is seasoned and the fish have to be captured at the time which may not correspond to the optimum production conditions. Hybridization is practiced to achieve either of the favourable outcomes: these are heterosis or hybrid vigor, which is defined in a broad sense as increased performance value of progeny above the average of the parental performance value (Ojutiku, 2008).

Many natural fish hybrids have been reported and numerous others have been artificially produced in the laboratory. Several research workers have recognized that

hybridization success is co-related with phylogenetic relationships. More closely related species should be expected to have greater success at producing young (Hester, 1970).

Significant result has been achieved in both research and applied genetics for the culturing of fresh water fish by the method of hybridization (Denton, 1973; Klaus, 1987). Interspecific hybridization was first obtained in carp culture (Kirpichnikov, 1970). Since then many other species have been hybridized with useful results in growth performance. The culture potential of *Clarias gariepinus* and *Heterobranchus longifilis* was discussed by Micha (1973); Legendre (1983); Hogendoorn (1983, 1984), Haylor (1989), and Nwadukwe, (1995). In Nigeria, the demand for *H. longifilis* far exceeds that for *C. gariepinus* which attains earlier sexual maturity (Nwadukwe, 1995). Fish hybridization seems to achieve optimum culture characters from different species as a result of heterosis (Hickling, 1966; Moav, 1979; Kirpichnikov, 1981; Chevassus, 1983 and Nwadukwe, 1995).

Sahoo *et al.* (2003) reported that hybridization has a success of 78 to 86% hybrid fry survival. They also reported that the advantages of the significant increase in growth rate of hybrids over parent Clariid, and the complete absence of cannibalism in the hybrids at different stages of rearing and in pond culture indicate a lucrative and promising catfish culture. At Fleuren and Nooijen Fish Farms Ltd; the elite parents from *Clarias gariepinus* are used to cross with *Heterobranchus longifilis* to produce fry of fingerling hybrid as a final product for commercial purposes. The hybrid is named Heteroclarias. They cannot be used for reproduction by customers. They also have other growing advantages such as higher dressing yield and niche market product for better price. However, the hybrid exhibited aggressive behavior and large variation in body weight (Fleuren, 2008).

2.1. TAXONOMY

Heterobranchus longifilis as described by Teugels (1990), has a long and broad head, somewhat rectangular in dorsal outline. The snout is broadly rounded, eyes in supero-lateral position, with an inter-orbital distance which is generally less than half the head length. The frontal fontanel is long and narrow in small specimen with the anterior tip reaching the line connecting the anterior eye borders. The occipital fontanel is oval-shaped. The tooth plates are as wide as the width of the head. The premaxillary teeth are conical. Vomerine and mandibular teeth are subgranular to granular. The skull is entirely covered with bone plates. The suprabranchial cavity is completely filled with a well-developed dendretic accessory air-breathing organ.

The distance between dorsal fin origin and the supra-occipital process is about 7 per cent of the Standard Length (S.L.). The adipose fin originates just behind the last dorsa fin ray and extends to the base of the caudal fin. Its length is equal to or slightly shorter than the rayed dorsal fin length. The pectoral fin extends from behind the operculum to the origin of the first dorsal fin rays. It has a pectoral spine which is serrated on the anterior and slightly smooth on the posterior side. The pelvic fins reach the first anal fin rays. The anal fin is situated below the posterior half of the dorsal fin. The lateral line is a thin white line that extends from the posterior end of the head to the middle of the caudal fin base. The color of the body is generally grey-brown or grey to dark-grey, with the belly and lower surfaces of the paired fins whitish to light brown (Teugels, 1990).

In a comparative study on morphology of *Clarias gariepinus* and *Heterobranchus longifills,* Legendre *et al* (1992), showed that the main difference between the two species is the large adipose fin of *Herterobranchus longifilis* which

is absent in *Clarias gariepinus*. This adipose fin is supported by 21 to 27 extended neural spines.The rayed dorsal fin of *H. longifilis* is shorter than that of *C. gariepinus*. The number of gill rakers on the first branchial arch is 16 – 29 in *H. longifilis* as against 24 – 110 in *C. gariepinus*.

2.2. DISTRIBUTION

H. longifilis is a fresh water species which can withstand salinity of up to 7‰ (Legendre, 1986). It is widely distributed in Nigeria's freshwaters from Lake Chad to the Niger River, Cross river, Lagos Lagoon and even the relatively transparent Ethiope River. It is present in other parts of Africa: Lake Kariba, the Zambezi below the Victoria Falls, North African rivers through most of the Congo River system, Lake Edward and Lake Tanganyika. *H. longifilis* is not found in rivers of the east coast of Africa (Dale and Degan, 1983). Although *H. longifilis* is widely distributed, it is not an abundant species. In an experimental netting in the Naodza and Gache estuaries in 1975 – 1976, *H. longifilis* formed only 0.27% of the total catch by numbers (Dale Degan, 1983). *H. longifilis* grows to a size of up to 55 kg Bodyweight (BW). The more abundant size ranges between 1.5 kg and 3.5 kg BW. *C. gariepinus* is more abundant and is distributed in all the above named regions including the middle East (Okoro, 1995).

2.3. BREEDING PROSPECTS IN FISH

Huisman (1985) gave that compared with farm animals fishes have a number of advantages. The most important are:

► High fertility in most species, enabling very accurate evaluation of breeding values by sib or progeny testing and high selection intensities among the brood stock tested.

► Pronounced genetic variability in most traits especially in growth traits, which is significantly higher than in farm animals indicating the chance for high selection responser.

► The extrauterine fertilization, which in combination with the high fertility, is of great advantage for cross-breeding programs, and facilitates genetechnology, the latter being of special importance of fish breeding.

2.4. POTENTIAL INTEREST OF HYBRIDIZATION

2.4.1. Hybrids

A proper evaluation of the potential interest of hybrids for aquaculture requires primarily a good description of their characteristics. Their value may in fact result from one or from an adequate combination of several of these characteristics.

2.4.1a. Viability of Hybrids

Blanc and Chevassus, (1979, 1982) reported the survival rate of the ”tiger trout” (brown trout, *Salmo trutta* and brook trout, *Salvelinus fontinalis)* is about 10 to 20 of that of the control 2 months after hatching. Blanc and Poisson (1983) for “*tiger trout*” reported variations from 0 to 43% in the survival rate at the end of the yolk reabsorption period between the offspring of different parents. When the exogenous feeding period is reached, the survival rate of the hybrids is mostly intermediate between that of the parent species (Chevassus, 1983). One of the aims of hybridization is to delay or even to inhibit sexual maturation. Such an effect may have positive consequences on the survival rate, as in Salmonids of the genus *Oncorhynchus* (Susuki and Fukuda, 1973).

2.4.1b. Growth Potential of Hybrids

In spite of old assertions on their obvious superiority (Skinner, 1938), the growth of hybrids mostly appear to be intermediate between that of the parental species. Susuki and Fukuda, (1972) in Salmonids; Burtzev and Serebryakova (1973) in acipenserids reported that the fast growing species transmit at least partly this potential to the hybrid. The hybrid may be of interest when one of the parental species is domesticated and liable to supply of eggs easily, the sperm being collected in a wild, fast growing species, for example the attempt to hybridize the female Plaice (*Pleuronectes platessa)* with the male halibut *(Hippoglossus hippoglossus* (Purdom and Lincoln, 1974).

2.4.1c. Reproduction

In fish hybrids, sexual maturation may be affected in several ways. Some hybrids present a normal maturation of both sexes, allowing production of F2 or back crosses. For instance, *Oncorhynchus keta* X *Oncorhynchus nerka* hybrids in Salmonids (Terao *et al.,* 1965), *Huso huso* X *Acipenser ruthenus* hybrids in acipenserids (Burtzev and Serebryakova, 1973), *Sarotherodon massambicus* X *S. hornorum* hydrids in Cichlids (Hickling, 1960). Some others only exhibit atrophic gonads with a few sexual cells at an early stage of gametogenesis.

2.4.1d. Hybridization and Sex Ratio.

One of the frequently recorded characteristics of hybrid populations is a deviation from the theoretical sex ratio of 1:1. For instance, Lincoln (1981a) reported an excessive number of males among trploid plaice X flounder hybrids; Bakos *et al*., (1978) found no males among 300 common carp X silver carp hybrids (but 25% of the animals showed a more or less developed testicular area within the ovaries).

2.4.2 Research Trends in Hybridization

2.4.2.1 Basic Research

2.4.2.1a Cytogenetics of Hybrids

i. The Problem of Aneuploidy – The possibility of incomplete karyogamy has to be considered, as an intermediate event between parthenogenesis and full karyogamy. Such a study assumes the development of karyological methods to detect the lack of a small number of chromosomes, and even the lack of parts of chromosomes. The presence of paternal characters in animals resulting from a hybrid gynogenesis might result from such a partial karyogamy (Stanley *et al* 1976).

ii. The cytogenetic aspect of meiosis in hybrids, especially the relationship between zygotic sterility and production of unbalanced gametes seems to be well established in some cases in *Salmo Solar* X *Salmo trutta* hybrids (Nygren *et al*., 1975) but the fertility of hybrids from crosses between species exhibiting very different Karyotypes such as *Oncorhynchus keta* (2n = 74) X *oncorhynchous gorbuscha* (2n = 52) hybrids seems by contrast to be a paradox. Moreover, the implication of meiotic disturbances as a casual factor of gametic or gonadic sterility has not yet been clearly demonstrated (Terao *et al.,* 1965).

2.4.2.1b. Hybridization and Classification

In most hybridization experiments, the authors are only presenting their results and they are not able a priority to estimate the potential viability and fertility of the different hybrids. However, Hubbs, (1970); Hester, (1970) suggested using these results as estimates of the phylogenetic distance between species, and thus as a tool

for classification. Chevassus (1979), reported using salmonids that systematics of a family do not allow one to predict either what would be the viable and inevitable hybrids or the existence of a large difference between the results of two reciprocal hybridization. Purdom and Lincoln (1974) suggested the study of the size of the spermatozoa and that of the micropyle but it does not appear to be very promising. A more accurate analysis (by biochemical or Karyological methods) of the phylogenetic relationships between species might lead to better prediction.

2.4.3 APPLIED RESEARCH

2.4.3.1 Production of New Hybrids

In addition to any study aimed at obtaining diploid hybrids, it seems to be very important to test systematically the possibilities of producing polyploid hybrid. Kirpichnikov (1981); Chourrout (1982a) stated that in the case of triploid hybrids, the induced diploidization of the material stock by retention of the second polar body may be obtained in several species. Capanna *et al.,* (1974); Lincoln (1981a&b) observed that an increased viability often appears and the sterility (at least the zygotic one) of these allotriploids seems to be well established.

More recent methods (Streisinger *et al.,* 1981; Thorgaard *et al.,* 1981; Chourrout 1982c) allow the production of tetraploids in some species by induction of an endomitosis. Adaptation of these methods to production of allotetraploids would lead to creation of fertile and stable "new species" as the meiotic pairing of their chromosomes would occur between the duplicated ones. Such "Species" may lead to production of triploid hybrids without additional treatment and even, if viable, to the production of "3 – way" hybrids.

2.5 Genetic Approaches in Hybridization

Genetic variation in natural population is considered to be an important resources that should be conserved for the use in aquaculture and provide the basis for new adaptations to a changing environment (Jorstad *et al*., 1994).

Intraspecific, interspecific and intergeneric crosses are employed in fish hybridization. These crosses may exhibit such qualities as rapid growth, higher food conversion, better utilization of food from the environment, viability, resistance to diseases, higher dressing values, better taste and nutritional quality (Aluko 1999b).

Hybridization helps in the production of monosex (all males) progeny as in the case of some tilapia species or in the production of sterile (aneuploid/triploid) hybrids, such as the hybrids produced by crossing females of common carp (*Cyprinus carpio var. commuris*) with males of Indian major carps (rohu, catla and Mrigal) or females of Chineses grass carp with males of bighead carp (*Hypophthalmichthys nobilis*) respectively. Sterility helps in controlling unwanted reproduction and consequently over population in a culture medium and in some species sterile individuals may also exhibit faster growth (Reddy, 1999).

2.6 ADVANTAGES OF HYBRIDIZATION

Hybridization possess two important advantages. It avoids inbreeding and it avoids contamination of the environment with sex hormones (Wohlforth, 1994). Advantages of hybridization include, increased vigor and phenotypic uniformity in the crossbred progeny (Seagrant, 1987). Tave (1993) reported that processing plants and consumers often desire uniform products, and hybridization is the most efficient method of producing uniform progeny. Aluko (1999a) reported that catfish hybrids have advantageous qualities like fast growth, better food conversion, higher survival,

and resistance against unfavourable environmental conditions and diseases. He reported remarkable growth performance of 250g for parent *C. anguillaris* and 900g for *H. longifilis* and 1,800g, for the hybrid of both parents. Aluko (1999c) also reported hybrid growth increase of about 25%. Plumb *et al*., (1975) showed that hybridization improved resistance to channel catfish virus disease. While Tave (1993), found that some hybrids improved yield by 10-18%. He also found that hybridization improved seinability and food conversion

The production of monosex fish through hybridization help in the control of unwanted reproduction and also one sex may be more valuable than the other. Hickling (1968) explained that a very skewed sex ratio sometimes results from hybridization, and in cases where hybridization gives 100 percent of one sex, the hybrids are hypothetically as useful as sterile fish for stocking with the added advantages of population control.

2.7 DISADVANTAGES OF HYBRIDIZATION

Hybrid lines cannot be maintained by allowing hybrids to reproduce, they must be produced from parental lines in every generation. Seagrant (1987) stated that the genetic uniformity and extreme heterozygosity of hybrid is lost after a single generation or random mating. Aluko (1999a) reported that the F1 hybrid produced as a result of intergeneric hybridization is fertile which aquaculture practioners in Nigeria have exploited by using the hybrids for breeding purposes. He explained that the potential hazards of the indiscriminate use of the F1 hybrids as breeders on the catfish gene pool are obvious. It could lead to the genetic loss of the pure parent species. Producing sterile F1 hybrids could only solve the problem of fertility of the F1 hybrid.

2.8 FUTURE AREAS OF RESEARCH IN HYBRIDIZATION

In the growing industry of aquaculture future development will depend largely on building up a genetically promising seed supply. High fecundity and high genetic variability in almost all-key performance parameters may well pave the way to extra ordinary selective improvement in aquatic animals (Langhotz, 1985).

Tolerance to disease and environmental stress are complex factors of importance for future research programmes (Chevassus and Coche, 1986).

Aluko (1999a) reported that the future of commercial catfish hybridization lies in the production of infertile hybrids with superior traits. He listed future trends in catfish hybrid production.

1. Research efforts at producing fingerlings of catfish hybrids all-year round through administration of environmentally friendly hormonal implant (drug)
2. On-going research efforts at producing transgenic catfish hybrid by DNA technology.
3. On-going research efforts to improve the indigenous parent stocks of catfish hybrids through application of biotechnology tools and selective breeding.

Biotechnology has opened a new window for development of genetic resources in aquaculture. However, the application of biotechnology and genetics to aquaculture in Africa has been constrained by various factors (Mugabe, 2002, Changadeya *et al*., 2003). In all, the future of aquaculture lies in production of species that are able to grow fast, adapt to changing climatic conditions and also remain capable of withstanding the rigors of an enhanced production system (Ataguba *et al*., 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SOURCES OF FISH

Breeders of the catfish clariidae (*Heterobranchus longifilis* and *Clarias gariepinus*) were obtained from the experimental tanks of Fish Biotechnology Programme, National Institute for Freshwater Fisheries Research, (NIFFR), New Bussa.

3.2 EXPERIMENTAL SITE

The experiment was performed at the fish Biotechnology Research Laboratory of National Institute for Freshwater Fisheries Research (NIFFR), located in New Bussa, Niger State. This falls within the Kainji Lake Basin which is located between 9^o 50' to 10^o 55'N and 4^o 23' to 4^o 45'E. Annual average rainfall is 900 to 10000mm mainly from May to early October. Temperature during peaks of breeding season ranges from 22^oC to 29^oC and as low as 16^oC in the harmattan season (Nlewadim, 2002).

3.2.1 RECORD KEEPING

Date of experiment, Tank numbers, species of fish, sex, weight of fish before inducement and after stripping, standard length of the fish, Hormone dose, time of Injection, Water Temperature, and Date spawned and Date hatched were all taken. The Record of weight and length of the fry and fingerlings were also taken during the experiment.

3.2.2 TANK PREPARATION (SPAWNING TANKS)

Twelve glass aquaria tanks with dimensions of 60cm x 30cm x 30cm were cleaned and filled with clean water to 2/3 of its volume and potable aerators fixed into

each to aerate the water. Clean kakaban to serve as substrate for the eggs were spread in each aquarium where fertilized eggs were spread.

3.3 SELECTION OF BROODERS

The brooders were selected on the basis of their morphological characteristics e.g Total length, weight.

3.3.1 FEMALE BROODER SELECTION

Two female brooders of 300g body weight of *Clarias gariepinus* and two female brooders of *H. longifilis* of 350g were selected and they exhibited soft, highly distended abdomen with reddish vent, and a few eggs were stripped upon application of slight pressure on the abdomen.

3.3.2 MALE BROODER SELECTION

These brooders exhibited a prominent vascularized, reddish, pointed genital papilla. Two male brooders *C. gariepinus* of 200g each and two male brooders *H. longifilis* of 300g each were used.

3.4 INDUCED BREEDING

The broodstocks were weighed and injected inter-peritoneally with ovaprim spawning hormone at the rate of 0.5ml per kilogram of fish and allowed for a latency period of twelve and fifteen hours for *C. gariepinus* and *H. longifilis* respectively at 27^{o}c.

The fish after induction (injection with ovaprim hormone) were kept separately in a big rubber bowl and covered with wooden tray measuring $30cm^2$ and each tray has a chicken wire which prevented the experimental fish from jumping out of the bowl.



Plate III: Weighing of fish

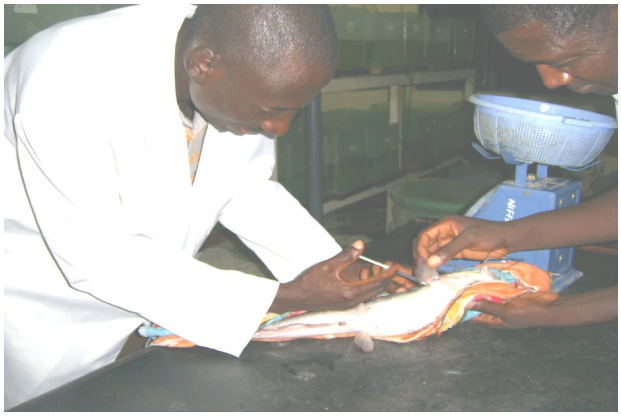


Plate IV: Injection of fish with the spawning hormone

3.5 MILT AND EGG COLLECTION

After the latency period of 12 hours for *Clarias gariepinus* and 15 hours for *Heterobranchus longifilis*, testes were collected by sacrificing the males. The two lobes of the testes of each were removed, the weight and length of the right and left testes were taken using a sensitive weighting balance for the weight and a measuring ruler for the length and then kept in a Petri-dish. The females were also stripped of eggs after gentle application of Pressure on the abdomen to release the eggs into a Petri-dish. Their weight were taken after stripping to know their spent weight.



Plate V: Removal of Testis from the male fish





Plate VI a: *Heterobranchus* Testis **VI b:** *Clarias* Testis

3.6 FERTILIZATION

The testes were macerated with care using a razor blade to release the milt. Milt and eggs for each cross were put together in a labeled Petri-dishes and were properly mixed with the aid of a feather. Saline (0.9% NaCl) solution was added to ensure maximum fertilization.

The parental and intergeneric mating combinations were carried out in triplicate as shown below:-



Plate VII: Stripping of the gravid female to collect the eggs

MATING COMBINATIONS:

Parental crosses

1. ♀Cl x ♂Cl
2. ♀Hl x ♂Hl

Intergeneric crosses

1. ♀Cl x ♂Hl
2. ♀Hl x ♂Cl

Where:

Cl is *Clarias gariepinus*

Hl is *Heterobranchus longifilis*

♀ is female

♂ is male

3.7 INCUBATION

Fertilized eggs of each cross were spread separately on the kakaban (egg nest) and were incubated under consistent aeration in aquaria ($60x30x30cm^3$) under ambient temperature ranging between 25^oC to 27^oC.



Plate VIII: Dispensing of the fertilized eggs on the kakaban in the incubating tanks

Percentage hatchability was calculated using:

$$\text{Percentage hatchability} \quad \frac{\text{No of hatchlings}}{\text{Total no of eggs fertilized.}} \times 100$$

3.8 GROWTH PERFORMANCE

After the yolk absorption, one hundred fry from parental and intergeneric crosses were stocked in triplicate in 60x30x30cm^3 aquarium tanks after taking pooled weight and length measurements.

The fry were fed ad-libitum with mixed zooplankton collected from NIFFR Natural Fish Food Production Unit, using zooplankton net.

At the end of 14 days (two weeks) in-door rearing period, the fry length and pooled weight were measured and then transferred to the experimental outdoor 2x2x1m^3 concrete tanks at a stocking density of 100 fry per tank in duplicate of the various mating combinations.

The fry were fed with 0.2mm – 0.3mm, 0.3mm – 0.5mm, 0.5mm – 0.8mm and 0.8mm – 1.2mm COPPENS feed for 8 weeks outdoor. A random sample of 50 fry/tank of each mating group were collected weekly for length and weight measurements for 8 weeks in outdoor concrete tanks during which time survival was also recorded.

The weight gain and specific growth rate (SGR) were also calculated using the following formular adopted by (Fagbenro, 1996).

Weight gain = Wt – Wo

$$\text{Specific growth rate} = \frac{\text{Ln Wt} - \text{Ln Wo}}{t} \times 100$$

Where

Wo = Initial weight of the fish

Wt = final weight of the fish

t = period of the experiment

3.9 WATER QUALITY PARAMETERS.

The water quality parameters monitored during the study includes temperature using thermometer, pH (hydrogen ion) using pH. Meter and dissolved oxygen through titration.

3.10 STATISTICAL ANALYSIS

The data obtained were subjected to statistical analysis, using one-way analysis of variance (ANOVA) in SPSS version 10.0 for windows and where significant difference were observed, means were separated with Duncan's Multiple Range Test at 5% level of significance.

CHAPTER FOUR

4.0 RESULTS

4.1 PERCENTAGE HATCHABILITY

The fertilized eggs in all mating groups hatched within 24-25 hours at the temperature of about 25^{o}C – 26^{o}C under laboratory condition. Figure 1: shows the percentage hatchability in the four mating combinations involving parental and Intergeneric crosses. The highest percentage was recorded in the Intergeneric cross between female *Clarias gariepinus* and male *Heterobranchus longifilis* (56%), followed by the parental cross of *Heterobranchus longifilis* (51%) and the lowest hatchability was recorded in the parental cross of *Clarias gariepinus* (40%).

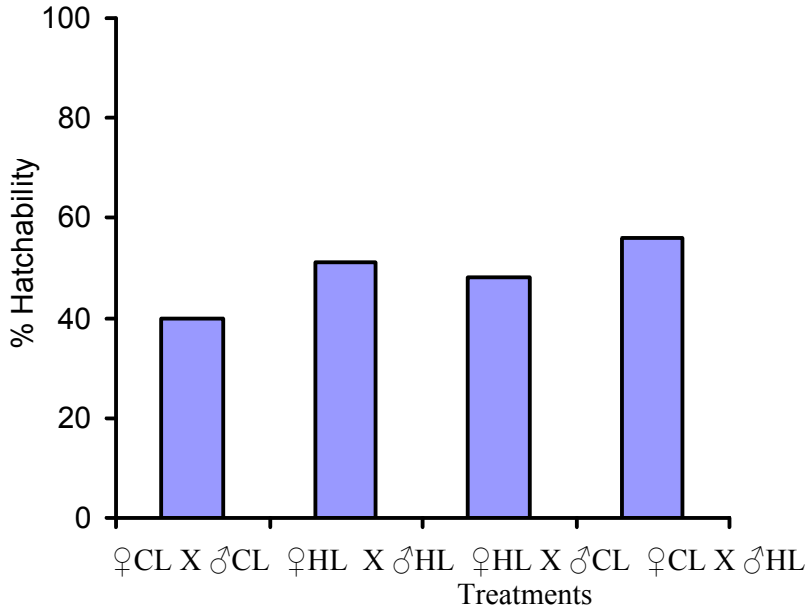


Figure 1: Percentage hatchability of the various mating combinations

4.2 PERCENTAGE SURVIVAL INDOOR

Figure 2 shows the percentage survival of fry of the various mating combinations reared for 14 days indoor in aquaria tanks measuring 60 x30x 30cm^3. The intergeneric crosses of both female *Heterobranchus longifilis* with male *Clarias gariepinus* and female *Clarias gariepinus* with male *Heterobranchus longifilis* had the highest survival percentage of 86% and was closely followed by the parental cross of *Clarias gareipinus* (81%) while the least was recorded for the parental cross of *H. longifilis* (75%) at the end of 14 days indoor rearing.

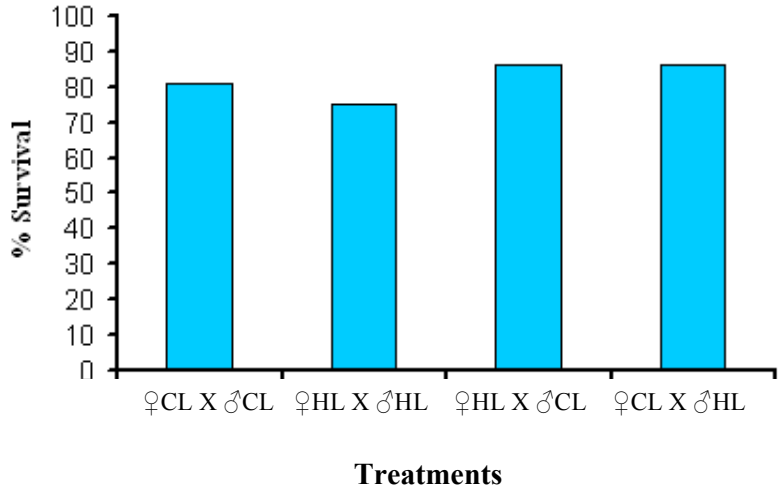


Figure 2: Percentage survival of fry of the various mating combinations for the in-door experiment (14 days).

4.3 PERCENTAGE SURVIVAL OUTDOOR

In the 8 weeks outdoor period, depicted in figure 3, the intergeneric crosses exhibited higher percentage survival. The mating combination of female *Clarias gariepinus* and male *H. longifilis* gave the highest survival outdoor (91.5%). The parental crosses still gave the least survival outdoor with 71.5%.

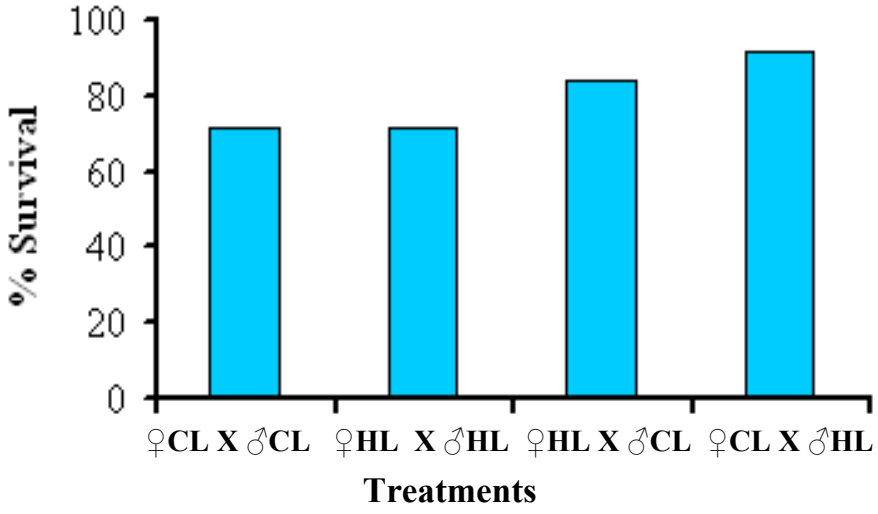


Figure 3: Percentage survival of juveniles of the various mating Combinations for the outdoor experiment (8 weeks)

4.4 GROWTH PERFORMANCE INDOOR

Table 1 shows the growth performance parameters of the various mating combinations for a period of 14 days indoor rearing. There was significant difference ($P<0.05$) of weight gain of ♀*C. gar.* x ♂ *C. gar.* compare to that ♀*H. long.* x ♂ *H. long and* ♀*H. long.* x ♂*C. gar* respectively. There was no significant difference ($P>0.05$) in the SGR of all the mating combinations. The parental cross of *Clarias gariepinus* at the end of 14 days had the highest pooled weight (9.0g) with the SGR of 30.50% wt/day while the least weight was recorded in the parental cross of *H. longifilis* (4.57g) but coincidentally with the highest SGR of 31.19%) wt/day.

Table 2 shows length parameters in all mating combinations. There was no significant difference ($P>0.05$) in the mean values of the mating combinations after the 14 days indoor rearing.

Table 1: Growth Performance of the Fry of the Various Mating Combinations at 14 days Indoor

Mating combination	Initial pooled wt. (g)	Final pooled wt (g)	Wt gain (g)	SGR (%. Wt./day)
♀*C. gar.* x ♂ *C. gar.*	0.12	9.0^{a}	8.88^{a}	30.50^{a}
♀*H. long.* x ♂ *H. long*	0.06	4.57^{b}	4.51^{b}	31.19^{a}
♀*H. long.* x ♂*C. gar*	0.09	5.0^{b}	4.91^{b}	29.02^{a}
♀C. *gar.* x ♂*H. long*	0.14	5.9^{b}	5.76^{ab}	26.90^{a}

Means with the same superscript within column are not significantly different ($P>0.05$).

H. long - *Heterobranchus longifilis*
C. gar - *Clarias gariepinus*

Table 2: Length Parameters of the Fry of the Various Mating Combinations at 14 days Indoor

Mating combination	Initial pooled l (cm)	Final pooled l (cm)	Length gain (cm)	SGR (%l/day)
♀*C. gar.* x ♂ *C. gar.*	3.03	10.7[a]	7.67[a]	8.80[a]
♀*H. long.* x ♂ *H. long*	2.8	9.2[a]	6.4[a]	8.32[a]
♀*H. long.* x ♂*C. gar*	2.96	9.1[a]	6.14[a]	7.98[a]
♀C. *gar.* x ♂*H. long*	2.96	10.6[a]	7.64[a]	9.10[a]

Means with the same superscript within column are not significantly different (P>0.05).

4.5 GROWTH PERFORMANCE OUTDOOR

The same parameters that were measured indoor for the growth performance was measured for the outdoor growth performance for a period of 8 weeks. Table 3 shows how the various mating combinations faired for the outdoor. The mean values showed no significant difference (P<0.05) between the mating combinations. The parental cross of *H. longifilis* recorded the highest final pooled weight (293.75g) and SGR of 10.14% wt/day at the end of 8 week, Table 4 shows the length parameters for the 8 weeks study. The mean values were however non-significant. (P>0.05).

Table 3: Growth Performance of the Fingerlings of the Various Mating Combinations at the End of 8 Weeks of Outdoor Rearing

Mating combination	Initial pooled wt. (g)	Final pooled wt. (g)	Wt. gain (g)	SGR (%. Wt./day)
♀*C. gar.* x ♂ *C. gar.*	17.20	288.15[a]	270.95[a]	5.04[a]
♀*H. long.* x ♂ *H. long*	11.25	293.75[a]	282.50[a]	5.88[a]
♀*H. long.* x ♂*C. gar*	13.05	219.00[b]	205.95[a]	5.04[a]
♀C. *gar.* x ♂*H. long*	13.50	249.20[ba]	235.70[a]	5.20[a]

Means with the same superscript within column are not significantly different (P>0.05).

Table 4: Length Parameters of the Fingerlings of the Various Mating Combinations at the End of 8 Weeks of outdoor Rearing

Mating combination	Initial pooled l (cm)	Final pooled l (cm)	Length gain (cm)	SGR (%l/day)
♀*C. gar.* x ♂ *C. gar.*	3.68	9.81[a]	6.13[a]	1.74[a]
♀*H. long.* x ♂ *H. long*	2.95	9.49[a]	6.54[a]	2.10[a]
♀*H. long.* x ♂*C. gar*	3.10	8.46[a]	5.36[a]	1.79[a]
♀C. *gar.* x ♂*H. long*	3.25	8.89[a]	5.64[a]	1.81[a]

Means with the same superscript within column are not significantly different (P>0.05).

4.6 DESCRIPTION OF THE HYBRID FISH

Clarias gareipinus does not have the adipose fin which is prominent in *Heterobranchus longifilis.* It is interesting to observe in this study that the hybrid of female *H. longifilis* and male *C. gariepinus* (plate 9) has shorter adipose fin and narrower and less flattened head, while the reciprocal hybrid, female *C. gariepinus* and male *H. longifilis* (Plate 10) possess longer adipose fin and the head is broad and flat.



Plate IX: Hybrid of female *H. longifilis* cross with male *C. gariepinus*

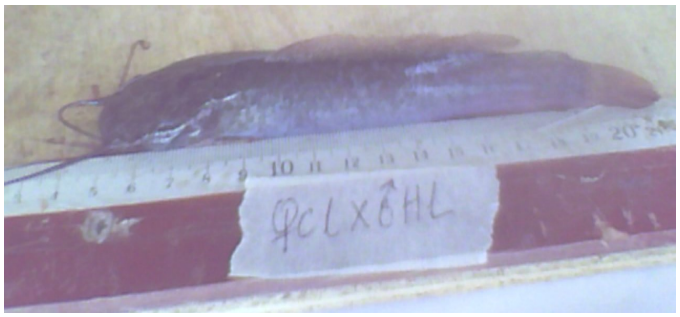


Plate X: Hybrid of female *C. gariepinus* cross with male *H. longifilis*



Plate XI: Fingerlings of the mating combinations produced

4.7 WATER QUALITY PARAMETERS

The results of physico-chemical parameters showed that temperature ranges between 25^{o}C to 27^{o}C, pH ranges between 6.9 – 7.5 and dissolved oxygen fluctuated between 5.0 – 7.4mg/l^{-1}.

CHAPTER FIVE

3.0 DISCUSSIONS

Aquaculture unlike capture fisheries requires deliberate human intervention in the organism productivity and yields that exceeds those from the natural environment alone. Aquaculture genetics shows immense potential for enhancing production in a way that meets aquaculture development goals for the new millennium (Akinwande, 2009).

The lack of good quality seed has been a major set back to the development and expansion of aquaculture in Nigeria and Africa in general. This opinion has been reiterated by many authors especially Haylor (1992) and Olufeagba (1999). The

result of this work clearly showed that the intergeneric mating between female *C. gariepinus* and male *H. longifilis* gave the best hatchability in the intergeneric cross. While in the intrageneric cross, the parental *H. Longifilis* gave the best hatchability. These findings are similar to that of Ollevier *et al.,* (2000) who in a similar field work, by comparing the fertilization, hatching, survival and growth ratio in reciprocal crosses of two strains of *Clarias anguillaris* Linnaeus 1758. Olufeagba *et al.,* (2005) in their studies on hatchability, growth and survival rate in Holand, *H. longifilis* and *C. gariepinus.* Ataguba *et al*., (2009) who reported the same in their work on Induced breeding and early growth of progeny from crosses between two African Clariid fishes. Again, in this study, the low hatchability for the Parental cross of *C. gariepinus* and the intergeneric cross involving male *C. gariepinus* may be due to the quality and condition of the milt. This result compares favourably with De Graat *et al.,* (1995) and Moses *et al.,* (2005) who reported 28.4% and 59.1% average hatching percentage in *C gariepinus* and *C anguillaris* respectively. Generally, in this work the hatchability percentage agrees with the value reported by Nwadukwe (1995) who recorded hatchability percentage lower than 50% and also recorded 40- 85% hatching success in *H. longifilis.*

The high survival rate found in the intergeneric crosses for the indoor and out door rearing throughout the period of study attested to the superiority of the hybrids. Legendre *et al.* (1992) had earlier recorded high survival rates in hybrids of intergeneric cross, after 15 days of larval rearing in recirculating tap water. Also in the same trend, Nwadukwe (1995) and Madu *et al,* (1999) both reported that the hybrids of intergeneric cross had superior values (hybrid vigour) than either parents. This survival potential found in the hybrid fish is expected to translate to all round performance as the fish matures. Mustafa and Rahman, (1999) also stated that intra-

population genetic diversity provides a range of genotypes, thereby presenting more opportunities for survival and evolution under changing environmental conditions. In all, in this study the treatments have a good survival rate which agrees with Nlewadim (2002) who stated that survival rate and production were inversely related to rearing duration.

Growth rates of fish depend on a number of factors among which are availability of food and density of the fish within available space. Even though production may increase with density, crowded populations suppress increase in weight and the size of the individuals (Bardach *et al*, 1972).

The growth performance observed for both the indoor and the outdoor experiment in this work in the hybrids (intergeneric crosses) as compared to the parent crosses, seems that the heterotic effect of hybridization (hybrid vigour) had not yet manifested at this early stage in the hybrids. Hence this compares well with Madu *et al*. (1993) who explains that the superior growth characteristics of the hybrids will not be manifested until after 12 months culture period from fingerlings to adult. Nwadukwe and Nawa (2000) noted that mean weight was directly related to length of rearing period, and this also explains the relative low weight of the hybrid compared with the parent cross. In this studies *H. longifilis* has higher SGR than the other treatments while it has the least final weight. This may be due to lower initial weight. It might also be a promising characteristics feature in the species at the long run as showed in the outdoor performance In addition, the hybrid vigour of growth was latent at the early stage of the hybrid. But in general performance of survival and growth in the hybrid (especially the cross between female *C. gariepinus* and male *H. longifilis*) seem to be the best aquaculture candidate.

CHAPTER SIX

4.0 CONCLUSIONS AND RECOMMENDATIONS

The results achieved by this work is to increase productivity of warm water fish species especially *C. gariepinus* through improved feed conversion and utilization leading to better growth performance and improved progeny performance i.e. fingerling survival, growth and resistance to disease can be improved upon, as it is clearly shown by this work, the hybrid cross between female *C. gariepinus* and male *H. longifilis* has the best hatchability and survival, and gave the best growth performance in the intergeneric crosses. It is therefore, concluded within the confines of this work that the hybrid female *Clarias gariepinus* and male *H. longifilis* produced through intergeneric cross remains the best combination for production of good quality hybrid seeds. However, the best performance of *H. longifilis* in parental group shows its superiority over *Clarias gariepinus* in terms of growth.

Even though it is known world-wide that hybridization work is intensive, especially at the early stage, I recommend that Government should make enough funds available for hybridization work especially to Academic Institutions and research Institutes to carry out research towards improving our commercially important and culturable species. This will eventually bring the high yield that is needed in our fisheries subsector to enable us stop fish importation. I also recommend to hatchery operators that the result of this work could be used as basic information for the production of aquaculture seeds.

REFERENCES

Akinwande, A.A., Moody, F.O. and Umar, S.O. (2009). Growth Performance and Survival of *Hererobranchus longifilis, Heterobranchus bidorsalis* and their reciprocal hybrids. *African Scientist* vol.10, no.1. pp15-18.

Aluko, P.O. (1999a). Genetic Approaches in catfish Hybridization: Efforts of NIFFR, Nigeria. National Institute of Freshwater and Fisheries Research Newsletter, Vol.16, Issue 2, May-August, 1999.

Aluko, P.O., D. Woru and A. Aremu (1999c). Growth performance of Intreaspecific hybrids of three strains *of Heterobranchus longifilis*. National Institute of Freshwater and Fisheries Research Annual report 1999.

Aluko, P.O., D. Woru and A. Aremu (2003). Development of Triploid Breeding Line in *Heterobranchus Clarias* Hybrid used in Aquaculture. *Nigerian Journal of Fisheries* 1: 1-10.

Ataguba, G.A., Annune, P.A., and Ogbe, F.G. (2009). Induced breeding and early growth of progeny from crosses between two African Clariid fishes, Clarias gariepinus (Burchell) and *Heterobranchus longifiis* under hatchery conditions. *Journal of Applied Biosciences* 14:755-760.

Brutan: M.N., Skeltan, P.S. and Jackson P.B.N. (1982). Pocket Gurde to the Freshwater Fishes of Southern Africa. Centaur, Cape Town. Academy of Science Publishers PP 77 - 78

Bakos, J., Krasznai, Z. and Marian, T., (1978), Cross-breeding experiments with carp, tench and Asian phytophagous cyprinids. *Aquaculture* Hung (Szarvas), 1:57 – 57.

Blanc, J.M and Chevassus, B., (1979). Interspecific hybridization of Salmonid fish. I. Hatchingand survival up to the 15th day after hatching in F1 generation hybrids. *Aquaculture*, 18:21-34.

Blanc, J.M and Chevassus B., 1982. Interspecific hybridization of Salmonid fish. II. Survival and growth up to the 4th Month after hatching in F1 generation hybrids. *Aquaculture,* 29:383-387.

Blanc, J.M and Poisson H., (1983). Parental sources of variation in hatching and early survival rates of *Salmo trutta* X *Salvelinus fontinalis* hybrid. *Aquaculture*, 32:115-122.

Bardach, J.E., Ryther, J.H. and Mc Larney, W.O. (1972). *Aquaculture: the farming and husbandry of freshwater and Marine Organisms*. Wiley-Inter Science, New York, London, 868pp.

Burtzev, I.A. and Serebryakova, E.V., (1973). A hybrid beluga X sterlet (*Huso huso* (L) X *Acipenser ruthenus* L., Pisces): Karyology, gametogenesis and Potential status. Abstracts Proceedings 13th International Capricorn *Genetics*, 74:35.

Capanna, E., Cataudella, S. and Volpe, R., (1974). Un ibrido intergenerico tra trota iridea e Salmerino di fonte *(Salmo gairdneri* X *Salvelinus fontinalis*). *Boll. Pesca Piscic. Idrobiol*., 29:101-106.

CGIAR, (2006). Consultative Group on International Agricultural Research. Improved Tilapia Benefits Asia. Science Council Secretariat, a unit of the CGiar System Office. C/o FAO viale delle Terme di Caracallce, 00153 Rome, *Italy Science Council Brief Standing Panel on Impact Assessment* number 6:1-6.

Changadeya, W., Malekano, L.B., and Ambali, A.J.D. (2003). Potential of genetics for aquaculture development in Africa NAGA 26 (3):31-35.

Chevassus, B., (1979). Hybridization in Salmonids: results and Perspectives. *Aquaculture*, 17:113-128.

Chevassus, B., (1983). Hybridization in fish. *Aquaculture*, 33: 245-262.

Chevassus, B. and Coche, A.G. (eds) (1986): Report of the Symposium on Selection, Hybridization and Genetic Engineering in Aquaculture of six and Shellfish for consumption and stocking. Bordeaux, France, 27-30 1986. EIFA Tec. Pap./Doc. Tech,., CECPI, (50): 52p.

Chourrout, D., (1982a). La gynogenese Chez les vertebres: revue bibliographique. *Rep Nutr. Dev.,* 22:713-734.

Chourrout, D., (1982c). Induction of Tetraploidy in rain bow trout *(Salmo gairdneri* R.) by heat shocks. *Rep. Nutr. Dev*., 22:569-574.

Clay, D., (1977). Biology of the Tropical Catfish (Family Calriidae) with Special Emphasis on its Suitability for Culture. *Fish Marine Survey. Report*, N. 1458: pp 1 – 68.

Dale; L, and Degan., G. (1983): The influence of low density on food adaptation, cannibalism and growth of eels (*Anguilla unguilla*) Barmigdeh, *Bulletine For fish culture in Israel*, 35(2). Pp.53-60.

De Graaf G.J., Galemoni F., Banzoussi, B. (1995). Artificial Reproduction and Fingerling production of the African Catfish, *Clarias gariepinus* (Burchell 1822) in protected and unprotected ponds. *Aquaculture Research* Vol. 26, No.4, April 1995. ed. S.J. de Groot and R.J. Roberts. Black well Science.

Denton, T. E., (1973). Fish Chromosome Methodology (Charles Thomas Ed), Spring Field Illinois. Pp 150 - 152

Dunham, R.A; Majumdar, K; Hallerman, E; Bartley, D., Mair, G; Hulata, G; Liu, Z; Pongthana, N; Bakos, J; Penman, D; Gupta; M; Rothlisberg, P and Hoerstgen-Schwark, G; (2000). *Review of the status of Aquaculture genetics*. In: Aquaculture in the third millennium. Technical Proceedings of the conference on Aquaculture in the third millennium, Bangkok, Thailand, 20-25 February, 2000. 137-166pp. NACA, Bangkok and FAO, Rome.

FAO, (2007). *The State of World Fisheries and Aquaculture 2006*. FAO Fisheries and Aquaculture Department. Food and Agriculture Organization of the United Nations, Rome, 2007. Pp5-22.

Fagbenro (1996). Apparent Digestibility of crude and gross energy in some plant and animal-based feedstuff by *Clarias isheriensis* (siluriformes: Clariidae) (Sydenham 1980). *Journal of Applied Ichthyology,* 12:67 – 68.

Fleuren, W. (2008). Reproductive and Grow out Management of African Catfish in the Netherlands; in Ponzoni R.W.and N.H. Nguyen (eds) 2008. Proceeding of a workshop on the development of a genetic improvement program for African Catfish *Clarias gariepinus*. *World Fish Center Conference Proceedings number* 189. The World Fish Center, Penang, Malaysia, 130p.

Freund, F., H.S. Gabriele and H. Wolfgang (1995). Seasonality of the reproductive cycle of female *Heterobranchus longifilis* in tropical pond culture. *Aquaculture Living Resources.* 8: 297-304.

Haylor, G.S. (1989). A case for the African Catfish, *Clarias gariepinus* Burchell, 1822, Clariidae: A comparison of the relative merits of Tilapiine fishes, especially *Oreochromis niloticus* (L) and *C. gariepinus*, Burchell, for African Aquaculture. *Aquaculture Fish Management.*, 20: 279-285.

Haylor G.S. (1992). Controlled hatchery production of *Clarias gariepinus* (Burchell, 1822): An investigation of tank design and water flow rate appropriate for *Clarias gariepinus* in hatcheries. *Aquaculture and Fisheries Management* 23: 649-659.

Hester, F.E., (1970). Phylogentic Relationship of sunfishes as demonstrated by hybridization. *Trans American Fish society* Vol. 99:No.3.

Hickling, C.F., (1960), Malacca tilapia hybrids *Journal of Genetics*, 57:1-10.

Hickling, C.F. (1966). Fish Hybridization. Proc. of the World Symp. on Warm water Pond Fish Culture, Rome. 18-25 May. *Food and Agricultural Organization Fisheries Report*, 44(4):1-11.

Hickling, C.F., (1968). Fish Hybridization *Food and Agricultural Organization Fisheries Report*,
44:Vol. 4:1-11.

Hogendoorn, H. (1983). *The African Catfish (Clarias lazera C & V 1840)*, A new species for Aquaculture. Dissertation, Agric. Univ., Wageningen, the Netherlands, 135pp.

Hogendoorn, H. (1984). The African Catfish – A new species for Aquaculture. *Netherland Journal of Agricultural Science*, 32: 64-66.

Hubbs, C., (1970). Teleost Hybridization studies. *Proc. Calif. Acad. Sci*., 38:289 298.

Huisman, E.A., (1985). Aquaculture Research in the Africa Region. Proceedings of the African Seminar on Aquaculture organized by the International Foundation for Science (IFS) Stockholm, Sweden; Kenya 1985.

Huisman, E.A. and Richter, C.J. (1987). Reproduction, growth, heat control and Aquaculture potential of the African Catfish, *Clarias gariepinus* (Burchell, 1822). *Aquaculture* 63:1-14.

Jones, A., (1974). *World protein Sources*. Medical and technical Publication Co. Ltd. Lancaster, England. Pp 1 – 25.

Jorstad, K.E; Paulsen, O.I.; Naevdal, G. and Thorkildsen, S. (1994). *Genetics Studies of Cod, Gadus morhual*, in Masfjorden, Western Norway: Comparison between the local stock and released, artificially reared Cod Agriculture and Fisheries Management 1994, 25, Supplement 1, 77-91.

Kirpichnikov, V. A., (1970). Selective Breeding of carp and Interspecification of fish Breeding in ponds Hauchin 1. Inst. Kho. Leningrad.

Kirpichnikov, V.S., (1981). *Genetic Bases of Fish Selection*. Springer-Verlag (Books) Berlin, Heidelberh, 410 pp.

Klaus, T., (1987). Selection, Hybridization and Genetic Engineering in *Aquaculture* Vol. 1: pp. 304 – 309.

Lincoln, R.F., (1981a). Sexual maturation in triploid male plaice *(Pleuronectes platessa)* and plaice X Flounder *(Platichthys flessus)* hybrids. *Journal of Fish Biology,* 19:415 – 426.

Lincoln, R.F., (1981b) Sexual maturation in female triploid plaice, Pleuronectes platessa and Plaice x flounder, platichthys flesus, hybrids, *Journal of fish Biology*, 19: 499-507.

Langhotz, H.J. (1985). *Breeding Strategies in Fresh Water Fish.* In: Aquaculture Research in the African Region. Proceedings of the African Seminar on Aquaculture Organized by the International Foundation for Science (IFS), Stockholm, Sweden, held in Kisumu, Kenya, 7-11 October, 1985. Ed. E.A. Huisman. Pudoc Wageningen, 1986.

Legendre, M. (1983): Examen Preliminaire des potentialities d'un Silure Africain, *Heterobranchus longifilis* (Val. 1840). Pour l'aquacultuee en milieu lagunaire.

Document of *Science Centre for Research and Oceanography*. Abidjan, 14(2): 97-107.

Legendre. M. (1986): Seasonal changes in Sexual maturity and fecundity and HCG-induced breeding of the catfish, *Heterobanchus longifilis* Val (Clariidae) reared in Ebrie lagoon (IvoryCoast). *Aquaculture*, 55:201-213.

Legendre. M. (1992). A Comparative Study on the Morphology, Growth Rate and Reproduction of *Clarias gariepinus* (Burchell 1822): *Heterobranchus longifilis* Valencies, 1840 and their hybrids (Pisces, Clariidae). *Journal of Fish Biology*, 40: 59-79.

Ligia, V.A.S. (2002). Hazard Analysis Critical Control Point (HACCP), Microbial Safety and shelf Life of smoked Blue Catfish (*Ictaturus furcatus*). A Thesis submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College.

Madu, C.T., S. Mohammed, A. Mezie, J. Issa and E.O. Ita (1993). Comparative growth, survival and Morphometric characteristics of *Clarias gariepinus*, *Heterobrancus bidorsalis* and their hybrid fingerlings. National Institute of Freshwater and Fisheries Research 1992 Annual Report pp.56-61.

Madu, C.T. and Aluko, P.O. (1999). Hybrid Mudfish Production: comparative Growth and Survival of Hybrids and Purative parents. Proceedings of Biotechnologies Society (1999) 89-94.

Micha, J.C. (1973). Etude des populations piscicoles de l'Ubangui et tentatine de Selection et d'adaptation de quelque especes a l'etang de pisciculture. CFTC, Paris, 110pp.

Moav, R. (1979). *Genetic Improvement in Aquaculture industry*. In. T.R.V. Pillay and W.A. Dills (eds.), Advances in Aquaculture fishing News Books Ltd., Farnham, Surrey, England, pp.610-622.

Moses Y., Olufeagba, S.O. and Raphael, A.Z. (2005). Intra-specific Hybridization in Two Strains of *Clarias anguillaris* Linnaeus 1758. Unpublished research report of *Aquaculture and Biotechnology programme of* National Institute of Freshwater and Fisheries Research.

Mugabe, J. (2002). Biotechnology in the Sub-Saharan Africa: Towards a Policy Research Agenda. African Technology Policy Studies Network – Nairobi. http://knowledge.cta.int/en/content/downoad/624/6147.

Mustafa, S. and Rahman, R.A. (1999): *Marine genetic resources and Sustainable fisheries management.* In: genetics in sustainable Fisheries Management. Edited by Saleem Mustafa, Fishing News Books.

Nlewadim, A.A. (2002). Hybridization Studies in 3 Clariid fishes. A Ph. D. Thesis Submitted to Federal University of Agriculture, Umudike.

Nwadukwe, F.O., (1995). Hatchery Propagation of five hybrid groups by artificial hybridization of *Clarias gariepinus* (B) and *Heterobranchus longifilis* (VAL.) (Clariidae) using dry, powered carp pituitary hormone. *Journal of Aquaculture Tropical*, 10:1-11.

Nwadukwe, F.O. and Nawa, I.G. (2000). Effects of rearing periods on the production f hybrid catfish (*Clarias gariepinus x Heterobranchus longifilis*) fingerlings. *Journal of Aquatic Sciences 15*: 23-26.

Nygren, A., Nyman, L., Svensson, K. and Jahnke, G., (1975). Cytological and Biochemical studies in back crosses between the hybrid Atlantic Salmon X sea trout and its parental species. *Hereditas,* 81:55-62.

Ojutiku, R.O. (2008). Comparative Survival and Growth Rate of Clarias gariepinus and Heteroclarias Hatchlings fed Live and Frozen Daphnia. *Pakistan Journal of Nutrition* 7 (4): 527-529.

Okoro, E.A. (1995). Mass Production of *Heterobranchus longifilis, Clarias gariepinus* and their reciprocal hybrid fingerlings. A Ph. D. Dissertation Submitted to the Department of Biological Sciences, Univ. of Calabar.

Olaosebikan, B. D. and Raji, A., (1999). Field Guide to Nigerian Freshwater Fishes. Federal College of Freshwater Fisheries Technology, New – Bussa, Nigeria. 102 pp.

Ollivier, F., Nguenga, D; Teugels, G.G; (2000). Fertilization, hatching, survival and growth rates in reciprocal crosses of two strains of an African catfish, *Heterobranchus longifilis* Valenciennes 1840 under controlled hatchery conditions. *Aquaculture Research,* 2000. 31: 565-573.

Olufeagba, S.O. (1999). Induced triploid *Heterobranchus longifilis* Valenciennes, 1840 (familyClariidae0 and its aquaculture Potentials.

Olufeagba, S.O. and Yisa, M. (2005). Studies on Hatchability, Growth and Survival Rate in Holandis. National Institute of Freshwater and Fisheries Research New Bussa, Research Report Unpublished.

Plumb; J.A. (1975). Channel Virus experiments with different strains of channel catfish. *Trans American fisheries Society*. 104-143.

Purdom, C.E and Lincoln, R.F., (1974), Gynogenesis in hybrids within the Pleuronectidae. In: J.H.S Blaxter (Editor), The Early Life History of fish. Springer – Verlag, Berlin, Pp 537 – 544.

Reddy, P.V.G. (1999): Problems and prospects Fish Culture with special reference to developing countries. *Journal Fisheries Technology*. Vol. I. No.1 4-10, 1999.

Reed, W., Burchard, J., Hopson, A. J., Jennes, J., and Yaro, I., (1967). *Fish and Fisheries of Northern Nigeria*. Ministry of Agriculture, Northern Nigeria, pp. 78 – 79.

Salami, A. A., Balogun, N., Fagbemiro, S., and Edibite L., (1993). Utilization of Non Piscine Pituitary Extract in the Breeding of *Clarias gariepinus*. 10th Annual Conference *Fisheries Society of Nigeria*, Abeokuta 1992.

Sahoo, S.K., Giri, S.S., Sahu, A.K., and Ayyappan, S. (2003). Experimental hybridization between catfish *Clarias batrachus* (Linn.) X *Clarias gariepinus* (bur.) and performance of offspring in rearing operations. *Asian Fisheries Science* 16 (2003): 157-166.

Seagrant. J. (1987). *Genetic guideline for Fisheries Management*. Sea Grant Research Report Number 17, (Un Pub.) Minnesota University.

Skinner, H.J., (1938). Salmon and trout hybrids. Field, July, No. 30, P.258.

Stanley, J.G., Biggers, C.J and Schultz, D.E., (1976). Isozymes in androgenetic andgynogenetic white amur, gynogenetic carp and carp amur hybrids *J. Hered*., 67:129 – 134.

Streisinger, G., Walker, C., Dower, N., Knauber, D. and Singer, F., (1981). Production of clones of homozygous diploid Zebra fish *(Brachydanio rerio). Nature*, 291:293-296.

Susuki, R. and Fukuda, Y., (1972) Growth and Survival of F1 hybrids among Salmonid fishes. *Bull. Fresh water Fish Res. Lab.,* 21:177-138.

Susuki. R. and Fukuda, Y., (1973). Appearance and numerical characters of F1 \ hybrids among Salmonids fishes. *Bull. Freshwater Fish. Res. Lab*., 23:5-32.

Tave, D., (1993). Genetics for fish hatchery managers, second edition. An AVI Book.

Terao, T., Uchiyama, M., Kurahashi, S. and Matsumoto, H. (1965). Studies on the interspecific Salmonid hybrids between Chum Salmon *Oncorhynchus Keta* (Walbaum) and Kokanee Salmon *Oncorhynchus nerka* Var. *adonis* (Jordan *et* McGregor). III On some external characteristics and fertility of F1 hybrids. *Science Republic Hokkaido Fish Hatchery.* 20:29-36.

Teugels, G. G., (1990). A Systematic Revision of the African Catfish Genus Heterobranchus. (Geoffrey – Saint – Helaire 1809) (Pisces: Clariidae). *Zoological Journal of the Linnean Society.*, 98: 237 – 257.

Thorgaard, G. H., Jazwin, M.E., and Stier, A.R., (1981). Polyploidy Induced by heat shock in rainbow trout, *Trans American Fisheries Society*., 110:546-550.

Venden. B., J.P. and Bernacsek, G.M., (1990). Source Book for the Inland Fishery Resources of Africa: 2. CIFA Technical Paper No.18.2, FAO Rome, 411pp.

Wohlforth, G.W, (1994). The Unexploited Potential of Tilapia Hybrids in aquaculture. *Aquaculture and Fisheries Management*. 25:781-788.

World Fish Center (2004). GIFT Technology Manual: An Aid to Tilapia Selective Breeding. World Fish Center, Penang, Malaysia, 56p.

APPENDIX

ANOVA Survival Indoor (14 days) and Hatchability

		Sum of Squares	df	Mean Square	F	Sig.
Survival indoor	Between Groups	246.000	3	82.000	.243	.864
	Within Groups	2696.000	8	337.000		
	Total	2942.000	11			
Hatchability	Between Groups	362.542	3	120.847	.306	.820
	Within Groups	3155.155	8	394.394		
	Total	3517.698	11			

ANOVA

Survival Outdoor (8 weeks)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	584.375	3	194.792	2.602	.189
Within Groups	299.500	4	74.875		
Total	883.875	7			

WEIGHT INDOOR

CLXCL	HLXHL	HLXCL	CLXHL
11.6	3.2	5.0	5.0
7.3	5.5	4.6	6.3
8.1	5.0	5.4	6.4

. ANOVA table

Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean square**
Treatments (between columns)	3	7407.2	2469.1
Residuals (within columns)	4	1237.5	309.39
Total	7	8644.8	

F = 7.981 = (MStreatment/MSresidual)

LENGTH IN-DOOR

CLXCL	HLXHL	HLXCL	CLXHL
9.5	9.9	9.3	11.3
11.2	9.9	9.0	11.2
11.3	7.9	8.9	9.4

ANOVA table

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments (between columns)	3	6.793	2.264
Residuals (within columns)	8	7.087	0.8858
Total	11	13.880	

F = 2.556 =(MStreatment/MSresidual)

CLXCL	HLXHL	HLXCL	CLXHL
11.6	3.2	5.0	5.0
7.3	5.5	4.6	6.3
8.1	5.0	5.4	6.4

. ANOVA table

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments (between columns)	3	7407.2	2469.1
Residuals (within columns)	4	1237.5	309.39
Total	7	8644.8	

F = 7.981 = (MStreatment/MSresidual)

WEIGHT OUT-DOOR

CLXCL	HLXHL	HLXCL	CLXHL
274.2	309.0	212.1	261.2
302.1	278.5	225.9	237.2

ANOVA table

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments (between columns)	3	7407.2	2469.1
Residuals (within columns)	4	1237.5	309.39
Total	7	8644.8	

F = 7.981 =(MStreatment/MSresidual)

LENGTH OUT-DOOR:

CLXCL	HLXHL	HLXCL	CLXHL
9.26	9.88	9.22	8.84
10.36	9.10	7.70	8.94

ANOVA table

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments (between columns)	3	2.189	0.7295
Error (within columns)	4	2.069	0.5174
Total	7	4.258	

F = 1.410 =(MStreatment/MSresidual)