TILAPIA GENETICS:

Survival, Growth and Sex differentiation

A thesis submitted to the University of Stirling

for the degree of Doctor of Philosophy

by

Patrick M. K. Chipungu

INSTITUTE OF AQUACULTURE

University of Stirling

May 1987

TEXT
CUT OFF IN THE
ORIGINAL

PAGE
NUMBERS
CUT OFF
IN THE
ORIGINAL

MISSING

PRINT

Dedication

To my wife Jane and children

Delina, Enala and Amanda

Declaration

I declare that this thesis embodies results of research conducted by me in the Institute of Aquaculture, University of Stirling, during the period 1984 - 1986. The thesis has been composed by myself. All previous work consulted has been cited, and where appropriate, collaborative help has been acknowledged.

This work has not been submitted for any other degree.

Candidate: P. M. Chipungu

Supervisor:

Date:

Acknowledgements

I wish to gratefully acknowledge the help and invaluable guidance rendered by my supervisor, Dr B J McAndrew, throughout the study period.

Dr K Majumdar suggested alternative approaches to the study and those formed a useful basis for the work. Dr T Singh enthusiastically advised on the design of the hormone treatment experiments. Drs J Muir and K Rana gave me useful tips on hatchery management and fry rearing. My colleague, Dr Wahab occasionally helped with water analysis for which I am very grateful.

I also wish to express my sincere appreciation for help received from technical staff of the Tropical Aquarium, namely Ian MacGowan, Keith Ranson and Willie Hamilton; to the staff of the Histology preparation room, namely Paul and to Ian McEwan of the Genetics laboratory for initial guidance with enzyme electrophoresis.

My training in Britain was sponsored by the Commonwealth Scholarship Comission and later the Commonwealth Secretariat. To these I am indeed grateful. I am indebted to the Zambian Government for support and to the British Government for unreserved hospitality. The research facilities were provided by the Institute of

Aquaculture under the directorship of humorous Professor R J Roberts.

Mention is also due to Dr R Richards, Dr C Sommerville, Dr J Webster, Innocent Adikwu, Joe Wong, Sam Adgebe and other members of staff and fellow students in the Institute for making smiling possible even during difficult times. My wife Jane and children Delina, Enala and Amanda provided infinite psychological inspiration and living reasons for perseverance.

Finally, I wish to express my gratitute to Mrs Kay Anderson for diligently typing the manuscript.

Abstract

Production of all-male tilapia for aquaculture is assuming an increasingly important role.

An important pre-requisite to repeated obtainment of monosex tilapia is a clear understanding of the mechanisms underlying sex differentiation.

Histological observations on gonadal morphorgonesis and sex differentiation provided basic data for hormonal sex manipulation in four commercially important species. Results indicate that gonadal morphogenesis starts at different times ranging from eight days after hatching in O. mossambicus to 17 days in O. niloticus. Sex differentiation followed a similar pattern, and ranged from 22 days in O. mossambicus to 36 days in O. niloticus.

The effects of subjecting fish to different rearing temperatures was assessed. No significant influence was found on sex ratio of treated fish.

Observations on offspring sex ratio in intraspecific breeding and interspecific hybridization demonstrated that significant differences between batches are a common occurrance and their regularity cannot be adequately explained on the basis of sex chromosome theory alone.

Treating fish with synthetic androgen (17 α-methyltestosterone) and synthetic oestrogen, (17 α ethenylestradiol) resulted in species specific and dosage dependant differences in sex ratios. Results also revealed significant differences in sex ratios of different batches of fish subjected to the same treatment, thus demonstrating that success rate in sex inversion varies not only between species and between stocks, but in sib groups as well.

Results of intraspecific and interspecific breeding suggest that sex determination in tilapia is under the influence of multiple factors. Results of hormone treatments indicate variations in inversion rate at batch level, thus demonstrating presence of individual differences in lability. On the basis of results from these four experiments, it is hypothesized that sex in tilapia is influenced by multiple genes and the fishes' propencity to change sex varies in individual fish.

Progeny testing oestrogen sex inversed fish indicates that on the basis of the chromosome theory of sex determination, S. galileaus and O. niloticus are female homogametic, while O. macrochir is female heterogametic.

The implications of the results obtained in this study for production of all-male tilapia are briefly discussed.

CONTENTS

Page

CHAPTER 1

General Introduction and Literature Review

INTRODUCTION

Sex may be defined as the division of the individuals of a species into the two contrasted but complementary types known as male and female. Male is the term applied to the sex that is equipped to produce male-type gametes, while female applies to the sex equipped to produce female-type gametes.

In fish the two types of gametes are both physiologically and morphologically different: the male-type is relatively small, lean and actively motile. The female type is relatively large, basically spherical in shape and passive.

In fish, sexuality has great significance because there are differences in growth rate, behaviour pattern, body colour, shape and size between male and female in each species. For example in tilapia males often grow faster and to a larger size than females. In salmonids, females usually grow to a larger maximum size than males. Fish breeders or culturists often want to breed males and females separately or to achieve a monosex culture depending on biological or economic traits. An understanding of the sex determining mechanisms would increase our ability to regulate sex differentiation in favour of a sexual type offering the most benefits in aquaculture.

Sex categories in fish

In fishes, the expression of sexuality is greatly diversified and includes various types of sex chromosome mechanisms, gynogenetic reproduction, differentiated and undifferentiated gonochorism, synchronous, protandrous and protogynous hermaphroditism.

Most fish species can readily be distinguished into two sexes through one or a combination of the following morpho-genetic characteristics.

i anatomy of the external genitalia

ii secondary sexual characters such as sex specific colouration during courtship

iii anatomy of the accessory sexual apparatus such as ducts concerned with transference of the products of the gonads to the site of fertilization.

iv the anatomy and physiology, of the gonads, i.e. milt and ova.

v Sex chromosome constitution

vi Sex specific enzyme polymorphism.

The biology of sex determination and sex differentiation in vertebrates including fish has been studied quite extensively. (Reviews by Bacci, 1965; Reinboth, 1975 and Price, 1984).

The following sexual forms are generally recognized as representative among fishes.

i hermaphroditism

ii unisexualism

iii gonochorism

The literature reveals that

(a) hermaphroditism occurs in a few fish.

(b) unisexualism occurs in even fewer fish

(c) gonochorism with genotypic sex determination is the norm

(d) heteromorphic sex chromosomes are uncommon among fishes

There is a prevailing view that remote ancestors of vertebrates were hermaphroditic, that the earliest origins of gonochorism involved environmental control of sex determination, and that genotypic sex determination was established later with the gradual evolution of sex chromosome heteromorphism, (Mittowich, 1965; Reinboth, 1975; Bull, 1980). Witschi (1957) attempted to date the origin of genotypic determination in tetrapod vertebrates and proposed that it was established 150 million years ago.

Hermaphroditism

This is a form of reproduction which can be defined as presence of male and female reproductive tissues on the same individual. It is regarded as functional if the individual functions reproductively both as a male and as

a female during its life. Hermaphroditism can be described as normal in a species if it occurs in most or all the adults of a species. Hermaphrodites have no fixed chromosomal sex determiners, they can develop as protogynous, protandrous or synchronous hermaphrodites in response to internal physiological conditions often influenced by an external stimulus.

Hermaphrodites are said to be synchronous or simultaneous when both eggs and sperm mature at the same time. They are asynchronous or sequential when the fish functions as either a male or a female at any one time.

Hermaphroditism is widespread among invertebrates. Among vertebrates it is normal only among fish (Ghiselin, 1969). About 130 cases of hermaphroditism have been reported in various species of fish (Yamazaki, 1983). Hermaphroditism is most widespread among marine fish where more than 100 species from 15 families have been reported (Reinboth, 1970; Choat and Robertson, 1975).

Simultaneous hermaphroditism is very rare, but examples are known among members of the family Serranidae, the sea basses, for example Serranus subligarius (Clark, 1965) and Hypoplectras nigricans (Barlow, 1975; and Fischer, 1981).

A simultaneous hermaphrodite may be self-fertilizing as in S. subligarius or may 'trade' gametes as in H. nigricans.

Sequential harmaphroditism is the more common and two forms are generally recognized.

1. Protogyny, where a female fish reverses sex and becomes a male e.g. Labroides diminiata (Robertson, 1972) and Anthias squamipinnis (Fishelson, 1970)

2. Protandry, when a male changes functionally into a female e.g. Cobitis taenea (Reinboth, 1962; Lodi, 1967) and Gonostoma gracile (Smith, 1975).

Of the two, protogyny is the more commonly occuring form. The process of sex reversal is often under social control. Robertson, (1972) showed experimentally that a female Labroides diminiatus could be induced to reverse sex by removing an only male from a social group containing one male and 9-16 females. Fishelson (1970) made similar findings with Anthias squamipinnis.

Protandrous life cycles are rare in fish. Two well studied examples are Cobitis laenea (Lodi, 1967; Ghiselin, 1969; Smith, 1975) and the little coral reef fish Amphiprion which lives in monogamous pairs within the shelter of a sea anemone's tentacles (Fricke and Fricke, 1977).

Although some plants and invertebrates can change sex in both directions, sequential hermophroditism is a one way process in all fish in which it is exhibited (Freeman et

al, 1980). Sex reversal in vertebrates is a complex and time consuming process. Daly and Wilson (1983) reported that a month or more was required for gonads of a sequentially hermaphroditic fish to convert into functional producers of the alternative gamete type. In both A. squamipinnis (Shapiro, 1977) and L. diminiatus (Robertson, 1972) the complete reversal process takes 14 days. The time required for a complete sex change in Monopterus albus ranged from 3 - 5 months (Chan, 1970). A male Amphiprion bicinctus changed to a female and laid eggs after 26 days (Fricke and Fricke, 1977).

Depending on species, when a female fish changes sex, she may undergo striking changes in external colouration, morphology, general and reproductive behaviour and possibly a thorough reorganisation of the gonad. Histologically a gonad of a sex reversing individual (e.g. female) shows widespread degeneration of the ovarian tissue, and active proliferation of testicular tissue. This observation has been demonstrated experimentally in Monopterus albus (Chan, 1970), in Thalassoma bifasciatus (Reinboth, 1970) and in Anthias squamipinnis (Shapiro, 1977b).

Diandry is perhaps the most unusual form of sex inversion. The only example found in the literature was Thalassoma bifasciatus. In this species, Reinboth observed that some males differentiate directly as males while others

from the same brood passed through a functional female stage before transforming into males.

Occasionally functional hormaphrodites may occur in species which are normally bisexual, for example, certain domesticated strains of Xiphophorus helleri (Lodi, 1980) and in Onchorhynchus keta (Hikita and Hashimoto, 1978; Honma, 1980)

Experimentally induced synchronous hermaphroditism was achieved in Rainbow trout by administration of esterone to undifferentiated fry (Jalabert, et al, 1975).

Evidence for sex inversion in hermaphrodites consists of three observations:-

i. One sex predominates in a larger size range than the other sex. Often the alternative sex is not found at all in the small size ranges. (Choat and Robertson, 1975; Warner et al, 1975; Brusle and Brusle, 1975; Shapiro, 1977).

ii Gonadal histology changes dramatically with an increase in size of individual fish (Lodi, 1967).

iii In simultaneous hermaphrodites, gonadal histology shows presence of both male and female gametic tissue.

The instability of hermaphroditism has been interpreted by some researchers to mean that hermaphroditism is a process of sex differentiation rather than of sex determination (Gold, 1979; Kirpichnikov, 1981).

Most reports on hermaphroditism are descriptive and do not help to clarify the mechanism of natural sex determination or sex reversal. However, Lepori (1980) has proposed that the most important feature of the gonad in hermaphrodites is a genetical factor which makes the primary sexualizing substances produced by male and female parts of the gonad non diffusable. This enables hermaphroditism to occur by preventing any interaction between ovarian and testicular tissue.

Though often enclosed in a single capsule, the ovaries and testes of simultaneous hermaphrodites are well separated and have separate ducts to the exterior of the body (Reinboth, 1962; Smith, 1965; both cited by Fischer, 1981). The separation of the gonadal tissue makes internal self fertilization very unlikley.

Harrington (1967), Choat and Robertson (1975) and Fischer (1981) have demonstrated experimentally that eggs of normal self-fertilising simultaneous hermaphrodites (Rivulus marmoratus and H. nigricans) can only develop after contact with milt and that they cannot, therefore, be simple parthenogens. It is, however, quite likely that these fish may be reproducing gynogenetically.

Fischer has also demonstrated that H. nigricans trade eggs, i.e. giving up eggs to be fertilized by another individual in exchange for an opportunity to fertilize those of another individual. This behaviour would in part account for the evolutionary stability of hermaphroditism by providing a fecundity advantage similar to that of parthenogens over sexual organisms (Ghiselin, 1969; Fischer, 1981).

In hermaphroditic or undifferentiated species, sex reversal is rather complicated because the sex determining genes are labile and remain active until later stages of the gonadal development have been attained.

The recent successes on induction of functional synchronous hermaphroditism suggests the involvement of sex steroids in naturally occurring hermaphrodites.

Unisexual fishes

Unisexuality in fish is very uncommon, however examples are known whereby females produce only female offspring. Hubbs and Hubbs (1932) described unisexuality in Poecilia (mollienesia) formosa. This species mates with male Poecilia latipinna or with male P. mexicanna but reproduces only female offspring which are phenotypically and genetically identical to the maternal parent type only.

Investigations have proved conclusively that the monosexuality is a result of gynogenesis. The male only provides a stimulus to activate development of the ovum without syngamy (Kallman, 1964a).

Natural gynogenesis has also been reported in triploid populations of two Poecilliopsis species (Schultz, 1967) and Carrassius auratus gibelio (Lieder, 1965, cited by Yamamoto, 1969).

Under experimental conditions, unisexuality may be induced by either gynogenesis or hybridogenesis. In gynogenesis, irradiated sperm of a closely related species is used to trigger development of the egg nucleus but does not fuse with it.

In hybridogenesis, gametic fusion occurs and the paternal genome is expressed, but only the haploid female genome is transmitted to the ovum, for example, in crosses between members of two different genera such as Tilapia zillii and Oreochromis mossambicus. Offspring from this cross are all female, but enzyme electrophoresis reveals that these offspring are genetic hybrids. (See also Schultz, 1971).

Bisexual fish (Gonochorists)

Gonochorism is the existence of either testes or ovaries in one individual fish. Most cultured species have this gonochorist type of sexuality. Bisexual fishes are often

divided into two categories depending on the extent of sexual separation.

In the undifferentiated gonochorist (also called intersexes), the gonad first develops into an ovary-like gonad then about one half of the individuals develop into males while the other half become female. For example the eel (Anguilla anguilla), rainbow trout (Salmo gairdneri), the herring (Clupea harengus and the minnow, Phoximus laevis.

In the differentiated gonochorists, the undifferentiated gonad directly differentiates into either testes or ovary (Yamazaki, 1983). For example Platyish Xiphophorus maculatus and the medaka Oryzias latipes. The number of bony fishes is estimated at 21 - 25,000 species (Ebeling and Chen, 1970; Chan, and Yeung, 1983), and these show marked sexual dimorphism.

Several different modes of sexuality have been identified among bisexual fishes. It has often been stated that separation of sexes is advantageous to the process of speciation and that if heteromorphic chromosome pairs ensure dimorphism by effectively isolating a different segment of chromosomes, then it follows that diverse teleosts should show an array of cytological sex-determining mechanisms, some primitive others elaborately specialized, (Lagler et al, 1962; Atz, 1964; Alston, 1967; and Ohno, 1967)

The following are among the better known and most reported sex determining mechanisms.

i environmental sex determination
ii heterogametic sex chromosomes
iii autosomal sex determination
iv multiple sex chromosomes
v polygenic sex determination

The methods employed to investigate sex determination in fish are varied and include the following:-

i analysis of sex ratios in intraspecific crosses

ii analysis of sex ratios in interspecific hybrids

iii segregation analysis of sex linked pigment genes

iv Cytological analysis of sex chromosomes

v analysis of progeny sex ratio in artifically sex reversed fish

vi analysis of sex ratio in fish obtained by artificial gynogonesis, androgenesis and artificial polyploidization.

vii analysis of sex linked enzyme heteromorphism.

In the present work, four techniques are used comparatively to investigate sex determining mechanisms in Tilapiine fishes.

Biology and history of tilapia culture

Members of the tribe Tilapiini, family Cichlidae, are warm water fish found naturally in Africa and parts of the Mediterranean region as far north as Syria (Trewavas, 1983). The group comprises over 70 species (Fukusho, 1968; Trewavas, 1983) conveniently grouped by Trewavas (1981 and 1983) into three broad subgenera according to their reproductive biology and brooding habits. In the present work, the tribal name tilapia is used frequently throughout the text. It can be distinguished from the generic name of one of the sub groups by the use of a small 't'. The genus Tilapia (with a capital 'T') implies the substrate spawning species, the genus Sarotherodon applies to paternal mouth breeders (e.g. S. melanotheron) and biparental mouth brooders (e.g. S. galileaus). The third genus, Oreochromis, comprises maternal mouth brooders such as O. macrochir, O. niloticus, and O. mossambicus. At the moment, Oreochromis species comprise about 40 species including most of the popularly cultured species. These three groups of tilapia exhibit parental care to varying degrees. For example Tilapia species jointly protect the egg nest and the developing larvae until larvae are capable of effectively fending for themselves and exhibiting escape mechanism. Some Sarotherodon species

jointly incubate the eggs in the mouth and will also protect larvae until they are fully developed (i.e. 7-10 days); Oreochromis species are mouth brooders. Only the female incubates eggs and broods larvae.

Rearing of tilapia in ponds is said to have been in practice in Egypt as far back as 2500 B.C. (Bardach et al, 1972). However, experimental culture of tilapia is reported to have started in Africa around 1924 (Balarin and Hatton, 1979; Ferreira, 1986).

Since 1938 some tilapia species have been introduced into most tropical and subtropical areas throughout the world. The most well documented of the introductions was that of O. mossambicus which was introduced into Java between 1938 and 1939 and was quickly spread throughout the sub region as a substitute for Asiatic carps which could not be imported from China during the occupation of the region by the Japanese (Hofstede and Botke, 1950; quoted by H.S. Swingle (1968). It was during this period that O. mossambicus earned its reputation as a 'wonder fish'. By 1950 O.mossambicus had been broadcast into several other countries including the Philippines, Taiwan, Sri Lanka, Malaysia, West Indies, India, South East Asia, Hawaii and the Southern U.S.A.

Tilapia have now been introduced into some temperate countries such as China, France, Germany and Britain under controlled conditions or for research and eventual

stocking in heated effluents of power stations (Kirk, 1972).

While the common carp (Cyprinus carpio) must still be considered the foremost pondfish in the world, O. mossambicus claims this position in tropical countries (Swingle, 1968).

Advantages of tilapia culture

The worldwide interest in tilapia culture stems from their great diversity and their generally favourable culture characteristics which include the following:-

1. Organoleptic characteristics (Crawford et al, 1978), Tilapia have a pleasing general appearance, firm textured flesh, and readily acceptable flavour, hence readily marketable.

2. Most tilapias have "high" reproductive capacity even under culture conditions. Generally all fresh water temperate zone fishes spawning in the Spring or early summer have gonadal recrudescence in winter or in Spring in response to long photo periods and warm temperatures (Harrington, 1957; Henderson, 1963; de Vlaming, 1972; Kaya and Hasler, 1972; Sundararai, 1976). On the contrary, most tilapias may spawn at frequent intervals throughout their reproductive life if conditions are suitable.

3. Adaptability to a wide range of environmental conditions including salt water and sewage ponds (Chimits, 1957; Balarin and Hatten, 1979; Trewavas, 1983). Most cultured tilapias can tolerate a wide range of environmental temperature conditions. For example Caulton (1975, 1977) observed that Tilapia rendalli practiced a marked diel movement from offshore into the shallow shoreline areas of tropical lakes. During these daylight movements, young T. rendalli often experience thermal changes varying from less than 20°C at night to 36°C during the day.

Balarin and Hatton (1979) recorded that the tilapia pH tolerance range is from 5 to 11. During the course of my work, pH as low as 3.5 was observed to be tolerable for a few hours in a recirculated tank.

Adaptability to salt water is also species specific and upper tolerance limits have been shown in different reports to vary from >50% (18‰) for O. niloticus, O. macrochir and T. rendalli, to 65% in O. spilurus and S. galileaus, and 100% or higher for O. mossambicus, O. aureus and T. zillii (Chervinski and Yashouv, 1971; Balarin and Hatton, 1979. Wolhfarth and Hulata 1981; Al-amouldi, 1982). Chervinski and Herring (1973) reported that T. zillii can survive, grow and reproduce in sea water; Kirk (1972) reports finding S. galileaus, O. niloticus and T. zillii in

Great bitter lakes of Egypt at salinities between 13.5 and 22.4‰.

Studies on oxygen demand in O. mossambicus and O. niloticus (Maruyama, 1958; Babiker, 1958) have shown that these fish can for a short time tolerate oxygen levels as low as 1mg/litre. This feat has been attributed to tilapia's ability to load oxygen to very low tensions (Denzer, 1967).

4. Wide diversity of food types and tilapias preparedness to take artificial diets.

The food habits of tilapia are very varied but for simplicity they could broadly be put into three classes as follows:-

(a) phytophagous species such as O. macrochir and S. galileaus have a capability to utilize blue green algae.

(b) macrophyte feeders such as T. rendalli and T. zillii are equipped with strong pharyngeal teeth and have the capability to secrete strong acids (Caulton, 1976; Trawavas, 1983).

(c) Omnivorous species are less specialized and include O. mortimeri, O. andersonii and O. aureus. Chi mits (1957) noted that O.

mossambicus is the only cultured fish known to take Euglena sanguinea.

5. Relatively rapid growth rates.

Some tilapias, especially those from the genera Sarotherodon and Oreochromis grow comparatively fast and can reach marketable size after six months. For example Galman (1986) reported growth rates of up to 1 kilogramme per year in O. niloticus. If supplied with an appropriate formula feed, the feed conversion ratio (FCR) can be as low as one (Chapters 4 and 5).

6. Other attractive aspects of tilapia culture include

(a) their potential for biological control of aquatic weeds and hence the control of malaria, and the use of specific tilapias to disrupt the life cycle of Schistosoma species by feeding on the host snails (Coche 1967)

(b) Tilapia have also been cultured for use as bait fish in Tuna fishing

(c) as a laboratory animal, tilapia are becoming popular in genetic experiments because of the ease with which they spawn in captivity and their tendency to breed relatively prolifically.

Limitations in tilapia culture

Despite all the favourable characteristics, commerical culture of tilapia is still in its infancy. The single most important reason for the slow development of tilapia culture is their prolific breeding which frequently results in over-crowding and stunted growth (Hickling, 1960; Bardach et al, 1972; Dadzie, 1982; Mires, 1983). In order to improve productivity in tilapia culture, several workers have proposed alternative means of increasing growth rate and ultimate fish size.

As a result of worldwide culture activities, good data base now exists on many aspects of Tilapia biology, and most of the research effort can now be directed towards improving growth performance.

Relative to salmonids and cyprinids, tilapia are far less fecund. They produce comparatively small batches of eggs (Oreochromis and Sarotherodon up to 2500 eggs per spawn), but because of a highly efficient parental care system which includes mouth breeding and subsequent fry protection, hatchability and survival is usually very high.

Lagler et al, (1962) have shown that fecundity in fish is inversely proportional to the degree of parental care in a species, and Welcomme (1967) showed that for the egg brooding O. leucostica, the total egg production is approximately equal to the square of the length, while for

the nest spawning T. zillii, egg production approaches the cube of the length.

The genus Tilapia comprises substrate spawners, these lay comparatively more eggs (up to 6000 per spawn) than either Oreochromis or Sarotherodon species. Although mouth brooding does not take place, the bond between the breeding pairs in Tilapia is much stronger than in the other two genera. The egg nest is aggressively defended by both parents and fry are tended until they are capable of leading a fairly independent existence. For the same size fish, Tilapia eggs are smaller, yolk material at hatching is also little and fry require to take food orally at a relatively early age (Lowe-McConnell 1955)

Frequent spawning coupled with high survival rates is the major cause of overcrowding, lowered growth rates and stunting, resulting in sexual maturity at a small size. Iles (1973) has suggested that such stunting probably represents an adaptive mechanism which enables tilapia populations to withstand high mortality rates.

In many developing countries, small fish are prepared whole in traditional dishes. However, it is clear that the ultimate objective in modern fish farming is to produce fish at a profit. Marketing small bony fish can therefore cause a problem to the catering industry, especially so in industrialized countries where protein availability is not

very limiting, and in areas which are readily accessible to large marine species. In such areas the demand for tilapia, and hence the market price will increase with fish size.

Basic and biological research has demonstrated that tilapia culture can become more productive if suitable techniques are developed to control sexual reproduction as a means to controlling overcrowding (Lowe McConnell, 1955c; R Welcomme 1964a; Fryer and Iles, 1972; Bardach et al, 1972; Heut, 1975; Coche, 1982). The technique popularly suggested involves physical and physiological disruption of the populations' reproductive capacity. Many reports have shown that in tilapia, males generally grow at a faster rate and to a larger size than females from the same brood (Hickling, 1967; Pruginin, 1967; Shell, 1967). This growth rate difference was as much as 50% in O. mossambicus.

Methods that have been tried for obtaining monosex fish for improving growth performance and for increasing fish size at sexual maturity include the following:

I Monosex culture of all male broods

1. Manual sexing of fingerlings when sex differentiation of the genital papillae is possible (Lovshin and Da Silva, 1976; Mires, 1977).

This technique has been extensively used in commercial farms in Israel, and by 1977, the number of hand sexed Oreochromis aureus was around 3 million per year (Mires, 1977).

Hand sexing is unlikely to become adopted universally because of the difficulty in manual sexing of fingerlings. The technique is labour intensive and tends to be very wasteful since a significant biomass of female fingerlings in which capital has been invested is discarded.

2. Interspecific hybridization has not always proved satisfactory because of the difficulty of obtaining pure strains of parent stocks for cross-breeding coupled with insufficient detail on the sex inheritance mechanism in the experimental animals.

Confirmed pure stocks of some popularly cultured tilapia species are only now becoming available (McAndrew and Majumdar, 1983), but it will be some time before these and any others can become universally available in research laboratories and fish farms.

Chapter VI of this work considers aspects of interspecific hybridization in sex determinism.

3. Endocrine control of sexual cycle by application of antigonadotropin substances and sex hormones (Eckstein and Spira, 1965; Clemens and Inslee, 1968; Guerrero, 1975; Dadzie, 1975; Yamazaki, 1976; Tayamen and Shelton, 1978).

Hormone induced sex inversion is still facing technical problems of applicability on a commercial scale (Mires, 1983). It would be very costly to raise several batches of tilapia according to age groups, and to feed them with a hormone added diet for several weeks. The unpredictability of the long term effects of the Hormone to the human consumer is another consideration yet to be adequately researched (Tsoi, 1969; Jones, et al, 1975; Johnstone et al, 1983).

Application of hormones in tilapia culture and their use in predicting genetic sex is the subject of Chapters VII and VIII of this work.

II Predator/Prey Interaction

Stocking of a predatory species with tilapia in the same ponds has been attempted with limited success. It was conceptualized that stocking ponds with predators would eliminate tilapia fry and fingerlings so that a low population density is maintained.

The probable reasons for the limited success are the lack of full understanding of predator prey interaction mechanisms, insufficient attention to the selection of ideal species for use, insufficient data on optimal stocking rates and inadequate appraisal of the ecological interactions in the culture environment.

III **Polyculture of different tilapia species as well as culture of tilapia species with other non predatory species such as carps. This technique has been adopted almost universally in extensive as well as in intensive system especially in Africa, Asia and Israel.**

One of the drawbacks with this method is its simplicity hence lack of proper investigations on its use. Basically the problems here are very similar to those stated for the predator/prey combination in II above. With a few exceptions (e.g. Israel) knowledge on optimum stocking rates is still lacking and often the position of species used in the pond food web are not adequately considered before stocking.

High stocking rates during the very early stages was suggested by Swingle (1968). The method does not seem to have been taken seriously by industry and no records of its application could be found in the literature. Clearly the technique could be self defeating since we do not know for sure what

fingerling stage stunting sets in. It is also a well known fact that crowding sets in when fish start reproducing, not from early larvae stages.

IV Cage Culture

Rearing of tilapia in cages would disrupt the process of nest building and inhibit the practice of mouth brooding due to total loss of gametes through the meshes. It is anticipated that even if some eggs were fertilized by chance encounter, the lack of parental care would preclude their normal development (Pagan-Font, 1975).

Tilapia cage culture has been used successfully in producing relatively large fish for marketing (Coche, 1976; Guerrero, 1977; Rifai, 1980).

Cage culture seems to offer partial refuge by effectively maintaining the population at a pre-determined density. However, the technique falls short of solving the problem since it does not eliminate the sexual dimorphism in growth between males and females which is manifested even more strongly following sexual maturation.

It is also known that even inside the cage, tilapias will indulge in spawning activity and sometimes even spawn, thus seriously reducing the proportion of

metabolic energy available to the fish for body growth.

V Chemosterilization

This technique makes use of chemical mutagens to destroy and disorientate chromatin in sperm. Generally this technique is poorly investigated and both application rates and success rates are not well documented. The technique is of practical interest because it is easier to apply to large amounts of sperm at the same time. Treatment could also be performed in the field without the need for sophisticated equipment (Balarin and Hatton, 1979).

Tsoi (1969) fertilized rainbow trout and Coregonus peled ova with sperm from fish treated with the mutagen dimethylsulphate and obtained embryos and fingerlings that were highly defective. Toluidine blue (Uwa, 1965) and ethyleneurea (Jones et al, 1975) have been applied directly to sperm which subsequently yielded gynogenetic embryos. More recent applications of chemicals to influence gametic sterility in fish has been reported by Stanley (1979), Donaldson and Hunter (1982) and Chourrout (1986). Chourrout used different concentrations of dimethylsulphate to induce haploidy in rainbow trout. Chemical concentrations and treatment durations were estimated. It was noted that haploid mortality in

the treatment occurred continually throughout the development period.

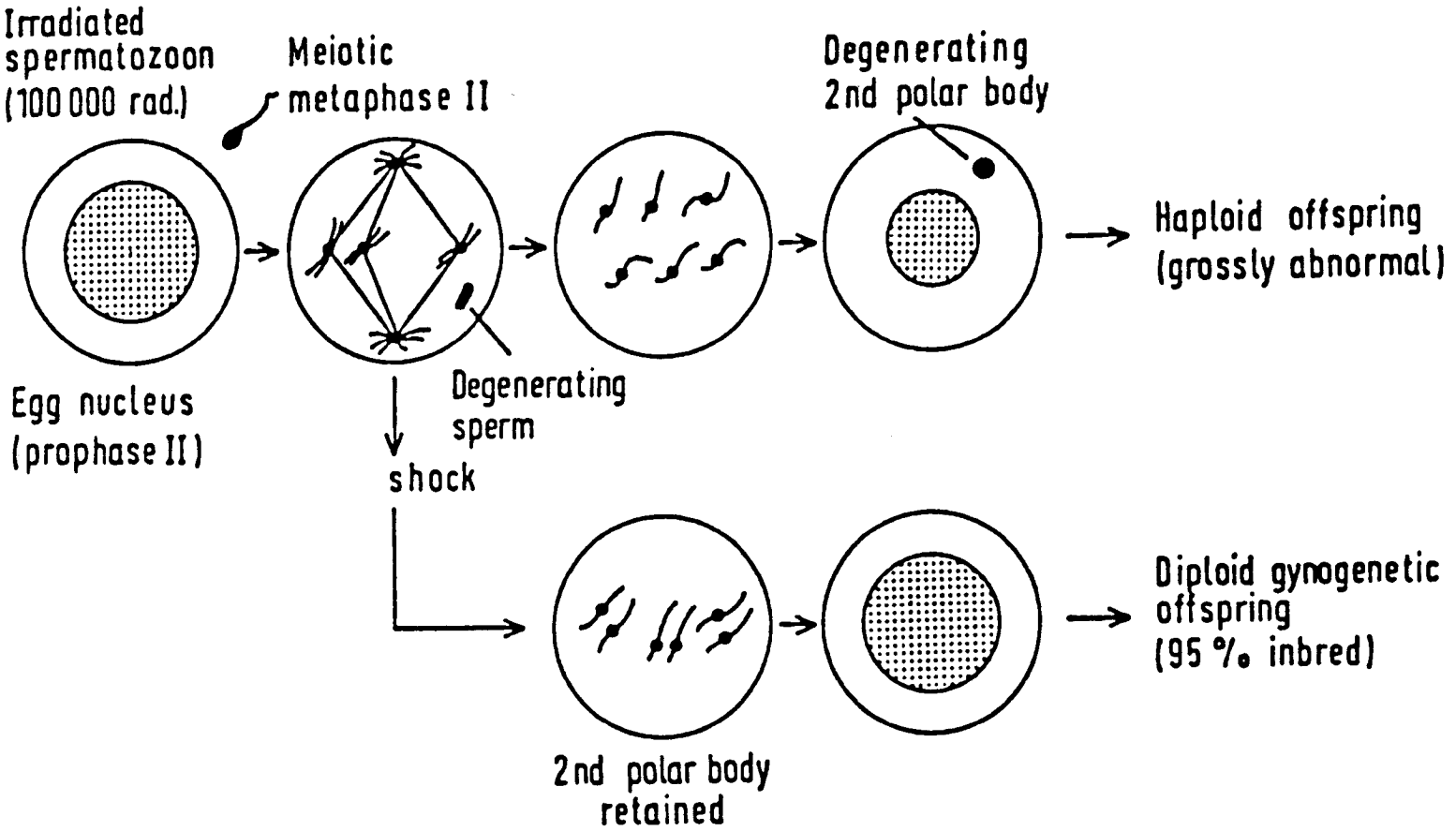
The use of chemical mutagens, cytotoxins and pesticides to induce sterility in fish could pose environmental problems (Donaldson and Hunter, 1982).

VI 1. Artificial gynogenesis

Gynogenesis is a rare form of reproduction in which embryonic development in eggs is induced by sperm without the sperm contributing its genetic material. The ovum nucleus develops with unreduced number of chromosomes (Thorgaard, 1983).

Artificially, gynogenesis is induced by fertilization of the eggs with irradiated sperm of the same or a closely related species. Following fertilization, temperature or high pressure shock is applied to restore diploidy in the zygote (Golovinskaya, 1972; Stanley, 1976a; Nagy et al., 1978; Johnstone et al., 1978; Refstie et al., 1982; Chourrout and Quillett, 1982, and Kowtal, 1986).

Gynogenesis is particularly useful for sex determination experiments in species in which no marker genes or heteromorphic sex chromosomes have been identified. Since gynogenetic



Diagrammatic representation of induced gynogenesis.

offspring receive only maternal genes, they may be all-female, or females and males depending on the genetic sex determining mechanism of the particular species.

If suppression of the first mitotic cleavage is used to diploidize the maternal set, the collected embryos are considered to be homozygous at all loci because they result from fusion of two mitotic products. If, on the other hand, diploidization of the maternal set of chromosomes is achieved by retention of the second polar body, the resulting embryo starts from two different end products of the same meiosis and so is not homozygous at all the loci. Such embryos are called heterozygous diploid gynogenetics. Failure of the second meiotic division brings together sister chromosomes in the zygote i.e. XX in species with female homogamety and either ZZ or WW in species with female heterogamety. Thus species with female homogamety are expected to yield only females and species with female heterogamety would yield half males with normal ZZ genotype and half super females with an unusual WW genotype (Stanley, 1976).

Gynogenesis was first observed in frogs by Hertwig in 1911 (Nagy et al, 1979). It is a

natural mode of reproduction in a few species of fish including Poecilia formosa (Hubbs and Hubbs, 1932) and Carrassius auratus gibelio (Cherfas, 1966).

Diploid gynogenesis was first introduced in Cyprinus carpio, Acipenser guldenstadti and Misgurnus fossilis by Romashov et al. in 1961 (quoted by A Nagy et al, 1978). Since then there have been many reports on successful inductions of gynogenesis in various species of fish (Golovinskaya, 1968; Purdom, 1969; Stanley and Sneed, 1973; Cherfas, 1975; Penman et al, 1986 and many others). Arising from the above experiments, the following species resulted in 100% female gynogenetic progeny:

Species	Reference
Cyprinus carpio	Golovinskaya, 1969
	Nagy et al, 1978
Ctenopharyngodon idella	Stanley, 1976
Salmo gairdneri	Chourrout and
	Quillet, 1982
	Refstie et al, 1982
Oncorhynchus kisutch	Refstie et al, 1982
Oreochromis niloticus	Penman et al, 1986
Oreochromis mossambicus	" " " "

The production of 100% females by gynogenesis is consistent with a female homogametic (XX) sex determining mechanism.

In other experiments, Purdom and Lincoln (1973) obtained 24 females and 14 males among gynogenetic plaice. Streisinger et al.,(1981), also obtained males and females in Zebra fish; Gervai et al., (1980) also found both males and females among gynogenetic paradise fish; and Penman et al.,(1986) obtained 90 females, 5 males and 3 intersexes among gynogenetic O. aureus.

The presence of males in these batches is indicative of female heterogamety or a polygenic sex determination. Comprehensive reviews on gynogenesis in fish have been written by Kirpichnikov (1981), Donaldson and Hunter (1982) and Purdom (1983)

Artificial induction of gynogenesis has generated a lot of interest among researchers because it is a very promising technique for rapid inbreeding, production of all female broods, and as a tool in sex determination.

Based on survival rates reported so far, gynogenetic techniques are poorly developed and

a lot more work is still required to improve yields.

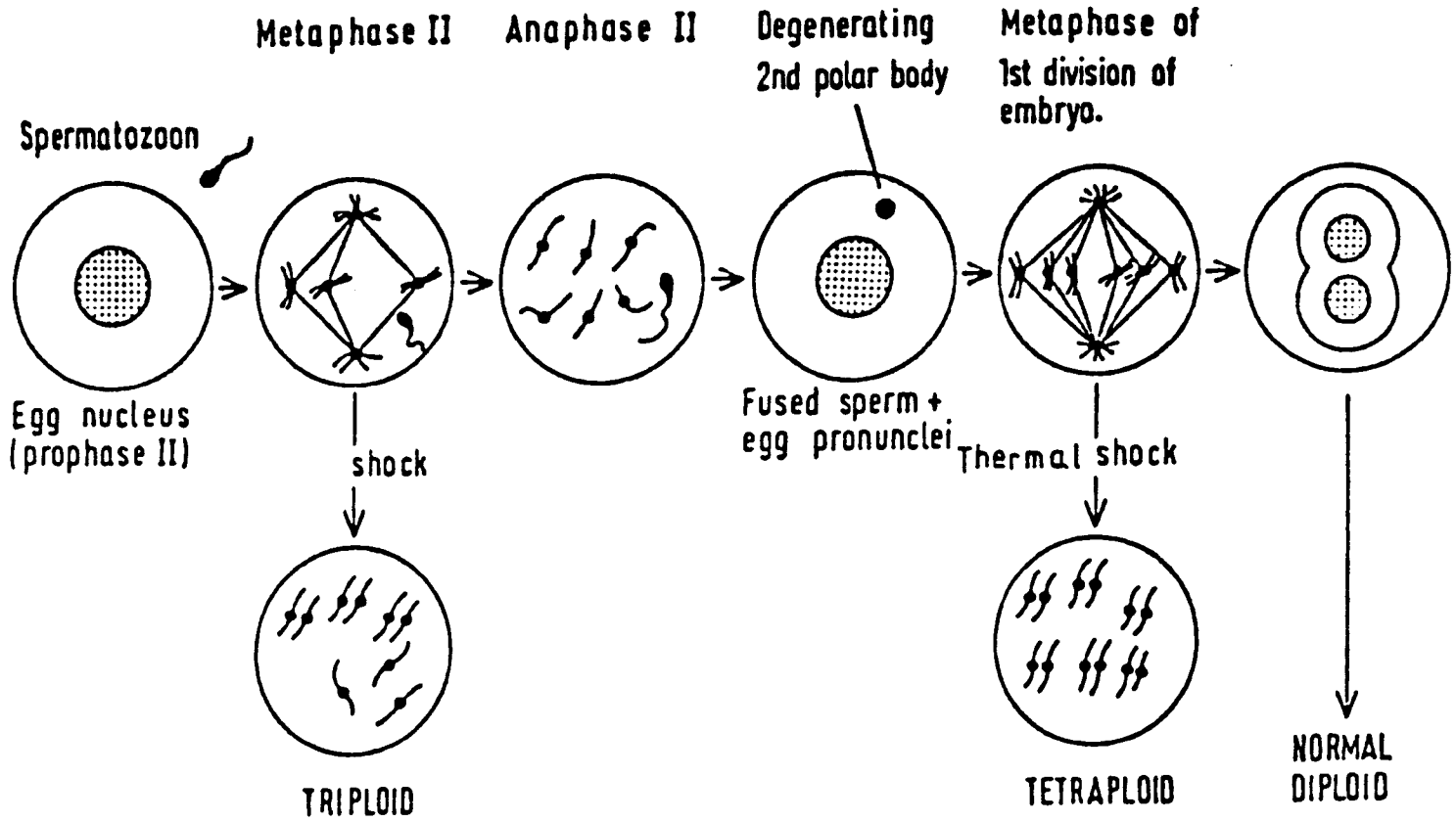
2. Androgenesis is a closely related concept to gynogenesis. Androgenesis can be induced by exposing eggs to radiation and then fertilizing them with normal untreated sperm. As for gynogenesis, diploidization is restored by applying either temperature or high pressure shock (Arai et al, 1978; Parsons and Thorgaard, 1984; Kowtal and Cherr, 1986).

Androgenesis offers potential for the production of all male broods in homogametic male, and a direct means of assessing sex determining mechanisms through sex ratio analysis.

Irradiation of egg chromatin for androgenesis proved especially tricky because of the thick Chorion, and obtainment of viable fry has so far been extremely unsatisfactory. The scope for research is still unlimited.

3. Triploidization

A number of natural triploids have been reported among fish. The most well documented of these are Carassius auratus gibelio (Cherfas, 1966); and some natural hybrids of Poeciliopsis (Cimino, 1972).



Diagrammatic representation of induced polyploidy.

Natural triploids often do not differ significantly from their diploid bisexual forms in viability, growth rate and final size (Nagy, 1986).

Hybridization of certain distantly related species has been reported to yield some triploid hybrids among the offspring, for example Pleuronectes platessa x Platichthys flesus (Purdom, 1972; Lincoln, 1981) and Ctenopharyngodon idella x Hypothalmichthys nobilis (Marian and Krasznai, 1978). This method of producing triploids did not work on attempted crosses of Oreochromis x Tilapia males.

Induced triploidization is achieved by physical treatment of the zygote shortly after fertilization of normal eggs with normal sperm. The early shock is intended to restore the second polar body. If the sublethal temperature or high pressure shock is applied shortly before first mitotic division, then endomitosis takes place and tetraploid embryos result.

Theoretically both triploids and tetraploid should grow faster and to a larger size than the normal diploid fish. Results so far have not

been in line with theory. In several reports triploids or tetraploids showed no significant difference from the normal fish. (Gervai et al, 1980; Nagy, 1986; Shah and Beardmore, 1986).

While triploids are theoretically sterile, tetraploids are not. The prospect for all sterile fish has so far been the basis for the interest on their production.

Triploids are easier to produce than gynogenetic fish and have been produced with relative ease, and much higher yields have been obtained than has hitherto been possible with gynogenesis.

Among the successes reported are rainbow trout (Thorgaard , 1983), pacific salmon (Utter et al, 1983), atlantic salmon (Johnstone, 1985), hannel cat fish (Bidwell et al, 1985) and tilapia (Chourrout and Itskovitch, 1983; Shah and Beardmore, 1986). Commercial production of triploid fish has been reported in grass carp (Hungary, USSR, USA) trout (France and Scotland).

Triploid sex ratios are directly useful in predicting genetic sex determination in fish.

VI Other techniques for controlling reproduction in tilapia for aquaculture.

The following techniques show good prospects but still need extensive investigation.

1. Selective breeding for specific characterisation. Kincaid (1986) defined a strain as a population that exhibits reproducible physiological, morphological or cultural performance characteristics that are significantly different from those of other fish populations of the same species or a broodstock derived from such a population and maintained thereafter as a pure breeding population. Differences in strain characteristics are a consequence of evolutionary or selection pressure, but may be accelerated by man.

Selective breeding is probably one of the oldest known, yet one that has rarely been applied consciously (Cherfas, 1975).

Significant strain differences between stocks were obtained from estimates of heritabilities and phenotypic variability of traits which led Langholz and Schwark (1986) to reiterate the importance of selection programmes for superior populations with regard to specific traits.

Other recent works demonstrating the importance of selection programmes include a report on intergrated fish culture in China (Li Sifa et al., 1986), a report on Chinook Salmon red colour inheritance in British Columbia (Withler, 1986), a report on growth rate inheritance in silver carp (Kronert et al., 1986) and growth rate and sex ratio in tilapia (Smitherman et al., 1984).

Tilapia characteristics likely to benefit from selection work include rapid growth, late maturation, higher dressed weight, adaptation to adverse environmental factors, resistance to disease and production of all-male progenies (Bardach et al, 1972; Tave and Smitherman, 1980; Wohlfarth et al., 1983).

2. Transplantation of growth hormones

Growth hormones are essential for normal growth and development of pre-adult fish. Information regarding growth hormones in fish is at the moment very scanty though not completely lacking. Extraction of growth hormones has been done in Acipenser guldenstadti (Farmer et al., 1981), and in common carp (Cook et al., 1983). The main objective in growth hormone extraction is to investigate its mode of action for eventual improvement of growth rates of fish

by molecular, biological approaches. Maclean et al. (1987) discuss technological aspects, implications and alternative techniques for introducing novel gene sequences into fish. They suggest splicing the coding sequence to a strong promoter such as mouse metallothion 1 gene as a means of increasing the chances of good expression. Although there are some difficulties in assaying expression of injected sequencies, Maclean et al. (1987) contend that genes with obvious phenotypic effects such as that of the growth hormone can easily be assessed from comparison with controls as was demonstrated by one of the authors on work with loach.

According to Chen et al. (1986) growth hormone is a single chain of polypeptide, produced by the somatotrophs, in the anterior portion of the pituitary gland. In their recent work, they report successful obtainment of a growth hormone by partially purifying a synthetic hormone from a cell extract they referred to as pAF51. This growth hormone was injected into rainbow trout at varying dosages. Preliminary results showed that after treatment with the semi-purified synthetic growth hormone for four weeks at 1µg/10 g of body weight, the weight gain in the experimental group was two times that of the

control group. These results clearly demonstrate the potential for utilizing the synthetic growth hormone in fish farming in order to promote rapid growth.

Although by no means exhaustive the foregoing review serves to put into perspective the variety of techniques which could be used for controlling reproduction in tilapia and for ultimate improvement to the gross yield in tilapia culture.

Most of the techniques reviewed in this section have not been fully investigated. Preliminary results do however indicate that the prospects for enhanced productivity among the tilapiine fish lies in the production of sterile fish or monosex fish of the male phenotype. In order for effective sex control to be realized, it is important to understand the underlying factors that influence sex differentiation.

The present study is aimed at comparing four alternative manipulations for predicting sex determining mechanisms in different species of tilapia. In view of their importance in commercial fish farming, survival and growth are evaluated along with sex differentiation.

CHAPTER 2

General Materials and Methods

GENERAL MATERIALS AND METHODS

1. Location of Facility

The experiments reported in this thesis were conducted in the tropical fish facility and laboratories of the Institute of Aquaculture, University of Stirling, Stirling, Scotland. The work was undertaken between July 1984 and September, 1986.

2. Experimental Fish

The fish used in the experiments were from three genera in the tribe Tilapiini as described in the preceeding section. The species were selected with regard to generic representation and popularity in culture practices as follows:-

i Genus Tilapia

For most of the time, the species in this genus show little or no sexual dimorphism. Size difference between males and females is not manifested until long after sexual maturity as demonstrated in T. mariae. Courtship related colouration is not strongly manifested except in T. zillii where both male and female develop deep red colour.

The two Tilapia species used in the present study were T. zillii and T. mariae. The former

Plate 2.1 Mature T. mariae male (M) and female (F). Papillae (P) are recessed and virtually indistinct in both sexes

has been used extensively as a weed controller in polyculture systems and in extensive integrated systems. On the other hand T. mariae is literally unknown outside the aquarium fish trade and in its native range in West Africa. Preliminary growth trials showed that by comparison, T. mariae exhibits faster growth rate and utilizes food more efficiently during the larval and juvenile periods. In culture systems this advantage, plus the very high survival rates would make it comparatively more productive.

Both species exhibited strong pair bonding long before onset of courtship. Courtship is prolonged and pair bonding is maintained through the incubation period, hatching and early feeding stages during which both male and female guard the eggs, and later tend the fry.

Both species are substrate spawners. the female lays a large number of sticky eggs which adhere to the substrate. The genital papillae in both male and female are small and recessed, becoming perceptible only during late stages of courtship.

11 Genus Sarotherodon

Only one species of the genus Sarotherodon was available for these experiments. The species used was the biparental mouth brooder, S. galileaus, originally from Lake Turkana in Kenya. The purity of the species was confirmed electrophoretically by McAndrew and Majumdar (1983).

Sexual dimorphism in this species was not discernable until fish were at least 50 g in weight. At this stage, males developed longer pectoral fins than females. In both sexes external genitalia are tiny and barely distinguishable between the two sexes. The situation improves as courtship advances and especialy shortly before spawning. There were no sex-linked colour changes during courtship and at spawning. Courtship was brief and eggs were extruded in one heap over a period that rarely exceeded two minutes. The egg batch was simulataneously taken up for mouth brooding by both parents. The eggs were larger than those of the genus Tilapia. But had only a vestigial layer of adhesive material. This layer helps to congregate eggs during spawning but it is broken up as soon as the eggs are taken into the mouth.

Pair bonding took place a few days before spawning, but the pair did not always maintain close contact especially during mouth brooding. Under stress, the male ingested the brood more readily and thereafter adopted an aggressive stance towards the still brooding female. Both male and female S. galileaus readily accepted a new spawning mate.

iii Genus Oreochromis

Most tilapia used in aquaculture are from this group. The genus comprises maternal mouth brooders. The species included in the present study were selected on the basis of their present or anticipated importance for fish farming. Specific details are given in each experiment.

The species used and their original source are as follows:-

Species	Source
O. macrochir	Botswana
O. mossambicus	Aquarist stock
O. spilurus	Tana river (Kenya)
O. aureus	Lake Manzala (Egypt)
O. niloticus	Lake Manzala (Egypt)

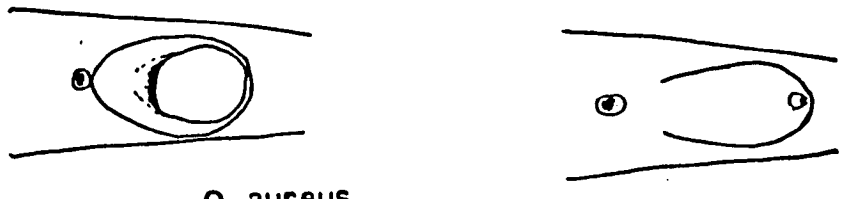
All the fish were previously electrophoretically checked for purity by McAndrew and Majumdar, (1983, 1984).

All the five species of Oreochromis used in the studies exhibited distinct form of sexual dimorphism. Unlike in the genus Tilapia and the genus Sarotherodon, Oreochromis males were invariably larger than their sib females. Urogenital papillae were comparatively larger and easily distinguished between males and females. Generally male pectoral fins in mature fish extended to or beyond the urogenital opening while those of females were noticeably shorter. Courtship colouration was species specific and for O. mossambicus, O. spiluris and O. aureus colouration was also sex specific.

All the fish exhibited varying degrees of pair bonding with O. spilurus and O. macrochir showing stronger bonds than the other three. Males manifested territorial behaviour, engaged in nest building activity and selected an ovulated female for spawning. Unlike in the genus Tilapia and the genus Sarotherodon, the male-female association is easily and frequently interrupted by other females visiting the nest site. Often the intruding females actively participate in parallel courtship although

FEMALE MALE

O.niloticus



O. aureus

O. macrochir

T.zillii

Figure 2:1 Diagramatic structure of genitatia of O. niloticus, O. aureus, O. macrochir and T. zilli - after McAndrew, 1985

simultaneous group spawning was never noticed.

Courtship period varied with size of fish, but for O. niloticus, O. aureus and O. mossambicus, the period from start of nest building to spawning rarely exceeds 3 - 4 hours. Spawning period also varies with size of fish and clutch. In large females (> 500 gr), the period can be as long as 30 minutes.

Eggs of all five species were free of any adhesive layer. The number of eggs laid per spawn is comparatively small by comparison with those of either Sarotherodon or Tilapia species. Oreochromis eggs were larger (2.8 - 4.2 mm) and, like those of S. galileaus, had large yolk reserves.

Once a female has completed spawning, the male usually drives her away from his territory in preference for an unspawned female. During the course of this study it was not unusual for a male to spawn with two or three females within the same day.

Experimental Systems

With the exception of the experiments on the influence of temperature on sex differentiation, all other trials were carried out in recirculating water systems. Temperature was thermostatically controlled and water quality was monitored weekly. Dissolved oxygen was maintained in The

range 6 - 8 mg/l by a constant supply of compressed air. pH was controlled at 6.0 - 7.0 by regular flushing of each experimental system when necessary. Local temperature variations are reported under the relevant sections.

i **Spawning arena**

Two x 2 metre diameter fibreglass tanks were used to obtain fry for hormone treatment studies reported in Section 5.2.1 and 5.2.2. The two tanks were part of an eight tank broodstock set up. The water temperature in the system was maintained at 27.5 ± 0.5°C by a 3 KW submersible thermostatically controlled heater.

ii **Glass tank spawning system**

A recirculating system comprising seven glass aquaria each measuring 100 cm x 40 cm x 30 cm was used for obtaining eggs used in artificial fertilization experiments. The header tank had a capacity of 220 litres and the four settlement tanks each had a capacity of 115 litres. The total capacity of the system was 1410 litres, thus the tank to volume ratio was 0.50. The four settlement tanks had plastic ring filters. A 1.0 H.P. centrifugal pump was used to circulate the water. (Plate 2)

Plate 2.2: A unit of 48 fry rearing cubicles for retention of first feeding fry during hormone treatment. The black polythene cover was used to control algal growth. Capacity of each tank is 3 litres

iii **Incubation System**

The incubation system comprised a recirculating set up with a maximum water capacity of 350 litres. The header tank, the settlement tank and the sump tank each had a capacity of 100 litres. There were eighteen incubation cubicles measuring 20 x 10 x 15 cms and arranged in parallel. Water flow to each cubicle was independently supplied through a 1 mm diameter perspex tubing. In addition to the plastic ring filters in the settlement tank, the system had four gravel filled filter trays positioned immediately above the settling tanks. The incoming water was passed through a 30 watt ultra violet sterilizer to destroy pathogens.

Water temperature was maintained at 26.0 ± 1.0° by a 200 watt heater. Compressed air was supplied through air stones placed in the header tank. A 0.5 H.P. pump was fitted to circulate water at approximately 9.5 litres per minute.

iv **Fry rearing system**

The fry rearing system used consisted of a 220 litre header tank, four x 220 litre settling tanks, a sump tank and six units of rearing tanks. Each unit of rearing tanks consisted of ten x 15 litre perspex glass tanks arranged in parallel roles of 5 tanks each side. A 1.0 H.P. centrifugal pump was used to maintain the circulation. The ten tanks in each

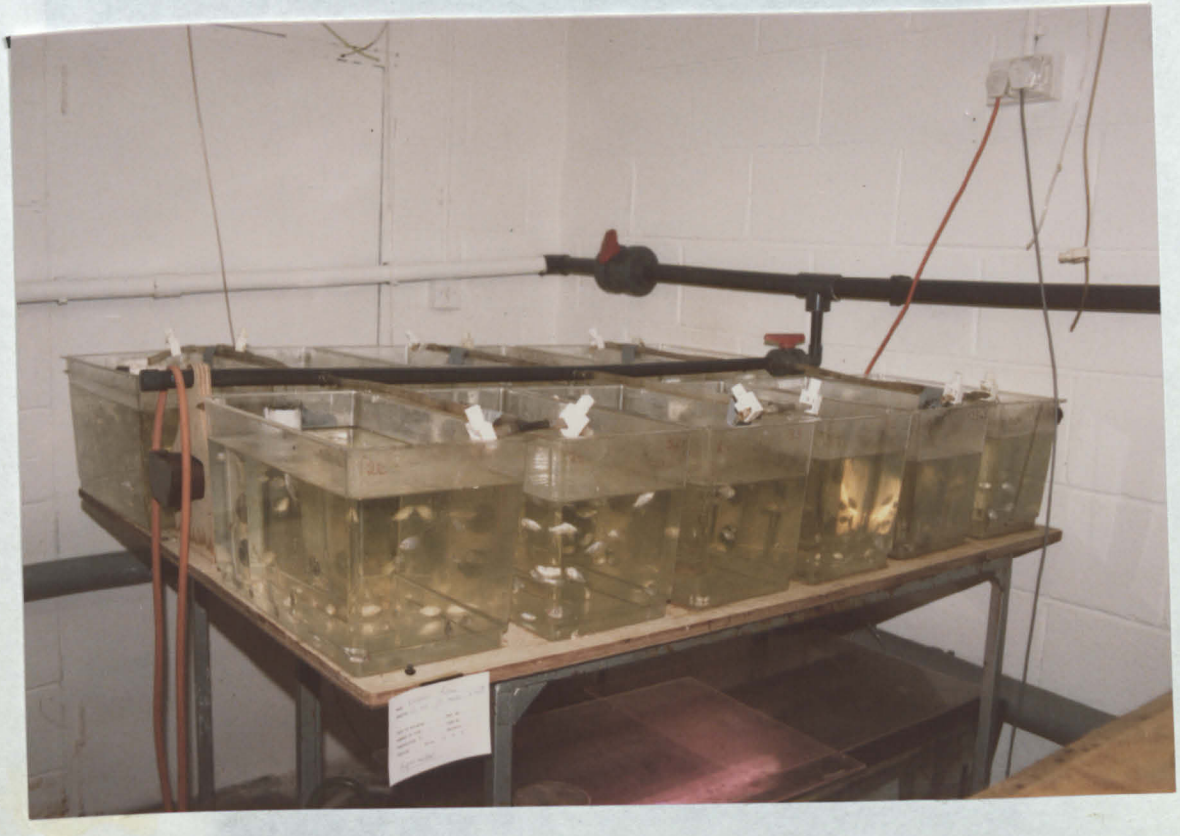


Plate 2.3 A fry rearing unit consisting of 12 x 15 litre glass tanks.

Plate 2.4 Part of a set of 16 glass tanks for controlled spawning

unit were independently supplied with water at approximately 1 litre per tank per minute through one or more fine holes leading out of a half inch diameter plastic pipe. (Plate 3)

Water temperature in the system was thermostatically controlled at 27.5 ± 0.5°C by a 3 KW submersible heater placed in the header tank.

Alongside the main system, a small set-up comprising 36 x 3 litre perspex glass cubicles was built to provide additional space for rearing individual groups of first feeding fry at a higher density. This facility was particularly useful in initial rearing of hormone treated fish where a high food to fry encounter was required. (Plate 4)

v **Grow-out system**

This set up consisted of three x 115 litre header tanks, seven x 115 litre settling tanks filled with plastic ring filters, and 24 x 60 litre rearing tanks arranged in double roles of six tanks each on a two tier system.

This system was used for growth studies of mature fish and later for keeping broodstock.

Additional filtration in this system was ensured through a stack of gravel filled trays situated below

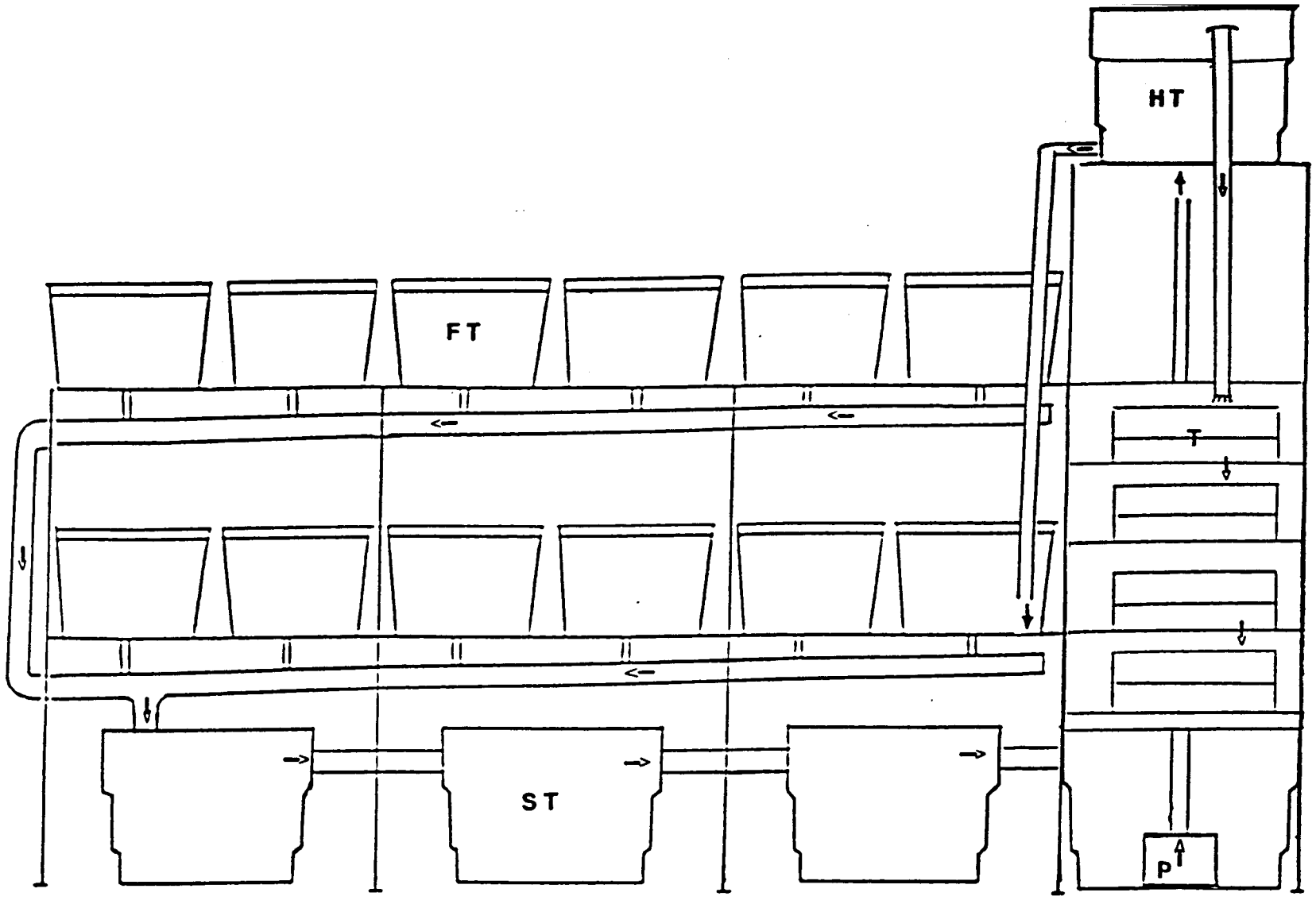


Figure 2.2: Diagramatic side view of the twenty four tank grow out system. The Header tank (HT), Pump (P), plastic fish tanks (FT) and settling tanks (ST) are shown

the overflow and immediately above the sump tank. The total capacity of the system was 2000 litres. Maximum tank capacity was 960 litres, and the tank to volume ratio was 0.48. A submersible pump discharging at 160 litres per minute was used to maintain circulation.

Water temperature was maintained around 26.5 ± 1.0°C by means of a thermostatically controlled 3 Kilowatt submersible heater.

4. Breeding Procedure

1. Natural spawning

Arrangement for spawning mates were made taking into account the observed specific behaviour as outlined earlier in this chapter.

In order to obtain natural spawns, O. mossambicus, O. niloticus and O. aureus were stocked as spawning families. The ideal ratios used were one male to five or six females in the 2 metre diameter spawning arena, and one male to three females in the large glass aquaria.

The strong pair bonding behaviour of T. zillii, T. mariae and to a lesser extent S. galileaus was assisted through careful selection and stocking of compatible partners.

Pair stocking was also used in Oreochromis species used in single pair spawning experiments (Chapter 5.I).

1.1 Obtaining eggs from natural spawns

Fish were fed at least twice daily at 2 - 3% body weight per day. Females were checked for spawning signs (i.e. inflamed papillae and mouth brooding) each day. 24-36 hours after spawning, Oreochromis and Sarotherodon eggs were removed from the mouth, examined under a microscope for embryonic development, counted and placed in incubation jars in the incubation system.

The eggs were kept gently rolling by an upwelling flow to emulate conditions in the brooders mouth and to minimize chance of fungal spore settlement and egg clumping.

Oreochromis eggs hatched in 4 - 5 days at 26 ± 0.5°C S. galileaus eggs hatched in four days.

After spawning naturally, eggs and larvae of substrate spawners (Tilapia) were left undisturbed until fry were actively swimming around. Generally eggs hatched in three days and were ready for first feeding by day six from spawning.

2. **In vitro fertilization**

Hydribization experiments and backcrosses required interference with the natural mode of spawning. A strict control of fertilization process was therefore implemented.

i Collection and storage of milt.

Under the favourable conditions prevailing in the tropical aquarium (controlled temperature, constant aeration and a 12 hour daylight) a large proportion of male broodstock were in ripe running state.

To collect milt for artificial fertilization a selected mature male was anaesthetized lightly in Benzocaine. The genital region was blotted with soft tissue to remove excess fluid. The belly of the fish was then gently squeezed starting immediately behind the pectoral fins, backwards to the genital pore. The stripping was repeated a few more times.

Milt samples contaminated with blood, urine or faeces were discarded. Dense properly collected milt was placed in small (1-2 ml) open vials and stored at 4°C for use within 24 hours.

ii Colletion of eggs

Potential breeding females were selected and stocked in large glass aquaria with an 'exciter' male. Two to three times each day, the genitalia of the females was visually checked to see if the papilla had swollen. If it had, then both the male and the female were kept under constant surveillance for courtship encounter.

The sequence for courtship leading to spawning as exemplified by O. mossambicus was as follows:-

The papilla of the female swells up indicating ovulation. The male establishes a terrirory in a quiet corner of the tank starts courtship display and nest building activity. The male then selects a receptive female that is ovulated and drives her into his territory. For some time the pair swim up and down in rhythmic style before the female joins in nest clearing. As courtship intensifies, both male and female make increasingly frequent sweeping dives into the nest followed by rolling dives, displaying the silver side. About 10 - 15 minutes later, the first batch of

eggs (about 15-20) is extruded into the nest.

In species from the genus Tilapia, and to a lesser extent in large Oreochromis specimens where the act of spawning takes fairly long, one can afford to wait until the first few eggs are dropped into the nest before removing the female for stripping. However, in S. galileaus, pair bonding is prolonged but courtship and spawning are quite brief. In this case the intensity of courtship and the frequency of visits to the nest by an ovulated female is sufficient indication of female readiness to spawn.

As soon as a female is presumed ready to spawn, she was gently fished out, mildly anaethetized and stripped by applying light pressure to the sides of the belly as described for sperm collection (2.1 above).

iii Artifical fertilization

Before fertilization, eggs were divided into sub-batches depending on the experiment to be carried out. Fertilization was carried out within fifteen minutes using either freshly collected sperm or sperm preserved

at 4°C in a refrigerator as described in 2(i) above.

Milt was added to the egg batch at a rate of 2 - 3 drops per 100 eggs. The mixture was left to stand for 15-30 seconds before adding a few drops of water at room temperature. The eggs and milt were then gently swirled with a soft paint brush and left to stand for a further 1½ - 2 minutes before adding more water (flooding) or transferring eggs to incubation trays in a water bath.

iv **Egg incubation**

Three to four hours after fertilization, eggs were transferred to incubation jars in the incubation system.

After hatching, fry were left undisturbed until they were ready for first feeding at 6 - 8 days old after hatching.

5. **Food preparation**

Throughout the experiment, the basic food used was the standard Ewos trout preparation. For small fish, number 3 diet was finely ground and graded to suit fish size as follows:-

Fish Weight	Food Particle Size/Number
< 1 gr	200 µ
1 - 2	500 µ
2 - 20	# 3
20 - 80	# 4
> 80	# 5

6. **Feeding regime**

Fish on a maintenance diet were fed according to the following schedule

Fish Weight (g)	**Feeding Rate % bwd**	**Frequency/ day**
< 10	10 - 7.5	3
10 - 40	7.5 - 3	3
> 40	3 - 2	2

Feeding rates and frequency in all experiments incorporating growth trials are reported separately under the relevant sections.

CHAPTER 3

Experiment I

Gonadal development and sex differentiation

3.1 INTRODUCTION

Production of monosex tilapia for aquaculture is assuming an increasingly important role. Of the several alternative techniques for the production of monosex tilapia, the use of steroid hormones either alone or in combination with other manipulations such as triploidy and gynogenesis seems the most potentious. Yamamoto (1969) suggested measures for improving efficiency of hormonal sex inversion. Among the most important factors, was the need to administer hormones starting at the undifferentiated stage and continuing through the differentiation phase of the gonads. Many workers after him have emphasized this fact and demonstrated that administration of hormones later than the onset of differentiation and failure to follow the treatment through the gonadal sex differentiation phase is a basic cause of failure to obtain complete functional sex inversion in tilapia (Shelton et al, 1978; Jensen et al, 1979).

Despite the importance of this fact, little experimental work has been performed and so far only a few reports have been devoted to investigations on the morphological process of sex differentiation (Eckstein and Spira, 1965; Nakamura and Takahashi, 1973; Yoshikawa and Oguri,

1977). The present study is aimed at providing basic information on histological development of the gonad and to serve as a base for controlled application of steroid hormones in experimental sex inversion of tilapia. The study also investigates the influences of temperature and of steroid hormones on the process of gonadal development and sex differentiation.

Temperature was chosen for investigation because of its key role as the environmental factor most often associated with change in physiological processes. Steroid hormones have been shown by many workers to have an influence on the balance of sexes, but their effect on gonadal differentiation as a process has only been reported once in O. aureus (Eckstein and Spira, 1965).

The species selected for this work were the same ones earmarked for use in hormone treatment experiments (Chapter VII). These are O. mossambicus, O. niloticus, O. aureus and S. galileaus.

3.2 MATERIALS AND METHODS

Experiments were carried out to determine the onset of sex differentiation in four groups of fish as follows:-

i Pure O. niloticus, O. aureus, O. mossambicus and S. galileaus.

ii First generation hybrids of O. niloticus x O. aureus.

iii O. niloticus, and O. mossambicus fed a diet treated with 17 α Methyltestosterone at 50 mg/kg

iv O. aureus and O. niloticus reared at 21.0°C, 27.5°C and 35°C

Fry used in the experiments were obtained by artificial fertilization as described in 2.4.2 and reared in the fry rearing system described under section 2.3.3 above. Each brood was transferred from the incubation vials into the experimental rearing system 6 - 8 days after hatching. Sampling started at the start of the experiments. Fifteen to twenty fry were taken from each tank every two to three days, weighed, measured and preserved in Bouin's solution for subsequent processing and histological sexing (Appendix 1 and 2). Sampling was continued until about fifty days after hatching.

Fish in i, ii, and iv were fed a normal micronized trout diet (200 - 500 μm) three times

daily at 10% body weight per day. Fish in group iii of the experiment were fed a diet to which 17 α-Methyltestosterone at 50 mg/kg had been added as detailed in Chapter II , Section 5.

Because of the necessity to maintain a constant temperature in several tanks, fish in experiment iv were kept in static tanks measuring 40 x 15 x 20 cm in a constant temperature room. To ensure good water quality, approximately 25% of the water was changed every two days and each tank was separately aerated.

The mid portion of each fixed specimen was cut in sections measuring 5 - 10 μm and stained as indicated in the results. Sections were examined for gonadogenesis using an Olympus microscope at a magnification of 250 or 400.

3.3 RESULTS

Gonads of both mature and immature fish were examined to establish their normal appearance and the appearance of sexually developing gonads. A back-tracking technique was used to locate sites of gonadal morphogenesis in undifferentiated specimens.

For the purpose of this study, gonadal development is used synonymously with gonadal morphogenesis, and refers to the stage when cells start to appear as paired gonads, and sex differentiation refers to the stage of development when morphological distinction between sexes becomes possible.

Location of gonads

The gonads develop in the posterior body cavity immediately ventral to the trunk kidney and gas bladder. The gonad consists of two lobes joined posteriorly. Each lobe is covered by a thick muscular tunica. Two short ducts extend from the posterior end of the gonad and join together to form the genital opening.

In developed gonads the posterior region of the testes is composed of branched coiled tubules. Developed ovaries are tubular in shape and are enclosed in fibrous connective tissue covered by mesothelium.

It is generally accepted that the primordial germ cells are the sole source of the definitive germinal elements (Hardisty, 1965; Yoshikawa and Oguri, 1978). Primordial germ cells differ from somatic cells in being round and relatively larger. Primordial germ cells have a clear cytoplasm and a large nucleus.

At the stage described here as gonadal development, the gonad appears in transverse section as a pair of oval lobes connected to the mid dorsal wall of the body cavity by short stalks. (Plate 7)

The gonadal lobes consist of delicate fibrous tissue which later lead to the formation of groups of deuterogonia of varying sizes within the undifferentiated gonad.

Further development leads to the gonad being lobulated and formation of nests or pockets of cells.

As early as 22 days after hatching, the potential ovaries were recognizable by the number, relative size and distribution of the primordial germ cells. The first testis distinguished in O. mossambicus at 25 days after hatching.

3.3.1 Sex differentiation in Sarotherodon galileaus

Observations were conducted in a recirculated glass system at 27.5 ± 0.5°C. Samples of about 15 fry were removed every 2 days starting on the fourth day.

Results have been arranged in five day interval groups and are presented in Tables 3.1 and 3.2.

Table 3.1 Distribution of sexually differentiating S. galilaeus fry by age class

Age-Class days	Sample	No sexually developed	differentiation ratio	male	female	undifferentiated
0 - 5	20	0	0	0	0	20
6 - 10	59	0	0	0	0	59
11 - 15	55	5	0,29	0	0	55
16 - 20	50	40	0,80	0	0	50
21 - 25	37	37	1,0	0	0	37
26 - 30	57	57	1,0	3	21	31
31 - 35	34	34	1,0	9	16	9
36 - 40	14	14	1,0	8	6	0

Table 3.2 Distribution of sexually differentiating S. galilaeus fry fed an MT impregnated diet

Age-Class days	Sample	No sexually developed	differentiation ratio	male	female	undifferentiated
0 - 5	19	0	0	0	0	19
6 - 10	39	5	0,26	0	0	39
11 - 15	17	15	0,88	0	0	17
16 - 20	36	35	0,97	0	0	36
21 - 25	29	29	1,00	9	1	19
26 - 30	14	14	1,0	11	0	3
31 - 35	16	16	1,0	11	0	5
36 - 40	19	19	1,0	14	0	5
41 - 45	17	17	1,0	15	0	2

Tables 3.1 and 3.2 show the distribution of sexually differentiated fry in normal fry and in fry raised on a diet with 50 mg/kg of Methyltestosterone.

The data in Table 3.1 and 3.2, and Fig 3.1 show that morphological development of the gonads in S. galilaeus starts between day 12 and day 14 and is complete by day 19.

Sexual differentiation (the morphological distinction between maleness and femaleness) was not observed until after day 22 in females and day 26 in males (Fig 3.2). Differentiation in female was complete by day 35 and in the male around day 40.

High correlation was found between sexual development rate with age, and sexual development rate with standard length.

3.3.2 Sex differentiation in O. mossambicus

Fry used in the two arrangements were obtained from a single spawn.

From Tables 3.3 and 3.4 it can be seen that gonadal development became morphologically discernable in 8 - 10 day old fry. Sexual differentiation became

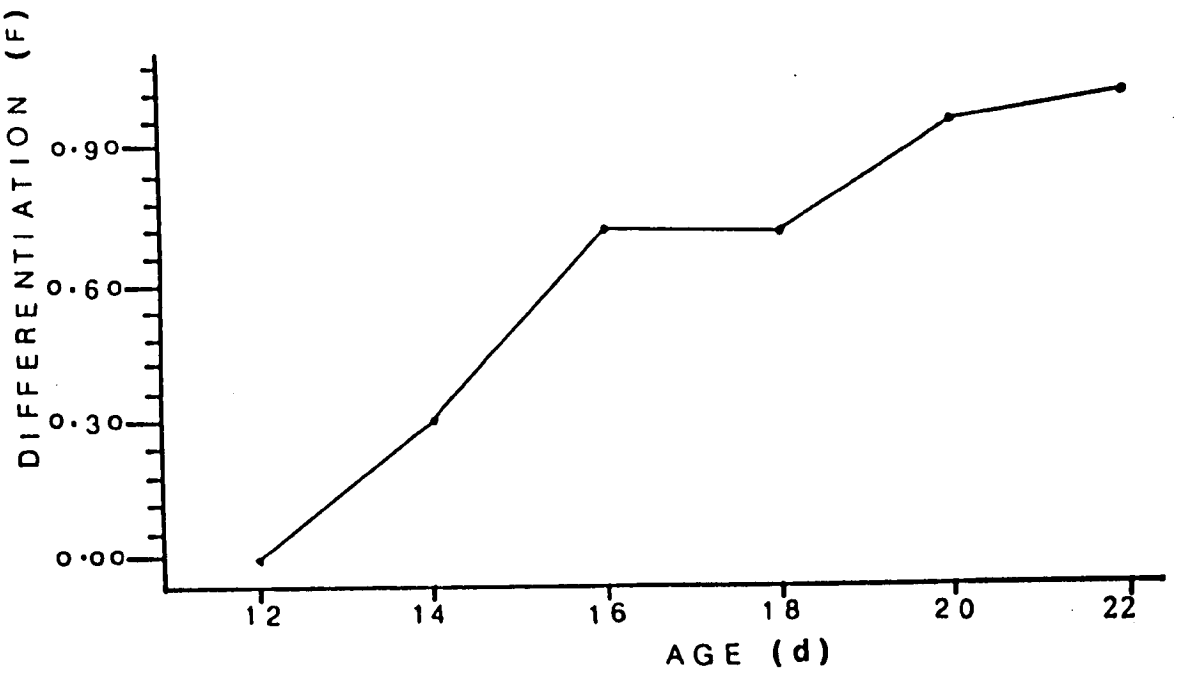


Figure 3.1: Distribution of sexually differentiating S. galileaus fry ... (proportion of total)

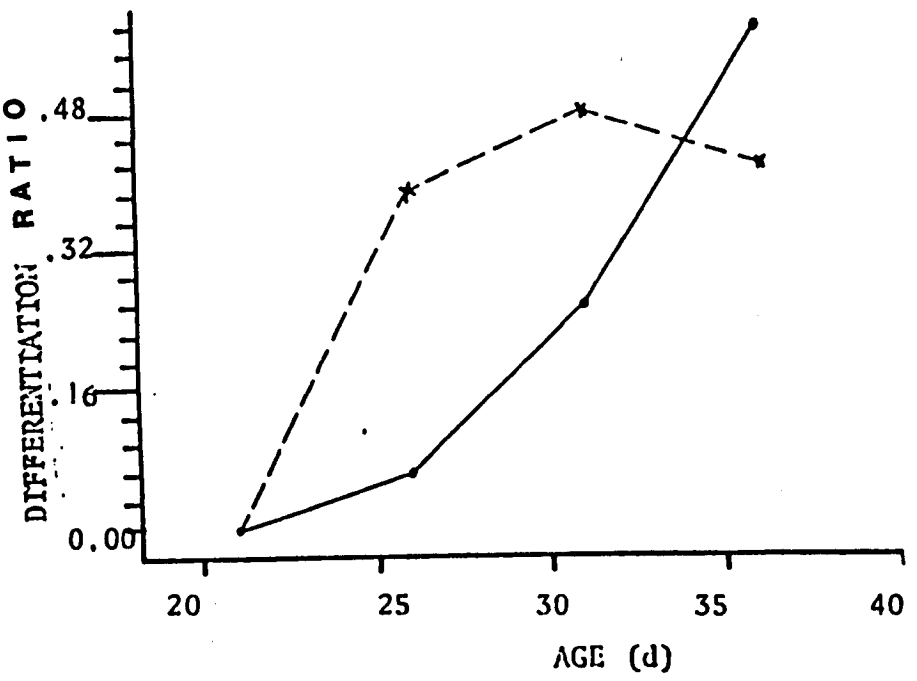


Figure 3.2: Comparative sex differentiation in male (——) and female (—x-) S. galileaus

Table 3.3 : Distribution of sexually developing O. mossambicus fry at 27.5 ± 0.5° C

Age-Class days	Sample	# sexually developed	Differentiation ratio	male	female	undifferentiated
0 - 5	20	0	0	0	0	20
6 - 10	37	8	0.47	0	0	37
11 - 15	18	17	0.94	0	0	18
16 - 20	34	34	1.0	0	0	34
21 - 25	35	35	1.0	4	10	21
26 - 30	19	19	1.0	4	9	6
31 - 35	15	15	1.0	8	6	1
36 - 40	17	17	1.0	9	8	0
41 - 45	19	19	1.0	11	8	0

Table 3.4 : Distribution of sexually developing hormone treated O. mossambicus fry

Age-Class days	Sample	# sexually developed	Differentiation ratio	male	female	undifferentiated
0 - 5	19	0	0	0	0	19
6 - 10	39	5	0.25	0	0	39
11 - 15	17	15	0.88	0	0	17
16 - 20	36	35	0.97	0	0	36
21 - 25	29	29	1.00	9	1	19
26 - 30	14	14	1.00	12	0	2
31 - 35	16	16	1.00	11	0	5
36 - 40	19	19	1.00	14	0	5
41 - 45	17	17	1.00	15	0	2

noticeable from the 22nd day in female and from the 25th day in male fry.

Figure 3.3 is a regression plot showing the onset and progression of differentiation in the male and female fry. There was little or no noticeable difference between the time for first appearance of males among normal and hormone treated groups.

The regression equations for male differentiation rate in the two sets of observations were:-

normal rate = -8.34 + 0.450 x age (R = 91.7%)
hormone treated rate = -6.57 + 0.520 x age (R = 89.6%)

The relationship between onset and progression of sexual differentiation in male and female O. mossambicus is as shown in Figure 3.4.

3.3.3 Sex differentiation in O. aureus

The fry used in the four treatments in this study were obtained from a single spawn.

Tables 3.5 to 3.7 are results of observations on sexual differentiation in O. aureus fry reared at three different temperatures. Table 3.8 shows

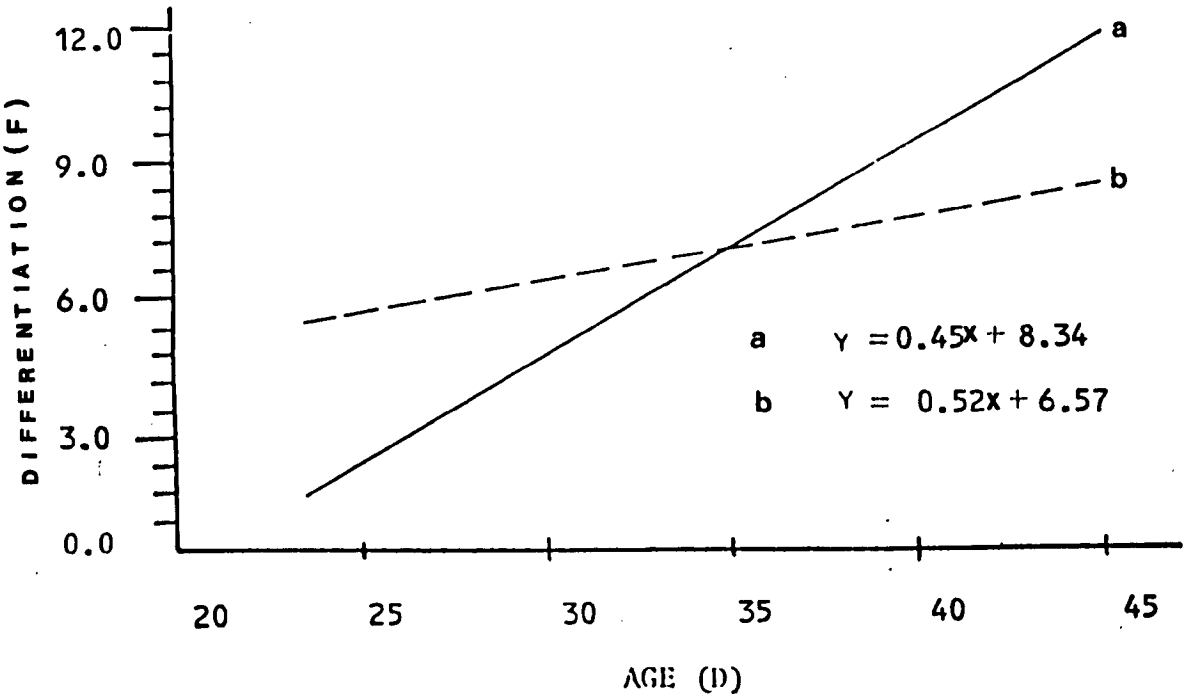


Figure 3.3: Regression plots of male (—) and female (---) O. mossambicus sexual differentiation

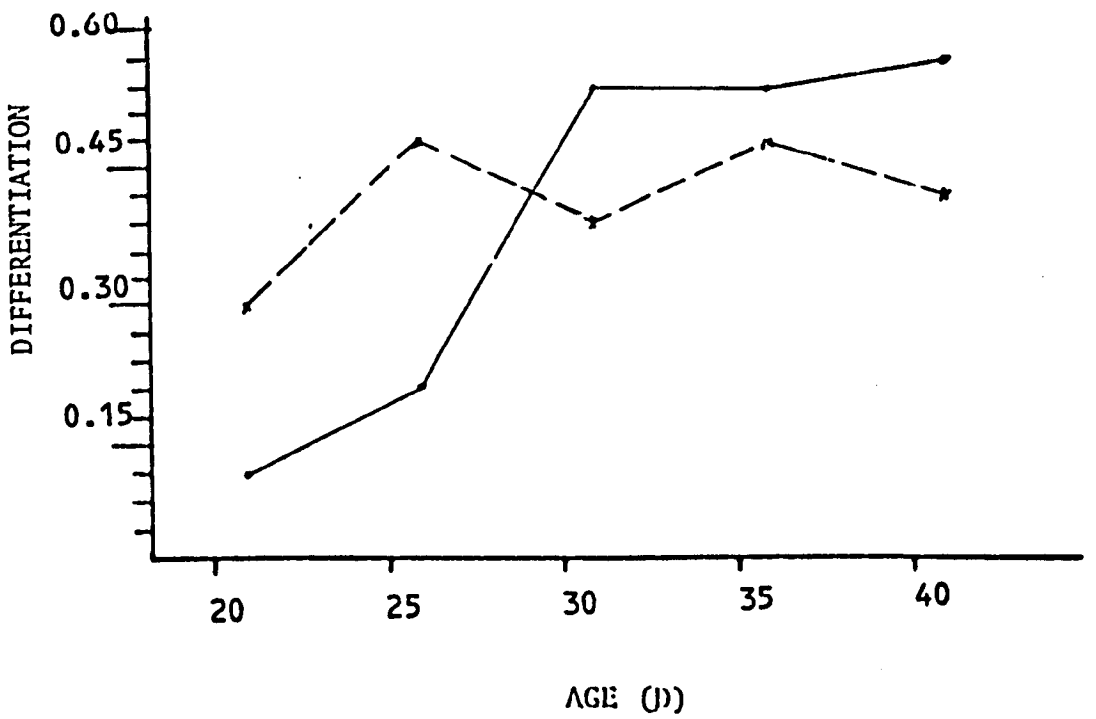


Figure 3.4: Comparative sex differentiation in male (—) and female (——) O. mossambicus

results in the group that was fed a diet with 50 mg/kg Methyltestosterone.

At 21°C development was discernable at 17 days of age, females were first identifiable at 25 days and males at 33 days after hatching.

At 27.5°C development was discernable after 15 days, females and males were first distinguished after 25 and 35 days post hatch respectively.

At 35.5°C gonadal development was discernable after 13 days, females and males became distinguishable after 29 and 33 days respectively.

In the treatment with 50 mg/kg of food, gonadal development was noticed after 16 days, males were identifiable from 31 days post hatch. Two females were identified in samples taken at 30 days one at 36 and two more at 45 days post hatch.

Correlation between gonadal differentiation and standard length was 0.90 , 0.89 , 0.93 and 0.937 for treatments at 21°C, 27.5°C, 35.5°C, and the hormone treated group respectively.

Correlation between gonadal differentiation and age for the four treatment groups, in the same order, was 0.94 , 0.99 , 0.97 and 0.97 . Giving

Table 3.5 : Distribution of sexually developing O. aureus fry at 21.0 ± 0.5°C

Age-Class days	Sample	# sexually developed	Differentiation ratio	male	female	undifferentiated
0 - 5	0	0	0	0	0	0
6 - 10	36	0	0	0	0	36
11 - 15	17	0	0	0	0	17
16 - 20	20	5	0.25	0	0	20
21 - 25	36	28	0.78	0	2	34
26 - 30	18	16	0.88	0	4	14
31 - 35	18	18	1.00	2	7	9
36 - 40	-	-	-	-	-	-
41 - 45	19	19	1.00	5	9	5
45 - 50	16	16	1.00	6	8	2

Table 3.6 : Distribution of sexually developing O. aureus fry at 27.5 ± 0.5°C

Age-Class days	Sample	# sexually developed	Differentiation ratio	male	female	undifferentiated
0 - 5	21	0	0	0	0	21
6 - 10	36	0	0	0	0	36
11 - 15	58	2	0.03	0	0	58
16 - 20	35	19	0.54	0	0	35
21 - 25	51	48	0.94	0	3	48
26 - 30	32	32	1.00	0	10	22
31 - 35	19	19	1.00	6	9	4
36 - 40	16	16	1.00	7	9	0

Table 3.7 : Distribution of sexually developing O. aureus fry at 35.5 ± 1.0°C

Age-Class days	Sample	# sexually developed	Differentiation ratio	male	female	undifferentiated
0 - 5	0	0	0	0	0	0
6 - 10	18	0	0	0	0	18
11 - 15	20	5	0.25	0	0	20
16 - 20	18	12	0.67	0	0	18
21 - 25	35	34	0.97	0	0	35
26 - 30	17	17	1.00	0	4	13
31 - 35	16	16	1.00	6	7	3
36 - 40	-	-	-	-	-	-
41 - 45	15	15	1.00	8	7	0

Table 3.8 : Distribution of sexually developing O. aureus fry raised on hormone treated diet at a temperature of 27.0°C

Age-Class days	Sample	# sexually developed	Differentiation ratio	male	female	undifferentiated
0 - 5	20	0	0	0	0	20
6 - 10	16	0	0	0	0	16
11 - 15	36	0	0	0	0	36
16 - 20	33	12	0.36	0	0	33
21 - 25	33	30	0.91	0	0	33
26 - 30	17	17	1.00	0	0	17
31 - 35	34	34	1.00	11	2	21
36 - 40	16	16	1.00	12	1	3
41 - 45	14	14	1.00	11	2	1
46 - 50	18	18	1.00	17	0	1

averages of 0.92 ± 0.009 (SE) and 0.973 ± 0.008 (SE) for standard length and age respectively.

Figure 3.5 shows regression plots for differentiation ratio against age of fish in the four treatment groups.

The corresponding regression equations were:

T21.0 di ra = 0.560 +0.0509 x age (R = 90.0%)

T27.5 = 0.6837 +0.0355 x age (R = 92.6%)

T35.5 = 0.944 + 0.0754 x age (R = 95.6%)

MT50 = 0.641 + 0.0706 x age (R = 94.2%)

The relationship between onset of sexual differentiation in male and female O. aureus at 27.5°C is shown graphically as figure 3.8

3.3.4 Sex differentiation in O. niloticus

Fish used in this study were obtained by pooling two clutches of eggs which were artificially fertilized with milt from a single male.

Tables 3.9 to 3.12 present results of studies on sex differentiation in O. niloticus fry at three different temperatures and one level of hormone treatment.

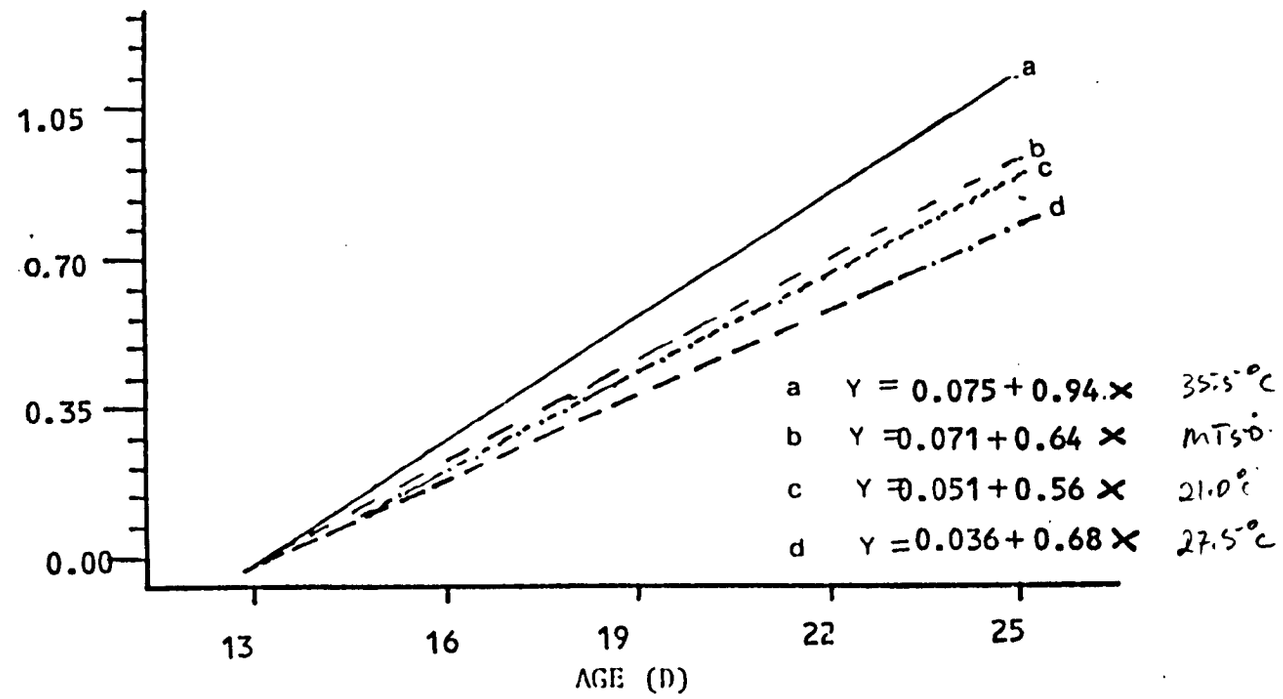


Figure 3.5: Comparative rate of sexual differentiation in O. aureus fry

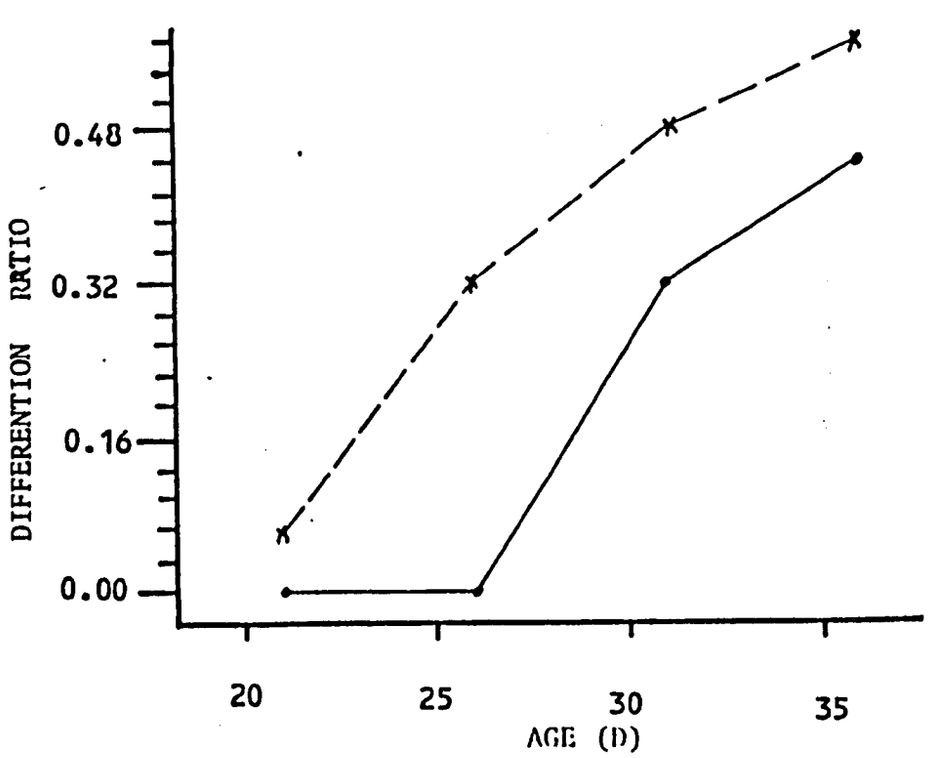


Figure 3.6: Comparative sex differentiation in male (—) and female (--) O. aureus fry

At 21°C, gonadal development became discernable 21 days from hatching. Sexual differentiation became identifiable after 35 days for the female and 46 days for males.

At 27.5°C, development became noticeable at 19 days and sexual distinction at 31 and 43 days for female and male respectively.

At 35.5°C gonadal development was noticed in 21 day old fry, while females and males became identifiable after 30 and 40 days respectively.

Sexual development in fry treated with MT was first observed in 16 day old fry, and male gonads became discernable after 38 days. No females were found in any of the samples examined.

Correlation rate and age were 0.94 , 0.95 , 0.93 and 0.94 for 21°C, 27°C, 35.5°C and for MT treated diet. The equivalent values for the relationship between rate of differentiation and standard length were 0.87 , 0.86 , 0.98 and 0.78 respectively.

Figure 3.5 gives regression plots for sexual differentiation within the appropriate age range.

Table 3.9 : Distribution of sexually developing O. niloticus fry at 21 ± 0.5°C

Age-Class days	Sample	# sexually developed	Differentiation ratio	male	female	undifferentiated
0 - 5	0	0	0	0	0	0
6 - 10	39	0	0	0	0	39
11 - 15	34	0	0	0	0	34
16 - 20	-	-	-	-	-	-
21 - 25	32	6	0.19	0	0	32
26 - 30	19	16	0.84	0	0	19
31 - 35	16	16	1.00	0	2	14
36 - 40	15	15	1.00	0	6	9
41 - 45	-	-	-	-	-	-
46 - 50	17	17	1.00	3	7	7

Table 3.10 : Distribution of sexually developing O. niloticus fry at 27.5 ± 0.5°C

Age-Class days	Sample	# sexually developed	Differentiation ratio	male	female	undifferentiated
0 - 5	0	0	0	0	0	0
6 - 10	40	0	0	0	0	40
11 - 15	18	1	0.06	0	0	18
16 - 20	37	6	0.16	0	0	37
21 - 25	35	31	0.89	0	0	35
26 - 30	18	18	1.00	0	0	18
31 - 35	37	37	1.00	0	5	32
36 - 40	16	16	1.00	0	7	9
41 - 45	18	18	1.00	4	7	7
46 - 50	15	15	1.00	8	6	1

Table 3.11 : Distribution of sexually developing O. niloticus fry at 35.5 ± 1.0°C

Age-Class days	Sample	# sexually developed	Differentiation ratio	male	female	undifferentiated
0 - 5	0	0	0	0	0	0
6 - 10	36	0	0	0	0	36
11 - 15	36	0	0	0	0	36
16 - 20	28	16	0,57	0	0	28
21 - 25	35	27	0.77	0	0	35
26 - 30	17	17	1.00	0	2	15
31 - 35	16	16	1.00	0	6	10
36 - 40	18	18	1.00	3	9	6
41 - 45	15	15	1.00	6	8	1
46 - 50	16	16	1.00	8	7	1

Table 3.12 : Distribution of sexually developing M.T. treated O. niloticus fry at 27.5°C

Age-Class days	Sample	# sexually developed	Differentiation ratio	male	female	undifferentiated
0 - 5	0	0	0	0	0	0
6 - 10	35	0	0	0	0	35
11 - 15	16	0	0	0	0	16
16 - 20	33	9	0.27	0	0	33
21 - 25	35	28	0.80	0	0	35
26 - 30	17	17	1.00	0	0	17
31 - 35	38	38	1.00	0	0	38
36 - 40	15	15	1.00	9	0	6
41 - 45	18	18	1.00	13	0	5
46 - 50	14	14	1.00	12	0	2

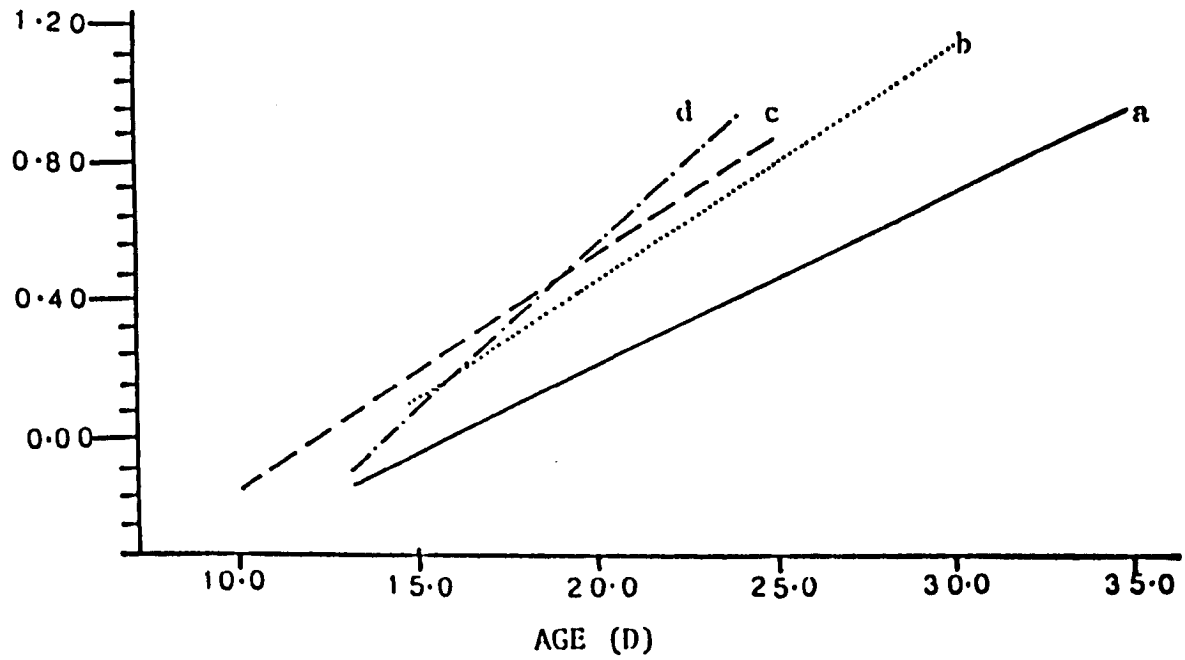


Figure 3.5 Comparative sexual differentiation in O. niloticus subject to different treatments

a - 21°C 0.0509 + 0.560X (R 90.0%)

b - MT50 0.0706 +0.641X (R 94.2%)

c - 27.5°C 0.0355 +0.684X (R 92.6 %)

d - 35.0°C 0.0754 + 0.944X (R 95.6 %)

3.3.6 Comparative sex differentiation between S. galileaus, O. mossambicus, O. aureus and O. niloticus

All the fish in this study were reared in similar tanks at 27.5± 0.5°C.

Table 3.15 and Figure3.9 show the effective period of gonad development in different species as defined earlier in this chapter. 47% of O. mossambicus fry were developing gonads by 10 days of age. Development was complete by day 16, 29% of S. galileaus had developing gonads by the 15th, and development was complete by the 22nd day. Development in O. aureus started around the 15th (3%) and was complete by the 23rd (94%). Development in O. niloticus was from the 16th to the 24th day.

Within the conditions of these experiments the duration for morphological sex differentiation in different species varied among species and with sex (Table 3.16, Figure 3.8). In S. galileaus differentiation was first observed when fish were 22-26 days old. In O. mossambicus from 22-25 days of age. In O. aureus differentiation was first observed at 25 days of age, and in O. niloticus 35 days.

Figure 3.8: Gonad development range from the first appearance of paired gonads to presence of paired gonads in 95% of the sample

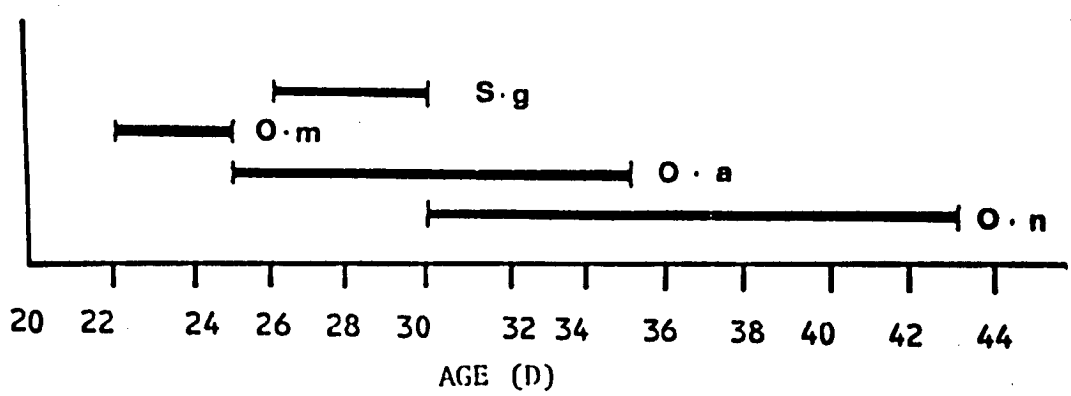


Figure 3.9: Sex differentiation range from first appearance of female fry to first appearance of male fry in the sample

Key

S.g - Sarotherodon galileaus
O.m - Oreochromis mossambicus
O.a - Oreochromis aureus
O.n - Oreochromis niloticus

Table 3.14 : Distribution of sexually developing F_1 hybrids of O. niloticus x O. aureus

Age-Class	Sample	# sexually developed	Differentiation ratio	male	female	undifferentiated
0 - 5	19	0	0	0	0	19
6 - 10	20	0	0	0	0	20
11 - 15	18	0	0	0	0	18
16 - 20	18	7	0.39	0	0	18
21 - 25	14	11	0.79	0	0	14
26 - 30	38	38	1.00	0	2	36
31 - 35	19	19	1.00	0	0	19
36 - 40	21	21	1.00	15	4	2

no more fish left.

Table 3.15 : Gonad development in S. galileaus, O. mossambicus, O. aureus and O. niloticus

AGE	S. galileaus	O. mossambicus	O. aureus	O. niloticus
0 - 5	0.00	0.00	0.00	0.00
6 - 10	0.00	0.47	0.00	0.00
11 - 15	0.29	0.94	0.03	0.06
16 - 20	0.80	1.00	0.54	0.16
21 - 25	1.00	1.00	0.94	0.89
26 - 30	1.00	1.00	1.00	1.00

Table 3.16 : Sex differentiation in S. galileaus, O. mossambicus O. aureus and O. niloticus

	S. galileaus		O. mossambicus		O. auraus		O. niloticus	
AGE	Male	Female	Male	Female	Male	Female	Male	Female
21-25	0.00	0.00	0.11	0.29	0.00	0.05	0.00	0.00
26-30	0.05	0.37	0.21	0.47	0.00	0.31	0.00	0.00
31-35	0.26	0.47	0.53	0.40	0.31	0.47	0.00	0.14
36-40	0.57	0.43	0.53	0.47	0.44	0.56	0.00	0.44
41-45	*	*	0.58	0.42	*	*	0.22	0.39
46-50	*	*	*	*	*	*	0.53	0.40

3.4 DISCUSSION

Morphological development of the gonads and sex differentiation of S. galileaus, O. mossambicus, O. niloticus, and O. aureus shows identical basic structures but with specific differences in timing of developments.

During the initial stages all larvae are in the undifferentiated phase and both sexes are homologous.

Primordial gonads appeared during successive days ranging from the 8th day in O. mossambicus to the 17th day in O. niloticus. S. galileaus and O. aureus were intermediate between the other two.

The pattern for gonadal differentiation in the four species followed the same trend established for the morphogenesis of primordal gonads which was as follows:

S. galileaus	12-14	days	post	hatch	22-35	; 26-40
O. mossambicus	8-10	"		"	>22	; 25
O. aureus	> 15	"		"	>25	; 33
O. niloticus	> 17	"		"	>35	; 46

In all cases females differentiated earlier than males. There was variation within each group and among sexes in the order of 10 to 15 days.

Yoshikawa and Oguri (1977) were able to distinguish testis from ovaries of Tilapia zillii at 15 days after hatching. The dates obtained in the present experiment for Oreochromis and Sarotherodon species were at least 10 days higher than that reported for T. zillii by the above authors, but relatively closer to the 16 to 20 days obtained by Nakamura and Takahashi (1973) for O. mossambicus. The differences in O. mossambicus (16 to 20 and 22 to 25) could be attributed to strain differences and differences in histological techniques.

The present study shows that the number of fish with differentiated gonads at a particular time was lower at the lower temperature (21 ± 1°C) than at either 27.5 ± 1° or 35 ± 1°C. However, no differences were observed in the duration it took for different groups of each species to develop primordial gonads.

In spite of their small size fish reared at 21°C differentiated at almost the same rate as those reared at higher temperatures, thus demonstrating that the effect of temperature on morphogenesis of primordial gonads was inconsequential at these temperatures (Table 3 and 4). For example in O. aureus, the first identifiable females were obtained between 21 and 25 days at both 21 ± 1 and 27.5 ± 1°C.

Using a different procedure, Shelton et al. (1978) investigated the effectiveness of sex inversion treatments in O. aureus at different temperature ranges. They obtained 90 - 95% male at 21 - 23°C, 98 - 100% at 25 - 27°C and 97% at 27 - 29°C. Not withstanding minor differences, their results seen to be in general agreement with the present finding that the process of gonadal development in O. aureus and in O. niloticus proceeds at a constant rate irrespective of treatment temperature.

As discussed elsewhere in this work, fish at the higher temperature (27.5 and 35°C) grew significantly faster ($p < 0.05$) than their counterparts at 21°C. Fukuyama and Takahashi (1983) found that gonadal development in the sand lamprey, Lampetra reissneri, corresponded to body growth (Length). This relationship was not found in the present experiment.

In the experiments by Shelton et al. (1978) growth averages attained for the three different temperatures after 3 weeks were 18 mm, 22 mm and 30 mm. However, as already indicated, this size variation did not seem to affect the success rate of sex inversion treatments. This result is in agreement with the present finding that gonadal development and sex differentiation in the four tilapia species are age dependant.

Fish treated with Methyltestosterone at a dose of 50 mg/Kg (MT 50) showed a mixed response to the treatment.

S. galileaus gonadal development started about 5 days earlier, but gonadal differentiation was almost unaffected at 25 - 30 days of age. In O. mossambicus and O. niloticus, gonadal differentiation closely corresponded with that at which males first appeared among the controls, which was approximately 6 - 10 days later than that of females.

Overall the effect of Methyltestosterone was a delay of up to 10 days in gonadal sex differentiation.

Eckstein and Spira (1965) observed significant delay in gonadal differentiation of O. aureus treated with 17α -Methyltestosterone at 1 mg/Kg diet even though no change in sex ratio was effected.

This study provides a guide for morphological sex differentiation in S. galileaus, O. mossambicus, O. aureus and O. niloticus. The point of physiological sex differentiation (i.e. a point at which a germ cell is sexualized) is not known but could be investigated around the ranges of gonadal

development and of gonadal sex differentiation estimated in the present experiment and others incorporating a similar approach.

CHAPTER 4

Experiment II

Environmental sex determination

INTRODUCTION

As reviewed in Chapter I there are several sex determining mechanisms which produce two sexes in a population. In some organisms, among them mammals, sex determination takes place by a heterogametic mechanism. There are, however, several others in which progeny sex is determined at a much later stage by the influence of the environment or other exogenic factors. This type of sex determination has been referred to by some authors as labile sex derterminatión (LSD) and by others as environmental sex determination (ESD) (Charnov and Bull, 1977; Bacci 1965).

The basis of ESD is that both male and female parents contribute autosomal genes equally to the next generation and that the sex of the offspring will be in direct response to physiological processes resulting from environmental pressures. In other words the sex ratio of the offspring will reflect the relative fitness of the two sexes in the habitat or patch. Under the circumstance, pre-determination of progeny sex might prove detrimental to survival of the stock.

Environmental sex determination is well documented in dioecious plants and in some lower animals such as parasitic copepodes, parasitic nematodes and parasitic isopods (Reinboth 1975). ESD has also been reported in

marine gastropods (Bacci 1965), in fish species such as Labroides dimidiate and in sequentially hemaphroditic coral reef fish (Robertson 1972).

Other documented examples of ESD in fish include the several anenome fish from the genus Amphiprion (Fricke and Fricke 1977) which demonstrate socially controlled sex change by aggressive dominance. Temperature dependent sex determination (TSD) is relatively common in turtles (Bull and Vogt, 1979), in a sea Lizard (Bull, 1980), and in an alligator (Ferguson and Joanen 1982). TSD is rather rare in fish it has been reported in Menidia mendia (Conover and Kynard, 1981) and in Rivulus marmoratus (Harrington, 1968). In all these, incubation temperature determines the course of sex differentiation and hence progeny sex ratio. For example in the Alligator mississippiensis, incubation temperatures below 30°C produce all females while temperatures above 34°C yield all males; In the sea turtle high (>30°C) temperatures produce all female while temperature between 27°C to 24°C produce all males.

In a number of teleosts, genetic sex determination involving sex chromosomes has been well established. However, early treatment with sex steroids has been shown to significantly alter phenotypic sex (Chapter VII) leading to questions as to whether genetic sex dermination could also be overridden by environmental

parameters with an influence on physiological processes.

On the basis of work done with M.menidia, Conover (1984) hypothesized that TSD provides a mechanism by which a sexual dimorphism can arise without sacrificing growth rate in either sex. He concluded that ESD should also occur in other fishes having a sexual dimorphism in size, prolonged breeding season and early maturation. Most species of tilapia mature in 6 - 12 months and at a fairly small size. Species from the genus Oreochromis also show sexual dimorphism in many morphological characteristics including size. Under suitable environmental conditions tilapia will breed frequently throughout the year. The present experiment was designed to test the hypothesis of ESD on two species; O.niloticus and O.aureus. The two species were chosen because they are among the most popular of the commonly cultured tilapia species; their reproductive biology is fairly well documented; their progeny sex ratios have frequently been reported in intraspecific breeding under standard culture conditions. The two species have responded differently to hormone induced sex inversion. The environmental factor chosen for this experiment was temperature; and mainly because of its generally accepted influence on physiological processes.

MATERIALS AND METHODS

The experimental fish were obtained from natural spawns of broodstock of pure O.niloticus and O.aureus kept in the tropical aquarium. The experiments were performed in static tanks kept in a constant temperature room. The temperature of water in each tank was thermostatically regulated by 100 watt microtronic water heaters.

The capacity of each tank was 10 - 12 litres and 25% of the water was changed every two days to prevent accumulation of organic waste and to control water quality. Oxygen levels in the water were maintained above 5mg/litre by aeration. Total ammonia was regularly monitored and controlled below 0.7 ppm by regular change of water and siphoning excess food particles and faeces. Initially fish were stocked at five temperature ranges. The experiment was later conducted in triplicate at 21.0± 0.5°C, 27.5± 1.0°C and 35.0± 1.0°C .Fish were stocked in tanks when they were 7 - 10 days old and prior to first supply of artifical diet. Fish were fed from the first day of experiment at a rate of 10% body weight per day (bwd) for 50 days, and thereafter at 7.5% bwd in 3 - 4 equal portions to ensure optimum consumption.

Sampling was done every seventh day throughout the experimental period; and after 50 days the remaining fish were transferred to on growing facilities at 27.5±

0.5°C. Fish were intensively fed and left to grow a further 4 - 5 weeks before being killed. Larger fish were sexed by examination of gonads while smaller fish were fixed in Bouin's solution and subsequently sexed by histological examination as in chapter three.

Data analysis

Performance of fish at the different temperatures was compared by analysis of survival, growth and sex ratio data. In each case, both sib groups and treatment categories were compared to evaluate inheritable variation as well as variation due to temperature influences. Survival data was compared by analysis of variance in conjunction with Duncan's multiple range tests (Duncan, 1955). Growth data were compared by analysis of variance on either SGR or final weight of fish. Other assessable parameters are presented in table 5 and used when they help to clarify marginal differences. Sex ratio of each batch of fish is assessed for proximity to a normal 1:1 sex ratio by chi-square test. Sex ratios between different groups of fish and between treatments are compared by analysis of variance and Duncan's multiple range test.

RESULTS

Results of the experiments are presented in tables 1 - 7 for O.aureus and 8 - 13 for O.niloticus. Final distribution of size is shown graphically in figures 1 and 2 for O.aureus and in 4 and 5 for O.niloticus at 35.0°C. Because of excessive mortality at 15°C and at 42°C these temperature ranges were discontinued.

Table 1 indicates that survival of different batches of O.aureus were affected by variations in treatment temperature. Overall survival in the first batch was significantly lower ($p<0.05$) than in the second and third batches. Results also show that within each batch, survival rates sometimes varied with temperature.

TABLE 4.1 Survival of O. aureus fry by sib groupings.

Temperature (°C)	Number of fish Initial	final	Survival % ± SE)*
21.0	125	69	55.20a
27.5	125	92	73.60b
35.0	160	119	74.38b
Total	360	300	67.72 ± 6.27a
21.0	75	58	77.33a
27.5	100	98	98.00b
35.0	110	93	84.55ab
Total	285	239	86.63 ± 6.06
21.0	75	54	72.00a
27.5	125	98	78.40a
35.00	150	114	76.00a
Total	350	266	75.47 ± 1.87

* Figures in treatment groups having same superscripts are not significantly different ($p>0.05$) and figures within each sib group showing different super scripts, are also not significantly different at 5%.

TABLE 4·2 Survival of O.aureus fry arranged by treatment type

Temperature °C	Number of fish Initial	final	survival (±SE)*
21.0	125	69	55.20a
"	75	58	77.33b
"	75	54	72.00b
Total	275	181	68.18 ± 5.44a
27.5	125	105	73.60a
"	100	98	98.00b
"	125	98	78.40a
Total	350	301	83.33 ± 6.09bc
35.0	160	119	74.38a
"	110	93	84.55a
"	150	114	76.00a
Total	420	326	78.31 ± 2.58^{ab}

* Figures within each treatment group showing different superscripts are significantly different from each other ($p<0.05$), and averages in column 4 shown with different superscripts are also significantly different from each other ($p<0.05$).

Table 2 shows that survival at 21.0°C is significantly lower than at 27.5°C but not at 35.0°C. Within treatment differences were also found on survival between fry at 21.0°C and at 27.5°C.

Table 3 shows the weight of fry at different times during the experimental period. Results show that differences in growth were significantly different from as early as seven days after the start of the experiment. Inspite of minor variations, growth rate of fry at 27.5°C and at 35.0°C was fairly uniform and no significant differences in weight were found by the end of the experiment (Table 4). However, the condition factor of fish reared at 35.0°C was significantly lower than that of fry reared at 27.5°C. The specific growth rate and food conversion rates of O.aureus at 21.0°C was significantly lower ($p<0.05$) than that of fry at either 27.5°C or 35.0°C.

The regression equations for length over the growth period was:-

$$Y = 0.206\ x + 0.185 \text{ at } 21.0°C$$

$$Y = 1.30X + 3.06 \text{ at } 27.5°C$$

and $$Y = 1.41\ x + 2.69 \text{ at } 35.0°C.$$

The relationship between age and weight of fish over the same period was:-

$$Y = 0.002\ x + 0.007 \text{ at } 21.0^{\circ}C$$

$$Y = 0.030\ x + 0.535 \text{ at } 27^{\circ}C$$

and

$$Y = 0.016\ x + 0.241 \text{ at } 35.0^{\circ}C$$

Where Y is the predicted weight of fish at age X.

In the early stages, fry growth is rapid and has an almost linear relationship with the age of fish. It thus appeared valid to use a linear regression for this growth study because of the relatively short experimental period (50 days) and because all fish would have been in the same part of the growth curve. (See Majumdar and McAndrew, 1987)

TABLE 4.3 Average weight of pooled samples of O.aureus fry at different ages.

Age (days)	mean weight at temperature 21.0°C (g)	27.5°C (g)	35.0°C (g)
7	0.009	0.009	0.009
14	0.019*	0.029**	0.026**
21	0.028	0.065	0.070
28	0.043	0.302	0.292
35	0.067	0.721	0.698
42	0.083	1.092	0.934
50	0.131*	1.546**	1.492**

Figures with different superscripts at day 50 are significantly different ($p<0.05$).

TABLE 4.4 Growth of O.aureus fry at three temperatures: pooled sample mean of 3 replicates.

Temperature sample mean weight (g)

°C.	Size	Initial	final	fcr	sgr	K
21.0 ± 0.5	145	0.009	0.131a	2.93a	3.45a	3.36b
27.5 ± 1.0	119	0.009	1.546b	1.17b	7.04b	3.58b
35.0 ± 1.0	125	0.009	1.492b	1.23b	6.50b	3.13a

Figures in columns, with different superscripts, are significantly different ($p < 0.05$).

Table 5 is a summary of pooled averages for a number of growth parameters assessed in O.aureus fry after 35 days. Results indicate that fish reared at 21.0°C were significantly different from fish reared at either 27.5°C or 35°C by every parameter. At 35 days, post hatch, fish reared at 35.0°C had on average a lower FCR than fish reared at 27.5°C inspite identical SGR values.

The average condition factor was 3.049 ± 0.08, 4.102 ± 0.06 and 3.546 ± 0.23 at 21.0°C, 27.5°C and 35.0°C respectively. The condition factor at 21.0°C was significantly ($p<0.01$) lower than that for either 27.5° or 35.0°C.

Table 4·5 Summary of growth parameters in 35 days old O.aureus

21.0°C

Parameters	Batch number 1	2	3	mean ± SE*
Initial wt (g)	0.010	0.007	0.009	0.009 ± 0.02a
Final wt(g)	0.073	0.061	0.067	0.067 ± 4.65^{-3a}
Absolute growth	0.063	0.054	0.058	0.059 ± 4.04^{-3a}
Relative growth	6.692	7.711	6.442	6.949 ± 0.04^{a}
SGR (44 days)	4.53	4.81	4.46	4.601 ± 0.01^{a}
FCR	1.35	1.54	1.41	1.435 ± 0.05

27.5°C

Initial wt. (gr)	0.010	0.007	0.009	0.009 ± 0.02
Final wt. (g)	0.860	0.627	0.677	0.721 ± 7.29^{-3b}
Absolute Growth	0.850	0.620	0.668	0.713 ± 7.89^{-3b}
Relative Growth	89.210	88.519	74.208	83.98 ± 0.55
SGR	10.00	9.99	9.60	9.86 ± 0.01^{b}
FCR	0.98	0.79	0.82	0.87 ± 0.07^{b}

35.0°C

Initial wt (grs)	0.010	0.007	0.009	0.009 ± 0.02^{a}
Final wt.(g)	0.630	0.751	0.713	0.698 ± 3.30^{-3b}
Absolute growth	0.621	0.744	0.704	0.689 ± 3.86^{-3b}
Relative growth	65.103	106.289	78.22	83.20 ± 1.29^{b}
SGR	9.314	10.39	9.72	9.80 ± 0.03^{b}
FCR	1.151	0.814	1.098	1.02 ± 0.01^{c}

* Identical superscript following figures in column 5 for corresponding paramaters in column number 1 are not significantly different (p>0.05).

Table Six is a comparison of sex differentiation between different sib groups of O. aureus juveniles. The results show that overall, one of the three groups was significantly different from the others in terms of progeny sex ratio. Proportion of males in group one was signicantly higher at 35.0°C than at 27.5°C. No difference in sex ratio was found in the other two batches of fish. Overall, the average proportion of the two sexes in the three batches of fish was about 1 male to 1 female.

TABLE 4.6 Comparative sex differentiation in O. aureus fry: by sib groups.

Temperature °C	Number of fry initial	sexed	%*	sex Male	female	sex ratios** M/f	% male
21,0	125	62	49,60	28	34	0,82	45,16^{a,b}
27,5	125	80	64,00	34	46	0,74	42,50^{a}
35,0	160	101	63,13	52	49	1,06	51,49^{b}
Total	360	243	67,50	114	129	0,80^{a} ±0,10	46,38 ±2,66
21,0	75	58	77,33	30	28	1,07	53,52^{a}
27,5	100	89	89,00	47	42	1,12	52,81^{a}
35,0	110	81	73,64	45	36	1,25	55,56^{a}
Total	285	228	79,99	122	106	1,15^{b} ±0,05	53,36 ±1,14
21,0	75	54	72,00	30	24	1,25	55,56^{a}
27,5	125	93	74,40	52	41	1,27	55,91^{a}
35,0	150	114	76,00	59	55	1,07	52,07^{a}
Total	350	261	74,13	141	120	1,20$_{b}$ ±0,06	54,41 ±1,33
Total	1045	732	70,05	377	355	1,07 ± 0,06	51,38 ± 1,56

* Percentage of fish sexed was calculated as

$$y = \frac{\text{number sexed}}{\text{number at start}} \times 100$$

** Figures in column seven (and in each of the three groups in column eight) followed by different superscripts, are significantly different from each other ($p<0.05$).

Table seven is a comparison of the influence of different temperatures on sex differentiation in O. aureus. Results show that there was no treatment related variation in sex ratio. However there was a significant difference in the sex ratio of fish from different groups grown at 27.5°C as shown in column seven, group two.

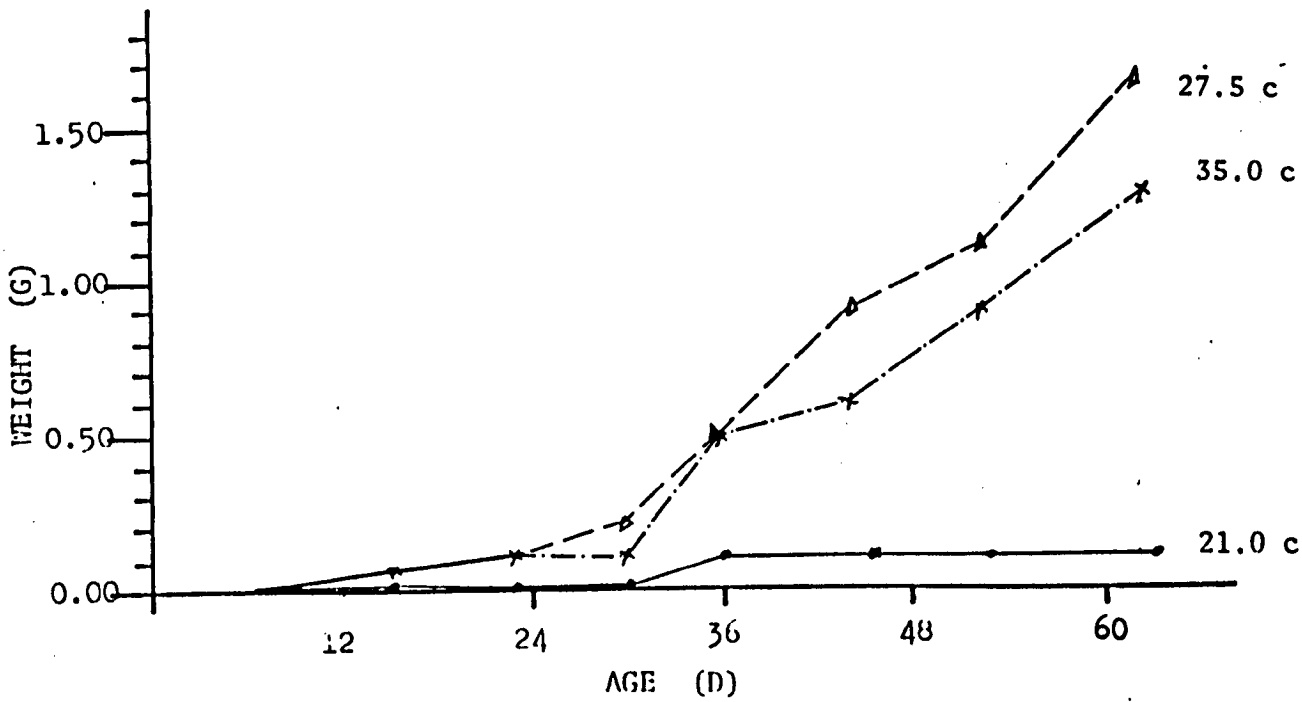


Figure 4.5: Comparative growth (wt) of O. Aureus fry at 3 different temperatures

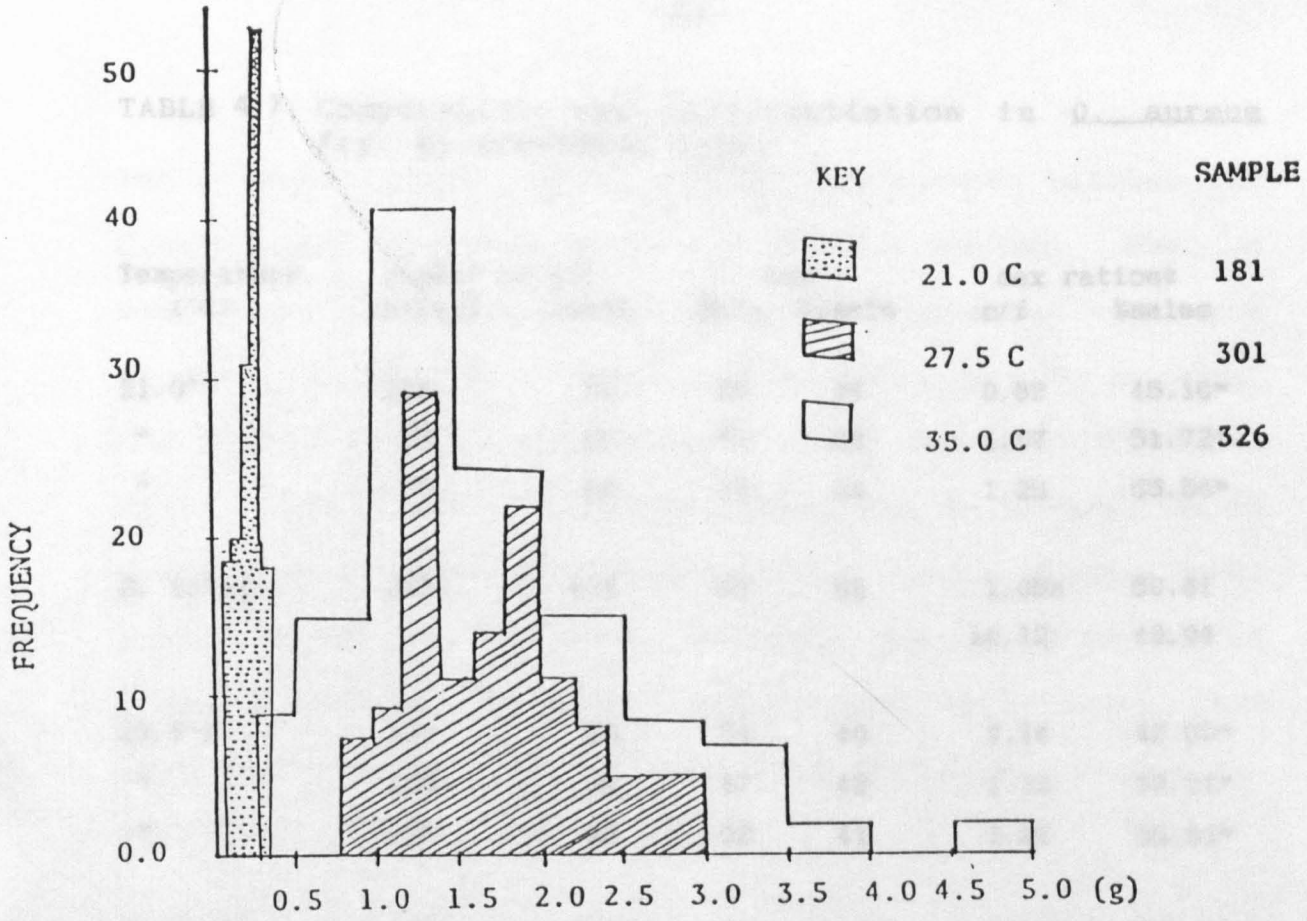


Figure 4.1: Weight frequency distribution of O. aureus reared at 3 different temperatures; 50 days after hatching

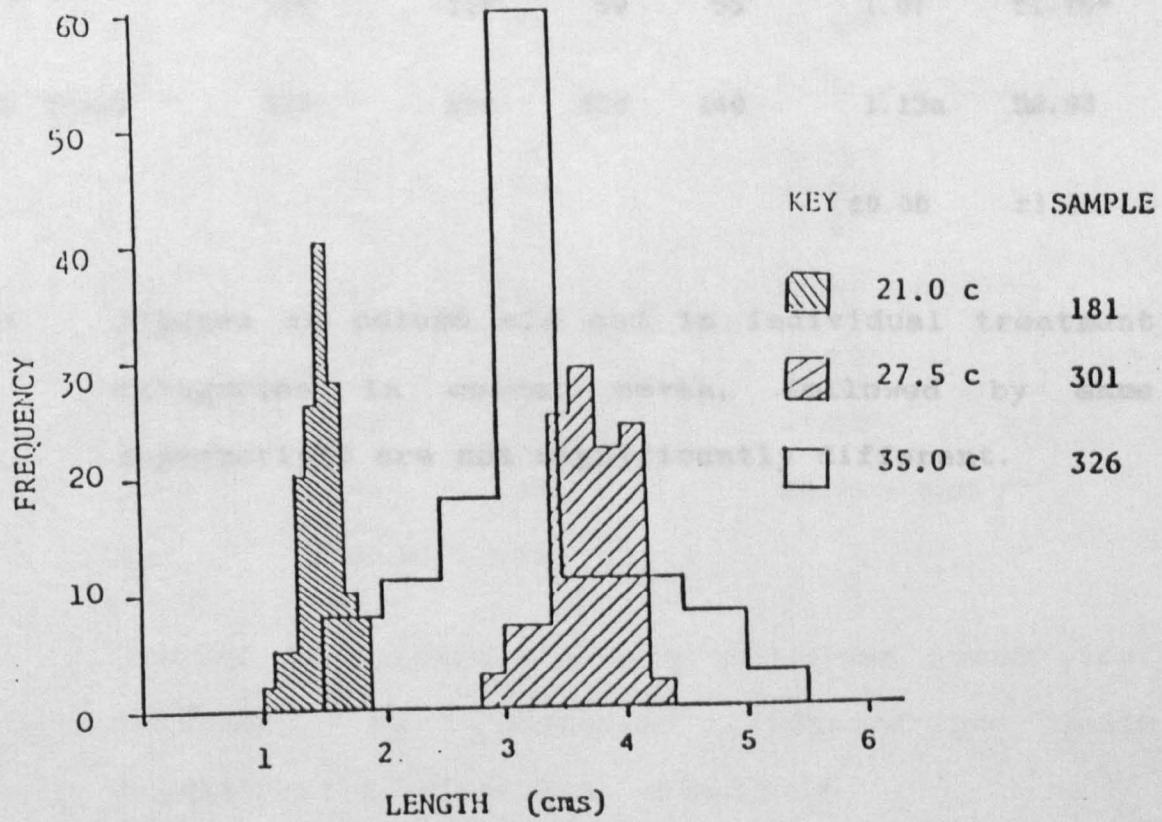


Figure 4.2: Length frequency distribution of O. aureus reared at 3 different temperatures; 50 days after hatching

TABLE 4.7 Comparative sex differentiation in O. aureus fry: by treatment type.

Temperature (°C)	number of fry Initial	Sexed	sex male	female	sex ratios* m/f	%males
21.0°	125	62	28	34	0.82	45.16[a]
"	75	58	30	28	1.07	51.72[a]
"	75	54	30	24	1.25	55.56[a]
S. total	275	174	88	86	1.05a	50.81
					±0.12	±3.04
27.5°C	125	80	34	46	0.74	42.50[a]
"	100	89	47	42	1.12	52.81[a]
"	125	93	52	41	1.27	55.91[b]
S. total	350	262	133	129	1.04a	50.41
					±0.16	±4.05
35.0°C	160	101	52	49	1.06	51.49[a]
"	110	81	45	36	1.25	55.56[a]
"	150	114	59	55	1.07	51.75[a]
S. total	420	296	156	140	1.13a	52.93
					±0.06	±1.31

* Figures in column six and in individual treatment categories in column seven, followed by same superscripts are not significantly different.

Table eight gives survival of different batches of O.niloticus fry after a 42 day rearing period. Results show that there was no difference in parental influence on survival of fry. In two of the three groups, survival was significantly affected by rearing temperature of the fish. This result is further confirmed by comparison of sex ratios by treatment temperature. (Table nine). Fish reared at 35.0°C had lower survival than fish reared at either 21.0° or 27.5°C. Significant differences were also found in survival of fish from different batches but reared at either 21.0° or 35.0°C.

TABLE 4·8 Survival of O.niloticus fry by sib grouping.

Temperature °C	number of fish Initial	Final	survival % (± S.E.)*
21.0	80	63	78.75[a]
27.5	80	69	86.25[a]
35.0	70	56	80.00[a]
Total	230	188	81.67 ± 2.32**
21.0	55	52	94.50[a]
27.5	65	60	92.31[a]
35.0	66	49	74.24[b]
Total	186	161	87.02 ± 6.42**
21.0	48	42	87.50[a]
27.5	75	64	85.33[a]
35.0	73	42	57.53[b]
Total	196	148	76.79 ± 9.65 **
Total	81.82 ± 3.72		

* Figures in different groups in column number four followed by different superscripts are significantly different ($p<0.05$).

Table 4.9 Survival of O.niloticus fry arranged by treatment type.

Temperature °C	number of fish Initial	final	survival (± SE)
21.0	80	63	78.75 ^{a}
"	55	52	94.50^{b}
"	48	42	87.50 ^{ab}
Total	183	157	86.92 ± 4.56 **
27.5	80	69	86.25^{a}
"	65	60	92.31^{a}
"	75	64	85.33^{a}
Total	220	193	87.96± 2.19**
35.0	70	56	80.00^{b}
"	66	49	74.24^{b}
"	73	42	57.53^{a}
Total	209	147	70.59 ± 6.74*

Figures with different superscripts within each treatment category are significantly different from each other at $p<0.05$.

Table ten shows terminal size of three batches of O.niloticus fry. Results show that within each group, fry reared at 21.0°C grew at a significantly slower rate than fish reared at the higher temperatures. No significant difference was found in growth rate of fish from the same batch reared at 27.5° and 35.0°C. Comparison of growth at different temperatures (Table eleven) confirms the significant difference in growth rate between fish grown at 21°C and at the higher temperatures.

The regression relationship between weight and length of fish in the size range of the experiment was

$$Y = 0.105X + 0.0634 \text{ at } 21.0°C$$

$$Y = 0.353X + 0.406 \text{ at } 27.5°C$$

and $$Y = 0.867X + 1.590 \text{ at } 35.0°C.$$

Fish at the higher temperatures grew very rapidly in the initial stages.

The average condition factor at the end of the experimental period was 3.38, 3.90 and 3.41 for 21.0°, 27.5° and 35.0°C respectively.

Table 4.10 Comparative growth of O.niloticus fry at 3 different temperatures: arranged in sib groups.

Temperature °C	sample size (number)	terminal size *	
		$\bar{x}$ wt ± SE (g)	$\bar{x}$ length ± SE (c)
21.0	63	0.08 ± 0.01[a]	1.2 ± 0.02
27.5	69	0.96 ± 0.01[b]	3.2 ± 0.03
35.0	56	0.88 ± 0.07[b]	2.9 ± 0.07
21.0	52	0.10 ± 0.02[a]	1.4 ± 0.01
27.5	55	0.78 ± 0.05[b]	2.8 ± 0.20
35.0	48	0.85 ± 0.17[b]	2.8 ± 0.23
21.0	42	0.07 ± 0.03[a]	1.3 ± 0.12
27.5	63	1.27 ± 0.12[b]	3.5 ± 0.21
35.0	42	1.11 ± 0.11[b]	3.2 ± 0.28

Within each sib group, figures bearing same superscripts are not significantly different ($p>0.05$).

Table 4·11 Comparative growth of O.niloticus fry by type of treatment.

Temperature	Sample size	Terminal size of fish*	
		$\bar{x}$ Wt ± SE	$\bar{x}$ Length ± SE
21.0	63	0.08 ± 0.01a	1.27 ± 0.02
"	52	0.10 ± 0.02a	1.43 ± 0.01
"	42	0.07 ± 0.03a	1.35 ± 0.12
S. total	157	0.08 ± 4.77^{-3}*	1.28 ± 0.05
27.5	69	0.96 ± 0.01^{a}	3.2 ± 0.02
27.5	55	0.78 ± 0.05^{a}	2.8 ± 0.20
27.5	63	1.27 ± 0.12^{b}	3.5 ± 0.21
S. Total	187	0.99 ± 0.10**	3.17 ± 0.13
35.0	56	0.88 ± 0.07^{a}	2.90 ± 0.07
"	48	0.85 ± 0.17^{a}	2.80 ± 0.23
"	42	1.11 ± 0.11^{a}	3.20 ± 0.28
S. total	146	0.95 ± 0.14**	2.97 ± 0.21

* Figures followed by different superscripts in column number three are significantly different ($p<0.05$).

Tables 12 and 13 compare the effect of temperature on sex differentiation between sib groups and between treatments respectively. Results show that the sex ratios of the three sib groups are significantly different from each other (Table 12) but are not significantly altered by rearing temperature (Table 13).

Table 4.12 Comparative sex differentiation in O.niloticus fry: by sib groups.

Temperature °C	number stocked	number sexed	sex male	sex female	sex ratio m/f	sex ratio % ma
21.0	80	60	27	33	0.82	45.00[a]
27.5	80	69	38	31	1.23	55.07[b]
35.0	70	55	29	26	1.12	52.73[a]
	230	184	94	90	1.05[a,b] ±0.12	50.93 ±3.04
21.0	55	52	27	25	1.08	51.92[a]
27.5	65	55	29	26	1.12	52.73[a]
35.0	66	48	28	20	1.40	58.33[b]
	186	155	84	71	1.20[b] ±0.10	54.33 ±2.02
21.0	48	39	18	21	0.86	46.15[a]
27.5	75	63	28	35	0.80	44.44[a]
35.0	73	42	20	22	0.91	47.62[a]
	196	144	66	78	0.86[a] ± 0.03	46.7x ±0.92
	612	483	244	239	1.04±0.06	50.44±1.52

* Figures in columns six followed by same superscripts are not significantly different. ($p>0.05$); and figures in each group of column number seven bearing the same superscript are also not significantly different.

Table 4.13 Comparative sex differentiation in O.niloticus fry; by treatement type.

Temperature °C	number of fry stocked	sexed	sex male	female	sex ratio m/f	* %male
21.0	80	60	27	33	0.82[a]	45.00
"	55	52	27	25	1.08[a]	51.92
"	48	39	18	21	0.86[a]	46.15
Total	183	151	72	79	0.92±0.08	47.69±2.14[a]
27.5	80	19	38	31	1.23[b]	53.07
"	65	55	29	26	1.12[b]	52.73
"	75	63	28	35	0.80[a]	44.44
Total	220	187	95	92	1.05±0.13	50.75±3.22[a]
35.00	70	55	29	26	1.12[a]	52.73
"	66	48	28	20	1.40[b]	58.33
"	73	42	20	22	0.91[a]	47.62
Total	209	145	77	68	1.14±0.14	52.89±3.09[a]

* Figures in column number seven followed by same letter are not significantly different from each other ($p > 0.05$), and figures among groups in column number six followed by same superscript are also not significantly different.

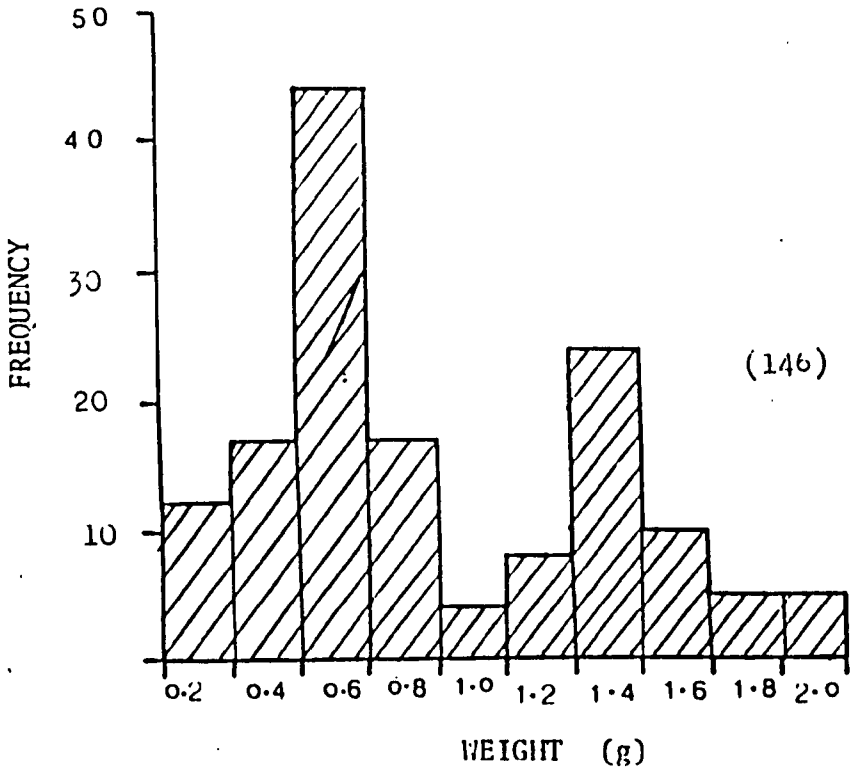


Figure 4.3: Weight frequency distribution of O. niloticus fry reared at 35.0°C; 50 days after hatching.

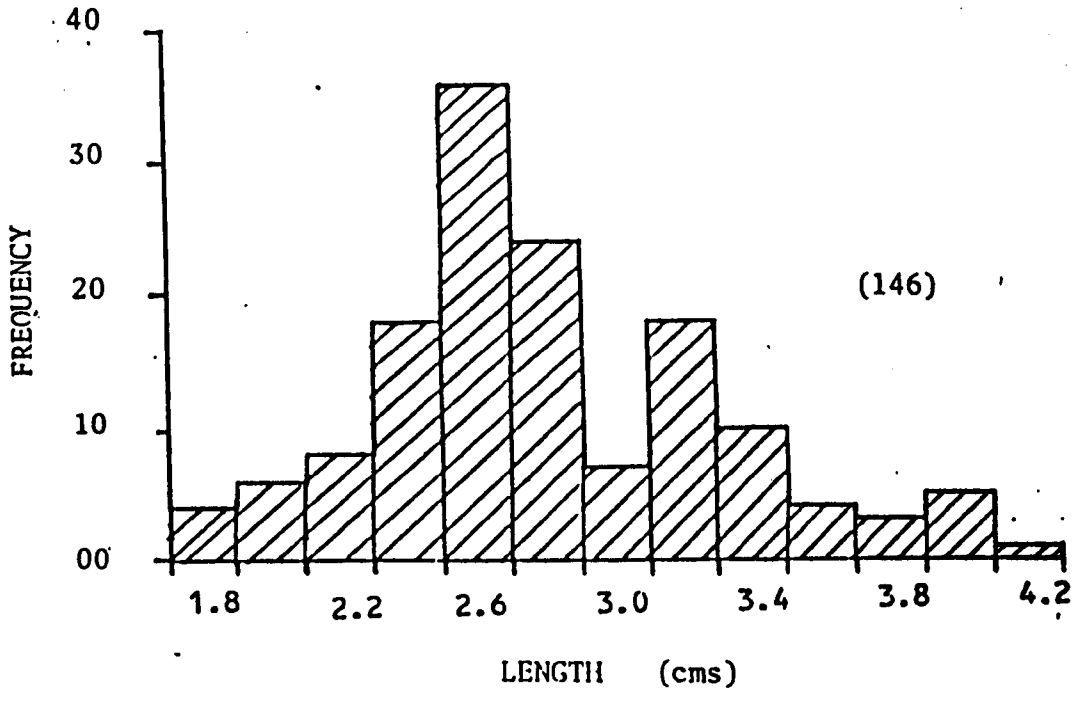


Figure 4.4: Length frequency distribution of O. niloticus reared at 35.0°C for 50 days

DISCUSSION

Survival

In the initial set up, each spawn was divided into 5 groups for treatment at 15, 21, 27.5, 35, and 42°C. Because of hyperactivity and early mortality in O. niloticus (about 12 hours) and in O. aureus (less than 8 hours) reared at 42.0±1.0°C trials at this temperature were discontinued. Fish maintained at 15°C were immotile, did not feed very actively and in time grew darker in colour and became highly emaciated. About half of the O. niloticus died during the first 10 days of treatment. O. aureus survived longer, fed comparatively more actively but did not show much growth. About half of them died within 21 days.

Balarin and Hatton (1979), reviewed literature on survival of tilapia species. Survival of O. mossambicus in nature was reported to be in the 16.5 to 39.0°C range in Lake Sibaya South Africa (Bruton, 1983). Allanson (1966) reported that O. mossambicus subjected to a cold shock at 15°C suffered a chill coma with no sign of obvious cold adaptation.

Lower lethal temperatures for tilapia have been reported for several tilapia species (Chimits 1957; McBay 1961; Bishai 1965; Shafland and Pestrak, 1982). In most of these there has been little or no agreement on the exact

lower lethal point. However, a practical range of 9-12°C seems generally accepted. Even in nature, reports on lower survival temperature are not uncommon, for example 80% survival of O. niloticus x O. aureus hybrids at 8°C has been reported in a commercial situation in Israel (Wohlfarth et al., 1983). Incidental observations during the present study demonstrated that a chill coma inflicted in the lower sub lethal range (12-10°C) for short periods of time was recoverable, and fry were able to resume feeding within a few hours.

There has been much less unanimity on the causes of low temperature mortality. Villaluz (1983) suggests that mortality could be a result of nutritional stress caused by the depressive effect of low temperature on appetite, Allanson and Noble (1971) histologically investigated fish mortality at low temperature and concluded that mortality is a result of irreversible degradation of parenchyma tissues.

Several attempts have also been made to determine the upper lethal temperature in tilapia (Allanson and Noble 1964; Welcomme 1964; Coe, 1966, Caulton, 1977; Al-amouldi 1982; Nyambi., 1982). In the present trials, fish maintained at 42°C died within 8 - 12 hours while fish reared at 35.0 ± 1.0°C survived and grew satisfactorily through the full course of treatment (42 - 50 days). A survey of the cited literature in conjunction with present

observations indicates that the upper lethal range of most commercially cultured tilapias is 37.5 - 42.0°C.

The present experiments indicates that survival of different batches of O. niloticus not significantly different. However, within each batch temperature variations have a significant influence on survival. Generally mortality is significantly higher at 35.0°C when compared with either 21.0°C or 27.5°C. Within each treatment, survival was constant at 27.5°C but fluctuated significantly at both the higher and the lower temperatures.

Survival of O. aureus fry showed significant differences between sib groups. Differences within each batch were generally related to temperature variations. Survival at 21°C was significantly different from that at 27.5°C but not significantly different from that at 35°C.

Overall, survival of the two species was not significantly different ($p>0.05$). However, a comparison of survival at different temperatures shows that O. niloticus juveniles are more sensitive to higher temperature than O. aureus. At 35°C, survival of O. niloticus was significantly lower than at other treatment temperatures (Table 9). On the contrary, O. aureus was not adversely affected by the higher temperature. Instead significant difference was observed at 21°C.

Adedire (1983) found that an increase in acclimation temperature from 18° to 24°C resulted in doubling of diffusional water permeability and that the consequences of acute temperature change from 10°C to 30°C was an exponential increase in water permeability coefficient. This observation demonstrates that an attempt is made by the fish to adapt to adverse environmental changes and renders support to Bishais (1965) observation that acclimation temperature does not alter the lethal temperature but causes a marked increase in the median resistance time.

From the literature, it would seem that the basic mechanism for mortality in fish is the same at both low and high temperature. For example it has been stated by a number of workers that mortality of fry at high temperature is due to high maintenance requirement which by implication means net starvation; and the major cause of fry mortality at low temperature is said to be low appetite induced starvation.

Observations during the present experiments did not indicate significant loss of appetite among fish maintained at 21°C. However mortality was a slow process implying a form of wasting. The fact that the mean condition factor of fish at the low temperature remained favourable through the experiment is evidence that feeding and food utilization was about normal. It is thus probable that mortality at the lower temperature was

caused by histological changes as observed by Allanson and Noble (1971). At high temperature wide, size variation was observed in both species. In view that feeding was carefully done by hand and at regular intervals it would seem unlikely that size variation was exclusively a result of starvation. It is also worth noting that the most active swimmers were the larger fish. It is probable therefore that at the high temperature, mortality occurs mainly in the docile emaciated lagards whose homeostatic processes may have broken down irretrievably.

An important outcome from this part of the experiment is the fact that interspecific differences in response to temperature induced strain have been highlighted. Their existence serves as evidence that genetic differences exist which should be considered in commercial fish culture.

Growth

In natural habitats. growth of fish is greatly influenced by seasonal and diel temperature fluctuations. Generally the influence is physical and results from redistribution of food on the one hand, low intake and reduced utilization on the other. Temperature may also exert physiological influence through its role in controlling metabolic rates. For example populations of fish associated with areas with relatively homothermal water fed by a spring, were found by Fryer and Iles (1972) to be invariably dwarfs while fish of the same species living

in adjacent but more natural aquatic environments usually grew faster and to a larger size.

In the present studies, O. niloticus and O. aureus fry were, in the first instance stocked at five different temperature ranges. As reported in the previous section, all fish stocked in water at 15.0 ± 0.5°C and at 42.0 ± 1.0°C did not survive the experiments. In the other three treatment categories, fish reared at 21°C had lower food intake and grew very little by comparison with those reared at higher temperature.

Previous studies have also demonstrated that tilapia reared in water temperatures below 15°C do not eat or grow (Bardach et al, 1972). Allanson (1966) and Allanson and Noble 1971) investigated histological changes in liver, kidney, stomach and intestine of O. mossambicus exposed to low temperature. They found that at temperatures below 15°C most fish did not digest the food ingested and ended up with swollen stomachs. Livers of these fish developed extensive cellular disruption, while kidneys became extensively vacuolated and pulpy. Five days into exposure at 14°C, fish started to die.

In the present experiments, fish kept in water at 21°C showed little growth (Tables 3, 4, 10 and 11). This was probably due to lower digestion rates and retarded utilization as was reflected by the higher food conversion rate (Table 4). Generally food conversion ratios in the

present trials followed the same pattern as the final weight, and was lowest in fastest growing fish, which in the present experiments was 27.5°C during the first 42 - 50 days. High food conversion ratio is indictative of either low acceptability of the diet or low utilization.

The FCR obtained in the present experiments for O. aureus at 27.5°C was identical to that obtained by Barash (1984) at a corresponding temperature. No comparable data were found for the higher or lower temperature treatments.

Specific growth rate (SGR) was calculated weekly to reflect upon differences at the start of each treatment interval. The cummulative mean SGR indicates that O. aureus reared at 27.5°C and at 35.0°C grew approximately twice as fast as fish reared at 21°C and ended up at least seven times as large after 50 days. Similar results were found in O. niloticus where fish maintained at 27.5°C and at 35.0°C were on average about 9 times as heavy as fish kept at 21°C. Although no significant difference was recorded between SGR in fish reared at 27.5°C and at 35.0°C, the SGR in fish reared at the lower temperature was slightly higher and at the end of the experiment fish were slightly longer and heavier. the results also show that for both species, initial growth was more rapid at 35.0°C, but this later slowed down as the size variation within the treatment widened. For example length variation in O. aureus was 15mm to 50mm at 35.0°C and 28 - 42mm in fish reared at 27.5°C (Figure 1 and 2). These

differences are indictative of genetic variation in individual response to thermal stress as reflected through metabolic and growth performance.

Similar growth variation found in O. kisutch (Ejike and Schreck, 1980 Jobling and Wandsvik, 1983) and in T. zillii (Saclauso, 1985) was attributed to the effects of positive aggressive behaviour. This theory does not seem to apply in the present circumstance where first feeding fry were stocked at relatively low densities and fed regularly at 10% body weight per day.

Present studies indicate that in terms of productivity (weight gain), optimum rearing temperature; for both O. aureus and O. niloticus, is about 27.5°C. This estimation is in general agreement with recent findings by other workers using a range of tilapia species. For example Saclauso (1985) estimated optimum growth in T. zillii to be around 30°C, Ross et al. (1978) obtained optimum growth in 7 - 40 gr at 25°C; Nyambi (1982 reported SGR of 8.88 at 31-32°C and 7.62 at 34-35°C in O. mossambicus. In a wider ranging trial, Sun Ru-yung and Zhang Yu Shu (1983), experimented with 8 - 12 gramme Oreochromis at temperatures ranging from 22 - 36°C and obtained optimum growth at 28 - 30°C. These workers further noted that from 22 - 28°C growth rate of Oreochromis increases with temperature where as from 32 - 36°C an inverse relationship results. Sufferin et al.

(1978) investigated growth in hybrids and obtained optimum growth for F_1 O. mossumbicus x O. hornorum at 32°C.

Saclauso (1985) has suggested that low growth rate at lower temperatures could be a result of food not containing adequate protein to provide optimum growth. Earlier Allanson (1966) observed that at low temperature, food movement in the gut ceased. Considering the constitution of the diet used in the present experiments (i.e. number 3 trout pellet) it would seem highly unlikely that protein content was limiting. In the circumstance, it would appear most likely that the low growth rate at 21°C was due to either greatly reduced food assimilation or a reduced food intake consequence upon lowered digestive rate. The relatively high condition factor obtained in fish reared at 21°C could therefore be partly attributed to the presence of undigested or partially digested food in the gut.

The effect of high temperature on food utilization by juvenile tilapia has been considered in some detail by Caulton (1977). He concluded that at higher temperature, fish assimilate food more efficiently but that after considering the metabolic costs, net energy gain may not be the highest because processing costs are also proportionately higher. In the present experiments, growth of juvenile fish at the higher temperature was almost identical to that at 27.5°C. However, as noted earlier, growth of fish declined markedly with time. This

would appear to indicate that a feed rate of 10% bwd was less than optimal for the faster growing albeit more active fish.

Influence of temperature on sex differentiation

In the introduction to this chapter, environmental sex deterimination was defined as a mechanism in which an individual's sex is determined in the embryo or in the undifferentiated larvae largely in response to some external stimulus. The basis for the hypothesis would be that alleles on each locus have a weak influence on whether the zygote becomes a male or a female. The best example of this mode of sex determination has been reported in turtles where high incubation temperature invariably results into females while low incubation temperatures result in males. (Bull, 1980).

Environmental sex determination largely depends on the organism's sex differentiaion in response to the temperature.

Among bisexual fish, ESD is a relatively rare phenomenon but one that nevertheless has been demonstrated. For example, Van den Hurk and Lambert (1982) found that in rainbow trout, a temperature shock of 25°C for 3 hours at day 44 yielded 59% females while the same shock applied after day 57 yielded 61% male. In the same paper, it was reported that Van der Hurk and Lambert obtained an all male brood in the African catfish, Clarias lazera by

applying a 3 hour temporary raise in temperature from 30 to 39°C during the incubation period.

In the present study an attempt has been made to influence sex ratio by varying rearing temperature in sexually undifferentiated tilapia of the genus Oreochromis.

Results show that sex differentiation in different crosses of O. aureus varies significantly in different batches (table 6). Variation within a brood was marginally influenced by rearing temperature in only one group. (Table 6, column 8). Overall, varying rearing temperature did not significantly alter sex ratio in the three treatments (column 6, Table 7). It can be concluded therefore that rearing temperature has no sex determining role in O. aureus, and that the observed variation in sex ratio in fish reared at 27.5°C is a reflection on inherent variation found in different broods.

Sex ratio in O. niloticus broods differed significantly in two sib groups (Table 12, C6). There was no temperature influenced significant difference observed in the three treatments. It is therefore probable that observed sex ratio differences between groups given the same treatment are a reflection of differences observed between different batches of fry as observed in O. aureus.

In 1974, Mires could not find any colaborative evidence to prove that the high proportion of males among offspring

of captive O. niloticus (80 - 100%), O. aureus, and O.N. vulcani (55 - 70%) were temperature influenced.

The only other reported investigation of the influence of temperature on sex in tilapia was by Shelton et al. (1978). In this investigation, O. aureus treated with androgens 17 α-Ethynyltestosterone were maintained at three temperature ranges. Results showed that the proportion of males obtained at 21° - 23°C was 90 - 95%; at 25 - 27°C it was 98 - 100%, and at 27 - 29°C it was 97% the sex ratios were not significantly different from each other, thus demonstrating that gonadal differentiation proceeded normally at the three temperature ranges.

In other studies, Ashby (1959) and Lucas (1968) reported no significant variation in sex ratios of Betta splendens subjected to different temperature treatments.

Positive influence of temperature on sex differentiation has been reported in a number of fish species. For example van Doorn (1962) and Lindsey (1962) were cited by Mires (1974) to have found significant correlation between temperature and sex ratio of cyprinids Epilatys chaperi and Gastersteus aculeatus respectively. Chan and Yeung (1983) cited Padoa (1939) who reported that Salmo gairdneri reared at 17 - 20°C differentiated bisexually without the intermediate female state that had been observed at a lower rearing temperature (8 - 13°C).

Conover and Kynard (1981) experimentally demonstrated the positive influence of temperature on sex ratio of progeny in Menidia menidia females.

Harrington (1967) conducted combined effect investigations on the influence of temperature, light intensity and salinity on sex differentiation in Rivulus marmoratus. Results showed that in sea water, low tempertaure (18 - 20°C) and bright light, results in all-male offspring but the mortality was in excess of 90%. In fresh water, low temperature and dim light resulted in all males at a survival rate of approximately 75%. At a higher temperature (30°C) only hermaphrodites were obtained in both fresh and salt water, and survival was 47% and 60% respectively. In experiments involving only temperature, Harrington (1968) demonstrated that eggs of R marmoratus incubated during the phenocritical period at 21.1 ± 0.2°C yield 100% hermaphrodites with no mortality while eggs incubated at 19.8° ± 0.5°C or less, yielded up to 92% males depending on the stage at which exposure to cold treatment is initiated.

One of the most frequently investigated species has been the European eel, Anguilla anguilla D'Ancona (1959) concluded that in sexually undifferentiated fish, high temperature favours sexual development into males while in already differentiated females, high temperature may trigger sex inversion. Wiberg (1983) translated these observations into pond culture and postulated that the sex

ratio of 75 - 90% males often obtained in pond cultured eels is a reflection upon the high water temperature (27°C) to which the elvers are subjected. However, Chan and Yeung (1983) found that in this species, sex ratios are influenced significantly by water quality.

The other environmental factor reported to have a significant effect on sex ratio is the age of parents as in B. Splendens (Lucas 1968).

Most studies on the influence of temperature on sex ratio end at documenting the sex ratio and make no effort to explain the underlying mechanisms. However, in A. anguilla, many investigators have assumed presence of heteromorphic sex chromosomes (See review by Wiberg 1983). Chan et al (1975) observed that for an extrinsic factor to exert its effect, the stimulus itself must be in some way recognizable to the animal and must be transmitted into biological signals which in turn control the biochemical process of sex differentiation. This statement implies that a neuroendocrine pathway operates to translate the external stimulus into an internal sex determinant.

Results of the present experiments, and analysis of the literature cited, shows that susceptability of a fish to environmental influences on sex differentiation is species specific and is probably genetically influenced. In other words sex phenotype (in those fish in which the environment has a positive effect) is, a result of

interaction between genetic constitution of the fish and the environment. The failure to influence sex determination through exertion of an external stimulus indicates that sex determinism in tilapia species is genetically stable.

CHAPTER 5

Experiment III

Single pair breeding and sex differentiation

INTRODUCTION

Sex differentiation in fish has been a matter of interest from the first decade of the century. According to Harrington (1974) in the first decade, sex was in general found to be inherited as a Mendelian trait. The existence of sex chromosomes in fish was first demonstrated by Aida in 1921 in Orzias latipes by sex linked colour inheritance (Harrington 1974;) Almost 30 years later Nogusa (1955) demonstrated the presence of sex chromosomes cytologically in the Gobiid Mqgrunda obscura. In the 25 years that followed sex chromosomes were identified in about 100 different species (Kirpichnikov, 1970, 1981 see also Chapter VI). In view that almost 25,000 species of fish are known, the number with cytologically identified chromosomes is very small.

In 1911, Goldschmidt (cited by Kosswig, 1964) proposed a gene balance theory of sex determination which has since been popularized as the multiple gene hypothesis (Kosswig, 1964; Mittwoch, 1969). Other workers such as Yamamoto (1969) have approached the hypothesis cautiously, suggesting that the theory is too abstract to reconcile with observations. This has recently been disproved by experiments (Bull, 1983, Kallman 1984; Majumdar, 1984). Kirpichnikov (1981) considers polygenic sex determination as the most primitive mechanism from which a sex chromosome system gradually evolved.

In tilpaia, interest in sex determination began when Hickling (1960) reported the possibility for producing all-male tilapia hybrids by interspecific hybridization. Most research effort since then has been concentrated on production and testing of interspecific hybrids (Chen, 1969; Jalabert et al., 1974; Shelton et al., 1978; Smitherman et al. 1984). Although this effort has met some successes in experimental conditions, large scale application of interspecific hybridization has yielded satisfactory returns in only a few cases, for example, in Israel (Mires, 1977). Understanding the genetic basis for variability in progency sex ratios remains therefore an important prerequisite to sex ratio manipulations in tilapia.

It is generally accepted that the ability of the individual parent to transmit a particular phenotype to the next generation lies in a complex inheritance mechanism operating in these fish (Majumdar and McAndrew, 1983; Shelton et al, 1983). Genetic variation exists when more than one form of a gene is present. In the individual, parental genotypes influencing progeny sex are not known but could be infered from progeny sex ratio. In order to make reasonable inferences, the sex ratios must be obtainable repeatedly. One way to reduce variability is to use inbred lines or populations that have been kept together for several generations. By the same argument, variability can be increased through

interbreeding of stocks, for example in interspecific hybridization.

In the present experiment, pure species and their interspecific hybrids have been used to study the progeny sex structure of single pair spawning tilapia with a view to assessing the underlyinging sex determining influences.

Previous work of particular relevance to this chapter is summarized in Table 5.1 for progeny sex ranges in intraspecific crosses and in 6.1 for interspecific hybrids.

Table 5.1 Range of progeny sex ratios obtained in intraspecific breeding experiments.

Species	% male	Reference
O.mossambicus	49 - 70	Hickling 1960
	41 - 51	" 1960
	33 - 77	" 1968
	44 - 53	Majumdar 1984
O.spilurus	41 - 48	Majumdar 1984
	42 - 46	Present work
O.niloticus	42 - 69	Jalabert et al. 1974
	45 - 65	" 1974
	46 - 57	Pruginin et al. 1975
	47 - 84	Hsiao 1980
	31 - 77	Shelton et al. 1983
	45 - 52	Majumdar 1984
	40 - 74	Smitherman et al. 1984
	41 - 59	present work
O.aureus	47 - 56	Hsiao 1980
	42 - 59	Pinto 1982
	28 - 100	Shelton et al. 1983
	52 - 54	Majumdar 1984
O.hornorum	53 - 61	Hickling 1960

5.2 MATERIALS AND METHODS

5.2.1 Tank set up and fish stocks

The experiments were carried out in rectangular glass tanks in a recirculating system (Section 2.3.2). The tanks measured 100 cms x 40 cms x 30 cms and were each filled with approximately 100 liters of water at 26.0 ± 1°C.

The fish used in the experiments were

i genetically pure O.niloticus, O.spilurus and O.mossambicus. The species were chosen because of their popular use in aquaculture (Pullin 1983).

ii F_1 hybrid O.mossambicus x O.spilurus. These were selected for comparison with their parental types and because of their readiness to breed in culture conditions as demonstrated in Chapter 5.2.

The broodstock used in the experiments were about one year old and ranged in weight from 150-200 grammes at the start of the experiments. Each tank was stocked with one pair of tagged fish at a time. Fish were fed (3% bwd.) regularly and at least twice a day. Spawning took place naturally and

eggs were removed for artificial incubation within 24 hours after spawning.

After spawning both members of the pair were returned to the stock tank and replaced by a fresh pair. Males and females were kept in separate stock tanks and spawning pairs were returned to spawning tanks after at least twenty one days. The process was repeated 2 or 3 times for each pair.

Following hatching, fry were on grown for 84 - 96 days. Sex of fish was determined by examination of external genitalia. Doubtful cases were confirmed by examination of gonads, the entire experiment lasted 6 months.

Fry were fed at a constant rate of 7.5% body weight per day using ground trout pellets.

5.2.2 Analysis of results

Comparison of results was done by Analysis of Variance (Anova) using the Minitab computer package (Minitab Pensylvania State University), followed by Duncan's multiple range tests (Duncan, 1955). Sex ratio for each batch was analysed by chi-square test. Except where specified, all statistically significant differences are given at 5%.

5.3 RESULTS

5.3.1 Influence of parental genotype on progeny survival

A total of 49 single pair spawns were obtained from 17 of the 25 participating pairs. The schedule for successful spawns included in the analysis was as follows:-

species	spawning fish female	male	spawning success (Days from start of experiment)		
O.mossambicus	4106	4105	2	45	62
	4110	4109	8	27	57
	4123	4121	24	50	78
O.niloticus	4873	4107	14	45	79
	4874	4138	5	28	71
	4139	4142	15	42	86
	3882	4146	10	33	-
	4893	4870	0	62	78
O.spilurus	4118	4116	13	36	67
	4125	4126	18	43	-
	4101	4119	16	47	95
O.mossambicus	4130	4116	31	55	70
x O.spilurus	3759	4126	22	45	93
	3765	4119	26	57	77
O.niloticus	4120	4105	10	50	81
O.mossambicus	4128	4109	13	44	73
	4104	4121	19	38	76

Viable fry obtained during the period were approximately 9400 as shown in Table 2.

Results show that survival of fry ranged from 57.44% to 92.67% in individual pairs and averaged 73.82 ± 1.47% for the entire experiment. There was no significant difference between averages of the different crosses (Table 5.2).

Among the pure species, O.mossambicus survival was 70.70 ± 2.25%, O.niloticus 77.77 ± 2.43, %, and O.spilurus 75.07 ± 2.71%. Both hybrid crosses had identical survival to O.mossambicus at 70.43 ± 4.30% and 70.79 ± 3.50% for O.mossambicus x O.spilurus and O.niloticus x O.mossambicus respectively.

Table 5.2 Comparative survival of juvenile tilapia obtained in single pair pawning.

species	number of Pairs	Spawns	number of fish Initial	final	survival % ± SE.
O.mossambicus	3	9	1669	1180	70.70±2.25
O.spilurus	3	8	1046	799	75.07±2.71
O.niloticus	5	14	3079	2424	77.77±2.43
O.mossambicus x O.spilurus	3	9	1275	901	78.43±4.30
O.niloticus x O.mossambicus	3	9	2345	1646	70.79±3.50
Total	17	49	9414	6950	73.82±1.47

Table 5.3. shows survival of fry from three pairs of O. mossambicus. The total number of fry was 1669 and survival 84 days after hatching ranged from 67.30% to 82.50%. The species average was 70.70±2.25%. As shown in column 6, survival in different batches from the same pair of parents was significantly different in two of the three pairs. No significant difference was found between averages in the three pairs.

Table 5.4. shows survival of progeny from three pairs of O. spilurus. The total number of fry was 1046. Survival in different batches ranged from 63.23 to 86.18% there was significant difference between means in pairs 3 with pairs one and two there was no significant difference in survival in sib groups of pairs one and two as shown in column 6.

Table 5.3 Survival of *O.mossambicus* fry obtained in single pair spawning.

Parental code Female	male	Batch	number of fish initial	Final	survival # % ± SE
4106	4105	1	150	114	76.00[b]
		2	200	165	82.50[b]
		3	200	125	62.50a
			550	404	73.67 ± 5.89*
4110	4109	1	185	143	77.30b
		2	200	123	61.50[a]
		3	123	74	60.16a
			508	340	66.35 -± 5.50*
4123	4121	1	136	92	67.65[a]
		2	175	142	81.14[a]
		3	300	202	67.33[a]
			611	436	72.04 ± 3.71*
Total		9	1669	1180	70.70±2.25

different superscripts in sib batches denote significant difference between batches ($p<0.05$). Asterisks on group averages denote significant difference between progeny from different pairs of parents ($p<0.05$).

Table 5.4 Survival of O.spilurus fry obtained in single pair spawning.

Parental Code female	male	Batch	number of fish initial	final	survival % ± SE
4118	4116	1	147	119	80.95[a]
"	"	2	106	78	73.58[a]
"	"	3	152	131	86.18[a]
			405	328	80.24 ± 3.65**
4125	4126	1	65	52	80.00[a]
"	"	2	34	26	76.47[a]
			99	78	78.24 ±1.76**
4101	4119	1	167	124	74.25[b]
		2	220	145	65.91[a]
		3	155	98	63.23[a]
			542	367	67.80±3.32*
Total		8	1046	773	75.07 ± 2.71

different superscripts in each set denotes significant difference between sib batches ($p<0.05$) Asterisks on pair mean denote significant difference between spawning pairs.

Table 5.5 shows survival of fry from O. niloticus spawning pairs. Fry production from the five pairs ranged from 200 to 600 per batch. The total number obtained in the experiments was 3079. Survival 96 days after hatching ranged from 63.74% to 92.0%. In one pair, survival differed significantly with the other four. Within each sib group, significant variation between batches was found in the second and in the fifth pairs (column 6).

Table 5.5 Survival of O.niloticus fry obtained through single pair breeding.

Parental code Female	male	Batch	number of fish initial	final	# Survival ± SE
4873	4107	1	200	148	7.40 ^{a}
"	"	2	200	179	89.50^{a}
"	"	3	300	254	84.67^{a}
sub-total			700	581	82.72± 4.58**
4874	4138	1	200	163	81.50^{b}
"	"	2	220	178	80.91^{b}
"	"	3	250	169	67.60^{a}
sub-total			670	510	76.67 ± 4.54**
4139	4142	1	250	183	73.20^{a}
"	"	2	250	210	84.00^{a}
"	"	3	250	212	84.80^{a}
sub-total			750	605	80.67± 3.74**
3882	4146	1	250	172	68.80^{a}
"	"	2	182	116	63.74^{a}
sub-total			432	288	66.27±2.53 *
4893	4870	1	166	131	79.00^{a,b}
"	"	2	275	253	92.00^{b}
"	"	3	86	56	65.10^{a}
sub-total			527	440	78.68±7.77**
Total		14	3079	2424	77.77± 2.43

different superscripts in each set in column 6 denote significant difference between sib groups ($p<0.05$); different asterisks in column 6 denote significant differences between parents.

Table 5.6 shows fry survival in three pairs of O.mossambicus x O.spilurus. The average number of fry collected was 142 per spawn, and the total from the 9 batches was 1275. Survival range was 52.31 to 92.67 %. The overall mean was 70.43 ± 4.30%. Survival in the first pair differed significantly with fry survival in the second pair (Column 6). Sib batches in pairs one and two also demonstrated significant difference within each set.

Table 5.6 Survival of O.mossambicus x O.spilurus fry obtained in single pair spawning.

Parental code female	male	batch	number of fry initial	final	survival # % ± SE
4130	4116	1	130	68	52.31^{a}
"	"	2	85	48	56.47^{a}
"	"	3	120	84	70.00^{b}
			335	200	59.59±5.34X
3759	4126	1	170	108	63.53^{a}
"	"	2	150	139	92.67^{b}
"	"	3	85	72	84.71^{b}
			405	319	80.30±8.69Y
3765	4119	1	220	160	72.73^{a}
"	"	2	150	114	76.00^{a}
"	"	3	165	108	65.45^{a}
			535	382	71.39±3.12XY
TOTAL		9	1275	901	70.43±4.30

different superscript in each set in column to denote significant difference between sib groups. Averages in column 6 followed by letters X and Y are significantly different from each other ($p<0.05$).

Table 5.7 gives the results of progeny survival for O.niloticus x O.mossambicus hybrids. The total number of fry obtained was 2345. Survival range from, 57.44% to 84.55% and averaged 70.79 ± 3.50%. There was no significant difference between average survival for each pair. However significant difference was found in different sib batches of the first and of the third pair.

Table 5.7 Survival of O.niloticus x O.mossambicus fry obtained in single pair spawning.

Parental code female	male	Batch	number of fish initial	final	survival # % ± SE
4120	4105	1	80	60	75.00 ^{b}
"	"	2	312	179	57.44^{a}
"	"	3	315	254	80.63^{b}
			707	493	71.02 ±5.70*
4128	4109	1	210	125	59.52^{a}
"	"	2	430	288	66.98^{a}
"	"	3	200	143	71.50^{a}
			840	556	66.00±3.49*
4104	4121	1	360	260	72.22^{a,b}
"	"	2	218	151	69.27^{a}
"	"	3	220	186	84.55^{b}
			798	597	75.35 ± 4.68*
TOTAL		9	2345	1646	70.79 ± 3.50

different superscripts in each set of solumn 6 denote significant difference between sib batches. Figures followed by an asterisk are not significantly different (p>0.05).

5,1,3,2 Influence of parental genotype on progeny sex differentiation.

Table 5.8 is a comparative summary of the results presented in Tables 8 - 12 for each cross. A total of 6886 fish from 49 spawns were sexed when they were between 12 and 16 weeks old. Of these, 3308 (49.35 ± 1.28%) were male. The proportion of males among the five crosses ranged from 33.29 ± 2.79% to 56.60 ± 1.51%. There was considerable variation in progeny sex ratios in different batches.

Table 5.8 Comparative progeny sex in different single pair crosses.

Cross	number of fish sexed	male	female	Ratio m/f	% male ± SE#
O. mossambicus	1148	589	559	1.05	51.09 ± 1.21 *
O. spilurus	799	455	344	1.32*	55.723 ± 2.03*
O. niloticus	2424	1213	1211	1.00	50.04 ± 1.61*
O. mossambicus x O. spilurus	901	510	391	1.30*	56.60 ± 1.51*
O. niloticus x O. mossambicus	1614	541	1073	0.50**	33.29 ± 2.79 **

Superscripts in column denote significant difference between sexes within a cross at * = $p<0.05$, and ** = $p<0.01$.

Superscripts in column 6 denote significant difference between different crosses.

Table 5.9 gives sex ratios in 9 spawns of O. mossambicus. The species mean was 51.09 ± 1.21 % male. the range for individual groups of fry was 44.14% to 55.81% there was no significant difference in average proportion of males in the three sets of sib batches.

Table 5.9 Progeny sex in single pair spawning *O. mossambicus*.

Parent code female	Parent code male	number of fish Sexed	male	female	sex ratio m/f	% male ±SE
4106	4105	111	49	62	0.79*	44.14ª
"	"	165	84	81	1.04	50.91ª
"	"	125	58	67	0.87	46.40ª
		404	191	210	0.90 ±0.13	47.15 ± 1.99*
4110	4109	143	75	68	1.10	52.45ª
"	"	123	66	57	1.16	53.66ª
"	"	74	39	35	1.11	52.70ª
		340	180	160	1.12 ± 0.03	52.94 ± 0.37*
4123	4121	93	48	45	1.06	51.61ª
"	"	142	74	68	1.09	52.11ª
"	"	172	96	76	1.26*	55.81ª
		407	218	189	1.14 ± 0.11	53.18 ±1.32*
Total		1148	589	559	1.05±0.14	51.09±1.21

Superscripts in column 6 denote significant difference between the two sexes superscripts within each set in column 7 denote significant difference beteeen sib groups. Asterisks in column 7 denote significant difference between pairs of spawners.

Table 5.10 gives progeny sex ratios of O.spilurus. The total number of fish sexed was 799 of which 455 (55.72 ± 2.03%) were male. The proportion of males in each of the 8 batches ranged from 42.31 to 62.18%. there was significant difference between sib batches in two sets. There was no significant difference between averages in different pairs. The overall sex ratio showed that the number of males is significantly higher than that of females ($p<0.05$). Progeny sex ratio in this cross was skewed in favour of males.

Table 5.10 Progeny sex in single pair spawning O. spilurus.

Parent code female	male	number of fish sexed	male	female	sex ratio m/f	% male ±SE
4118	4116	119	74	45	1.64**	62.18[b]
"	"	78	43	35	1.23	55.13[a]
"	"	131	72	59	1.22	54.96[a]
Sub-total		328	189	139	1.36±0.11	57.42* ± 2.38
4125	4126	52	22	30	0.73*	42.31[a]
"	"	26	15	11	1.36*	57.69[b]
sub-total		78	37	41	0.90 ± 0.22	50.00 ± 7.69
4101	4119	151	88	63	1.40*	58.28[a]
"	"	98	53	45	1.18	54.08[a]
"	"	144	88	56	1.57**	61.11[a]
Sub-total		393	229	164	1.38 ± 0.09	57.82* ± 1.67
Total		799	455	344	1.32 ± 0.09	55.72 ± 2.03

Superscripts in column 6 denote significant difference between males and females in the same batch * = $p<0.05$; ** = $p<0.01$. Superscripts within each set in column 7 denote significant difference between sib groups ($p<0.05$).

Asterisks in column 7 denote significant difference between pairs of spawners ($p<0.05$).

Table 5.11 shows sex ratio of O.niloticus offspring obtained from fourteen spawns The proportion of males in these spawns ranged from 40.56 to 57.30%. Significant difference was found in sex ratio of 4 of the 14 batches of fry. Averages within sib groups did not differ significantly in any of the five groups. There were significant differences in progeny sex ratio of the different pairs of spawners as indicated by superscripts X, Y, and Z in column 7.

Table 5.11 Progeny sex in single pair spawning *O.niloticus*.

Parent code female	male	number of fish sexed	male	female	sex ratio	ratio#
4873	4107	148	77	71	1.08	52.03a
"	"	179	106	73	1.45*	59.22a
"	"	254	141	113	1.25	55.51a
		581	324	257	1.26±0.09	55.59 ±2.08Z
4874	4138	163	85	78	1.09	49.53a
"	"	178	102	76	1.34*	57.30a
"	"	169	94	75	1.25	56.62a
		510	281	229	1.23±0.06	54.82Z ± 2.48
4139	4144	183	75	108	0.69*	40.98a
"	"	210	92	118	0.78	43.81a
"	"	212	86	126	0.68*	40.56 a
		605	253	352	0.72±0.03	41.79 ± 1.02X
3882	4146	172	76	96	0.79	44.19a
"	"	116	55	61	0.90	47.41a
		288	131	157	0.83±0.03	45.80 ±1.611XY
4893	4870	131	64	67	0.96	48.86a
"	"	253	130	123	0.06	51.78a
"	"	56	29	27	1.07	51.79a
		440	224	216	1.04 ±0.03	50.81±0.98YZ
Total		2424	1213	1211	1.03 ±0.06	50.04±1.61

Superscripts in column 6 denote significant difference between males and females in the same batch ($p<0.05$)

Superscripts within each set in column 7 denote significant difference between sib groups Figures followed by letters X,Y,Z, in column 7 denote significant difference between pairs of spawners ($p<0.05$).

Table 5.12 gives sex of offspring in three pairs of O.mossambicus x O.spilurus hybrids. A total of 901 fish from 9 spawns were successfully sexed. 510 (56.62 ± 1.51%) were male. In all three brood pairs, progeny sex ratios were biased in favour of males. 4 of the 9 batches of fry had sex ratios that differed significantly (column 6). Two group averages resulted in significant differences in proportions of males and females. Significant difference was found in sex ratios of two of the three sib groups (i.e. group 2 and 3). There was no significant difference between sex ratios of the three groups represented by asterisks in column 7.

Table 5.13 shows progeny sex in three of the five pairs of O.niloticus x O.mossambicus hybrid crosses. Of the 1614 fish sexed, 541 (33.29 ± 2.79 %) were male. The lowest sex ratio obtained was 13.60% and the highest 43.08%. progeny sex ratios in all batches were biased in favour of the female phenotype. there was significant difference between average sex ratios of the second parental pair compared with the first and third pairs (column 7). As shown in column 6, all batches gave significantly lower proportion of males.

Table 5.12 Progeny sex in single pair spawning *O.mossambicus* x *O.spilurus*

Parent code female	male	number of fish sexed	male	female	sex ratio m/f	% male ± SE
4310	4116	68	37	31	1.19	54.41[a]
"	"	48	26	22	1.18	54.16[a]
"	"	84	46	38	1.21	54.76[a]
		200	109	91	1.19±0.01	54.45 ± 0.17 *
3759	4126	108	62	46	1.35*	57.41a
"	"	139	87	52	1.67**	62.59a
"	"	72	34	38	0.89	47.22b
		319	183	136	1.31*±0.39	55.74*± 4.71
3765	4119	160	95	65	1.46*	59.38a
"	"	114	67	47	1.43*	58.77[a]
"	"	108	56	52	1.08	51.85[a]
		382	218	164	1.32*±0.21	56.67*±2.41
Total		901	510	391	1.30* ±0.23	56.62 ±1.51

. Superscripts in column 6 denote significant difference between males and females in the same batch *=p<0.05; **=p<0.01. Superscripts in each set of fish in column 7 denote significant difference bwteen sib groups (p<0.05). Asterisks in column 7 denote significant difference between parental pairs (p<0.05).

Table 5.13 progeny sex in single pair spawning O.niloticus x O.mossambicus.

Parent code female	male	number of fish sexed	male	female	sex ratio # m/f ±SE	% male ±SE
4120	4105	60	24	36	0.67*	40.00a
"	"	148	58	90	0.64*	39.12a
"	"	253	103	150	0.69*	40.71a
		461	185	275	0.67*	39.97±0.4#
4128	4109	125	17	108	0.16***	13.60a
"	"	288	80	208	0.38***	27.78b
"	"	143	41	102	0.40**	28.67b
		556	138	418	0.33***	23.35±3.99**
4104	4121	260	112	148	0.76*	43.08b
"	"	151	42	109	0.39***	27.81a
"	"	186	64	122	0.52***	34.41ab
		597	218	379	0.58*	35.10±4.42*
Total		1614	541	1073	0.50***	33.29±2.79

superscripts in column 6 denote significant difference between male and female in the same batch 8 = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

Superscripts in each group of column 7 denote significant difference between sib groups ($p<0.05$). Asterisks on group averages in column 7 denote significant difference between parental pairs ($p<0.05$).

DISCUSSION

5.4.1 Influence of parental genotype on survival of tilapia.

Survival rates of single pair spawned O.mossambicus, O.spilurus and O.niloticus are presented in Tables 5.3, 5.4, and 5.5 respectively. Survival rates from single pair spawned F_1 hybrids of O.mossambicus x O.spilurus, and O.niloticus x O.mossambicus are presented in tables 5.6 and 5.7 respectively. results show that survival of O.mossambicus broods averaged 70.70 ± 2.25%. There was no significant difference found between different pairs of brood fish. In O.spilurus, average survival obtained was 75.07 ± 2.71%. As in the case of O.mossambicus, no significant difference was found between the different pairs of participating parents. However, significant differences were found among some sib groups. In O.niloticus, survival of fish in 14 different batches averaged 77.77 ± 2.43%. Variation between sib groups was generally not significantly different although survival in one of the batches was very high. As in the other two species, there was no significant difference in four of the five sib groups.

Overall, the results seem to indicate that under closely controlled environmental conditions, survival in all three species is stable, thus suggesting that it may be under the control of genetic factors. Inspite some intraspecific variations among sib batches there was no significant difference found in survival of the three species, the slightly lower survival of O.mossambicus is probably a result of increased aggressive tendencies realized after sexual maturation which was becoming apparent towards the end of the experimental period.

Survival in the 9 batches of O.mossambicus x O.spilurus hybrids averaged 70.43 ± 4.30 %. Significant differences were obtained in sib batches as well as between pairs of parents. Among the F_1 broods of O.niloticus x O.mossambicus, survival averaged 70.79 ± 3.50%. There was no significant difference found among the different pairs of parents. Only one of the three sib groups resulted in significantly different sex ratio with each other.

Results from the hybrid crosses are identical to survival rates in O.mossambicus in both crosses. This result suggests that survival of progeny from these two crosses are about the same as that of the lower of the two species irrespective of which sex

is used. Although no significant difference was obtained between the different species and their interspecific hybrids, intraspecific variations were found in each of the crosses used. This is probably an indication that inbreeding has not taken place in these purebred species.

The extent to which survival is genetically controlled is not well known. However, a number of studies have demonstrated that resistence to induced mortality is species specific (Balarin and Hatton, 1979; Al-aMoudi , 1982). Maruyama (1975) compared survival of O.niloticus fry at two different temperature ranges and found that survival was higher at the lower temperature (14 - 16°C) than at the higher temperature (30 - 34°C) even though fish were actively feeding and demonstrated satisfactory growth rate at both temperature ranges.

Genetic control of survival has been infered through comparative studies under similar or carefully manipulated conditions as in the present experiments. In a comparative study, Andryasheva (1970) found that there was no significant difference in survival of carp larvae and the hybrids at a higher temperature but that lowering temperature resulted in 41% survival of pure species but 78.1% survival of the hybrids. Hybrid

advantage was also noted in a series of experiments involving four generations of hybrids (Andryasheva, 1970; Kirpichnikov, 1970). More recently; Smitherman et al. (1984) obtained higher survival for different strains of channel catfish than from parental groups. in tilapia, Smitherman et al. (1984) reported that survival in hybrids of O.niloticus x O.aureus averaged 96% compared to 72% in the pure bred parental types.

The present work does not show hybrid vigour for survival rate. The absence, of significant difference between pure species and their interspecific hybrids suggests that in breeding may not have reached significant proportion in the three pure species used in the present work. It seems likely therefore that the hybrid vigour reported by Smitherman et al. (1984) may be a result of overcoming inbreeding depression in the domesticated stocks used by the authors. (McAndrew, personnal communication). The observed variations in survival also indicate that inheritance of characters for enhanced resistance to genetically manifested mortality (e.g. reistance to disease and physiological stress) is inherited differentially in individuals.

5.4.2 Influence of parental genotype on progeny sex differentiation.

The average progeny sex ratio obtained in 9 broods of single pair spawning O.mossambicus varied from 44.14% to 55.81% male. The overall mean for the species was 51.09 ± 1.21% the difference between males and females was thus not statistically different. In repeated spawns, the mean sex ratio for each pair of parents did not differ significantly from the overall mean for the species.

The results obtained in the present experiments are identical to those obtained by Majumdar and McAndrew (1983) who found that the sex of offspring from single pair spawning in this species ranged from 43.75 to 52.99% male. Hickling (1960, 1968) obtained different ranges in experiments conducted between 1960 and 1968 as shown in Table 5.11.

The obtainment of a statistically balanced sex ratio indicates either a balanced sex determining system or an inbred stock for the sex determining factors.

In O.spilurus broods obtained in 8 spawns from 3 pairs of parental fish yielded a mean sex ratio of 1.32 males to a female. This ratio indicates significant difference in numbers of the two sexes.

Significant difference was also observed in sib groups. The range 42 - 62% male observed in the present work straddles results obtained by Majumdar and McAndrew (1983) who obtained 41 - 48% male and a significantly lower overall mean. In the present work the sex ratio was significantly skewed in favour of males. these contrasting observations in fish from the same stock suggest high diversity in sex determining factors in this stock and further serves to underline the observation made on survival that the stock is not inbred for these characteristics.

Sex ratio in 14 broods from 5 pairs of O.niloticus gave an average of 50.04% males with a range of 40.56 to 59.22% Significant differences were found between sexes in different pairs of spawners as shown in column 7 of Table 5.10. Inspite the wide range, the overall mean ratio for this species resulted in an almost equal number of males and females. In four of the broods relative numbers in the two sexes were significantly different with each other. A brief review of previous work on this species shows that only three reports specifically looked at progeny sex in single pair spawned O.niloticus (Shelton et al, 1983; Majumdar, 1984. Smitherman et al, 1984). However other ratios have been reported by various workers and those giving a range of results are included in

Table 5.1. For example Jalabert et al (1974) obtained a low of 42% male and a high of 69% male using the same stock of fish. Pruginin et al (1975) and Hsiao (1980) obtained a low of 46% and 47% male and a high of 57% and 87% male in their respective works. Shelton et al (1983) used single pair spawning technique to analyse progeny sex ratios in O.aureus and O.niloticus. From 126 progeny groups, they noted that 25% of O.niloticus and 20% of O.aureus were significantly different from the expected 1:1 sex ratio. They also noted that 3 batches of O.aureus were all-male and 4 batches of O.aureus were all-female. The lowest sex ratio was 31% male while the highest was 77% males. in their experiments, Shelton et al (1983) obtained a predominance of males (54.70%). Majumdar (1984) used genetically pure stocks and obtained different sex ratios. The lowest was 43.75% male and the highest 52.99% male. These results are comparable with the presently obtained sex ratios (41-59% male) and the present overall average does not differ significalty from that obtained by Shelton et al (1983). Smitherman et al (1984) obtained different proportions of males in a stock of O.niloticus. The lowest was 40% male and the highest 74% male. the overall ratio was skewed in favour of males.

The results of the present experiments and others cited here and in table 5.1.1 indicate that significantly different sex ratios are very common in O.niloticus. The general pattern seems to show a minimum of about 40% males, but a much wider ranging upper limit. These results seem to suggest that sex ratio determination in this species is much less stable than in the other two species.

Among broods from the cross O.mossambicus x O.spilurus, the average sex ratio was 1.30:1 male to female. This ratio is about the same as that obtained in pure O.spilurus. Chi-square analysis indicates significant difference between the two sexes ($p<0.05$). Significant differences were also found between sexes in the sib groups of some spawning pairs (column 7 Table 5.12) and between individual batches of offspring.

Results suggest that sex ratios in this cross are highly unstable. There is a general skewness in favour of males. The close similarlity in sex ratio between O.spilurus and this hybrid indicates that O.spilurus exerts a higher sex determining influence on the hybrid than does O.mossambicus. This result contrasts the sex ratios obtained in the same cross by Majumdar (1984). This author reported obtaining consistently lower sex ratios. Considering that the experiment was carried out under identical conditions with the present series,

these contrasting observations can only be justified in terms of individual variations in strength of sex determining factors in the particular fish used. In the present experiments, the same males that spawned with pure O.spilurus were used to fertilize eggs in the hybrid cross and the subsequent sex ratios appear to be closely linked. The similarity of the observed sex ratios (Tables 5.12 and 5.12) further seems to suggest that strength of the sex determining factors in both females (O.spilurus, table 5.10 and O.mossambicus Table, 5.12) are identical and that males in O.spilurus had an overriding effect over female sex determining factors.

In the cross between O.niloticus x O.mossambicus, the average sex ratio obtained was 33.29 ± 2.79 % male. This proportion of males was significantly different on the chi-square test. Significant difference was also found in sex ratios among sib groups. In the cross utilizing pure O.mossambicus male and female, the sex ratios fluctuated about the 1:1 ratio probably suggesting an even balance of sexualizing factors. On the other hand, the same male did not maintain a balance of sexes when crossed with female O.niloticus. In the cross utilizing pure O.niloticus males and females, the sexes were evenly balanced. The result of the cross O.niloticus x O.mossambicus seems to suggest

that the balance of factors in the two sexually neutral species has been tipped in favour of females; i.e. in the cross between O.niloticus x O.mossambicus, the female determining factors are predominant. Majumdar (1984) also obtained lower proportion of male in this hybrid than in either of the parents.

Results of the present study are in general agreement with previous studies that have demonstrated significant sex ratio variation in both intraspecific and interspecific hybrids (Shelton et al, 1983; Majumdar, 1984; Smitherman et al, 1984). As demonstrated in the present work, in general single pair crosses result in sex ratios that differ significantly from 1:1; but pooling batches of fry from several different parents tends to even the balance of sexes.

It is clear that few of the individual sex ratios obtained in the present work can be explained on the basis of single locus sex determination alone (i.e. XX/XY or ZW/ZZ). faced with the same dilema, some previous authors have infered that a large number of sex genes with male or female potencies are involved in sex determination. (Bull, 1983; Majumdar and McAndrew, 1983; Shelton et al, 1983; Kallman, 1984). According to this theory, sex genes are scattered over many if not all

chromosomes. As a result each gene in a set has a tendency and sex in a zygote is determined according to the total value of tendencies. In a simple formulation, a single threshold is specified, so that total values larger than the threshhold are one sex and values less than the threshold are the other sex (Kallman, 1968; Nayudu, 1979; Bull, 1983). In a population the tendencies tend to even out, so that the larger the sample examined is, the higher the likelihood of getting an even sex ratio in the population.

Such a sex determining mechanism seems more demonstable in species such as guppies where colour patterns seem a viable tool for predicting sex inheritance. Such colour patterns are in turn controlled by either X-linked or Y-linked genes (Mittwich, 1959; Kallman, 1965).

Criteria and possible models for the analysis of polygenic sex determination have been suggested by a number of authors (Kosswig, 1964; Kirpichnikov 1981; Bulmer and Bull 1982; Kallman, 1984; Majumdar, 1984).

An alternative theory that has proved useful in explaining high variability in sex ratio is the mutiple sex chromosome theory. this theory may be described as one in which two or more sex

chromosome pairs can be identified in the karyotype. (Uyedo and Miller, 1971; Thorgaard, 1978; Filho et al., 1980; De Almeida Toledo et al., 1984). Such a mechanism has been demonstrated for example by Campos (1972) in

Stephanolepis cirrhefer $X_1X_2X_2/X_1X_2Y$
Callichromous bimaculatus $X_1X_1X_2X_2/X_1X_1X_2$
Hoplias spp XX/XY_1Y_2 by Thorgaard (1978) in
Oncorhynchus nerka $X_1X_1X_2X_2/X_1X_2Y$ and by DeAlmeida Toledo et al (1984) in Eigemanannia species XXAA/XYAA.

While it appears relatively easy to demonstrate the mutiple sex chromosome theory cytologically, the theory, may not be easy to demonstrate in tilapia where no heteromorphic sex chromosomes have so far been found. (Majumdar & McAndrew, 1984) However, dealing with a finite number of variables might assist to explain some of the frequently observed sex ratios in tilapia.

On the basis of the present results and others cited in this chapter, it would seem that the absence of fixed sex ratios favours the slightly more abstract concept of polygenic sex determination. Recent application of experimental data to the mathematical models seem to support the practicability of a polygenic sex determining mechanism in tilapia (Bull, 1983; Kallman, 1984; Majumdar, 1984).

CHAPTER 6

Experiment IV

Interspecific breeding and sex differentiation

INTRODUCTION

As a biological concept the term species denotes a group of closely affiliated mutually fertile individuals, showing constant differences from other groups. Interspecific differences especially in courtship anatomy, and breeding behaviour are considered important barriers to natural hybridization.

Generally, the close genetic relationship between tilapia species increases the possibility for successful mating between individuals from different species, especially under duress (Lowe McConnell, 1959; Whitehead 1960; Lovshin, 1982; Trewavas, 1983).

In nature interspecific hybridization is a rare phenomena, and because hybrids are often morphologically intermediate between parental species, the few that are encountered have often been difficult to identify. Some early discoveries of natural hybrids include crosses between Pleuronectes platessa x Platichthyes flesus (Sick, Frydenberg and Nielsen, 1963), Salmo salar x Salmo trutta (Payne, Child and Forrest 1972) and Char x Brook Trout (Suzuki and Kato, 1966).

Natural intergeneric hybrids have been identified between Chub x Roach (Wheeler and Easton, 1978), Silver bream x Roach, and Silver bream x Bronze bream (Swinney and

Coles, 1982). Families in which natural hybrids have so far been identified include Ichtaluridae, Poecilidae, Salmonidae, Cyprinidae, Siluridae and Cichlidae (Hubbs, 1955, Krasznai 1986; Hoorbeck and Macphee, 1986; and Naevdal and Dalpadado 1986).

Among Tilapiine fishes, natural hybrids are less frequently encountered. The main reason is probably their close morphological and meristic resemblance which makes idenitification of hybrids in nature much more difficult. Cases of natural hybridization have been reported in areas with introduced species (Lowe McConnell, 1958, Yashouv and Chervisky, 1959; Whitehead 1962; Welcome 1963; 1966). Recent improvements in investigative techniques such as the use of electrophoresis have shown that hybridization in nature is probably much more common than previously presumed (Galman and Avtalion, 1983; Taniguchi et al, 1985).

In nature, the frequency of hybridization is influenced by ecological factors such as scarcity of one species contrasted with abundance of another. Generally natural hybridization has been noted more frequently in fresh waters than in salt water because there is greater opportunity for chance encounter of egg and sperm and also due to frequent desruptive change in the environment. Hubbs, 1955; Wohlfarth and Hulata, 1981; Ferreira, 1986). For example, Hubbs reported that in one small pool, he observed six or more species of minnows

breeding violently on a simple small patch which he presumes was a nest of a larger species.

Artifical hybridization has been successfully performed in many species. A prerequisite of practical application, is that the progeny should have a reasonably high degree of survival at all stages in the life cycle, (Naevdal and Delpadado, 1986). Hybrid sterility and inviability could prevent the process of hybridization between species. Although presumably rare, genetic incompatability can be a major hinderance to the successful execution of a potentially important hybrid cross such as in 'remote' hybridization for induction of gynogenesis, or production of gametic sterility in intergenic crosses of tilapia (personal observation).

Hybrid steiility is a result of disruption of the normal processes of meiosis. This may be due to the structual differences that prevent pairing of homologous chromosomes or, could be due to hybrid developmental abnormalities affecting the reproductive tissues. Inviability is probably a result of a disruption of the integration of growth and development processes in the individual organism due to a failure of the parental genome to co-operate in directing growth and development synchronously or harmoniously. Inviability is synonymous with maldevelopment death, and may occur at any stage from blastula to adult.

Hybrid characteristics are generally intermediate between the parental species and are of considerable interest from both taxonomic and phylogenetic view points (Chevassus, 1983; McAndrew and Majumdar, 1983; Krasznai, (1986);

Numerous viable interspecific crosses between different groups of fish including tilapias have been reported. Intergeneric hybrids have been produced albeit much less frequently. For example, viable allotriploid hybrids of Cyprinus carpio x Ctenopharyngodon idella and C. Carpio x Hypophthalmichthys molitrix (Krasznai, 1986). Viable gynogenetic and androgenetic progenies from Common carp x grass carp and viable sterile hybrids of grass carp x big head carp have been reported by Stanley and Jones (1976) and Beck et al. (1980) respectively.

Several artifical interspecific hybrids of tilapia have been produced. Of particular interest to aquaculture is the reproductive incapacity of these hybrids. Some of the hybrids develop normally in both sexes, others develop as monosex broods, while others may be sterile in both sexes. A list of crosses that resulted in all-male fish is given in table 1.

Generally interspecific crosses produce normal fertile offspring while intergeneric tilapia hybrids, are either sterile or show highly skewed sex ratios (Whitehead, 1960; Crapon de caprona, 1986; Rana, personal

Table 6.1 Reported hybrid crosses of tilapiine fishes yielding all-male offspring.

Female	Male	Reference/Source
Oreochromis niloticus	O.aureus	Fishelson 1962 Pruginin et al.1975 Hsiao 1980 Hulata 1981
O.aureus	O.niloticus	Loushin and De Silva 1975
O.niloticus	O.hornorum	Pruginin, 1968; Bard, 1969 Jalabert et al.1971; Lovshin et al.1974; Pinto 1978; Hulata et al.1981 Wohlfarth et al.1983.
O.niloticus	O.variabilis	Pruginin 1967
O.niloticus	O.macrochir	Lessent 1967; Jalabert et al.1971 Majumdar and McAndrew, 1983
O.mossambicus	O.hornorum	Hickling 1968; Chen 1969; Bard 1975; Hulata et al.1981 Wohlfarth et al, 1983
O.mossambicus	O.macrochir	Majumdar 1984
O.nigra	O.hornorum	Pruginin 1967
O.spilurus	O.macrochir	Majumdar 1984
O.aureus	O.hornorum	Pinto 1978
O.hornorum	O.aureus (97%)	Hulata et al, 1981
O.hornorum	O.niloticus	Hulata et al, 1981
T. zillii	O.andersoni	Ibrahim 1976

communication) Arai (1984) has demonstrated karyologically that in intergeneric hybrids of Salmonids, genomic incompatibility between species is a likely cause of abnormal development. Among the tilapias high viability was realised when eggs of substrate spawning Tilapia zillii were fertilized with milt from either Saratherodon or Oreochromis species (Rana, personal communication), but the reciprocal crosses involving Oreochromis females with males of T.zillii, T.mariae, and T. buterkoferi were not successful (personal observation).

A major reason for the growing interest in interspecific hybridization is the possibility of obtaining monosex broods for aquaculture without the necessity for contamination with steroid hormones, or by using laborious hand sexing techniques (Hickling, 1960; Chen 1969: Jalabert et al. 1971; Bardach et al, 1972). Previous studies have shown that monosex broods are more likely to be obtained if fish with alternative sex determining mechanism are crossed (Chen 1960; Jalabert et al, 1974; Avtalion and Hammerman, 1978), For this reason, attention has recently been focussed on positively establishing genetic sex determining mechanisms to simplify the task of monosex fish production for aquaculture; the simplicity with which interspecific hybrids are obtained, coupled with recent improvements in in-vitro fertilization techniques, has created fresh opportunities for better planned interspecific breeding.

The possibility for artificially obtaining monosex hybrids in tilapia was first hatched by Hickling (1960) when he crossed female. O.mossambicus x male O.hornorum and obtained all male offspring. Since then interspecific hybridization has been practised with increasing enthusiasm. Of nearly 100 crosses reported during the past 2½ decades, about 30 have resulted in all-male offspring (Table 1) and many more yielded at least 90% males. One motivating factor for production of monosex tilapia is the anticipated growth benefits which include hybrid vigour. A review of the literature indicates that of the nearly 100 crosses, production of all-male broods has only been realized with 6 females and 6 males as follows:-

Females	Males
O.mossambicus	O.hornorum
O.niloticus	O.macrochir
O.nigra	O.variabilis
O.aureus	O.aureus
O.hornorum	O.niloticus
O.spilurus	O.andersoni

Monosex broods have been obtained more frequently when either O.hornorum or O.macrochir are used as sperm donors. The use of O.aureus also yields high proportion of male offspring. The other three (O.niloticus, O.andersoni and O.variabilis) have each been reported once as donors of sperm that yielded all-male broods.

Table 6·2 Range of sex ratios reported for first generation interspecific hybrids in the genus Oreochromis.

Cross	% male (Lowest/highest)	Source/reference
O.mossambicus x O.aureus	49.16	Majumdar, 1984
	91.00	Hsiao, 1980
O.aureus x O.mossambicus	71.6	Avault and Shell 1968
	80.0	Hulata et al, 1981
O.mossambicus x O.niloticus	15.00	Kuo 1968
	64.00	Hsiao 1980
O.niloticus x O.mossambicus	3.57	Majumdar, 1984
	72.00	Hsiao 1980
O.mossambicus x O.hornorun	49.6	Hicking 1960
	100	Hickling 1960
O.hornorum x O.mossambicus	48.00	Hickling 1960
	90.00	Hulata et al, 1981
O.mossambicus x O.spilurus	30.61	Majumdar 1984
	64.13	Present report
O.spilurus x O.mossambicus	26.92	Majumdar 1984
	44.44	Majumdar 1984
O.mossambicus x O.macrochir	100	Majumdar 1984
O.niloticus x O.macrochir	56.52	Majumdar 1984
	100	Jalábert et al, 1971
O.macrochir x O.niloticus	68.00	Lessent 1968
	77.00	Lessent 1968
O.spilurus x O.macrochir	94.11	Majumdar 1984
	100	Majumdar 1984
O.spilurus x O.niloticus	47.92	Majumdar 1984
	50.00	Majumdar 1984
O.niloticus x O.hornorum	100	Pruginin 1967
	100	Garcia 1982
O.hornorum x O.niloticus	50.00	Hulata et al, 1981
	100	Huluta et al, 1981
O.hornorum x O.aureus	77.00	Pinto 1982
	97.00	Hulata et al, 1981
O.aureus x O.hornonum	100	Garcia 1982
O.aureus x. O.niloticus	33.00	Hulata et al. 198
	88.29	Majumdar, 1984
O.niloticus x O.aureus	50.00	Garcia 1982
	100	Pruginin et al, 1975
O.vulcani x O.aureus	52	Pruginin et al, 1975
	98	Pruginin et al, 1975

Commercial production of all-male broods through interspecific hybridization has been realised in Israel where about 80% of the total production of tilapia from fish farming is obtained through this technique (Mires 1983). Elsewhere the technique has not been so successful. The failure to obtain consistent monosex broods in commercial tilapia culture can be attributed to the observed irregular progeny sex ratios in the crosses even under experimental conditions (table 2). As can be seen in Table 2, variant sex ratios are found even within the same stocks as demonstrated by Hickling (1960), Lessent (1968) Pruginin et al. 1975, and in crosses between certified pure species Majumdar (1984). Majumdar and McAndrew (1983) carried out 27 interspecific crosses using five genetically pure species of tilapia. Results showed sex ratio variation in crosses between O.spilurus x O.mossambicus (26.92 - 44.44% males) and (30.61-36.23%) in the reciprocal cross. The cross O.niloticus x O.aureus resulted in progeny sex ratios ranging from 52 - 85.71 % males. These results help to dispel the erroneous assumption that sex ratio variations encountered during interspecific hybridization are probably due to use of impure stocks. In order to try and explain sources of variation in progeny sex ratio of interspecific hybrids, a number of theories have been suggested. In 1960, Hickling carried out experiments with O.mossambicus x O.hormorum and obtained sex ratios ranging from 49.6 to 100% male offspring. He

concluded that a lethal factor was probably killing off female eggs. In 1969, Chen carried out a series of crosses using O.mossambicus and O.hornorum. In repeated experiments, he showed that while mating within each species gives a 1:1 sex ratio, interspecific crosses between female O.mossambicus with male O.hornorum results in 100% male offspring. He further demonstrated that a reciprocal cross between the two species results in a Mendelian sex ratio of 1:3 female to males. Earlier, Gordon (1947) had found that natural populations of platyfish in Mexico had a homogametic female (XX), and that the a heterogametic male (XY),and the Honduran population of the same species had a heterogametic female (WY) and a homogametic male (YY) system. Gordon had reported that when a homogametic male (YY) was crossed with a homogametic female (XX), all-males were produced, and that when a reciprocal cross of heterogametic fish was performed, a 1:1 ratio was obtained. Thus Gordon explained the results by a three sex chromosome theory with W,X. females and Y. male. On the basis of Gordon's theory, Chen (1969) interpreted his results to mean that O.mossambicus has a homogametic (XX) female and heterogametic (XY) male; on the other hand, O.hornorum was assessed as having a homogametic male (ZZ) and a heterogametic female (WZ). However, further crosses with presumed heterogametic hybrids failed to yield the predicted Mendelian ratios in F2 crosses and reciprocal back crosses. Chen concluded that differential mortality might have occurred.

Jalabert et al, (1971), Lovshin et al (1975) and Lee (1979) conducted similar experiments to those of Hickling (1960) and Chen (1969). By counting eggs prior to fertilization, they were able to demonstrate conclusively that the theory of selective mortality was inadequate for explaining the observed sex ratio variations. Jalabert et al. (1971) used O.niloticus x O.macrochir and O.macrochir x O.niloticus parents and obtained 0:1 and 1:3 females to males respectively. These sex ratios were in agreement with Chen's hypothesis. However, reciprocal back crosses of O.macrochir x O.niloticus hybrids with O.niloticus male yielded 1:2, 1:3, 1:1.47, 1:1.63; and 1:1.78 female to male while the back cross with O.macrochir male resulted in 1:1, 1:2, 1:1.18 and 1:0.63 female to male. These sex ratios could not reasonably be explained on the basis of Chen's reversed role hypothesis.

They concluded that autosomes were probably influencing sex determination in some hybrid crosses. The theory of autosomal sex determination was first proposed by Winge (1934). This theory supposes that in addition to the sex chormonsomes, there are also autosomes with male and female determining potencies (Kosswig, 1964; Kallman, 1968) but that these autosonal factors are not sex genes per se. On the basis of this theory, Avtalion and Hammerman (1978) and Hammerman and Avtalion (1979) proposed a model of a pair of autosome complements. According to their theory, each species has three

gonosomes complemented by two autosomes so that each chromosome is independent of the other and sex is determined in an individual by their additive effect. The model enabled them to work out eighteen different genotypes each with a pair of autosomes (AA, Aa, aa) and two sex chromosomes (WX, WY, WW, XY, XX, or YY). The theory enabled them to predict eight sex ratios (0:1, 1:3, 3:5, 1:1, 9:7, 5:3, 3:1, and 1:0) female to male. Avtalion and Hammerman (1978) applied this model to Chen's 1969 data and found that they were able to explain 3 additional sex ratios (5:3, 9:7 and 3:5) that could not be predicted on the basis of Chen's four chromosome theory alone. However even this theory was not able to justify all the observed results.

The foregoing review demonstrates the array of sex ratios found in tilapia hybrids. Despite the effort, so far no one hypothesis seems to provide an acceptable solution to the problems encountered in analysing sex ratios obtained when different species of tilapia are hybridized in different sequences. It is obvious that without a proper understanding of the underlying mechanism for sex determination, production of monosex broods for aquaculture will remain as a chance encounter.

One of the objectives of the present experiments is therefore to investigate the sex determining mechanisms in three pure species of Oreochromis (namely O.niloticus

O. mossambicus, and O. spilurus) by analysis of hybrid progeny sex ratios.

In a previous study involving pure species, Majumdar (1984), and Majumdar and McAndrew (1987) analysed sex ratios in first generation hybrids. The present study has been extended to include second generation hybrids and back crosses. No attempt was made to obtain reciprocal crosses with the parental species as these were included in an earlier analysis using the same broodstock (Majumdar, 1984; Majumdar and McAndrew, 1987).

MATERIALS AND METHODS

Pure species of O.spilurus, O.mossambicus and O.niloticus were used in the experiment (McAndrew and Majumdar 1983). The following crosses were carried out in order to compare survival rates and proportion of the two sexes among the offspring. Throughout the present work, the female parent is given before the male.

O.niloticus x O.mossambicus F_1
O.mossambicus x O.spilurus F_1

O.niloticus x O.mossambicus F_2
O.mossambicus x O.spilurus F_2

(O.niloticus x O.mossambicus) F_1 x O.niloticus
(O.niloticus x O.mossambicus) F_1 x O.mossambicus

Yields from these crosses were compared against each other and with pure species obtained under identical treatment.

Each of the above crosses was replicated two or three times using different pairs of parents. F_2 hybrids were obtained by using sibling groups of the F_1 groups as broodstock. Similarly backcrosses were obtained by artificially fertilizing sub-batches of eggs from F_1 hybrids female with sperm from parental species males.

Fry were fed microtonized trout diet at 10% body weight per day; and reared in 60 liter plastic tanks of a re-circulated water system until ready for sexing by examination of the urogenital papillae when about 96 days old. To highlight the morphological distinction between the sexes, the genital papillae were touched with a dye (crystal violet or Methylene blue) before examination under a magnifying glass. Fish not satisfactorily identified by the external features were killed and had their sex determined by visual examination of the gonads.

Results were compared by one way analysis of variance (ANOVA) followed by Duncan's multiple range testing. Individual sex ratios were analysed by Chi-square testing using a minitab computer package.

RESULTS

Survival

Table 3 is a summary of survival data from all crosses performed, and comparative survival of the parental species.

Average survival in 3 spawns of O.niloticus was 82.72 ± 4.58%. Survival of O.niloticus was 82.72 ± 4.58%, Survival of O.mossambicus was 73.67 ± 5.89%. Survival from four spawns of the F_1 hybrids between the two species was 89.61 ± 1.83 %. Hybrid survival range was 85.60% to 93.20%.

There were significant differences between survival of different batches of pure O.niloticus and pure O.mossambicus. However, no significant difference ($p>0.05$) was found in survival between the four batches of hybrid fish. Overall survival of the hybrids was significantly higher than that of O.mossambicus and higher than that of O.niloticus though not significantly different.

Survival of the F_2 hybrids and the backcross with male O.niloticus was about the same as that of pure O.niloticus and did not differ significantly from the F_1 hybrids.

Survival of the back cross with male O. mossambicus was significantly lower than that of the F_1 hybrids, but about the same as in parental O. mossambicus.

Survival of pure O. mossambicus and O. spilurus did not differ significantly from that of their F_1 and F_2 hybrids which were almost the same as that for O. spilurus. Marginal difference was found in survival of fry in the F_1 groups (Table 4).

Table 6·3 Survival of progeny in O.niloticus and O.mossambicus, and between their F_1 and F_2 backcross offspring.

Cross female	male	number of fish initial	final	survival % ± SE
O.mossambicus	O.mossambicus	150	114	76.00[a]
		200	165	82.50[a]
		200	125	62.50[b]
		550	404	73.67 ± 5.89x
O.niloticus	O.niloticus	200	148	74.00[a]
		200	179	89.50[b]
		300	254	84.67[b]
		700	581	82.72 ± 4.48XY
O.niloticus	O.mossambicus	162	151	93.20[a]
		107	94	87.50[a]
		115	106	92.17[a]
		264	226	85.60[a]
		648	577	89.61 ± 1.83Y
O.nil x Omos	O.nil x O.mos	140	124	88.57[a]
		75	60	80.00[a]
		180	148	82.22[a]
		67	53	79.10[a]
		462	385	82.47 ± 2.14XY
O.nil x O.mos	O.mossambicus	265	212	80.00[a]
		195	128	65.64[b]
		4.60	340	72.82 ± 7.18X
O.nil x O.mos	O.niloticus	74	67	90.54[a]
		155	117	72.48[b]
		229	184	81.51 ± 9.03XY

Figures within each group in column 5, followed by letters a, ab, b are significantly different from each other ($p<0.05$).

Group averages in column 5 followed by letters X, XY, or Y are significantly different from each other ($p<0.05$).

Table 6.4 Survival of progeny in O.mossambicus and O.spilurus, and of their F_1 and F_2 hybrids.

Cross Female	Male	number of fish initial	final	survival % ± SE
O.mossambicus	O.mossambicus	150	114	76.00[a]
		200	165	82.50[a]
(As in table 3)		200	125	62.50[b]
		550	404	73.67 ± 5.89X
O.spilurus	O.spilurus	147	119	80.95[a]
		106	78	73.56[a]
		152	131	86.18[a]
		405	328	80.24 ± 3.65X
O.mossambicus	O.spilurus	124	92	74.19[a]
		176	147	83.52[ab]
		137	119	86.85[b]
		437	358	81.92 ± 3.79X
O.mos x O.spil	O.mos x O.spil	80	68	85.00[a]
		124	108	87.09[a]
		220	160	72.73[a]
		424	336	81.61 ± 3.17X

* Average survivals in column 5 followed by a Letter X are not significantly different from each other (P>0.05).

Survival figures within each group of fish, followed by the same letter are not significantly different at (p>0.05)

SEX DIFFERENTIATION

Tables 5 and 6 are summaries of offspring sex ratios obtained through interspecific breeding of O.niloticus with O.mossambicus and of O.mossambicus with O.spilurus respectively.

Data from batches of intraspecific breeding of parental species is included for comparison.

Results show that on the basis of Chi square tests, sex ratios in some batches of offspring differ significantly (columns 3,4, and 5). Both the frequency and magnitude of differences is highlighted by hybridization but moderated in back crossing.

Within each cross, no significant difference was found between sex ratios of O.mossambicus and O.niloticus (Column 6, table 5) but significant differences were found in sex ratio of different batches of fish in the same cross of F_1 F_2 and the back cross with male O.mossambicus. Significant sex ratio differences were also found between group averages of some crosses as shown by different superscripts in column 6 (table 5).

The trend in table 6 is similar to that in table 5. Most batches of O.spilurus and the F_1 and F_2 hybrids differ significantly in proportion of male to female progeny. With the exception of F_2 progeny. Overall sex ratios of

different batches of fish within a cross do not differ significantly with each other (superscripts X and Y in column 6, Table 6). The proportion of males in O.spilurus and both F_1 and F_2 hybrids was significantly higher than that obtained in O.mossambicus.

TABLE 6.5. Sex of offspring in O.niloticus and O.mossambicus, and their F_1, F_2 and reciprocal backcrosses.

Cross female	male	Progeny Sex female	male	Sex ratio * m/f	% male ± SE
O.mossambicus	O.mossambicus	62	49	0.79*	44.14[a]
		81	84	1.04	50.91[a]
		67	58	0.87	46.40[a]
		210	191	0.90±0.13	47.15±1.99Y
O.niloticus	O.niloticus	71	77	1.08	52.03[a]
		73	106	1.45*	59.22[a]
		113	141	1.25*	55.51[a]
		257	324	1.26 ±0.09	55.59±2.08Z
O.niloticus	O.mossambicus	119	32	0.27***	21.19[a]
		71	23	0.32***	24.47[a]
		67	39	0.58**	36.79[b]
		141	85	0.60**	37.61[b]
		398	179	0.44±0.17***	30.02±4.21W
O.nil x O.mos	O.nil x O.mos	63	61	0.97	49.19[c]
		36	24	0.67*	40.00[cb]
		90	58	0.64*	39.19[b]
		40	13	0.33***	24.53[a]
		229	156	0.65 ±0.26**	38.22±5.10WY
O.nil x Omo	O.mossambicus	126	86	0.68*	40.57[a]
		59	69	1.17	53.91[b]
		185	155	0.93±0.35	47.24±6.67Y
O.nil x O.mo	O.niloticus	39	28	0.72*	41.79[a]
		68	49	0.72*	41.88[a]
		167	77	0.72*	41.84± 0.83XY

*Numbers in column five followed by an asterisk are by chi-squared analysis significantly different

* = $p<0.05$
** = $p<0.01$
*** = $p<0.001$

Superscripts on numbers of each batch within each cross in column six are significantly different from each other ($p<0.05$).

Group averages in column six followed by superscripts W.X.Y and Z are significantly different ($p<0.05$).

TABLE 6·6 Sex of offspring in O.mossambicus and O.spilurus and their F_1 and F_2 hybrids.

Cross female	male	Progeny female	Sex male	Sex ratio.* m/f	%male± SE
O.mossambicus	O.mossambicus	62	49	0.79*	44.14[a]
		81	84	1.04	50.91[a]
(as in table 5)		67	58	0.87	46.40[a]
		210	191	0.90±0.13	47.15±1.99[X]
O.spilurus	O.spilurus	45	74	1.64***	62.18[a]
		35	43	1.23*	55.13[a]
		59	72	1.22*	54.96[a]
		139	189	1.36±0.11	57.42±2.38Y
O.mossambicus	O.spilurus	33	59	1.79***	64.13[a]
		69	78	1.13	53.06[a]
		45	74	1.64***	62.18[a]
		147	211	1.52±0.35***	59.79±3.41Y
O.mos. O.spil	O.mos. x O.spil	31	37	1.19	54.41[a]
		21	87	4.14***	80.56[b]
		65	95	1.46**	59.35[a]
		117	219	2.26±1.63	64.78±8.02Y

* Figures in column 5 followed by an asterisk were significantly different by chi-square analysis.

* = $p<0.05$
** = $p<0.01$
*** = $p<0.001$

Figures in each cross followed by letters a,b or c are significantly different from each other ($p<0.05$).

Group averages in column six, followed by superscripts X,Y are significantly different ($p<0.05$).

DISCUSSION

SURVIVAL

In general, hybridization is practised in order to combine desirable traits from two or more species into a single culturable type. Thus in successful hybridization, in heritable characters generally show intermediate inheritance (Victorovsskii, 1970; Wheeler and Easton, 1978). Identification of hybrids can therefore be done by analysis of inheritable characteristics in which the parental types differ. These include morphological, biochemical and physiological aspects (Magee and Phillip, 1982. Marion et al 1986). Success of hybridization is measured by the degree to which the desirable character has been inherited in viable hybrids.

The present work compared survival in different combinations of the hybrids. Results show that O.mossambicus, O.niloticus, and O.spilurus hybridize readily and yield viable offspring. The hybrids between O.niloticus x O.mossambicus and O.mossambicus x O.spilurus are fertile in both male and female and will inturn successfully outcross with each other and with their parental types. For example in an incidental three way spawn, F_1, O.niloticus x O.mossambicus eggs were successfully fertilized by a male F_1, O.mossambicus x O.spilurus; 85% of the fertilized eggs hatched normally and a survival of 93% (480 fry) was noted after 21 days when the fry were destroyed.

The results further show that survival of hybrids may vary in different batches of the same species and sometimes between batches within a cross. Variations in survival may be a result of genetic and environmental factors; In the present work environmental factors were seemingly minimized by rearing fish under closely monitored conditions in a recirculated system. It is therefore highly probable that the observed variations are largely indicative of genetic factors.

Genetic factors may be manifested through chromosomal recombination and incompatability of crossover events, or by differences in quality of both sets of gametes (Arai, 1984). Generally the more phylogenetically distant the species are, the lower the chances of successfully producing compatible gametes and hence viable offspring. This point has been illustrated in the example of hybridization attempts between species from the genus Oreochromis with species from the genus Tilapia and by Arai's report on intergeneric hybrids between Salmonids. As early as 1927, Winge noted that one of the unusual aspects of sex chromosomes is that for most of their length, they appear to be undifferentiated, and yet crossover events often take place. In this regard, the locus for a lethal factor cannot easily be identified by microscopic examination.

Recent reports have demonstrated that not all viable offspring from hybridization are hybrids. For example some

crosses between common carp and silver carp are gynogenetic fish (Makeeva, 1975; Stanley and Jones, 1976; Bakos et al. 1978) and Chevassus (1983) stated that attempted hybridization may result in either successful production of hybrids with diploid triploid or tetraploid genomes or may result in parthenogenetic development leading to gynogenesis or androgenesis. These reports seem, to suggest differences in capabilities of paternal genomes to penetrate the maternal cytoplasm and effect combination of the two parental haploid genome.

Survival of F_1 hybrids of O.niloticus with O.mossambicus was significantly higher than that of O.mossambicus and demonstrably higher than in O.niloticus. In the crosses O.mossambicus x O.spilurus, there was no significant difference found in survival between parental species and the two hybrids (F_1 and F_2). Survival was higher than in O.mossambicus and about the same as that obtained in parental O.spilurus. Obtainment of heterosis in survival of hybrids is probably due to an additive effect by genes controlling survival or restoration of heterozygosity.

Enhanced survival in fish has been reported in a number of interspecific hybrids including those of common carp (Andriyasheva, 1970) and in Salmonids (Naevdal and Delpadado, 1986). Among the tilapias, Avault and Shell (1968) obtained 94% and 86% survival in reciprocal hybrids of O.mossambicus with O.niloticus. These values were much higher than the 80% and 79% obtained for parental

O.niloticus and O.mossambicus respectively and Smitherman et al. (1984) reported F_1 hybrid survival averaging 96% compared with 72% in parental species when they crossed O.niloticus x O.aureus.

Heterosis is of great potential practical application in fish culture, clarification of the mechanisms involved would be useful in planning selection experiments. Hybrid superiority in survival is probably realised through faster growth in the early stages, thus ensuring better utilization of food. It is also probable that hybrids by some crosses are more viable and better able to withstand unfavourable environmental changes and are more resistant to disease than the parental types. Such advantages can be related to the underlying objective of hybridization which is to combine favourable dominant factors from different individuals into a single animal. Andriyasheva (1970) suggests that heterosis may be a result of higher heterozygosity of the hybrid which is manifested through biochemical enrichment of the underlying mechanism.

Sex Differntiation

All the six hybrid crosses attempted (2 x F_1 ; 2 x F_2; 2 x backcross) proved viable and yielded survival rates similar to or higher than those of the parental species.

All the crosses yielded mixed sex progeny which were fertile in both sexes. Progeny sex ratios were as shown in table 7. In all, 18 treatments were used. From the six

Table 7 Summary of sex ratios obtained in F_1, F_2 and backcrosses of O.niloticus x O.mossambicus and in O.mossambicus x O.spilurus.

Cross		Sex ratios	mean
female	male	female/male	
O.niloticus	O.mossambicus	4:1; 3:1: 7:4	7;3
O.nil x O.moss	O.nil x O.moss	1:1; 3:2; 3:2;	3:1 3:1
O.nil x O.moss	O.mossambicus	3:2; 7:6 - -	1:1
O.nil x O.moss	O.niloticus	7:5; 7:5 - -	7:5
O.mossambicus	O.spilurus	5:9; 1:1; 3:5	3:5
O.mos x O.spil	O.mos x O.spil	1:1; 1:4; 2:3	5:9

Sex Ratio	4/1	3/1	7/4	3/2	7/5	7/6	1/1	2/3	3/5	5/9	1/4
Frequency	2	2	1	3	2	1	3	1	1	2	1

Batch ratios 11; Treatments 18; crosses 6.

From the six crosses, eleven different sex ratios were obtained. The highest was 4 females to a male, and the lowest 4 males to a female.

Results clearly demonstrate that even within each cross, sex ratios of progeny are rarely constant.

Review of Literature demonstrated that sex ratios from interspecific hybridization show a wide range of values (Table 2). In the cross O.niloticus x O.mossambicus, KUO (1969) obtained 15% male while Hsiao (1980) reported 64% male. The reciprocal cross of the two species was even more varied with a low value of 3.57% male obtained by Majumdar (1984) against a high of 72% male obtained by Hsiao (1980).

In the cross O.mossambicus x O.spilurus the range of sex ratios was 30.61% male (Majumdar 1984) to 64.13% male

obtained in the present experiments. The reciprocal cross yielded 26.92% to 44.44% male, both obtained by Majumdar (1984). Sex ratios of F_2 hybrids are not frequently reported and the few that were found were spot values i.e. single mean value. However, the sex ratios of F_2 O.niloticus x O.mossambicus and of the reciprocal F_2 progeny obtained by Avault and Shell (1968) were 92.4% and 89.5% males. These treatments were not replicated and can only serve as a guide. F_2 sex ratio for O.niloticus x O.mossambicus in the present experiment had a range of 25% to 50% male. No comparable F_2 values could be found for the cross O.mossambicus x O.spilurus. The range for F_2 sex ratio in the present experiments was 54 - 81% male.

Interspecific hybridization has been used successfully in research for investigating phylogenetic relationships, recombination of desirable culture characteristics, for production of monosex broods, and many other uses. Hybridization has also been used as a means for investigation of sex determining mechanisms, particularly when stable sex ratios can be obtained. The main interest of hybridization in tilapia culture is to produce all-male broods for pond stocking without further treatment or sorting. Although some crosses resulted in the successful production of all-male broods (Table 1) most attempts have shown that the predicted sex ratios are not always obtainable (Table 2). The following examples will help to highlight the point. The results were calculated on the

basis of the most popularised sex determining mechanism as proposed for tilapia species by Chen (1969).

1. Avault and Shell (1968) obtained 71.6% and 70.6% male in reciprocal hybrids of O.niloticus xO.mossambicus; and 92.4 and 89.5% male in the respective F_2 progenies.

   These results are peculiar in that the species used are both presumed to have female homogametic sex determining mechanism (XX), and would therefore be expected to yield a 1:1 sex ratio.

2. Ibrahim (1976) reported a successful cross between T. zillii and O.andersoni which resulted in all-male broods. Although the mechanisms for sex determination are yet to be reported for both species, other intergeneric hybridization reports indicate that Ibrahim's result is rather unlikely (Chervassus 1983; John and Reddy, 1986; and personal observation, present work).

3. Garcia (1982) obtained 90% male in backcrosses of (O.aureus x O.hornorum) with O.aureus female. Pinto (1978) obtained all-male progeny from a cross between O.aureus female with O.hornorum male.

   These two species have been reported to have male homogametic sex. On the basis of Chen's (1969) chromosome theory, the cross should have yielded equal numbers of males and females.

4. Pruginin and Hulata (1981) obtained equal numbers of males and females in a cross between female O. mossambicus with male O. aureus. Theoretically all-male offspring were expected since O. aureus is presumed to have male homogamety.

The foregoing examples along with other sex ratios summarized in table 2 cast doubts on the validity of a homo-heterogametic sex chromosome arrangement for tilapiine fishes.

Most sex ratio variations in tilapia are generally attributed to

1. Use of impure stocks.

2. Mistaken identity of species.

3. Sex influenced differential mortality.

Majumdar and McAndrew (1983) screened the species using modern biochemical techniques before using them in experiments. Results demonstrated that sex ratio variations occur in genetically pure stocks with equal randomness.

As will be explained later in this section the basis for chromosomal sex determination is a set of four chromosomes

that can be rearranged in a fixed number of permutations, so that irrespective of species used, the four chromosomes can only be rearranged into three sex ratios namely 1:1; 1:3; and 0:4 females to males. This means that even mistaken identity should not alter sex ratios away from the above three if the four chromosemes are the sole sex determinants.

The presumed influence of sex dependent mortality has been investigated by Lovshin et al. (1975) and Lee (1979) and found not to have a significant influence on progeny sex ratio.

In the present work 18 batches of fry from 6 crosses were analysed. A total of 11 different sex ratios were obtained (table 7). As reviewed in the introduction to the present chapter, many previous workers obtained widely ranging sex ratios that could not be reasonably reconciled with the sex determining mechanism proposed by Chen (1969). For this reason a number of researchers have in the past proposed alternative hypothesis. In the following section, some key hypothesis will be reviewed in conjunction with results obtained in the present experiments.

Chromosome sex determination.

In diploid sexually reproducing species, the genetic information responsible for intimating the various manifestations of sexual differentiation is often restricted to one pair of homologous chromosomes thus, sex

is determined at fertilization through the combination of chromosomes derived half from the ovum and half from the sperm. These chromosomes are known as sex chromosomes. In many instances, evolution has led to a visible structural modification of one of these homologs while the other has remained basically unchanged. The sex that carries the changed pair of chromosomes (XY or ZW) is termed heterogametic. The opposite sex with two similar chromosomes (XX or ZZ) is said to be homogametic.

Although evidence is often indirect, both male and female heterogamety are known to occur. Individual fish chromosomes tend to be difficult to distinguish because.

i. Chromosomes are very small

ii. They are numerous

As recently as 1966, Nayyar (cited by Ebeling and Chen, 1970) reaffirmed that only two of the cytologically known species had been reported to possess sex chromosomes. Of the 25,000 species of bony fish known (Ebeling and Chen 1970), about 1,000 species have had their chromosome numbers and karyotypes investigated (Yamazaki, 1983), and of these about 100 have been found with hetermorphic sex chromosome pairs (Chen 1969; Ebeling and Chen, 1970; Kirpichnikov, 1981; Bull 1983; Yamazaki, 1983). A sample list is given in Appendix 3.

The majority of fishes have morphologically undifferentiated sex chromosomes. The only reported finding of heteromorphic sex chromosomes in tilapia is by Nijjhar et al. (1983) in O. niloticus, O. multifasciatus and T. busumana. Their claim that all three have homogametic male is in direct conflict with the established findings in O. niloticus (from hybridization) and casts doubts on the authenticity of the new finding.

Genetical and developmental experiments featuring cyprinodontid fishes suggest that chromosomal sex determination may vary both within and between species, and can occasionally allow considerable flexability in ultimate sexual expression (Winge, 1934; Aida, 1936; Gordon, 1954; Schultz, 1961; Harrington, 1967).

Ebeling and Chen (1970) suggested three criteria for the confirmation of heterogamety in fish.

i. the invariable occurance of a heteromor phic pair of chromosomes in one sex but not in the other sex.

ii. the presence of an atypically behaving bivalent at meiosis - usually in end to end chromatin association.

iii. the presence of two different karyotypes in secondary spermatocytes each containing a single member of the heteromorphic pair.

Because there are no confirmed descriptions of heteromorphic chromosomes in tilapiine fish, the morphological technique for investigating sex determining mechanisms is not applicable.

The technique commonly used is the sex chromosome mechanism as detailed in the introduction to this chapter. Gordon (1947) suggested the technique to explain the observed sex ratios in the platyfish Xiphophorus maculatus. It was later adopted by Chen (1969) to explain observed sex ratios in tilapia hybrids.

According to this hypothesis, some fish have a homogametic female (XX) and heterogametic male (XY) while alternative species have a heterogametic female (ZW) and a homogametic male (ZZ). The offspring from each species will inherit gonosomes from the parents in equal assortment so that the outcome will always be a sex ratio of 1:1. on the other hand, when a homogametic (ZZ) male is crossed with, a homogametic (XX) female, the offspring will be of a single genetic constitution and will be all-male. Alternatively, when a heterogametic male (XY), is cross bred with a heterogametic female (ZW), the offspring will consist of three different genetic constitutions (¼ will be female homozygotes, ½ will be male heterozygotes, and the rest will be male homozygotes). Over the years, this hypothesis has generally been accepted albeit with some reservations.

In practice. sex ratios from, fish species presumed to have alternative sex determining mechanisms do not always yield the predicted 0:1 or 0:3 sex ratios. Breeding experiments have also shown that even within each system i.e. species sharing common sex determining mechanisms, a 1:1 sex ratio appears as an exception rather than a rule (tables 2,5 and 6).

For the lack of a similar simple to understand hypothesis, the sex chromosome theory has been applied extensively to justify observed sex ratios in fish including tilapia. Often variations from the expected sex ratio (1:1, 3:1, 1:0 male to female) are ignored or attributed to differential mortality, stock impurities, and stock misidentification so that the assumed characteristic of this hypothesis is it's presumed coherance.

On the basis of this theory, only three of the eleven sex ratios obtained in the present experiments can be explained. (Table7) These are as follows:-

i. One batch of the F_2 O.niloticus x O.mossambicus

ii. One batch of O.mossambicus x O.spilurus F_1,

iii One batch of O.mossambicus x O.spilurus F_2, all yielded 1:1 progeny sex ratio and would be presumed either homogametic female x heterogametic male, or heterogametic female x homogametic male. In short both male and female in each cross are of the same genetic sex determinism. the rest of the results can not be explained on the basis of this theory.

Because of a growing need to produce all-male broods for aquaculture, sex ratios not fitting the chromosome theory with exactitude have had to be investigated further to establish a more explainable sex control mechanism. As explained earlier in this chapter, Jalabert et al. (1974), crossed O.niloticus with O.macrochir in both directions and obtained at least 10 different sex ratios in different parental and hybrid pairings, their results could not be explained by the chromosome theory so they suggested that maybe an unknown number of autosomes was influencing sex differentiation.

Avtalion and Hammerman (1978) and Hammerman and Avtalion (1979) attempted explaining Chen's (1969) results by proposing a model where by sex chromosomes operate in conjunction with a pair of autosomes (see introduction). Their model supposes that there are 3 gonosomes (W, X, Y) and two autosomes (A,a) involved in sex differentiation. Within a species, the autosome pair is identical (AA or aa). Using this model, they calculated possible sex ratios as follows:

0:1, 1:3, 3:5, 1:1, 9:7, 5:3, 3:1, and 1:0 female to male. Using this model they were able to explain most of the sex ratios obtained by Chen (1969). In addition the model predicted 3 sex ratios (9:7, 3:5, 5:3) which could not be explained by the homo-heterogametic theory.

While this hypothesis seemed good enough for most practical purposes, it did not adequately explain sex ratios in F_2 and backcrosses obtained in Jalabert et al. (1974).

Fitting the present results into this hypothesis has helped to explain 6 of the values in table 7 (1:1, 3:1: 3:5). The model also caters for 3 of the six group sex ratios. However, the model does not fully explain all the sex ratios obtained in the present series of experiments and in previously reported studies some of which are listed in tables 1 and 2.

In a monofactorial sex determining system such as the XY chromosome system, the sex ratio is generally fixed at 1:1, and even in hybridization, instances of atypical sex differentiation are extremely rare. This would be indictative that sex chromosomes, are supreme and the genetic mechanism is stable. Such a situation has been reported in Xiphophorus maculatus, X. helleri, X variatus, X. signum, X. milleri and X.alvarezi (Gordon, 1947, 1951; Kallman, 1968, 1982 Price, 1984). However, in addition to the present study, several others using different species have shown that the ideal sex ratio is not always closely approximated. The observed correllation between the 3 gonosome x 2 autosome theory and the sex ratios in Chen (1969), Jalabert et al. (1974) and the present work, is an indication that multifactorial theories such as that hypothesized by Avtation and Hammerman (1978) and later authors are probably a little more realistic.

In a polyfactorial system, two or more genes occuring on a number of chromosomes probably contribute additively to the sexual differentiation of an individual (Nayudu 1979): Where these genes exist on autosomes, the sex chromosomes X, Y or W, Z, may also be present. In such a combination of factors, strength of individual sex determinism may vary. This may result in fluctuations in progeny sex ratios when individuals are mated with different partners or when an attempt is made at hormone induction of sex reversal.

The situation in single pair mating was discussed in the previous chapter, and the case of hormone sex reversal is the subject of the next two.

The possibility of polygenic sex determinination in fish was first advanced by Kosswig (1964). He hypothesized that in species such as Colisa Lalius (with female ZO) and Galaxias platei (with male XO), sex determining genes may be localized in autosomes and that sex determining genes for the homozygous individual would be in the available chromosome. For example in G. platei, female X_f X_f A_m A_m and male X_f OA_m A_m. Such that 2F is stronger than 1F. By assuming three male determining genes at four loci, Kosswig (1964) predicted 16 different allelic combinations.

Nayudu (1979) tried to explain sex ratio variations in the guppy on the basis that an unknown number of autosomal

sex influencing genes would collectively exchange the balance of sex and override the sex chromosomes. He hypothesized that in some individuals, there is accumulation of these autosomal sex influencing genes toward one sex or the other.

Majumdar (1984) adopted Kosswig's (1964) theory in investigating sex determination in tilapia. He demonstrated theoreticaly predictions of sex ratios based on 5 males and 4 females from the 16 allelic combinations predicted by Kosswig. From these 5 males and 4 females, Majumdar obtained 6 different sex ratios and predicted 1 to 4 sex ratios for each of the 5 males when, crossed with the four females. Applying this hypothesis to sex ratios in a single male tilapia spawned with 2 females, Majumdar obtained 1:2 females to males in one, and 20 females to a male in the other, thus confirming that individual allelic strength of the two females played a key role in determining progeny sex ratio.

From the above examples, it can be concluded that the strength of individual alleles determines sex in a polygenic system (Kosswig 1964; Nayudu, 1979; Majumdar, 1984), so that within a population, some individuals are genotypically stronger for one sex while others are stronger for the opposite sex. Individuals of each type could be identified by checker board selection spawning.

From the experimental work presented in this chapter and data quoted from previous reports, it seems improbable that observed hybrid sex ratios of tilapia can be fully justified on the basis of hypothesis advanced by Chen (1969) Jalabert et al (1974) and Avtalion and Hammerman (1979). On the other hand, a polygenic sex determining system offers wider scope for justifying highly variable sex ratios without the need for adjustments. There is, further, likelihood that the allelic balance for sex determination varies with species.

CHAPTER 7

Experiment V

Application of hormones in Tilapia I: Direct feeding

Application of hormones in tilapia culture

7.0 INTRODUCTION

In common with other vertebrates, the reproductive process of teleosts is under the direct control of the pituitary glands (Barr, 1963; Arai, 1967; Liley and Stacey, 1983). However, the precise mechanism of the control has not been established in fish and detailed information on the role of the pituitary on gonadal sex differentiation is sparse.

Sex in fish is assumed to be genetically determined at fertilization by assortment of genetic material and restoration of the diploid state. At hatching many fishes are not clearly differentiated into either male or female. Gonadal development passes through a labile undifferentiated phase prior to phenotypic expression as an ovary or a testis (Persev, 1975; Bull, 1984).

Potentially therefore, the sex of fishes can be artificially manipulated by affecting any link in the sequence of events controlling the differentiation processes. The ability to influence the process of sex differentiation is the basis for artificial induction of sex inversion.

A number of previous writers have demonstrated that generally, even in species such as trout where sex chromosomes have been positively identified, administration of androgens to sexually undifferentiated larvae favours the production of males, while administration of oestrogens, favours the production of the female phenotype (for reviews see Yamamoto, 1969; Schreck, 1974; Lam 1982; Donaldson and Hunter, 1982).

In recent years, tilapia has gained popularity in aquaculture. However, efforts to increase yields through intensive aquaculture have not always proved successful. This is mainly because, under favourable environmental conditions, tilapia tend to reproduce at frequent intervals throughout the year. The ability to reproduce almost uncontrollably results in over population which leads to slow growths, stunting and attainment of sexual maturity at a small size. In order to alleviate this problem, culture of monosex populations is growing increasingly popular.

Several techniques that would theoretically yield a monosex population are briefly summarised in the introductory chapter of this thesis (Chapter I). One of these techniques, involves administration of steroid hormones to sexually undifferentiated embryos and larvae in order to physiologically influence gonadal development and differentiation.

The general benefits that would accrue from culture of monosex and sterile fish have been reviewed in Chapter I and in references therein.

Advantages of using steroid hormones for the production of monosex fish have been reiterated by several reports, some of which appear in reviews by Yamamoto (1969); Shelton et al (1978); Hunter and Donaldson (1986). The following are among the advantages I consider most important.

1. Synthetic hormones are relatively inexpensive, easy to administer by a selection of techniques using relatively low level technology.

2. Steroid hormones can be administered to first feeding fry at a high density, hence less wastage of tank space and food resources.

3. Theoretically, administration of anabolic substances to juveniles should increase their growth rates.

4. Unlike other techniques, survival rates of treated fish are usually high and all hormone sex inversed fish are potentially useful for human consumption.

5. Basically the procedure for producing males is the same as that for obtaining females.

6. Unlike other techniques such as gynogenesis the use of hormones does not instantaneously increase in breeding.

7. For most practical purposes, pure species are used for production of brood stock. This helps to avoid inherent problems of introgressive hybridization such as inconsistent productivity.

8. Any species or hybrid can be used.

From the above it can be noted that the use of steroid hormones for obtaining monosex fish has some unparallelled advantages over the other treatments. Despite these and other favourable aspects, it has been suggested that use of hormones for production of monosex broods could result in a number of problems (Fernandez, 1986) of which the following are probably most important.

1. low viability of masculinized fry especially at high dosages

2. hormones could produce unpredictable effects on growth rates

3. there is a possibility that several fish would have to be killed in order to obtain gametes from broodstock from which gametes cannot be freely obtained due to malformation of either the gonads or the genitalia

4. that since only half of the treated fish would be genetically sex inversed, the batch would include broodstock that would produce a 3:1 male to female ratio when mated with normal broodstock.

By comparison with those of other techniques reviewed in Chapter I, the setbacks in steroid hormone use are relatively insignificant. Various reports have demonstrated fairly convincingly that under suitable conditions of treatment, high efficacy of the hormones coupled with low mortality more than compensates these concerns.

However, there are two other objections which seem to influence the general acceptability of steroid hormone usage. These are:

1. environmental considerations involving probability of unacceptable quantities of hormones being released into the environment.

2. ethical considerations regarding possible deleterious effects of steroid hormone residues in fish destined for human consumption.

The possible effects of hormone residues to human consumers has recently been taken up for investigation in trout and tilapia (Johnstone et al. 1977, 1978, 1983). These studies have demonstrated that in both groups of

fish, steroids are in part secreted via faeces. For example Johnstone et al. (1983) showed that visceral levels of the steroid 17α-Methyltestosterone declined to less than 3% of their original value in less than 50 hours and in both trout and tilapia, the total radioactive 17α-Methyltestosterone fell to less than 1% in about 100 hours.

Johnstone et al.(1977) found that in Rainbow Trout and in Atlantic salmon, 95 - 99% of the injected 19β-estradiol was eliminated from all tissues within 72 hours, and that the liver, which had accumulated 33% of the total injected radioactive dose during the first four hours had less than 0.1% of the dose after three hours.

Pankhurst et al. (1986) considered the relative influence of method of hormone administration in Goldfish, Carassius auratus and concluded that injected steroid is more rapidly absorbed and cleared whereas release from pellets is lower but of longer duration. They supplied 17β-estradiol to fish in suspension of peanut oil, saline, cocoa butter and in pellets of solid silastic. In all treatments, the disappearance rate was initially very rapid, probably due to removal of the most accessible or soluble steroid. their results showed, that at 20 mg/Kg body weight, the half life of 17β-estradiol is 1.40, 1.37, and 3.66 days if administered in peanut oil, saline or cocoa butter respectively. At 50 mg/Kg, the half life of the hormone in silastic is 16.98

days. The authors further demonstrate that the hormone levels in all treatments returned to basal levels after 4.9, 4.8, 10.5, and 26.8 days respectively.

In addition to these direct studies, Guerrero (1975) observed that both ethynyltestosterone and methyltestosterone are widely used in human medicine, and that despite their long term therapeutic use, no carcinogenic effects have been reported. Johnstone et al. (1977) report that according to Klopper, the half life of estradiol in higher vertebrates is very low and that the hormone is easily cleared through polar metabolites. The half life of 17β-estradiol, for example, was given as 10 hours in rainbow trout and three minutes in women.

Of the four techniques used to administer hormones in fish, oral administration by incorporation in the diet is the most popular. In tilapia, the treatment is given for up to 50 days and in Salmonids up to 90 days after hatching. If intended for human consumption, tilapia are reared to market size in about six months and trout in about 18 months.

Although only a few studies have so far been conducted, the findings are quite consistent and show that in fish, degradation of hormones takes a relatively short time. when this fact is considered vis-a-vis the observations that higher hormone dosages (10 - 50 mg/Kg) are applied in human medicine (Johnstone et al . 1983), and for longer

periods without observable ill-effects (Guerrero, 1975), it can be concluded that the present fears for human health are seemingly unfounded.

The term sex reversal is often applied to situations where an individual's phenotypic sex has developed in apposition to that indicated by sex chromosome constitution.

Reinboth (1970) and some other research workers prefer the term sex inversion which sounds more descriptive. These authors argue (perhaps correctly) that the term sex reversal has also been applied to situations in which changes in the sexual phenotype occur secondarily after the original process of sex differentiation has been accomplished, for example in sequential hermaphroditism.

The fact that complete sex inversion leading to the development of fertile females is possible in most species, as well as the viability of YY males, suggests that the X and Y chromosomes do not differ markedly with each other (Reinboth, 1975).

A common strategy in sex inversion is to apply steroids to juvenile fish in order to induce the redirection of sex differentiation to the desired gonadal sex. The time of treatment depends primarily on the species-specific time of phenotypic sex differentiation (See Chapter III). Other experimental data obtained from sex

inversion in fish indicate that sex differentiation in oviporous fish commences after hatching either before or after the initiation of feeding (Yamamoto, 1969; Shelton et al, 1978).

Upon reaching maturity, the sexually inverted homogametic individuals are then spawned with normal fish. If the untreated individuals have a heterosomic sex determining mechanism, the progeny are all homogametic and will have the same phenotypic sex.

The first reported study on artificial induction of permanent sex inversion in fish was by Yamamoto (1953). Since then artificial sex inversion has been carried out in several species by using heterologous sex steroids (androgen as male inducer and oesterogen as female inducer). Yamazaki (1983) and Hunter and Donaldson (1983) list several examples of fish in which hormone induced sex inversion has been successfully achieved. Yamamoto (1969) achieved complete sex inversion by means of mammalian steroids. He demonstrated that administration of oestrogens to young O. latipes with gonads still in the indifferent stages caused the chromosomal males to develop into females and that these (XY) females when crossed with normal males (XY) produced YY males.

In tilapia, both androgens and oestrogens have been applied to juvenile fish to induce maleness (Clemens and

Inslee, 1968; Jalabert et al, 1974; Guerrero, 1975; and others). Oestrogens have been successfully used to induce sex inversion in O. niloticus, O. aureus and O. mossambicus (Majumdar and McAndrew, 1987).

Hormonal sex inversion has mainly been used for the direct obtainment of monosex fish for aquaculture or as a technique for obtaining homogametic broodstock of the alternative phenotypic sex. However, the growing interest in production of monosex fish for aquaculture now demands a more permanent and more reliable means of producing monosex broods. One way to do this is to identify the underlying factors in sex determination. The ease with which the sex of fish is functionally inversed has made the application of hormones an attractive technique for evaluating their sex determining mechanisms. The information obtained would in the long run ensure that monosex stocks are available more readily using simple technology and without the necessity for further hormone treatment, manual sexing or irradiation.

A principal objective of the present work is to assess the effectiveness of an androgen (17α-Methyltestosterone and an oestrogen 17α-ethynylestradiol) in altering the sex of pure tilapia, and of some interspecific hybrids of aquacultural interest.

7.1 Materials and Methods

Fish Stocks

Six pure species of tilapia and two F_1 hybrids were used in the three experiments.

i The following were treated with 17α-methyltestosterone

Tilapia zillii, *T. mariae*, *Oreochromis niloticus*, *O. mossambicus*, *O. aureus* and *Sarotherodon galileaus*.

System failure resulted in excessive mortality of *O. aureus* in all three replicates and led to their abandonment.

ii The following were treated with 17α-ethynylestradiol

O. niloticus, *S. galileaus* and F_1 hybrid of *O. niloticus* x *O. macrochir*.

Except in the case of the interspecific hybrids where artificial fertilization was used, all other broods were from natural spawning in glass aquaria.

7.1.1 Procedure

Eggs were removed from the brooding parents within 24 hours of spawning and transferred to incubation jars. Six to eight days after hatching, fry were counted,

sample weighed, and divided into sub groups according to the required treatments. Each brood of O. niloticus, S. galileaus and T. mariae were divided into 4 nearly equal groups for treatment at 3 hormone inclusion levels and a control. O. mossambicus for androgen treatment was divided into two groups only (one hormone treatment level) to provide brood fish for progeny tests. Because hybrid broods were small, only one treatment level was used.

Fry were reared in a micro fry rearing system at 27.5 ± 0.5°C. Each treatment was replicated at least twice.

7.1.2 Food Preparation

Eight treatment feeds were prepared by the alcohol evaporation method (Guerrero, III 1975; Shelton et al. 1978). For each treatment feed, one kilogramme of Ewos #3 trout pellet was finely ground before being thoroughly mixed with the appropriate hormone solution. Hormone stock solutions were prepared by dissolving 30 mg, 45 mg and 60 mg of 17α-methyltestosterone in one litre of 95% alcohol. Stock solutions for the oestrogen treatments were prepared by dissolving 35 mg, 50 mg, and 65 mg of 17α-ethynylestradiol in a litre of 95% alcohol.

After mixing thoroughly the food was oven dried at 50°C. The thoroughly dried food was packed in self sealing plastic bags and kept in a refrigerator or a cooler.

Control diet was prepared in the same manner but without including the hormones.

7.1.3 Feeding procedure

Fry were fed treatment diets at 10% body weight per day given in three equal portions. Feeding was started when fish were six to eight days old and was continued until 40 - 45 days after hatching in accordance with latest gonadal differentiation stages established in sex differentiation experiments (Chapter III).

At the end of the hormone feeding period, fry were supplied normal untreated diet for a further two weeks to increase size. About half were then killed and fixed for histological sexing as outlined in appendix 1 and 2. The remaining fish were retained and sexed when they became externally sexable or had developed easily identifiable gonads by inspection.

A random sample of about 25 - 30 females from each of the oestrogen treated species were retained for progeny testing experiments. At an opportune time, these were stocked in spawning tanks with normal untreated fish of the opposite sex.

Sampling

Sampling was done at 7 day intervals by mildly anaesthetizing about 30 fry, before weighing and measuring them.

7.1.4 Analysis and terminology

For the purpose of this work, the phenotypic sex of a sex inversed fish is as named post partum. For example, a 'sex inversed' (or reversed) female refers to a genotypic male whose sex has been altered functionally to produce ova; and a 'sex reversed' male is a genotypic female whose sexual phenotype has been altered functionally to produce sperm.

Unless stated otherwise, the term normal fish refers to a fish that has not been consciously exposed to either type of sex hormones and is not a product of brood fish that have previously been subjected to sex inversion treatment. In short, the sexual phenotype of a normal fish corresponds to its sexual genotype in a natural way.

The term 'sex inversion' is used synonymously with 'sex reversal'.

Analysis of results was done on both androgen and oestrogen treated fish to evaluate

i the effect of the hormone on survival
ii the effect of the hormone on growth
iii the influence of the hormone on sex ratio of treated groups.

The variations in survival, growth, and sex ratio due to different concentrations of the hormone were compared by

Analysis of Variance tests (ANOVA) in conjunction with Duncan's new Multiple range tests (Duncan, 1955). Chi-square tests were performed to detect significant differences in numbers between sexes in each treatment. Chi-square calculations were also done by the minitab computer package. T-test was used to compare twin values.

7.2 Results

Results of hormone application experiments are presented and discussed in three separate sections as follows:-

I Influence of 17α -methyltestosterone on survival, growth and sex differentiation in T. mariae, S. galileaus, O. mossambicus and O. niloticus.

II Influence of 17 α-ethynylestradiol on survival, growth and sex differentiation in S. galileaus, O. niloticus and F_1 hybrid O. niloticus x O. macrochir

III Progeny test ratios in fry obtained from oestrogen treated fish.(Chapter 8)

To minimise size differences at the start of the treatments, identical size broodstock were used in I and II. Each batch of fry was sample weighed before being divided into treatment groups. There was therefore little or no size variation between treatment groups at the start of each set of treatment.

7.2.1 Effect of 17 α-Methyltestosterone (17 α - MT) on survival of *T. mariae* fry

Table 1.1 shows number of fry at the start and 45 days after hatching. The highest survival was 80.58% obtained in batch number 2 at a treatment of 45 mg/Kg diet. The lowest was 54.08% obtained in the same batch at a hormone dose of 60 mg/Kg diet.

Significant difference was found between average survival of the control with that of 60 mg/Kg. Survival in different sib groups was identical for all groups.

7.2.2 Effect of 17 α-Methyltestosterone on survival of *S. galileaus* fry

Table 1.3 shows the initial and final numbers of fish surviving up to 45 days post hatch. Survival of treated fish in the sib groups did not show a particular survival trend in group one. In the second and third batches, survival was significantly lower at 45 mg/Kg. The highest survival was 91.50% in the third batch, at 30 mg/Kg. The lowest was 60.71% in the second batch, at 45 mg/Kg diet. Table 1.4 shows that there was no significant difference between averages of the different hormone dosages. Survival means at the four levels of treatment were 82.43 ± 3.04, 85.54 ± 3.81, 70.21 ± 6.58 and 75.89 _ 2.11% for the control, 30 mg/Kg, 45 mg/Kg and

Table 7.1 Comparative survival of T. mariae fry 45 days after hatching

Hormone dose mg/Kg diet	Number of fish Initial	Final	Survival # %
0	300	225	75.00 b
30	200	136	68.00 ab
45	230	178	77.39 b
60	220	147	66.82 a
0	250	186	74.40 b
30	240	144	60.00 a
45	242	195	80.58 b
60	260	138	53.08 a
0	280	198	70.71 b
30	300	219	73.00 b
45	380	203	53.42 a
60	320	209	65.31 b

Superscripts within each sib group in column 4 denote significant difference with each other ($p < 0.05$)

Table 7.2 Effect of 17α-MT dose on survival of T. mariae fry

Hormone dose mg/Kg diet	Number of fish Initial	Final	Survival # % ± SE	
0	300	225	75.00 a	
0	250	186	74.40 a	
0	280	198	70.71 a	
	830	609	73.37 ± 1.34	Y
30	200	136	68.00 ab	
30	240	144	60.00 a	
30	300	219	73.00 b	
	740	499	67.00 ± 3.79	XY
45	230	178	73.39 b	
45	242	195	80.58 b	
45	380	203	53.42 a	
	852	576	70.46 ± 8.57	XY
60	220	147	66.82 b	
60	260	138	53.08 a	
60	320	209	65.31 b	
	800	494	61.74 ± 4.35	X

Figures in each treatment group in column four differ significantly with each other ($p < 0.05$). Superscripts on treatment averages denote significant difference between treatments.

Table 7.3 Comparative survival of MT treated fish 45 days after hatching (*S. galileaus*)

Hormone dose mg/Kg	Number of fish Initial	Final	Survival # %
0	150	116	77.33 a
30	130	102	78.46 a
45	140	116	82.86 a
60	200	146	73.00 a
0	140	123	87.86 c
30	90	78	86.67 c
45	140	85	60.71 a
60	150	112	74.67 b
0	190	156	82.11 b
30	200	183	91.50 b
45	170	114	67.06 a
60	150	120	80.00 b

superscripts denote significant difference between treatments within sib groups ($p < 0.05$)

Table 7.4 Effect of MT dose on survival of S. galileaus fry

Hormone dose mg/Kg diet	Number of fish Initial	Final	Survival # % ± SE
0	150	116	77.33 a
0	140	123	87.86 a
0	190	156	82.11 a
	480	395	82.43 ± 3.04 *
30	130	102	78.46 a
30	90	78	86.67 ab
30	200	183	91.50 b
	420	364	85.54 ± 3.81 *
45	140	116	82.86 b
45	140	85	60.71 a
45	170	114	67.06 a
	450	315	70.21 ± 6.58 *
60	200	146	73.00 a
60	150	112	74.67 a
60	150	120	80.00 a
	500	378	75.89 ± 2.11 *

Similar superscripts on treatment averages are not significantly different ($p > 0.05$). Different superscripts on figures within the same treatment group are significantly different ($p < 0.05$)

60 mg/Kg diet respectively. Significant differences were found between treatments in sib groups two and three.

7.2.3 Influence of 17 α-Methyltestosterone on survival of O. mossambicus fry

Results in Table 1.5 show that in two of the three sib groups, survival of fry was adversely affected by the treatment. However, Table 1.6 shows that overall there was no significant difference in survival of fish in the treated groups when compared with the controls.

The survival range in the experiment was 68.85% to 90.00%. The mean survival in the treated group was 74.35 ± 3.776, and the controls was 81.72 ± 5.618%.

7.2.4 Influence of 17 α-Methyltestosterone on survival of O. niloticus

High survival rate was observed in the three batches of fry used in the experiment. Survival range for the experiment was 60.80% in fry treated at 60 mg/Kg to 93.00% in the third control group. (Table 1.7). Significant differences were found between treatments in batches one and three. Results in Table 1.8 indicate that survival of fry at 60 mg/Kg of the androgen

Table 7.5 Comparative survival of MT treated O. Mossambicus fry, 44 days after hatching.

Hormone dose mg/Kg diet	Number of fish Initial	Final	Survival # %
0	202	170	84.16 b
50	260	179	68.86 a
0	200	142	71.00 a
50	190	155	81.58 b
0	180	162	90.00 b
50	230	167	72.61 a

Different superscripts on figures within each sib group denote significant difference ($p < 0.05$).

Table 1.6 Effect of 17α-Methyltestosterone dose on survival of O. mossambicus fry

Hormone dose mg/Kg diet	Number of Fish Initial	Final	Survival # % ± SE
0	202	170	84.16 b
0	200	142	71.00 a
0	180	162	90.00 b
	582	474	81.72 ± 5.62 *
50	260	179	68.85 a
50	190	155	81.58 b
50	230	167	72.61 ab
	680	501	74.35 ± 3.78 *

Averages with similar superscripts are not significantly different ($p > 0.05$). Superscripts within each treatment group denote significant differences ($p < 0.05$).

Table 7.7 Comparative survival of 17α-MT treated O. niloticus fry 45 days after hatching

Hormone dose mg/Kg diet	Number of fish Initial	Final	Survival # %
0	80	72	90.00 c
45	90	70	77.78 b
60	125	76	60.80 a
0	150	129	86.00 a
45	120	106	88.33 a
60	120	94	78.33 a
0	130	121	93.00 b
45	120	109	90.83 b
60	105	73	71.43 a

Different superscripts on figures within a sib group are significantly different ($p < 0.05$)

Table 7.8 Effect of 17 α-MT dose on survival of O. niloticus fry

Hormone dose mg/Kg diet	Number of fish Initial	Final	Survival # % ± SE
0	80	72	90.00 a
0	150	129	86.00 a
0	130	121	93.00 a
	360	322	89.69 ± 2.05 **
45	90	70	77.78 a
45	120	106	88.33 ab
45	120	109	90.83 b
	330	285	85.65 ± 4.00 **
60	125	76	60.80 a
60	120	94	78.33 b
60	105	73	71.43 b
	350	243	70.19 ± 5.10 *

Different asterisks on treatment averages in column 4 denote significant difference between treatments ($p < 0.05$). Different superscripts on figures within each treatment category indicate significant differences ($p < 0.05$) between identical treatments.

increases mortality rate in O. niloticus fry. Significant differences between individual groups within a batch or within a treatment category indicate that fry response to androgen treatment varies with groups of fish.

7.2.5 Influence of 17α -Methyltestosterone on growth of T. mariae fry

Analysis of results in Table 1.9 based on data collected 71 days post hatch. There was no difference in starting weight as detailed in the introduction to this chapter. Marked size variation was noted at about 45 - 50 days after hatching. However the difference was not sustained, and by day 71 post hatch, no significant difference was found in all four groups

Regression equations for growth in relation to age was

$Y = 0.014 X + 0.246$ for the control
$Y = 0.014 X + 0.232$ for MT30
$Y = 0.012 X + 0.169$ for MT45
$Y = 0.014 X + 0.208$ for Mt60

Table 1.9 Mean individual weight of T. mariae fry fed varying amounts of hormone up to 45 days after hatching (17∝ Methyltestostorone)

Age	Control	mt30	mt45	mt60
6	0.006	0.006	0.006	0.006
12	0.025	0.024	0.024	0.027
19	0.038	0.046	0.046	0.045
26	0.056	0.065	0.068	0.080
33	0.101	0.093	0.142	0.153
45	0.171	0.187	0.265	0.369
51	0.307	0.338	0.388	0.438
64	0.745	0.729	0.558	0.752
71	1.035a	0.986a	0.822a	0.937a

The data is also presented graphically in Figure 1.1. Results show that within the age range of this experiment, T. mariae progressively gained weight. As can be seen in Figure 1.1, there was very little difference between the control and the three hormone treatments.

Mean food conversion rate (FCR) and instantaneous growth rate (SGR) calculated for the period up to 71 days after hatching was as follows

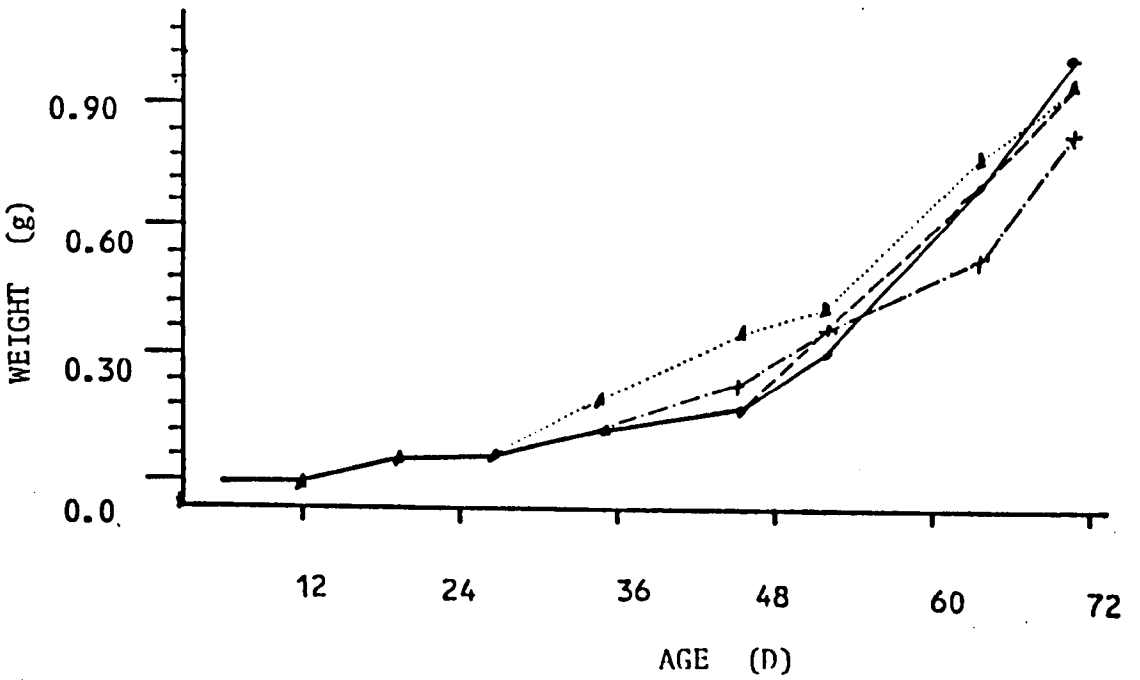


Figure 1.1: Comparative growth of T. mariae treated with MTC(—•—), MT30 (---), MT45 (-.-.-.) and MT 60 (...)

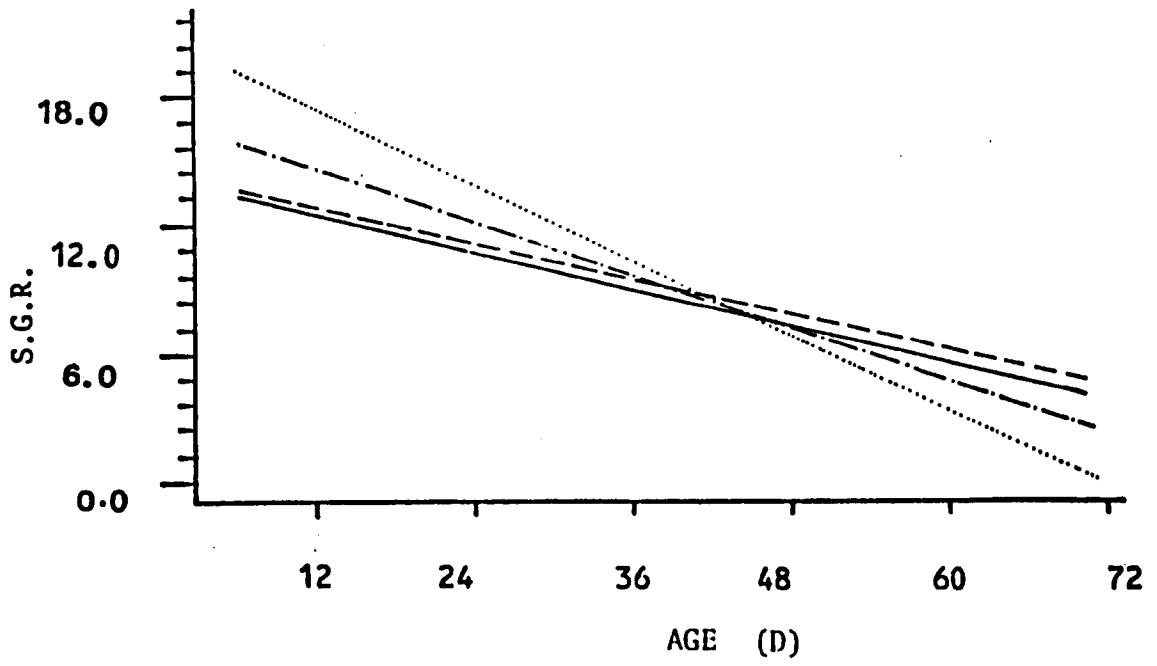


Figure 1.2: Graphic presentation of SGR in T. mariae fry treated with MTC (—•—), MT30 (——), MT45 (-.-.-.) and MT 60 (....)

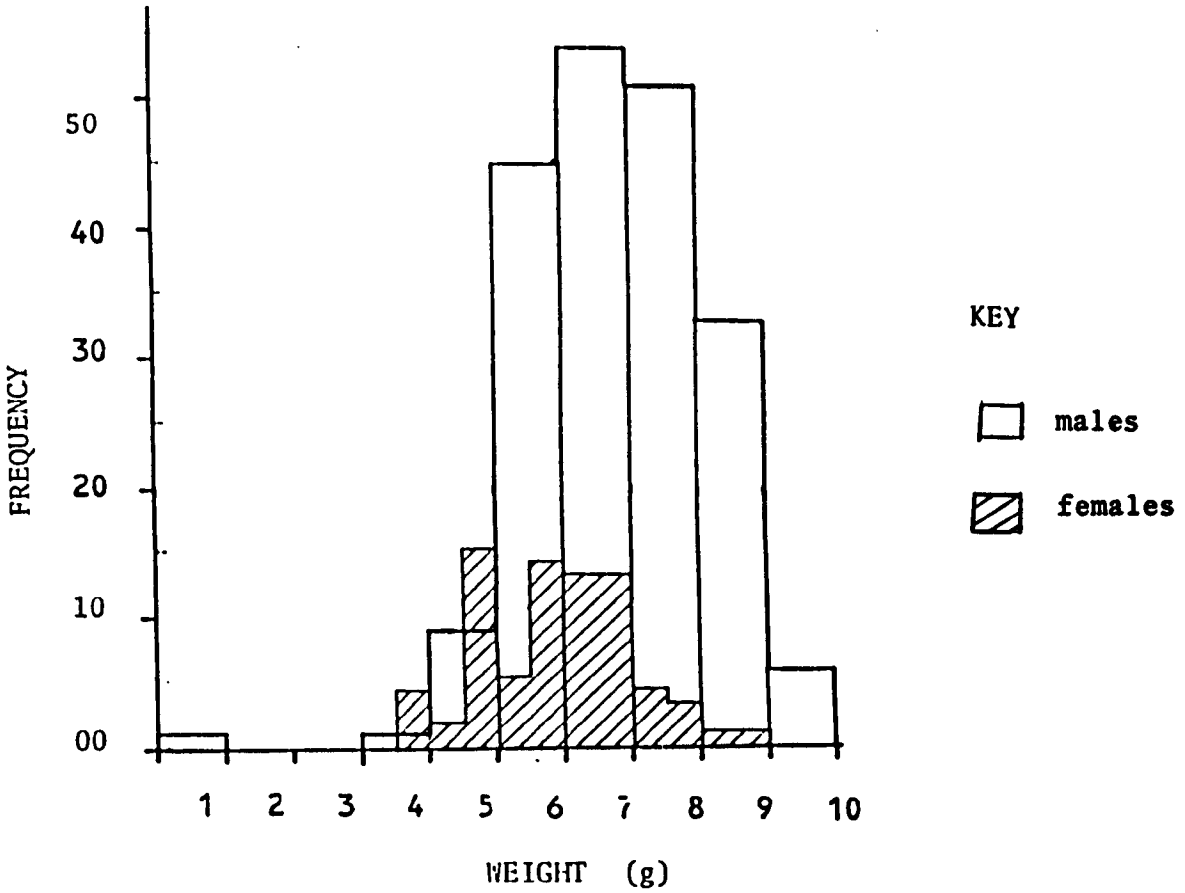


Figure 1.3: Frequency distribution of final weight in phenotypic males (a) and females (b)

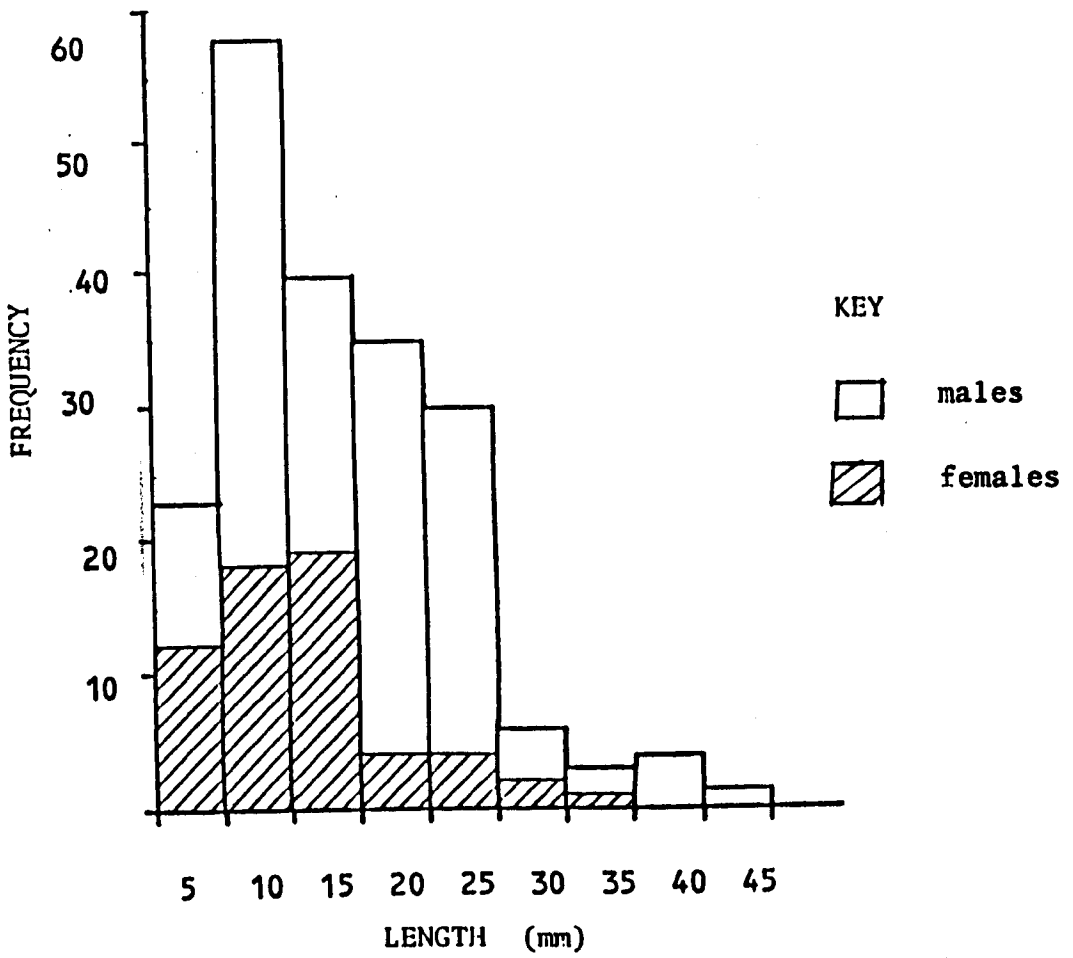


Figure 1.4: Frequency distribution of final standard length in a phenotypic male (a) and female (b) population of androgen treated T. mariae

hormone dose mg/Kg	FCR ± SE	SGR ± SE
0	1.964 ± 0.172 *	6.83 ± 1.11 *
30	2.294 ± 0.294 *	6.57 ± 1.21 *
45	2.357 ± 0.290 *	7.26 ± 1.60 *
60	2.198 ± 0.331 *	5.48 ± 2.20 *

No significant difference was found in either FCR or SGR ($p > 0.05$). However, fish that were retained and weighed 120 days after hatching showed that the hormone treated groups grew significantly faster than the controls. Overall, males grew 32.42% heavier than females. The largest difference was 68.77% attained in fry fed a diet with 30 mg/Kg (Table 1.10).

Table 7.10 : Weight of T. mariae treated in relation to sex : 120 days after hatching

hormone dose (mg/Kg)	NUMBER OF FISH				WEIGHT OF FISH			
	Total	Male	Female	% Male	Total	Male	Female	Difference
0	85	41	44	48,23	13,24a	13,96a	12,57a	11,06
30	51	29	22	56,86	16,76b	18,70b	11,08a	68,77
45	66	51	15	77,27	15,23ab	16,78b	10,04a	68,03
60	74	68	6	91,89	14,29ab	16,15b	12,07a	33,80

The smallest size difference between the sexes was found in the control groups where males were only 11.06% heavier than females.

Regression lines of SGR show, that in all groups, SGR declined rapidly with age. (Figure 1.2).

The regression relation for size (Weight/Length) for the sexes was as follows:

$$Y = 0.490\ X + 19.7 \quad \text{for males}$$

$$Y = 0.583\ X + 25.9 \quad \text{for females}$$

$$Y = 0.566\ X + 24.3 \quad \text{for the mixed}$$

No statistically significant differences were found in growth of groups fed different hormone levels (Table 1.10 column 6). Treated males (normal + sex reversal) grew significantly faster than the control males (genotypic males only). Hormone fed females did not differ significantly from the control in weight.

The result seems to indicate that female growth was unaffected by androgen treatment. On the other hand the androgen promoted growth significantly in phenotypic males.

Figure 1.3, and 1.4 show normal weight distribution in both male and female. This probably indicates that both normal and sex reversed males grow at the same rate. Figure 1.4 also indicates that, while growth in males is fairly uniform, that of females varies markedly and is probably influenced by selective response to androgen treatment. There was higher variation in condition.

7.2.6 Influence of 17 α-Methyltestosterone on growth of first feeding Sarotherodon galileaus fry

Feeding with hormone treated diet was terminated after 40 days. Analysis of growth is based on measurements taken 89 days post hatch.

Table 1.11 gives the average weekly weight gains for three sets of fish supplied with different amounts of 17 α-Methyltestosterone in the diet.

Starting weight in each trial was the calculated population batch mean at stocking. There was therefore no variation in starting weights between the treatments. Significant difference was found in the final weight as indicated by superscripts in the last row of Table 1.11. Figure 1.6 presents the results graphically. Growth was slow at first, but increased rapidly from about 40 days after hatching.

Table 7.11 Average weight of S. galileaus fry calculated at weekly intervals

Age (days)	Level of Hormone Inclusion (mg/Kg) 0	30	45	60
6	0.011	0.011	0.011	0.011
13	0.023	0.022	0.025	0.025
19	0.026	0.030	0.046	0.037
26	0.058	0.065	0.081	0.062
40	0.144	0.158	0.196	0.120
47	0.185	0.215	0.236	0.338
54	0.300	0.295	0.308	0.494
61	0.401	0.413	0.435	0.725
68	0.606	0.512	0.578	0.985
75	0.748	0.846	0.763	1.078
82	1.274	1.706	1.697	1.924
89	2.340 a	2.827 b	2.722 b	2.365 a

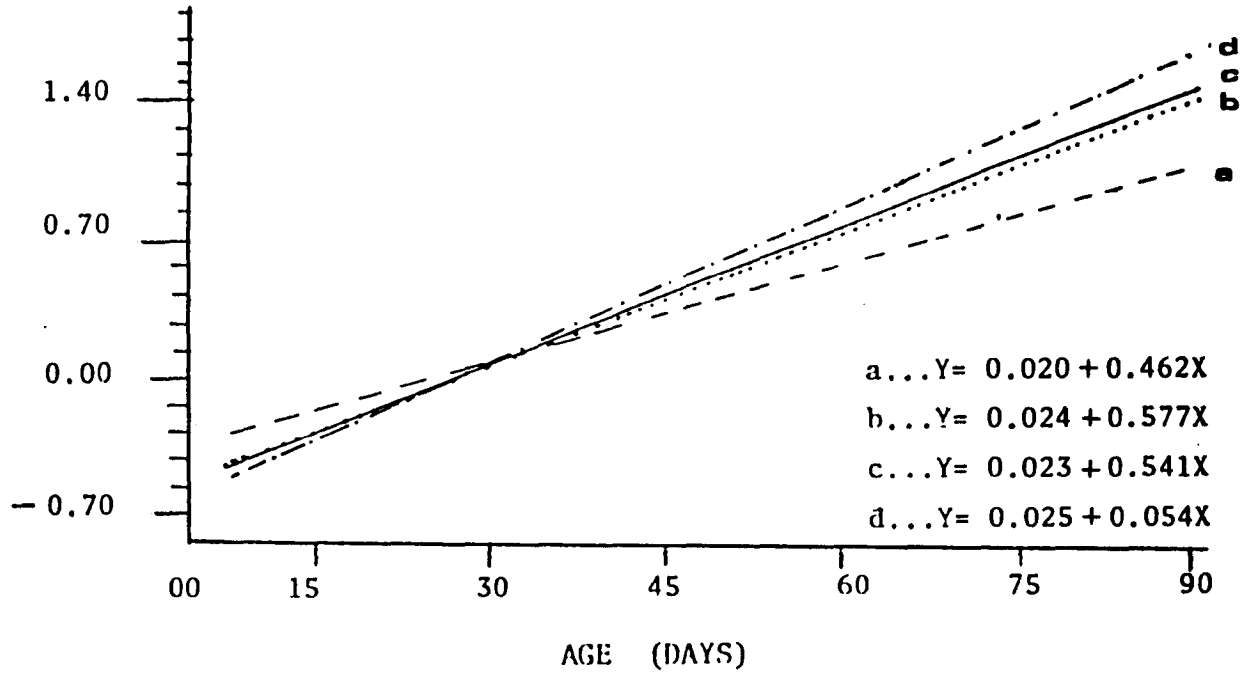


Figure 1.6 :Comparative growth (wt) of androgen treated S. galileaus fry at MT.C (——),MT 30 (....),MT 45 (---),MT60 (_._.)

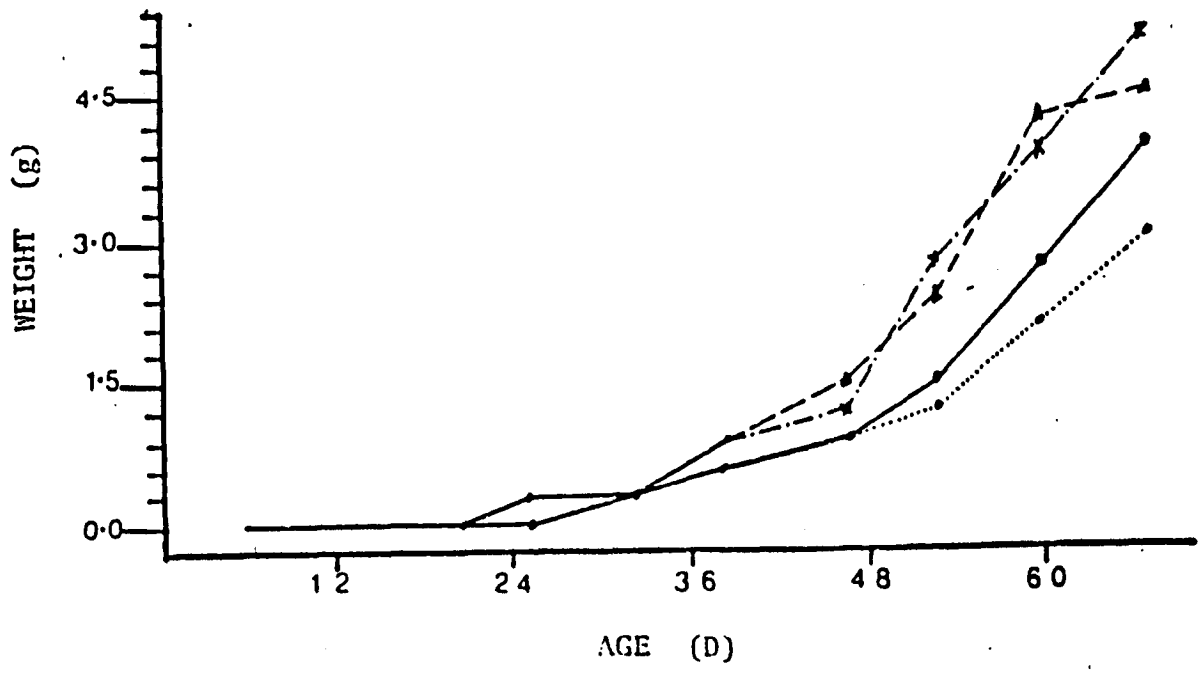


Figure 4.7: Comparative growth (wt) of estrogen treated S. galileaus fry

As indicated again elsewhere, the presence of an inflection point coincident with end of hormone feeding may not necessarily imply a postive influence of the untreated diet.

Food conversion rate and specific growth rate of fry in different treatments was as follows:-

Hormone level	FCR ± SE	SGR ± SE
0	1.807 ± 0.409ba	6.398 ± 0.422a
30	1.427 ± 0.218a	6.697 ± 0.798a
45	1.619 ± 0.275ab	6.090 ± 0.993a
60	2.166 ± 0.156b	6.628 ± 0.623a

No significant difference in SGR was found between different treatments. Food conversion at 30 mg/Kg was significantly higher than at 60 mg/Kg diet ($p < 0.05$).

7.2.7 Influence of 17 α-Methyltestosterone on growth of *O. niloticus* fry

Table 1.16 is a summary of growth (weight) in hormone treated *O. niloticus* fry. The data is also presented graphically in Figure 1.8. No significant difference was found at 98 days post hatch. However, observable differences in growth between the

Table 7.16 Average growth of 17∝ MT treated O. niloticus fry

Age	Cont	mt. 45	mt. 60
0	0.007	0.007	0.007
7	0.008	0.009	0.009
14	0.014	0.014	0.012
21	0.124	0.120	0.116
35	0.244	0.228	0.194
50	0.328	0.348	0.394
57	0.404	0.443	0.568
63	0.502	0.535	0.773
70	0.930	1.268	1.229
77	1.816	2.266	2.030
84	2.549	3.237	3.695
91	5.029	5.525	5.726
98	8.150 a	8.745 a	8.330 a

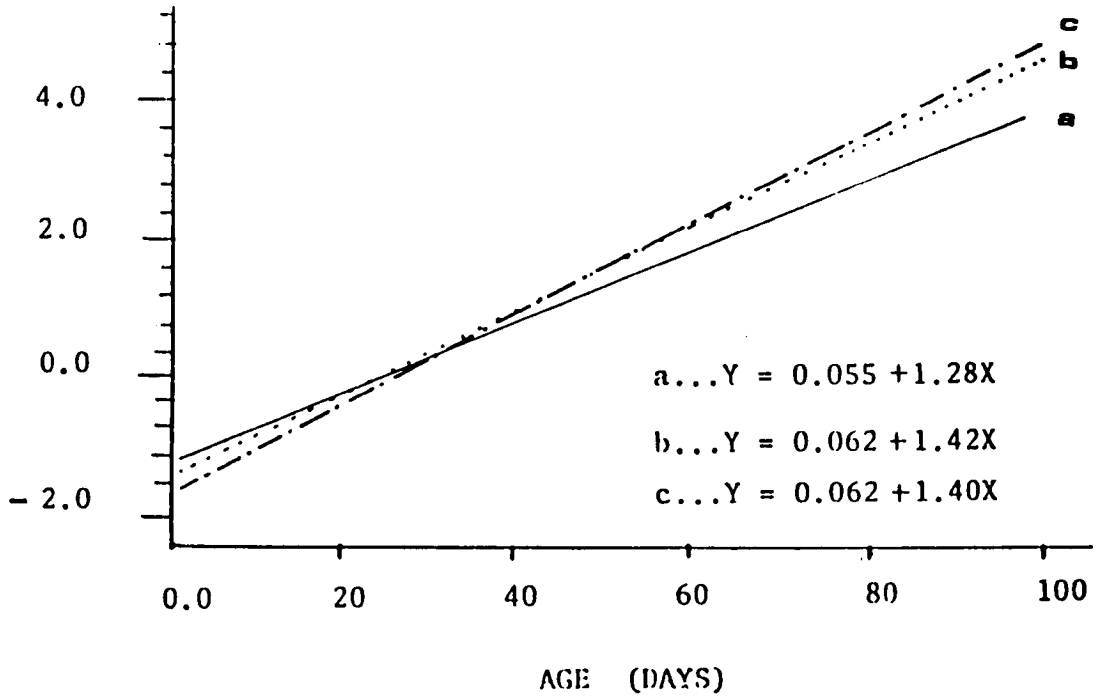


Figure 1.8 Comparative growth (wt) of androgen treated O. niloticus fry at MTC (—), MT45(---), and MT60(-·-·)

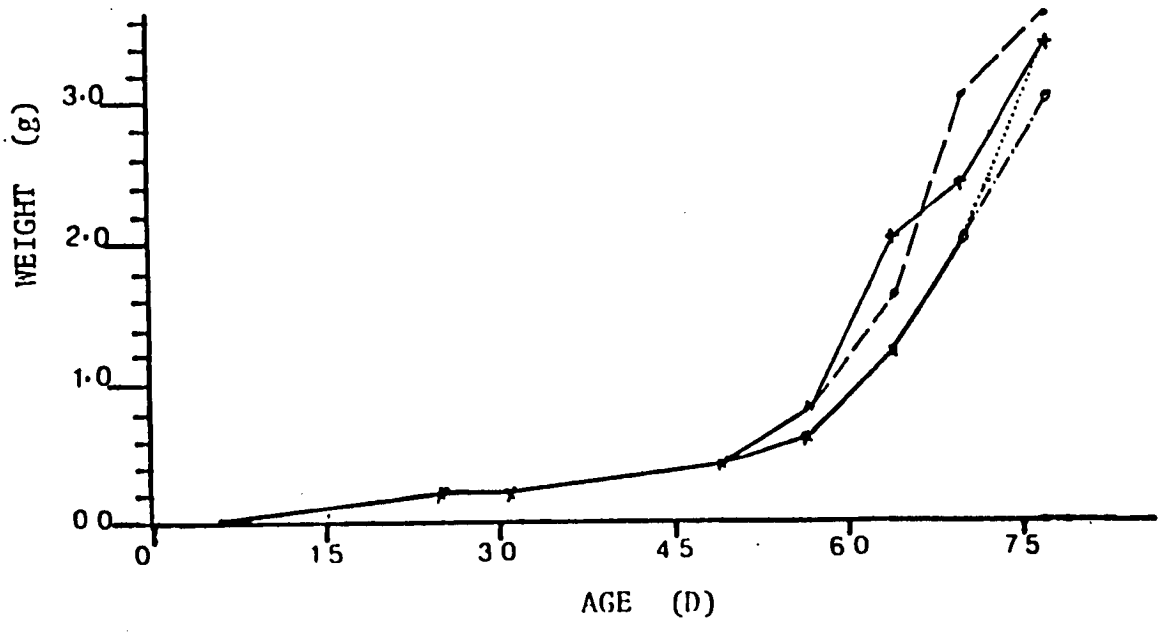
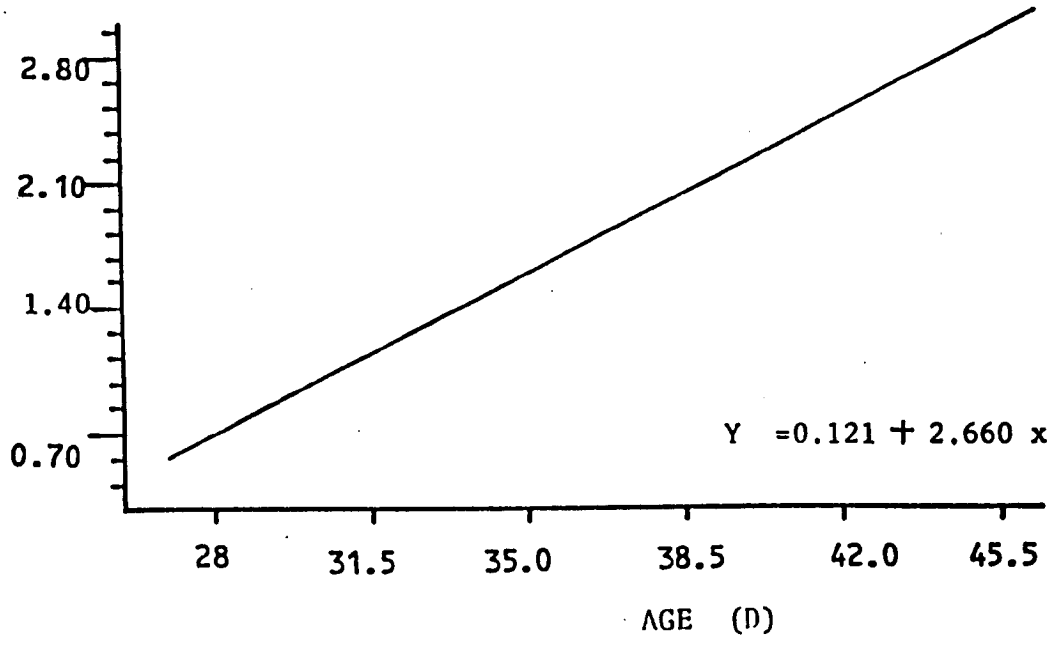
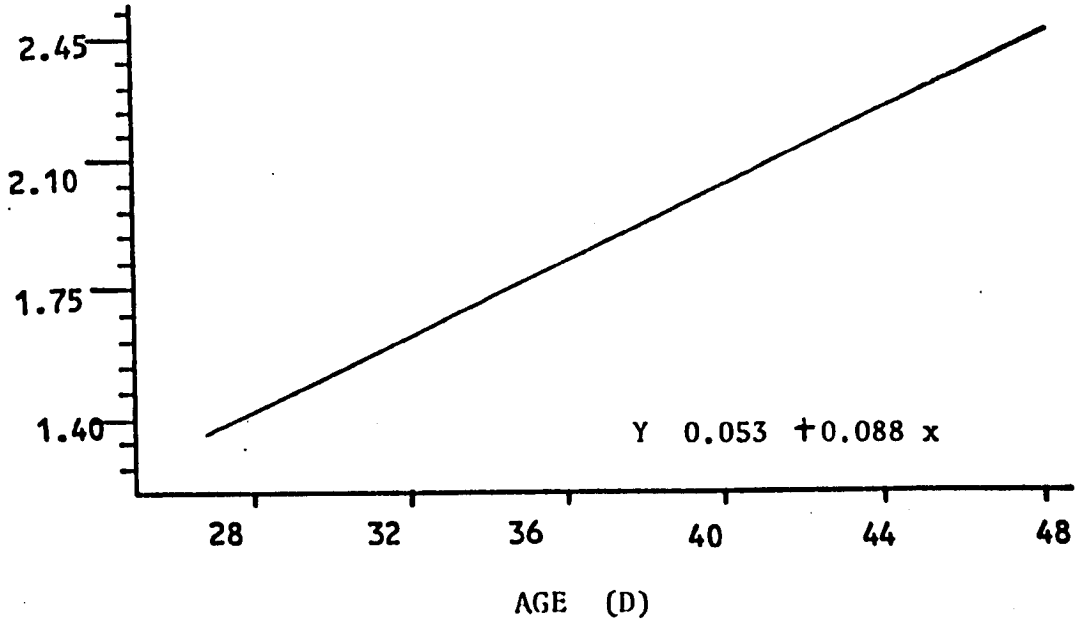


Figure 1.9: Comparative growth (wt) of S.galileaus treated with 17 ethynylestradiol at EE_c (—), EE_{35}(--), EE_{50}(·····), and EE_{65} (-·-·-)

Figure 2.2 : Graphic representation of growth (wt) in androgen treated O. niloticus (a) male (b) female





control and the hormone treated fish appeared from about 50 days after hatching (Table 1.16).

The average FCR and SGR derived from weekly means are summarized in Table 1.17. The gross mean FCR for the experimental period was 1.762 ± 0.202 and the value for SGR was 4.998 ± 0.319. There was no statistical significance found in SGR between the three treatment categories.

The FCR at 45 mg/Kg diet was significantly lower than in the controls.

Table 7.17 Group average FCR and SGR for O. niloticus fry treated with 17 α-Methyltestosterone

Hormone level	FCR ± SE	SGR ± SE
0	1.424 ± 0.377	4.711 ± 0.677
45	2.116 ± 0.349	4.893 ± 0.550
60	1.745 ± 0.321	5.391 ± 0.433

7.2.8 Influence of 17α-Methyltestosterone on phenotypic sex differentiation in Tilapia mariae

Table 7.18 Phenotypic sex differentiation in T. mariae fed androgen treated diet.

Dose	Sample	Male	% Male	Female	% Female	m/f	Undiff
0	100	48	48.00	46	46.00	1.04	6
30	128	63	49.22	58	45.31	1.09	7
45	99	52	52.53	44	44.44	1.18	3
60	102	99	97.07 ***	0	0.00	1.0*	3
0	100	48	48.00	52	52.00	0.92	0
30	48	25	52.08	22	45.83	1.14	1
45	99	68	68.69*	30	30.30	2.27	1
60	98	97	98.98***	0	0.00	1.0*	1
0	120	63	52.50	55	45.83	1.15	2
30	150	93	62.00*	54	36.00	1.72	3
45	98	72	73.47**	21	21.43	3.43	5
60	83	78	93.99 ***	4	4.82	19.50	1

different superscripts within each group in column 4 denote significant differences with the respective control groups (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.09$)

Table 7.19 Dose effect of 17α- MT on phonotypic sex differentiation in T. mariae

Hormone dose mg/Kg diet	Sample	Male	% male#	female	% female	M/F#	Undiff
0	100	48	48.00a	46	46.00	1.04	6
0	100	48	48.00a	52	52.00	0.92	0
0	120	63	52.50a	55	45.83	1.15	2
	320	159	49.50 ± 1.22	153	47.94 ± 1.6	1.04 ± 0.05a	8
30	128	63	49.22a	58	45.31	1.09	7
30	48	25	52.08ab	22	45.83	1.14	1
30	150	93	62.00b	54	36.00	1.72	3
	326	181	54.43 ± 3.16	134	42.38 ± 2.61	1.32 ± 0.17a	11
45	99	52	52.53a	44	44.44	1.18	3
45	99	68	68.69b	30	30.30	2.27	1
45	98	72	73.47b	21	21.43	3.43	5
	296	192	64.89 ± 5.17	95	32.06 ± 5.47	2.29 ± 0.53b	9
60	102	99	97.06a	0	0	1:0	3
60	98	97	98.98a	0	0	1:0	1
60	83	78	93.98a	4	4.82	19.5:1	1
	283	274	96.67 ± 1.19	4	1.61 ± 1.31	1:0c	5

Different superscripts within each treatment in column 4 denote significant differences between batches of fish. Superscripts on treatment averages in column 7 denote significant difference between treatments. ($p < 0.05$)

Results in Tables 1.18 and Table 1.19 indicate that sexually different batches of fish responded differently to the treatment. For example, a dose of 30 mg/Kg and 45 mg/Kg had no effect on sex differentiation in the first batch but significantly altered sex ratio in the third group (Column 4, Table 1.18). In the first and second batches no females were obtained at 60 mg/Kg diet. The proportion of the sexually indistinguishable fish was highest in the first batch and lowest in the second. There was no batch influenced sex ratio variation among the controls and in fish at a high dose level (60 mg/Kg). Treatment means differed significantly from the controls at 45 mg/Kg and 60 mg/Kg (Table 1.19, column 7).

Results indicate significant variation in response to 17 α-MT treatment by different batches of fish. The high number of fish not positively sexed is in part due to the difficulties in sexing T. mariae. Among the positively sexed fish, maldevelopment was restricted to ovarian duct constriction or formation of two or more compartments of the ovary.

7.20 Influence of 17α-MT on phenotypic sex differentiation in S. galileaus

Table 1.20 Phenotypic sex differentiation in adnrogen treated S. galileaus

Hormone dose mg/Kg diet	Sample	Male	% male#	female	% female	M/F#	Undiff
0	116	54	46.5517	58	50.0000	0.93	4
30	102	76	74.5098**	22	21.5686	3.45	4
45	116	114	98.2759***	0	0.0000	1:0	2
60	136	124	91.1765***	4	2.9412	31.00	8
0	123	67	54.4715	54	43.9024	1.24	2
30	78	55	70.5128*	23	29.4872	2.39	0
45	85	82	96.4706***	0	0.0000	1:0	3
60	105	99	94.2857***	0	0.0000	1:0	6
0	135	69	51.1111	66	48.8889	1.04	0
30	125	107	85.6000**	18	14.4000	5.94	0
45	114	104	91.2281***	0	0.0000	1:0	10
60	106	103	97.1698***	0	0.0000	1:0	3

Superscripts in column 4 denote significant difference with the respective control group at 5%, 1% and 0.01% for 1 star, 2 stars, and 3 stars respectively.

Table 7.21 Dose effect of 17 α-MT on phenotypic sex differentiation in S. galileaus

Hormone dose mg/Kg diet	Sample	Male Number	%	Female Number	%	Ratio M/F	Undiff
0	116	54	46.55a	58	50.00	0.93	4
0	123	67	54.47a	54	43.90	1.24	2
0	135	69	51.11a	66	48.89	1.05	0
	374	190	50.71±1.87	178	47.60±1.53	1.07:1a	6
30	102	76	74.51ab	22	21.57	3.45	4
30	78	55	70.51a	23	29.49	2.39	0
30	125	107	85.60b	18	14.40	5.94	0
	305	238	76.87±3.69	63	21.76±3.08	3.78:1b	4
45	116	114	98.28a	0	0	1:0	2
45	85	82	96.47a	0	0	1:0	3
45	114	104	91.23a	0	0	1:0	10
	315	300	95.33±1.73	0	0	1:0c	15
60	136	124	91.18a	4	2.94	31:1	8
60	105	99	94.29a	0	0	1:0	6
60	106	103	97.17a	0	0	1:0	3
	347	326	94.21±1.41	4	0.98±0.80	81.5:1c	17

Different superscripts in column 4 denote batch influenced significant difference within each treatment category ($p < 0.05$) Asterisks on group means in Column 7 denote significant differences between hormone dosages (p 0.05).

Results in Table 1.20 indicate that sex ratio in S. galileaus was significantly altered at all the three treatment levels.

Five of the six groups of fish treated with 45 mg and 60 mg of MT per Kg diet resulted in complete elimination of females (Table 1.20, column 5).

Table 1.21 shows that variation in sex ratio due to feeding androgens was constant in two of the three treatment levels (Table 1.21, column 4). The proportion of fish that could not be positively sexed was 1.60%, 1.31%, 4.76% and 4.09% in the control group and the three treatments respectively. The difference between treatments was significant.

The problem of positively distinguishing small testis encountered in T. mariae was also experienced in S. galileaus and heavy dependence on the microscope was made. The problem of ovaries with fluid, encountered in oestrogen treated S. galileaus was not found in androgen treated fish.

7.11 Influence of 17α-Methyltestosterone on phenotypic sex differentiation in O. mossambicus

Table 1.22. Phenotypic sex differentiation in androgen treated O. mossambicus

Hormone dose mg/Kg diet	Sample	Male	% male#	female	% female	M/F#	Undiff
0	170	89	52.35	81	47.6471	1.09877	0
50	160	160	100.00***	0	0.0000	*	0
0	142	75	52.817	67	47.1831	1.11940	0
50	151	147	97.35***	0	0.0000	*	4
0	162	82	50.617	79	48.7654.	1.03797	1
50	167	164	98.204***	0	0.0000	*	3

superscripts in column 4 denote significant difference with the respective controls ($p < 0.001$).

Results in Table 1.22 indicate that feeding O. mossambicus fry with 17 α-MT at 50 mg/Kg diet is highly effective in eliminating females. The table also shows that among the treated fish only 1.4% of the fish could not be positively sexed, while among the controls, the corresponding proportion was much less than one percent. In part, this is a reflection on the ease with which the sexes can be distinguished, but more importantly gonadal development and maturation in O. mossambicus is rapid (Chapter III).

Table 1.23 shows that the effect of hormone feeding was almost uniform in the three batches.

Table 7.23 Dose effect of 17α-MT on phenotypic sex differentiation in O. mossambicus

Hormone dose mg/Kg diet	Sample	Male Number	%	Female Number	%	Ratio M/F	Undiff
0	170	89	53.35a	81	47.65	1.10:1	0
0	142	75	52.82a	67	47.18	1.12:1	0
0	162	82	50.62a	27	48.77	1.04:1	1
	472	246	57.93± 0.55	227	47.87±0.39	1.08:1*	1
50	160	160	100	0	0	1:0	0
50	151	147	97.35a	0	0	1:0	4
50	167	164	98.20a	0	0	1:0	3
	478	471	98.52±0.64	0	0	1:0**	7

Similar superscripts in column 4 denote lack of significant difference between batches of fish subjected to same treatment ($p > 0.05$). Superscripts in column 7 denote significant difference between treatment averages.

1.12 Influence of 17α-Methyltestosterone on phenotypic sex differentiation in O. niloticus

Table 7.24 Sex distribution in O. niloticus fry treated with 17α-MT

Hormone dose mg/Kg diet	Sample	Male	% male#	female	% female	M/F#	Undiff
0	72	42	58.333	30	41.6667	1.40	0
45	70	66	94.286***	0	0.0000	1:0	4
60	76	73	96.052***	0	0.0000	1:0	3
0	129	69	53.488	57	44.1860	1.21	3
45	106	98	92.453***	0	0.0000	1:0	8
60	94	89	94.681***	0	0.0000	1:0	5
0	121	54	44.628	67	55.3719	0.80	0
45	109	98	89.908***	9	8.2569	10.89	2
60	75	75	100.000***	0	0.0000	1;0	0

Superscripts in column 4 denote significant difference with the respective controls at $p < 0.001$.

Table 7.25 Dose effect of 17∝-MT on phenotypic sex differentiation in O. niloticus

Hormone dose mg/Kg diet	Sample	Male Number	%	Female Number	%	Ratio M/F	Undiff
0	72	42	58.33b	30	41.67	1.40	0
0	129	69	53.49ab	57	44.19	1.21	3
0	121	54	44.63a	67	55.37	0.81	0
	322	165	52.15 ± 3.28	154	47.08 ± 3.44	1.07*	3
45	70	66	94.29a	0	0	1:0	4
45	106	98	92.45a	0	0	1:0	8
45	109	98	89.91a	9	8.26	10.89	2
	285	262	92.22 ± 1.04	9	2.75 ± 2.25	29:1**	14
60	76	73	96.05a	0	0	1:0	3
60	94	89	94.68a	0	0	1:0	5
60	75	75	100	0	0	1:0	0
	245	237	96.91 ± 1.30	0	0	1:0**	8

Similar superscripts in column 4 denote lack of significant difference between batches of fish subjected to the same treatment ($p < 0.05$). Superscripts in column 7 denote significant difference between treatment averages.

Tables 1.24 and 1.25 give results of treatment of O. niloticus fry with 17 α-Methyltestosterone at two feeding levels. Table 1.24 shows that this species is highly responsive to the treatment and resulted in elimination of virtually all females in 5 of the six treatments. The proportion of fish that could not be sexed was highest in batch number 2 (see column 8), and amounted to 4.86%.

Table 1.25 shows sex ratio of fish in relation to hormone dosage. Results show that among the controls, sex ratio in different batches differed significantly with each other. A similar observation was also made earlier (in Chapter V). The groups fed hormone treated diet did not differ significantly with each other at any given dosage (Table 1.25, column 4). The treated groups yielded significantly different sex ratios with the controls.

7.2.9 Influence of 17 α-ethynylestradial (17 α -εε) on survival of S. galileaus fry

Tables 2.1 and 2.2 show survival of S. galileaus first feeding fry given different amounts of the oestrogen. Results show that within each batch the amount of hormone supplied to the fish had a significant influence on survival (Table 2.1). Within each treatment category, significant difference was found in groups fed 50 mg/Kg and 65 mg/Kg diet. There was no significant difference at 35 mg/Kg diet and in the control groups. Between treatments, significant difference in survival was found between 65 mg/Kg and the other groups (Table 2.2).

Table 2.1 Comparative survival of S. galileaus fry fed on trout diet treated with 17 ∝-EE. Data arranged by sib group

Hormone dose mg/Kg diet	Number of fish Initial	Final	Survival # %
0	110	87	79.09b
35	150	124	82.67b
50	150	110	73.33b
65	140	79	56.43a
0	110	84	76.36b
35	110	93	84.55b
50	130	114	87.69b
65	100	73	73.00b
0	100	83	87.00b
35	190	176	92.63b
50	170	106	62.35a
65	160	103	64.38a

Different superscripts within each treatment denote significant difference between batches of fry and different superscripts between group averages denotes significant difference between treatments ($p < 0.05$).

Table 2.2 Comparative survival of S. galileaus fry fed on trout diet treated with 17 α-EE. Data arranged by type of treatment

Hormone dose mg/Kg diet	Number of fish Initial	Final	Survival # % ± SE
0	110	87	79.09a
0	110	84	76.36a
0	100	83	87.00a
	320	254	80.82 ± 3.19 cb
35	150	124	82.67a
35	110	93	84.55a
35	190	176	92.63a
	450	393	86.62 ± 3.06cb
50	150	110	73.33b
50	130	114	87.69c
50	170	106	62.35a
	450	330	74.46 ± 7.34ab
65	140	79	56.42a
65	100	73	73.00b
65	160	103	64.38ab
	400	255	64.60 ± 4.79a

different superscripts within each treatment denote significant difference between batches of fry and different superscripts between group averages denotes significant difference between treatments ($p < 0.05$).

7.2.10 Influence of 17 (x-ethynylestradial on survival of *O. niloticus* fry

Average survival for the entire experiment was 73.59 ± 3.54. The lowest survival was 46.00% obtained in one group treated at 65 mg/Kg diet. The highest was 87.06% obtained in 35 mg/Kg diet.

Within each batch of fry, significant difference was found between treatments in two of the groups as shown in Table 2.3, column 4. Significant difference was also found between survival of fish in 65 mg/Kg and the other three treatments as shown in column 4 of Table 2.4.

Table 2.3 Survival of O. niloticus fry fed on trout diet treated with 17 ∝ EE; arranged by sib group

Hormone dose mg/Kg diet	Number of fish Initial	Final	Survival # %
0	178	118	66.29a
35	85	74	87.06c
50	180	128	71.11b
65	150	69	46.00a
0	150	130	86.67b
35	200	144	72.00ab
50	120	74	61.67a
65	94	58	61.70a
0	120	106	86.33a
35	120	98	81.67a
50	110	90	81.82a
65	130	105	80.76a

Within each group, different superscripts denote significant differences between treatments ($p < 0.05$)

Table 2.4 Comparative survival of O. niloticus fry fed on trout diet treated with ethynylestradiol, arranged by type of treatment

Hormone dose mg/Kg diet	Number of fish Initial	Final	Survival # % ± SE
0	178	118	66.29a
0	150	130	86.67b
0	120	106	86.33b
	448	354	80.43 ± 7.04*
35	85	74	87.06b
35	200	144	72.00a
35	120	98	81.67ab
	405	316	80.24 ± 4.41*
50	180	128	71.11ba
50	120	74	61.67a
50	110	90	81.82b
	410	292	71.53 ± 5.82*
65	150	69	46.00a
65	94	58	61.70b
65	130	105	80.76c
	374	232	62.82 ± 10.05**
	1637	1194	73.59 ± 3.54

different superscripts within a group denote significant differences between batches. Different superscripts on group averages denote significant differences between treatments ($p < 0.05$).

Table 2.5 Comparative survival of *O. niloticus* X *O. macrochir* F_1 hybrids fed on trout diet treated with 17α-EE. Data arranged by sib group

Hormone dose mg/Kg diet	Number of fish Initial	Final	Survival # %
0	87	64	73.56b
50	87	76	87.36a
0	92	85	92.39b
50	115	91	79.13a
0	75	66	88.00b
50	96	72	75.00a
0	95	84	88.42b
50	84	58	69.05a

Within each group, different superscripts denote significant difference between treatments ($p < 0.05$).

Table 2.6 Comparative survival of O. niloticus x O. macrochir F_1 hybrid fry fed trout diet treated with 17α-EE. Data arranged by type of treatment.

Hormone dose mg/Kg diet	Number of fish Initial	Final	Survival # % ± SE
0	87	64	73.56a
0	92	85	92.39b
0	75	66	88.00b
0	95	84	88.42b
	349	299	85.59 ± 6.26*
50	87	76	87.36b
50	115	91	79.13ab
50	96	72	75.00ab
50	84	58	69.05*
	382	297	77.63 ± 5.69 *

different superscripts within each group denote significant differences between batches and superscripts on group averages denote significant differences between treatments. ($p < 0.05$).

7.2.11 Influence of 17 α-ethynylestradiol on survival of *O. niloticus* x *O. macrochir* F_1 hybrid fry

The average survival in the control groups was 85.59 ± 6.26%. The lowest was 73.56% and highest was 92.39%. The average survival among the hormone fed groups was 77.63% ± 52.69%. No significant difference was found between treatment averages (Tables 2.6 column 4) probably as a result of a moderating effect from the high survival of treated fish in the first batch (Table 2.5).

7.2.12 Influence of 17 α -ethynylestradiol on growth of first feeding *S. galileaus*

As shown in Table 2.7 and Figure 2.1, there was little difference in growth rate of *S. galileaus* subjected to different levels of hormone treatment. The FCR and SGR showed little variation between the three batches and was not significantly different at 5% (Tables 2.8 and 2.9). There was significant difference between weight of fish compared after 67 days. Fish fed the εε 65 diet grew significantly slower than the control group or fish fed εε 35 and εε 50 diets

Table 2.7 Mean weight of S. galileaus fry treated with ethynyl estradiol

Age (days)	Fish Weight # Control	EE 35	EE 50	EE 65
6	0.008	0.008	0.008	0.008
13	0.025	0.023	0.024	0.024
20	0.082	0.070	0.063	0.077
25	0.223	0.211	0.244	0.193
32	0.360	0.378	0.345	0.397
39	0.780	0.829	0.776	0.767
47	1.298	1.416	1.297	1.203
53	2.351	2.508	2.681	2.139
60	3.808	3.159	3.881	2.964
67	4.386a	4.589a	4.497a	3.508b

Different superscripts in the final weight row indicate significant difference between treatments ($p < 0.05$).

Table 2.8 Comparative F.C.R. and S.G.R. of S. galileaus fry fed a hormone treated diet: 40 days after hatching. (17∝ Methyltestosteron

Hormone dose mg/Kg	F.C.R. ± S.E.	S.G.R. # ± S.E.
0	1.28 ± 0.01	9.41 ± 0.18ba
35	1.48 ± 0.04	9.43 ± 0.16bb
50	1.36 ± 0.05	9.63 ± 0.07b
65	1.31 ± 0.08	8.85 ± 0.11a
0	1.36 ± 0.02	8.92 ± 0.05a
35	1.23 ± 0.01	9.68 ± 0.18b
50	1.39 ± 0.07	9.45 ± 0.13ab
65	1.22 ± 0.06	9.31 ± 0.18ab
0	1.27 ± 0.01	9.25 ± 0.11ab
35	1.78 ± 0.07	9.13 ± 0.08a
50	1.18 ± 0.11	9.75 ± 0.14b
65	1.53 ± 0.11	9.17 ± 0.11a

Different superscripts in column 3 indicate significant difference between treatments within a sib group ($p \leq 0.05$)

Table 2.9 Comparative FCR and SGR in S. galilcaus fry fed a hormone treated diet for 40 days; arranged by type of treatment

Hormone Dose	F.C.R.	±	S.E.	S.G.R.	±	S.E.
0	1.28	±	0.01	9.41	±	0.18
0	1.36	±	0.03	8.92	±	0.05
0	1.27	±	0.01	9.25	±	0.11
	1.30	±	0.01a	9.20	±	0.08ba
35	1.48	±	0.04	9.43	±	0.16
35	1.23	±	0.01	9.68	±	0.18
35	1.78	±	0.07	9.13	±	0.08
	1.50	±	0.05a	9.41	±	0.08ba
50	1.36	±	0.05	9.63	±	0.07
50	1.39	±	0.07	9.45	±	0.13
50	1.18	±	0.11	9.75	±	0.14
	1.31	±	0.04a	9.61	±	0.06a
65						
65	1.31	±	0.08	8.85	±	0.11
65	1.22	±	0.06	9.31	±	0.18
	1.53	±	0.11	9.17	±	0.11
	1.35	±	0.07a	9.11	±	0.10b
Total	1.37	±	0.05	9.36	±	0.08

Superscripts in Columns 2 and 3 denote significant difference between treatment averages ($p < 0.05$)

Table 2.10 Size composition of S. galileaus fry fed a diet treated with EE at 50 mg/Kg; arranged by sex

Hormone dose mg/kg	Number of Fish			Sx Ratio	Mean Weight			Differ %
	total	male	female	% female	total	male	female	M/F
0	68	31	37	54.4	1.77	1.57	1.94	19.07
50	66	0	66	100	1.96	-	1.96	-

Within the size range of this experiment, females comprised 54.4% by number and grew 19.07% faster than males. The level of hormone inclusion used in the feed was effective in producing an all female population repeatedly.

The treated groups grew relatively faster than the controls, but the final size of the female fish was about the same in both populations.

The relationship between weight and length in (a) male: (b) female S. galileaus was:-

$$Y = 0.121 + 2.660\ X \quad \ldots\ldots \text{(a)}$$

$$Y = 0.053 + 0.088\ X \quad \ldots\ldots \text{(b)}$$

and is presented graphically in Figure 2.2.

7.2.13 Influence of 17 ∝-ethynylestradiol on growth of first feeding O. niloticus fry

Data in Table 2.11 and the corresponding graph in Figure 2.3 show that within the age range of this experiment, 17 ∝-EE had no significant effect on fish growth rate. The growth rate of fish increased rapidly starting from about 50 days after hatching. although the inflection period coincided with the end of hormone feeding, it is unlikely that the hormone had anything to do with it since similar changes took place in the control group about the same time. No significant difference was found in mean weight of fish 78 days after hatching.

The lowest mean food conversion was obtained in fish treated with 50 mg/Kg diet and the highest was in the control groups (Tables 2.12 and 2.13). Marginal differences were found in FCR ratio in different treatments of each batch (Table 2.12) but no differences were found between averages of the different treatment groups (Table 2.13).

Table 2.14 and Figures 2.4 and 2.5 based on final data 78 days after hatching. The data shows that among the control groups, males were on average 8.68% heavier than females, but the difference was not significant ($p > 0.05$). Figures 2.4 and 2.5 show normal distribution of growth parameters in both sexes.

The treatment was effective in altering sex ratios significantly at all three hormone levels ($p < 0.001$). (Column 5)

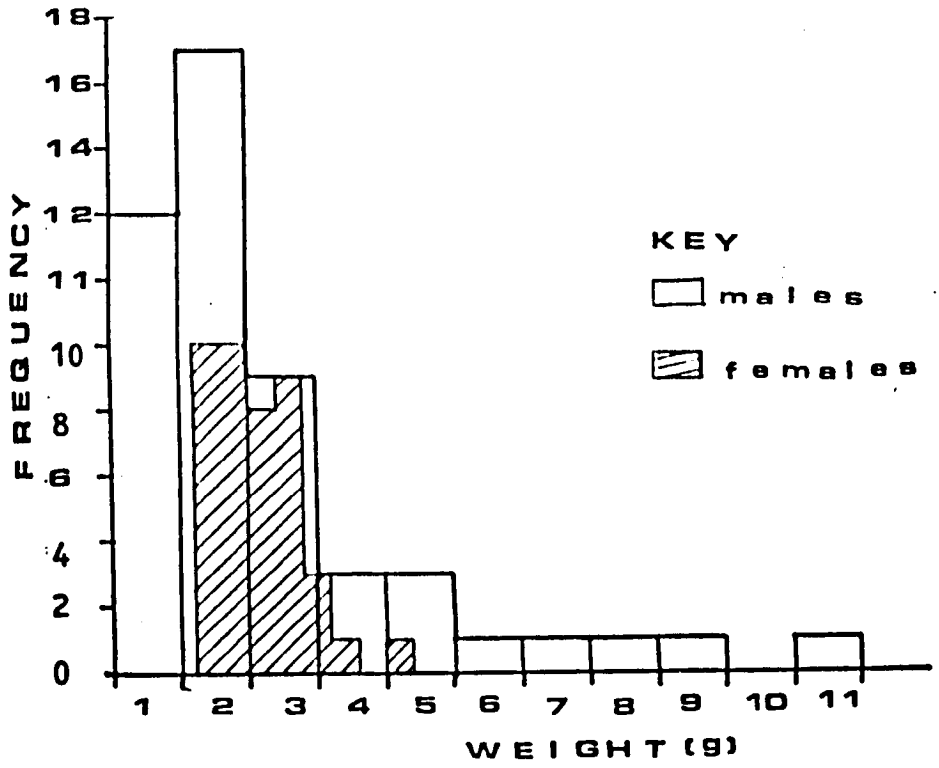


Figure 1.13: Weight frequency distribution of (a) male and (b) female

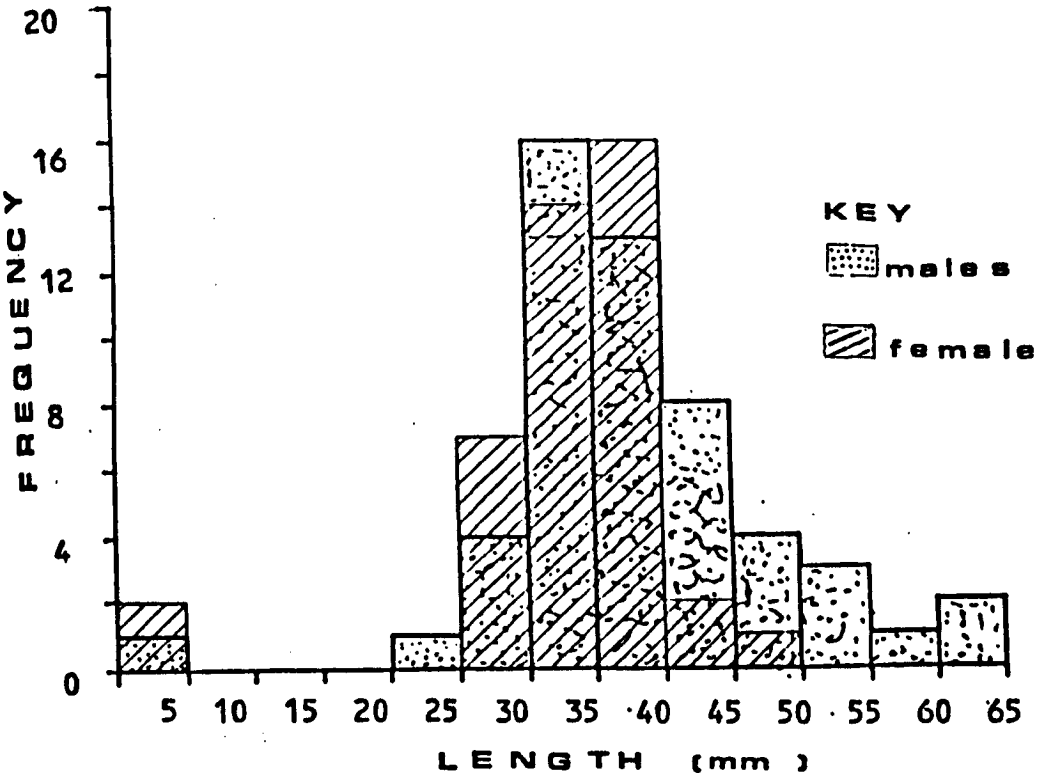


Figure 1.14: Length frequency distribution of (a) male and (b) female

Table 2.11 Mean individual weight of fish sampled at different times during the experiments (O. niloticus)

Age (days)	EEc	EE55	EE50	EE65
6	0.010	0.010	0.010	0.010
25	0.165	0.173	0.113	0.128
32	0.240	0.201	0.152	0.179
50	0.481	0.500	0.378	0.378
57	0.718	0.678	0.689	0.744
64	1.934	1.692	1.237	1.246
71	2.470	2.940	2.067	2.090
78	3.308*	3.572*	3.095*	3.327*

.12 Comparative F.C.R. and S.G.R. of hormone treated O. niloticus fry. Data arranged by sib groups

Hormone Dose mg/Kg diet	F.C.R. ± S.E.	S.G.R. ± S.E.
0	1.58 ± 0.06a	6.98 ± 0.17a
35	1.12 ± 0.08c	7.88 ± 0.21a
50	1.30 ± 0.03b	7.44 ± 0.08a
65	1.27 ± 0.06b	6.98 ± 0.14a
0	1.15 ± 0.02b	7.24 ± 0.08b
35	1.44 ± 0.08a	6.59 ± 0.13a
50	1.16 ± 0.06b	6.81 ± 0.15ab
65	1.34 ± 0.04a	6.36 ± 0.06a
0	1.34 ± 0.07a	7.31 ± 0.04a
35	1.19 ± 0.04c	7.38 ± 0.08a
50	1.22 ± 0.02bc	7.46 ± 0.13a
65	1.20 ± 0.05bc	7.49 ± 0.09a

Table 2.13 Comparison of F.C.R. and S.G.R. values in *O. niloticus* fry treated with 17α-ethynyl estradiol Data arranged by type of treatment

Hormone dose mg/Kg diet	F.C.R.# ± S.E.	S.G.R. ± S.E.
0	1.58 ± 0.06b	6.98 ± 0.17a
0	1.15 ± 0.02a	7.24 ± 0.08a
0	1.34 ± 0.07a	7.31 ± 0.04a
	1.36 ± 0.09*	7.17 ± 0.06*
35	1.12 ± 0.08a	7.88 ± 0.24b
35	1.44 ± 0.08b	6.59 ± 0.43a
35	1.19 ± 0.04a	7.38 ± 0.28b
	1.26 ± 0.03*	7.29 ± 0.21*
50	1.30 ± 0.03a	7.44 ± 0.18a
50	1.16 ± 0.06a	6.81 ± 0.25a
50	1.19 ± 0.02a	7.46 ± 0.23a
	1.22 ± 0.04*	7.24 ± 0.18*
65	1.27 ± 0.06a	6.98 ± 0.12ab
65	1.34 ± 0.04a	6.36 ± 0.06a
65	1.20 ± 0.05a	7.49 ± 0.09b
	1.27 ± 0.06*	6.94 ± 0.04*

treatment averages with the same asterisk are not significantly different ($p > 0.05$) from each other.

Table 2.14 Size (Wt) and sex composition of O. niloticus treated with 17 α -ethynylestradiol

Hormone dose	Number of Fish			Sex Ratio	Individual $\bar{X}$ Weight			
mg/Kg diet	Total	Male	Female	% Male	Total	Male	Female	% Diff
0	39	18	21	48.72	3.13	3.16	2.97	8.68
35	71	10	61	***14.08	3.16	3.21	3.08	4.22
50	94	2	92	*** 2.13	3.28	-	3.28	-
65	43	0	43	*** 0	3.33	-	3.33	-

Sex ratios with asterisks are significantly different from the control ($p<0.001$)

7.2.14 Influence of 17 α-ethynlestradiol on growth of first feeding O. niloticus x O. macrochir F_1 hybrids

As shown in Table 2.15, two sets of fry were used in growth studies. Results indicate that growth rate in the two control groups was significantly higher than in the hormone fed fish. Growth rate was

Table 2.15 Mean individual weight of O. niloticus x O. macrochir F_1 hybrids

Age (days)	Control (1)	Control (2)	EE50 (1)	EE50 (2)
6	0.009	0.009	0.009	0.009
30	0.160	0.219	0.162	0.236
38	0.299	0.398	0.267	0.340
43	0.398	0.426	0.315	0.441
50	0.617	0.851	0.415	0.518
57	0.731	1.166	0.603	0.630
69	1.635	1.957	1.163	1.371
76	2.390b	3.016c	1.668a	1.791a

generally low. The final weight in the treated groups was identical at the end of the experiment, but differed significantly in the two control groups. Overall there was significant difference in

Table 2.17 Comparative FCR and SGR in O. niloticus X O. macrochir

Hormone Dose mg/Kg Diet	F.C.R. # ± S.E.	S.G.R. ± S.E.
0	1.31 ± 0.08	7.34 ± 0.22ab
0	1.20 ± 0.10	8.18 ± 0.17b
0	1.36 ± 0.16	7.57 ± 0.20b
0	1.27 ± 0.07	6.87 ± 0.29a
	1.29 ± 0.09*	7.49 ± 0.17*
50	1.42 ± 0.13	6.87 ± 0.25a
50	1.71 ± 0.07	7.49 ± 0.09a
50	1.47 ± 0.10	6.81 ± 0.18a
50	1.32 ± 0.05	6.92 ± 0.10a
	1.48 ± 0.02**	7.03 ± 0.05*

Averages were significantly different at $P < 0.05$

FCR but not in SGR of the treated fish with the controls ($p > 0.05$) as presented in Table 2.17. Results show that control fish were more efficient food converters than the hormone treated group. The average FCR and SGR was identical in the two sexes as follows:-

Sex	FCR	±	SE	SGR	±	SE
Male	1.39	±	0.035	7.15	±	0.184
Female	1.37	±	0.114	7.37	±	0.307

Table 2.18 is a summary of relative weight between male and female at the end of the experiments.

Artificial hybridization of O. niloticus x O. macrochir resulted in all male broods in both crosses. Application of 17 α-EE at 50 mg/Kg was successful in altering sex ratio of fish significantly.

Table 2.18 Comparative weight by sex of hormone treated F_1 hybrids O. niloticus x O. macrochir

Dose mg/Kg diet	Number of fish total	male	female	% ma	Mean individual weight total	male	female	Diff %
0	42	42	0	100%	2,78%	2,78%	-	-
50	54	11	42	20,37%%	2,09%	2,80%	1,92	45,83

Table 2.18 also shows that the mean individual weight of males in the two groups did not differ. However, the weight of females was significantly lower than that of males both in the control group and among the hormone treated fish. Among the hormone treated fish, males were 45.83% heavier than females after 75 days.

Figure 2.8 shows combined size distribution by weight and by standard length of the F_1 hybrids of *O. niloticus* x *O. macrochir*

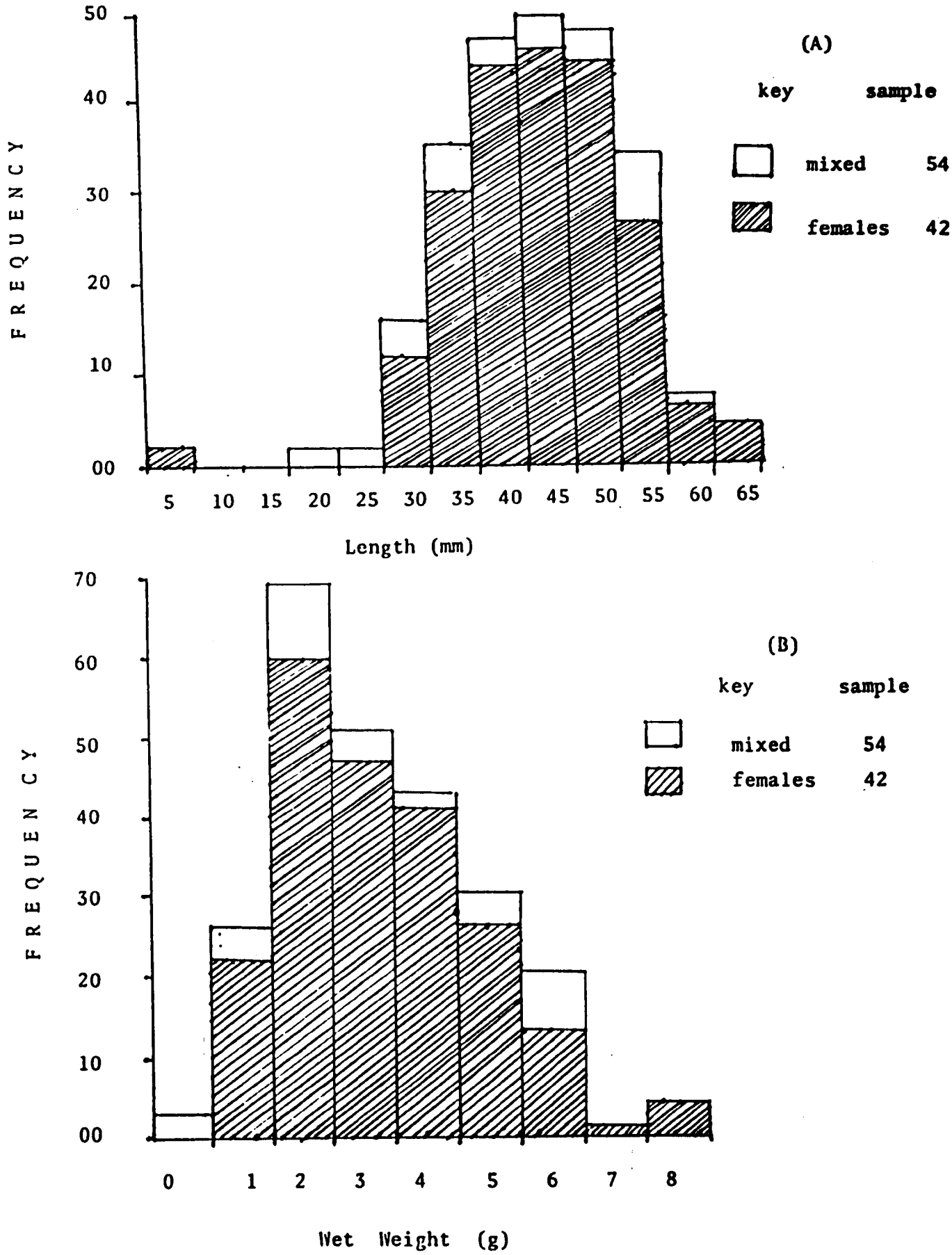


Figure 2.8; Weight frequency (A), and Length frequency (B) distribution of F1 hybrids O.niloticus X O.macrochir treated with 50mg/Kg of 17α-ethynylestradiol.

7.2.15 Influence of 17 α-ethynylestradiol on development of phenotypic sex in first feeding *S. galileaus*

Results in Tables 2.19 and 2.20 show that administration of 17 -EE to fry from the first feeding stage resulted in significant alteration on sex ratio at all three levels of treatment. Table 2.19 shows the response to treatment of different groups of fish from the same batch. Table 2.20 shows the same groups of fish re-arranged to compare the overall effects of different treatments. Results show that the average sex ratio among the control groups was 0.96 male to female. The sex ratios in the three groups of control fish did not differ significantly with each other. The effect of a hormone dose of 35 mg/Kg was varied, but in all cases differed significantly from the respective controls. The average proportion of females with this treatment was 72.57 ± 3.35. Hormone dosages of 50 mg/Kg diet and 65 mg/Kg diet were effective in totally eliminating males from the population. However, among the apparent phenotypic females almost 3% of the gonads were maldeveloped ovasacs, devoid of cellular components.

Table 2.19 Comparative effect of 17∝EE treatment in sib groups of S. galileaus

Hormone	Sample	S E X # Male	% Male	S E X # Female	% Female	Undifferential	MF Ratio #	
0	83	40	48.19a	43	51.81	0	0.09:1	
35	100	35	35.00b	65	65	0	0.54:1	**
50	110	0	0 c	110	100	0	0:1	***
65	75	0	0 c	74	98.67	1	0:1	***
0	84	39	46.43a	45	53.57	0	0.87:1	
35	91	19	20.87b	72	79.12	0	0.26:1	***
50	108	0	0 c	104	96.30	4	0:1	***
65	70	0	0 c	70	100	0	0:1	***
0	80	42	52.50a	38	47.50	0	1.11:1	
35	125	33	26.40b	92	73.60	0	0.36:1	**
50	100	0	0 c	102	100	0	0:1	***
65	100	0	0 c	95	100	0	0:1	***

Figures in column four having different superscripts are significantly different with each other. Superscripts in column 8 denote sex ratios in each row that differ from the control.

Table 2.20 Comparative effect of 17∝EE dosage on sex differentiation in S. galileaus

Hormone	Sample	S E X # Male	% Male	Female	% Female	Undifferential	MF Ratio #
0	83	40	48.19a	43	51.81	0	0.93
0	84	39	46.43a	45	53.57	0	0.87
0	80	42	52.50a	38	47.50	0	1.11
	247	121	49.04±1.47	126	50.96±1.47	0	0.96a
35	100	35	36.00a	65	65	0	0.54**
35	91	19	20.87b	72	79.12	0	0.26***
35	125	33	26.40b	92	73.60	0	0.36***
	316	87	27.42±3.36	229	72.57±3.35	0	0.38b
50	110	0	0	110	100 a	0	0:1 ***
50	108	0	0	104	96.30a	4	0:1 ***
50	100	0	0	102	100 a	0	0:1 ***
	318	0	0	316	98.77±1.01	0	0:1 c
65	75	0	0	74	98.67a	1	0:1***
65	70	0	0	70	100 a	0	0:1***
65	100	0	0	95	95 a	5	0:1***
	240	0	0	239	97.89±1.22	6	0:1c

In columns 4 and 6, figures with same superscripts do not differ significantly with each other within the group ($p < 0.05$). In column 8, figures with superscripts are sex ratios that differ significantly with their respective control groups.

* = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$

7,2,16 Influence of 17 ∝-ethynylestradiol on development of phenotypic sex in O. niloticus fry

Table 2.21 gives the distribution of the sexes in sib groups exposed to different dosages of the hormone. Table 2.22 shows the effect of dosage on sex differentiation. The average proportion of males in the control groups is 46.88± 0.69%. The average proportion of males in the groups supplied 35 mg/Kg diet of 17 ∝-EE was 4.35 ± 1.85%. No males were obtained at 50 and 65 mg/Kg. There was no significant difference in the effect of the hormone at each of the feeding levels. The incidence of undifferentiated or maldeveloped gonads in the hormone treated fish was less than 1 per cent.

Table 2.21: Comparative effect of 17 (X-EE in sib groups of O. niloticus

Hormone	Sample	SEX # Male	% Male	SEX # Female	% Female	Undifferential	MF Ratio #
0	118	56	47.66a	62	52.54	0	0.90:1
35	74	4	5.41b	70	94.60	0	0.06:1***
50	128	0	0 c	128	100	0	0 :1 ***
65	69	0	0 c	69	100	0	0:1 ***
0	136	60	44.12a	76	55.88	0	0.78:1*
35	124	11	7.63b	112	90.32	1	0.10:1***
50	74	0	0	68	98.55	6	0:1 ***
65	58	0	0	54	98.18	4	0:1 ***
0	106	52	49.06a	54	50.94	0	0.96:1
35	98	0	0 b	94	95.92	4	0:1 ***
50	90	0	0 b	90	100	0	0:1 ***
65	105	0	0 b	97	92.38	0	0:1 ***

Superscripts in column 4 indicate significant differences within a sib group ($p < 0.05$). Superscripts in column 8 indicate significant differences with the respective control groups.

Table 2.22: Dosage effect of 17 α-EE on sex differentiation in *O. niloticus* fry

Hormone	Sample	SEX # Male	% Male	SEX # Female	% Female	Undifferential	MF Ratio #
0	118	56	47.46	62	52.54a	0	0.90:1
0	136	60	44.12	76	55.88a	0	0.78:1*
0	106	52	49.06	54	50.94a	0	0.96:1
	360	167	46.88 ± 0.69	192	53.33 ± 1.19	0	0.87:1a
35	74	4	5.41	70	94.60a	0	0.06:1***
35	124	11	7.63	112	90.32a	1	0.10:1***
35	98	0	0	91	100 a	0	0:1 ***
	296	15	4.35 ± 1.85	273	94.97 ± 2.29	1	0.05:1b
50	128	0	0	128	100 a	0	0:1 ***
50	74	0	0	68	98.55a	1	0:1 ***
50	90	0	0	90	100	0	0:1 ***
	292	0	0	186	99.52 ± 0.39	1	0:1b
65	69	0	0	69	100 a	0	0:1 ***
65	58	0	0	54	98.18a	1	0:1 ***
65	105	0	0	87	100 a	0	0:1 ***
	232	0	0	210	99.39 ± 0.50	1	0:1b

In columns 4 and 6, figures with same superscripts do not differ significantly with each other within the group ($p > 0.05$). Superscripts in column 8 indicate significant difference with their respective control group.

Tables 2.23 and 2.24 show the results of feeding different batches of fish varying amounts of the hormone, 17 α-EE. The percentage of males in the four control groups ranged from 92.19 to 100%; the average was 96.65 ± 1.63% (Table 2.24). The proportion of males in the treated groups was 22.00% ± 2.05%.

The treatment was effective in altering the sex ratio in all batches. The effectiveness of the treatment varied little between groups (Table 2.24 columns 4, 6 and 8). The treatment was not effective in producing monosex broods. Constriction was the principle form of ovarian maldevelopment among the induced females. The number of fish with undifferentiated gonads was 2% and 6% in the control and treatment groups respectively.

Table 2.23 : Comparative Influence of 17 α-EE on sib groups in the F_1 hybrid O. niloticus x O. macrochir

Hormone	Sample	S E X # Male	% Male	Female	% Female	Undifferentiated	MF Ratio #
0	64	59	92.19	5	7.81	0	11.8:1
50	76	16	21.62	56	78.38	4	0.29:1***
0	85	83	100	0	0	2	1:0
50	91	21	23.33	63	70.00	9	0.33:1***
0	66	63	96.97	0	0	3	1:0
50	72	19	26.39	53	73.61	0	0.36:1***
0	84	78	97.44	6	7.14	0	13:1
50	58	9	16.67	45	83.33	4	0.2:1***

Superscripts in column 8 indicate significant difference in the treated groups when compared with the respective controls ($p < 0.001$)

Table 2.24: Dosage effect of 17 α-EE on sex differentiation in *O. niloticus* x *O. macrochir* F_1 hybrids

Hormone	Sample	SEX # Male	% Male	Female	% Female	Undifferentiated	MF Ratio #
0	64	59	92.19a	5	7.81	0	12:1
0	85	83	100 a	0	0	2	1:0
0	66	63	96.97a	0	0	3	1:0
0	84	78	97.44a	6	7.14	0	13:1
	299	283	96.65 $\pm$ 1.63	11	3.74 $\pm$ 1.87	5	26:1a
50	76	16	21.62ab	56	78.38	4	0.29:1***
50	91	21	23.33ab	63	70.00	9	0.33:1***
50	72	19	26.39b	53	73.61	0	0.36:1***
50	58	9	16.67a	45	83.33	4	0.20:1***
	297	65	22.00 $\pm$ 2.04	217	76.33 $\pm$ 2.90	17	0.30:1b

Superscripts in column 4 indicate significant difference in proportion of males in different batches ($p < 0.05$). Superscripts in the treated groups of column 8 indicate significant difference with controls ($p < 0.001$).

7.3.4 Discussion

7.3.4.1 Effect of steroid hormones on fry survival

Application of steroid hormones for induction of sex reversal works on the premise of differentially affecting the physiological process involved in sex differentiation. This selectivity raises the possibility of sexually influenced differential mortality in the animals being treated. For this reason, survival of the fish during and after a course of treatment is an important economic consideration to be assessed when evaluating suitability of a hormone for sex reversal.

7.3.4.2 Effect of 17 α-Methyltestosterone

Survival of T. mariae, S. galileaus, O. niloticus and O. mossambicus following treatment with hormones for up to 40 days after hatching is given in Tables 7.3.1, 7.3.2, 7.3.3, 7.3.4, 7.3.5 and 7.3.6 - 8.3.8 respectively.

Significant difference was found between the control and some of the hormone fed T. mariae groups. In two of the three batches, survival of fish at MT.60 was significantly lower than in the controls. A similar trend was observed in O.

niloticus and O. mossambicus. Although variations were observed in S. galileaus, the trend was not as clear as in the other three species. When results from the three batches in O. mossambicus and S. galileaus were each pooled, no significant difference was found between hormone fed fish and their respective controls (Table 7.3.4 and 7.3.6). However, pooled batches of O. niloticus and of T. mariae resulted in significant difference between fish given MT.60 and the controls. Lower hormone dosages had no effect on survival of all groups of fish treated (Table 7.3.2 and 7.3.8).

The results indicate that different batches of fish probably respond differently to treatment with Methyltestosterone.

In similar experiments, Guerrero III (1975) found no significant difference in survival of groups of O. aureus treated with 1-dehydrotestosterone acetate, 17 α-ethynyltestosterone and 17 α-Methyltestosterone at 15, 30 and 60 mg/Kg diet for 18 days. Guerrero treated fish for a relatively shorter period, but used equivalent hormone dosages with the present experiments.

7.3.4.3 **Effect of 17 α-ethynylestradiol (17 α-EE)**

Survival of S. galileaus, O. niloticus, and O. niloticus x O. macrochir hybrids treated with 17 α-ethynlestradiol is summarised in Tables 7.3.9, 7.3.10, 7.3.11, 7.3.12, 7.3.13 and 7.3.14 respectively. In sib groups, significantly lower survival was obtained in two of the three batches of S. galileaus fed EE65 and in all hybrid groups fed EE50.

When different batches were put together and compared, there was significant difference found between groups treated with hormones at 65 mg/Kg diet in all the three species of the fish. At 50 mg/Kg, the hormone did not have a significant effect on survival of either O. niloticus, or its hybrid with O. macrochir. Survival from four batches of the hybrid was 77.63 ± 5.69% and 85.59 ± 6.26% in the hormone treated fish and in the control respectively.

The above results demonstrate that feeding tilapia with 17 α-ethynylestradiol up to 50 mg/Kg in the diet did not result in significant negative effects on survival. However, feeding fry with 65 mg/Kg of the hormone resulted in significantly lower survival in both S. galileaus and O. niloticus.

The increase in mortality rate in some, but not all the treated fish suggests individual variations in resistance to the negative effects of these drugs. The pattern of reduction in survival further demonstrates that the effect of the hormones is probably dose dependent. The actual mode of action of the hormones is not obvious from the present experiments. However, in view that no lowering of feeding rates was observed during the treatment period, the observed lowering of survival could be due to direct toxic effects at the high dose rate.

7.3.4.4 Influence of steroid hormones on growth

As earlier stated, the objective of treating fish with hormones is to influence the direction of sex differentiation into production of single sex population. In tilapia, production of all-male broods is preferred because of their inherent growth superiority over females.

In the present thesis, growth comparisons were done to compare the effects of the hormones in both sexes.

7.3.4.5 Effect of 17 α-Methyltestosterone on growth

Growth data on T. mariae, S. galileaus and O. niloticus treated with 17 α-MT is presented in Tables 7.3.9, 7.3.15 and 7.3.19 respectively.

Results indicate that after 45 and 71 days after hatching, growth of T. mariae fed a hormone treated diet at 30 mg/Kg, 45 mg/Kg and 60 mg/Kg did not differ significantly with the controls. However, a sample of fish taken 120 days post hatch indicated significant difference between weight of some treated groups of fish with the controls' (Table 1.10). There was also significant difference between weight of males in the different treatments. Weight of female fish in all groups did not differ significantly.

These results suggest that the androgen had a significant positive influence on growth rate in males but not in females. Although not significantly different, the mean weight of females in groups fed a diet with 30 mg/Kg and 45 mg/Kg was noticeably lower than the control groups. In the present state of knowledge, it was not possible to distinguish sex reversed males from normal genetic males in the treated groups, so that the influence of the hormone on the two types of males could not be determined.

Comparative analysis of growth data in S. galileaus demonstrates significant difference in 89 day old fish (Table 11). Fish fed 17 α-Methyltestosterone at 60 mg/Kg grew at almost the

same rate as the controls, while fish fed at 30 mg/Kg and 45 mg/Kg grew significantly heavier.

In O. niloticus, the difference in weight between various treatments was not significantly different 98 days after hatching (Table 1.16). The significantly different weight in hormone treated males compared with males in the control group suggests that 17 α-Methyltestosterone has a growth promoting effect on T. mariae. No other studies were found in the literature on the effects of hormones in this species.

In mammals, androgens have a nitrogen retention effect and induce muscular development (Dorfman and Shipley, 1956). In fish it is not known whether the observed faster growth is as a result of androgen influenced growth promotion or as a result of enhanced appetite and increased food consumption rate as suggested by Yamazaki (1976).

Many reports in the literature indicate that administration of androgens significantly increases growth rate of fish. For example in tilapia, Yashouv and Eckstein (1965) found that maintaining tilapia fry in water to which small amounts of Methyltestosterone had been added increased growth. MacIntosh et al. (1985) obtained significant growth enhancement in wild

stocks of O. mossambicus fed a diet with 60 mg/Kg of Methyltestosterone treated fish 54% heavier. Majumdar and McAndrew (1987) found that MT treated O. niloticus, O. mossambicus and O. aureus grew approximately 25.7%, 14.7% and 37.8% heavier than the respective control groups. Hanson et al. (1983) compared growth rate of androgen treated O. niloticus with F_1 hybrids of O. niloticus x O.hornorum and found that androgen sex reversed fish grew faster than normal genetic males which in turn grew faster than the hybrids and genetic female O. niloticus. They concluded that the higher growth of male tilapia is related to the phenotypic sex rather than being a sole function of the genotypic sex. This finding implies that the effect of the Methyltestosterone may be stronger than that of genotype.

In the present work, significant weight gain was only obtained in T. mariae. Other species showed a general trend for increased weight gain but did not attain significant proportions. Katz et al. (1976) demonstrated that significant weight increase in O. niloticus treated with adrenosterone was only found in fry over 3 grammes in weight. In other studies such as Ufodike and Madu (1986) significant weight differences were consistently obtained by analysis of FCR, PER and mean growth rate in Methyltestosterone treated O.

niloticus fingerlings less than 900 mg in mean wet weight. They also reported that fingerlings immersed in 5 mg/l adrenosterone for three months were 61% heavier and 21% longer than the controls; hence confirming the anabolic effect of both MT. and adrenosterone.

One good example from outside the tilapia group is the report by Yamazaki (1976) that demonstrated the effect of hormone dosage on growth. It was reported that feeding Goldfish C. auratus on a diet to which 17 α-Methyltestosterone was added resulted in significant increase in growth rate at 1 ppm, no effect at 10 ppm, and a significant growth retardation at 30 ppm. Anderson and Smitherman (1978) found that treating O. niloticus and O. aureus fry with Ethynyltestosterone resulted in lowered growth rate.

The findings of the present thesis and the reports quoted above indicate that growth superiority in androgen treated fish is realized with such frequent regularity that the observed results are unlikely to occur by chance encounter.

Whilst most workers report positive weight gain, there have been a few notable ones on negative growth effects in androgen treated fish. These reports are important because they serve to

underline that not all androgenic compounds have growth promoting effects, and that the ability for androgenic compounds to override the role of sex genotype in growth may be dose dependent.

Donaldson et al (1979) have noted that when administered in high concentration, all androgens, particularly synthetic ones, will exert deleterious effects on various organs. They also suggest that when the hormone concentration reaches a critical level there will be interference with normal processes of the organs to the extent that they will cancel any gain or anabolic response. Examples in this regard have been given by Ashby (1957) in brown trout, McBride and Fagerlund (1973) in Coho Salmon, and Yamazaki (1976) in Goldfish.

7.3.4.6 **Effect of 17 β-ethynylestradiol on growth**

Growth response to treatment of S. galileaus, O. niloticus and the hybrid O. niloticus x O. macrochir is given in Tables 2.7 - 2.8, 2.11 - 2.12, and 2.15 - 2.16 respectively. Results show that 67 days after hatching, S.galileaus treated with ethenylestradiol at 65 mg/Kg were significantly lighter than the other groups. In the control group, female weight was 19.07% higher than that of males (Table 2.10). This difference was not significant ($p > 0.05$). Results also

show a non significant but positive weight gain in fish treated with 35 mg/Kg.

In O. niloticus, no significant difference was found in weight of male and female in all treatments at 78 days after hatching.

In the hybrid group, the control fish were significantly heavier than the hormone treated fish sampled at 76 days post hatch. There was no treatment related intra sexual weight difference.

The finding of relatively faster growth in S. galileaus females when compared with males seems to contradict the norm for tilapia species. Unfortunately no comparable report could be found on growth of hormone treated fry in this species. At the time of final sampling, the majority of fish had ovaries in an advanced development status.

The results of oestrogen treatment in S. galileaus, O. niloticus and the O. niloticus x O. machrchir F_1 hybrid seem to indicate that unlike 17 α-MT., ethynylestradiol has no growth promoting effects in tilapia. At low dose rate, no significant change in weight was observed. At higher dosage, significant retardation in growth was noted. From the observed trend, it seems

probable that 17 α-ethynylestradiol has a negative dose dependent effect. The observed retardation in growth at higher dose rate could be due to toxic effects, reducing food utilization and suppressing appetite. Buckley (1972) has suggested that in species where males normally grow at a slower rate than females (e.g. Salmonids), a feminizing effect on the male may result in net growth gain. The reverse could, under the circumstances be presumed likely in tilapia as observed in the present experiments.

With the exception of my observations on S.galileaus, other growth responses to oestrogen treatments in this thesis are in general agreement with reports by other authors. For example, Yashouv and Eckstein (1965) found that adding ethynylestradiol to the diet of 'tilapia' caused high mortalities and growth depression. Sanico (1975) found that treating O. aureus with estrone caused no significant growth between treated and control fish. Majumdar and McAndrew (1987) found no significant difference in growth between groups of O. aureus and O. niloticus fry treated with 17 β-Oestradiol and their respective controls. In the same series of experiments, they found that subjecting O. mossambicus fry to the same treatment resulted in significantly lower growth rate. Similar observations have been reported in

other groups of fish. For example, when fed DES at 0, 0.1, 1.0 and 10 mg/454 g diet, the catfish Ictalurus punctactus showed an inverse relationship to growth (Bulkley, 1972), and Yamazaki found that feeding Goldfish C. auratus, with 17 β-oestradiol ranging from 1-30 ppm showed no effect on growth, and that supplementation of the steroid in excess of 30 ppm resulted in retarded growth. Although female growth rate in Salmonids is generally higher than in males, Johnstone et al. (1977) obtained slower growth in oestrogen treated fish than in the controls. This example provides clear circumstantial evidence that oestrogenous compounds could have a negative effect on growth of treated fish.

7.3.4.7 Influence of steroid hormones on sex differentiation in tilapia

All developing vertebrate gonads pass through a state when they contain tissue for differentiation into either testes or ovary. In teleosts, the potential male and female tissue is intermingled and this indifferent gonadal tissue can readily be triggered to form either definitive ovary or testes by hormonal treatment (Matty, 1985). On the basis of histological evaluation Eckstein and Spira (1965) considered 18-22 mm to be a critical length range in the growth of O. aureus during which gonadal differentiation is completed. As

demonstrated in Chapter III of the present thesis, size attained by fish at a particular time depends on environmental factors such as availability of food, and environmental temperature. In the same Chapter it has been demonstrated that gonadal development and sex differentiation are more closely linked to age than to fish size. The present Chapter discusses the influence of 17 α-Methyltestosterone on sex ratio of treated fish.

7,3,4,8 **17α-Methyltestosterone on sex ratio**

Results of feeding T. mariae, S. galileaus, O. mossambicus and O. niloticus with MT treated diet are presented in tables 1.18-1.19, 1.20-1.21, 1.22-1.23, and 1.24-1.25 respectively.

In T. mariae, treatment of fish with 30 mg/Kg diet did not significantly alter the sex ratio from that obtained in the control. At both 45 mg/Kg diet and 60 mg/Kg diet, sex ratio was significantly altered from the control. The lower dose yielded 64.89 ± 5.17% male, while the higher dose yielded 96.67 ± 1.19% male. In two of the treatments at 60 mg/Kg diet, no females were found (Table 1.19).

In S. galileaus, all three levels of treatment resulted in significantly different sex ratio from

the control. Fewer than 22% females were found at 30 mg/Kg diet, no females were found at 45 mg/Kg and only four females were found in one of the batches treated at 60 mg/Kg diet. The proportion of abnormally developed gonads in this species was significantly higher than in the controls (Table 1.21).

In O. mossambicus, no females were found in any of the fish treated with 50 mg/Kg diet but 43.87 ± 0.39% of the control groups were females. A similarly high masculinizing rate was obtained in O. niloticus at 45 mg/Kg and 60 mg/Kg diet. In these species, the rate of abnormal development was about 1.5% and 3.0% respectively.

Results obtained in these experiments demonstrate that 17 α-Methyltestosterone is an effective androgen for sex reversing these four economically important species of tilapia. Differences in optimal dose rates were observed in T. mariae, S. galileaus and O. niloticus. At 35 mg/Kg and 45 mg/Kg, both S. galileaus and O. niloticus were more readily masculinized than T. mariae.

Studies on the effects of androgenic hormones in fish have been going on since the mid fifties when Yamamoto (1953) first obtained sex reversed Oryzias latipes and C. auratus. Progress in

utilizing hormones in sex reversal was slow until Egami and Arai (1964) observed that androgenic steroids in fish were markedly different from those of mammals, and Arai (1967) identified testosterone as the testicular androgen in teleosts. In the tribe tilapiine, Eckstein (1970) was the first to report in vitro biosynthesization of 11-ketotestosterone in ovaries of O. aureus, thus confirming that this steroid is the most active androgenic steroid in fish.

Comparative studies undertaken to compare androgenicity of hormones in fish have demonstrated existence of significant differences between them. For example Idler et al. (1961a) showed experimentally that androgenic activity of 11-Ketetestosterone is only 58% of that of testosterone propionate; Egami and Arai (1964) reported that Methyltestosterone and Methyltandrosteredione were about 1000 times more androgenic than testosterone. Yamamoto (1969) showed that in the Medaka, Ethynyltestosterone was about four times more potent than Methyltestosterone. He further suggested that synthetic androgens such as 17 α-Ethynyltestosterone and 17 α-Methyltestosterone are orally more active, while naturally occurring ones such as testosterone, androsterone, and

androstenedione are more potent when injected. Schreck (1974) summarized by stating that steroids with side chains at C17 ($COCH_3$ or $COCH_2OH$) such as the gestagens and corticoids have no effect on directing sex.

On the basis of the various findings, synthetic hormones have been used frequently and with high success rates. The androgen most commonly used for sex reversal is 17 α-Methyltestosterone.

In the present studies 17α-Methyltestosterone (MT) was used with a high rate of success in all four species. As noted earlier, optimal dose rates differed according to species with S. galileaus and O. niloticus responding favourably at 30 and 45 mg/Kg and O. mossambicus giving similar indications. On the other hand the best induction rate in T. mariae was around 60 mg/Kg diet.

Previous reports indicating complete androgen sex reversal in O. niloticus were made by a number of workers including Jalabert et al. (1974) Tayamen and Shelton (1978) Ufodike and Madu (1986); in O. mossambicus (by Clemens and Inslee, 1968; Nakamura, 1975; Guerrero, 1976b; Majumdar and McAndrew, 1987) and in O. aureus (Nakamura 1975;

Sanico, 1975; Guerrero III, 1975; Shelton et al, 1980).

As demonstrated in this thesis, not all attempts have been an unqualified success. For example in T. zillii, Guerrero (1976a) was unable to effect sex reversal, Shelton et al, 1978 obtained 90%, 97% and 100% male at MT15. MT25 and MT50 respectively, while Woiwode (1977) readily obtained 100% males with 50 mg/Kg of the same hormone.

Other species that have been successfully sex reversed with Methyltestosterone or ethynyltestosterone are O. hornorum (Smitherman et al, 1984) and O. macrochir (Jalabert et al. 1974). In the present thesis two additional species (T. mariae and S. galileaus) have been successfully sex reversed.

7,3,4,9 **Effect of 17α -ethynylestradiol on sex ratio**

Oestrogenic sex reversal of fish was first reported by Yamamoto (1953) in O. latipes. Since then a number of attempts in different species including tilapia have been made (Yamamoto, 1969; Shelton, et al, 1978; Mair et al, 1986).

In the present experiments, two pure species and one interspecific cross have been treated with

ethynylestradiol to test the efficacy of the hormone and to obtain fish for progeny testing. Using sex reversed females in progeny testing is convenient because:-

i several females can be stocked with the same male hence optimizing use of tank space and hastening testing

ii confirmed sex reversed homogametic males can be used directly as brood fish for production of all-male stocks.

Further the use of presumed monosex hybrids simplified the generally protracted process of confirming induction success.

Results of feeding 17α -ethynylestradiol to S. galileaus, O. niloticus and F_1 hybrids O. niloticus x O. macrochir are given in Tables 2.19 - 2.20, 2.21 - 2.22, and 2.23 - 2.24 respectively. In S. galileaus, all three levels of treatment were successful in significantly altering sex ratio from that obtained in the controls. At 35 mg/Kg diet, the yield of females was about 73%. At both 50 mg/Kg and at 65 mg/Kg no obvious males were found, and positively identified females were 98.77 ± 1.01% and 97.89 ± 1.22% respectively. At 50 mg/Kg two of the three treatments were all-

female and at 65 mg/Kg one of the three treatments yielded only females (Table 2.20).

In O. niloticus, 4.35 ± 1.85% males were obtained at 35 mg/Kg, and none at either 50 mg/Kg or 65 mg/Kg. These results were significantly different from the controls (Table 2.22).

In the hybrid groups 3.74 ± 1.87% females were found in the control group, but approximately 76% females were obtained in the group treated with 17 ∝-ethynylestradiol at 50 mg/Kg.

These results indicate high efficacy for this oestrogen in the species used. Although complete reversal was not realised in the hybrid group, the obtainment of 70% to 83.33% in different batches was highly significant in this cross.

Induction of sex reversal has not always been successful in different species or sometimes in different strains of the same species. For example androgen treatment did not appear to influence sex ratios in Lepomis macrochirus (Chew and Stanley, 1973), in gynogenetic Ctenopharyngodon idella (Stanley and Thomas, 1978) in T. zillii (Guerrero, 1976a) and in O. niloticus (Das et al, 1986). Even more common are reports of partial sex reversal in which only a percentage

of the brood changes sex. For example in this thesis, treatment of T. mariae with MT at 30 mg/Kg and among the hybrids treatment with ethenylestradiol at 50 mg/Kg diet.

The failure to obtain complete sex reversal is often attributed to the following:-

i choice of hormone in relation to fish species.

Some species are more responsive to hormone treatment even at low levels of inclusion. For example Yamamoto (1959) obtained 100% females in O. latipes with 50 mg/Kg of estrone, Nakamura (1981) obtained 100% female Masu Salmon by immersion of alevins in static water containing oestrogen, and Sinico et al (1986) obtained 100% female Ictalurus punctatus from 17 β-estradiol. In tilapia, Bulkley (1972) failed to induce significant change in sex ratio of O. hornorum, Sanico (1975) was also unsuccessful in O. aureus using estrone at 100 mg/Kg and Majumdar and McAndrew (1987) showed variation in responsiveness of three tilapia species to 17 β-oestradiol.

In a paradoxical response, Goudie et al, (1983) obtained only females at all levels of androgen treatment in Channel Catfish

ii Inappropriate administration techniques (Vallowe, 1957; Shelton et al, 1978).

Four hormone administration techniques are commonly used in fish. These are:-

(a) Interperitoneal injection
(b) Capsular implantation
(c) Immersion and bath treatments
(d) Oral administration through food

In tilapia, oral administration of hormones through the diet is generally used because it ensures economical utilization of the drug. The major drawback of this method is that variations in food acceptability may sometimes lead to reduced induction success.

iii Inappropriate timing of the treatment in relation to the age of fish and duration of treatment (see Chapter III).

iv Lability of a particular sex phenotype to manipulation. For example Yamazaki (1976)

was able to obtain 100% male populations in Zebra fish with every dose level of Methyltestosterone from 1 ppm to 100 ppm with no abnormal developments.

v Availability of alternative nutrients in the environment has often been given as a probable cause for failure to obtain complete sex reversal either way. However, McIntosh and Little (personal communication) have consistently obtained complete masculinization of O. niloticus in green water systems by feeding fish with MT 40.

This last observation seems to indicate that the amount of hormone comsumed is more critical than the presence of supplementary feed in the environment.

As indicated earlier, increasing dosage above optimal level does sometimes produce negative symptons. For example, I suggested ealier in this thesis that the increased mortality observed in T. mariae at high MT dose could be a result of toxicity. In S. galileaus high dosage of ethynylestradiol resulted in abnormal development of ovaries. While in MT it suppresed spermatogonial development in Salmonids.

Johnstone et al, 1978; Goetz et al, 1979), and in O. aureus (Guerrero, 1975). Incorporation of high dosages of MT at 1000 mg/Kg resulted in a paradoxical feminizing effect on the gonad of genetic males in O. mossambicus (Nakamura, 1975) and produced androgenic castration in both sexes of O. latipes (Yamamoto, 1958).

Other studies have shown that increased dosages or prolonged treatment with oestrogens sometimes results in development of atypical papillae with little or no change in sex ratio of the treated groups. For example, alterations in urogenital papillae have been reported in O. macrocephala (Aronson, 1951) O. mossambicus (Clemens and Inslee, 1968) O. niloticus and O. macrochir (Jalabert et al, 1974) and O. aureus (Jensen 1976). Jensen and Shelton (1979) warned that this anomally can be a major cause of misleading interpretation of the results if external sexing is the sole criteria for evaluating sex ratios from hormone treatments.

In order to avoid the necessity of applying high dose rates, it has been demonstrated that steroid hormone treatments can be supplemented with antigonadotropin substances such as Methallibure which will help to remove or suppress endogenous sex hormones during the period of gonadal

differentiation, or supplementing with a pituitary blocker such as Cyproterone acetate which inhibits development of secondary and accessory sex characters in mammals. (Bulkley, 1972; Shelton, et al, 1978).

For example. Hopkins et al. (1979) failed to influence sex reversal in O. aureus with EE 100, but got 90% female in a combination treatment with Methallibure, and Mair et al. (1986) got 85% female using the same combination of chemicals. The choice of steroids and the need for an antigonadotropin varies with species. Yamamoto (1969) noted that the potency of naturally occurring oestrogens arranged in increasing order are estrone, estriol and 17 β-estradiol. Comparative studies have shown that synthetic oestrogen such as 17 α-estradiol, diethystilbrestrol (DES) and ethynylestradiol (EE) are more potent than natural ones (Shelton et al. 1978; Tayamen and Shelton, 1978; Hopkins et al. 1979).

In the present experiment, the various problems associated with failure or low success rate in hormone treatment were as far as possible taken into account at the beginning.

Results show that a high success rate was realized in all five crosses. By using a range of dosages, for each of the two hormones used, it was possible to estimate optimal dose rate in three of the four pure species namely T. mariae, S. galileaus and O. niloticus, O. mossambicus and the hybrid cross were treated at a single dose level.

This study has demonstrated that inspite of careful planning at the beginning, some fish did not sex reverse. This tendency not to respond to treatment occurred in individual fish and in batches of fish as demonstrated by significant sex ratio variation. Some fish appeared to have developed normal external genitalia, but proved to have abnormally developed gonads. This was particularly true in oestrogen treated S. galileaus where some gonads developed into ovasacs devoid of germ cells or into compatimentalized ovaries with constricted oviducts.

This last observation gives an indication that in these fish, response to exogenous sex steroids is on individual basis. Results further demonstrate that different species respond to the stimulus provided by the hormone at different rates. From these two observations it can be concluded that the observed species response is the mean of individual responsorials such that in species

where indidivual mean is at a lower threshold, the net result will be induction of sex reversal at a correspondingly low dose, while in individuals whose mean threshold is high, the opposite will prevail.

The former seems to be the case in S. galileaus and O. niloticus and the later in T. mariae. The probable reason for incomplete realisation of sex reversal in the hybrid involving O. niloticus could thus be an elevation of the threshold by a recombination of genetic factors.

The formular by which polygonic sex determination is described is relative. As stated by Kosswig (1964), it can be imagined that in organisms with polymorphic sex-realization, there are two systems of polygenes: the one working towards maleness, and the other towards femaleness. The polygenes could interact in different ways by being either completely interdependent, partially interdependent, or completely independent by one allel of each pair working as male-determining and the other as a female determining gene. So that the expressed sex is the sum of genetic effects at many loci.

CHAPTER 8

Experiment VI

Application of hormones in tilapia II: Progeny testing

INTRODUCTION

In tilapia, males generally grow faster than females, and tilapia culture is likely to become more productive if all male fish are bred.

Our interest in establishing sex determining mechanisms is therefore based on the desire to acquire the ability to alter sex ratio in favour of the male phenotype as a means to improve productivity.

According to the Chromosome theory, sex of a fish is genetically determined at fertilization. However, the exact mechanism by which this happens is not well known. through observations on genetic manipulations such as hybridization, parthenogenesis, and progeny testing hormone sex inversed fish, the sex determining mechanism can be inferred.

Generally, progeny testing is used in

1. testing if sex inversed fish produce viable gametes and whether offspring from sex inversed females are viable.

2. identifying functionally sex inversed females for use in subsequent breeding programmes for the production of all-male populations (Jensen and Shelton, 1979). This method relies on sex reversing of the homogametic male and involves the crossing of sex-inversed females with normal male fish.

3. obtaining sex ratios upon which an assessment of the sex determining mechanisms can be reasonably predicted on the basis of Mendelian theory.

Theoretically, sex inversed female homogametic fish will produce all male offspring if eggs are fertilized with sperm from a normal homogametic male. The main advantages of obtaining all-male broods by this method are

(1) that the ethical objections of feeding hormones directly to fish intended for human consumption are avoided, and

(2) assuming that male growth superiority has a genetic basis, production of genetically and phenotypically all-male stocks would result in increased productivity.

Preliminary experiments indicate that this can be done with androgens (Clemens and Inslee, 1968; Jalabert et al, 1974) and with estrogens (Guerrero III, 1975; Hopkins et al, 1979).

Jalabert et al. (1974) treated fry of O. niloticus with Methyltestosterone and achieved complete and functional sex reversal. When they outcrossed some of these males with normal females the resulting offspring were all females. This experiment demonstrated that all the offspring must have had female XX genotype, thus demonstrating female homogamety.

Hunter et al. (1982) proved the usefulness of sex steroid treatment in coho Salmon sex determination by treating eyed eggs and alevins with various concentrations of 17 β-estradiol or 17α-Methyltestosterone

All female progenies were obtained in several trials. When these XY, females were fertilized with normal milt, the offspring produced were in ratios 2, 2.4 and 3.0 males to 1 female. These results were similar to those obtained in Rainbow trout by Johnstone et al. (1979). These results are sufficient evidence that both coho Salmon and rainbow trout are male heterogametic. Both teams of researchers are unanimous in concluding that a 3:1 male:female ratio indicates complete YY viability and thus the presence of the two lower sex ratios indicate variability in YY viability.

Similar work on Carassius auratus (Yamamoto and Kajishima, 1968), Oreochromis mossambicus (Clemens and Inslee, 1968) and Salmo gairdneri (Okado et al. 1979) demonstrated female homogamety in these species.

Guerrero III (1975) treated Oreochromis aureus with ethynyltestosterone and methyltestosterone and obtained up to 100% males. Some of these males were later outcrossed with normal females. At 2.1- 3.1 females to 1 male, the sex ratios obtained were significantly different from the expected 1:1 ratio. However, these results were in complete harmony with the suggestion by Yamamoto and Kajishima (1968) that an androgen induced WZ male mated with a normal WZ female would produce progeny having a sex ratio of 2-3 females to 1 male. In this respect, Guerrero's results provided conclusive evidence for female heterogamety in Oreochromis aureus.

The objective of the present work is to test the usefulness of progeny testing tilapia sex reversed with oestrogens.

i in pure S. galileaus

ii in a presumed homogametic hybrid O. niloticus x O. macrochir

MATERIALS AND METHODS

The broodfish used in this experiment were obtained from oestrogen treated broods (Chapter 7).

A random sample of 20-25 fish from high female yielding estrogen treatment groups of *S. galileaus* and F_1 hybrids of *O. niloticus* x *O. macrochir* were on grown to sexual maturity and subsequently used in progeny testing. Sections 1.1 and 1.2 present the results.

Statistical analysis using chi-square tests were carried out on the different sexes. Analysis of variance was done to compare ratios.

1.1 *S. galileaus* x *S. galileaus* (normal male)

Female fish used in this experiment were obtained by feeding normally obtained fry with a diet mixed with 50 mg/kg of 17α-ethynylestradiol

Twelve female fish obtained from the treatment were each paired with a normal male in sequence. the experiments were conducted in two glass tanks in a recirculating system over a six week period. Fish were left to spawn naturally and fry were removed after 7 days.

1.2 'Backcrosses' of F_1 O. niloticus x O. macrochir treated with 50 µg/g ethynylestradiol

Females used in this experiment were obtained randomly from among the hormone treated group (Section 7.1). Fourteen females were stocked in three separate tanks with normal untreated O. niloticus males at the rate of 4 females to one male.

Over a six week period, two females spawned naturally in the tanks. Eight others were prevented from spawning naturally, instead they were captured, stripped and each batch of eggs sub divided into two for fertilization with O. niloticus in one case and with O. macrochir in the other.

As in previous work of this nature, the following assumptions were made to assist in the analysis of the observed results:

1. Sex differentiation in tilapia is based on sex chromosomes. (Chen, 1969)

2. Sex reversal affects the phenotype and not the genotype of the fish (Yamamoto, 1969)

3. A sex ratio of 1:1 is the normal.

RESULTS

At the end of six weeks, five different spawns had been collected, three females had died and four were still in the tanks and were subsequently killed for examination of gonads. Survival of fry and progeny sex ratios of the five successfully reared groups are shown in Table 1.1

Table 1.1 Survival and progeny sex ratio of hormone treated S. galileaus

Pair No	Number of fry		Survival		Sex				Sex Ratio
	Initial	Final	%	SE	Male	%	Female	%	ma/Fe
4	136	94	69,11*		74	78,72**	26	21,28	2,80 b
5	120	82	68,33*		59	71,95**	23	28,05	2,60 b
7	90	73	81,11*		38	52,05	34	46,58	1,12 a
9	83	60	72,29*		33	55,00	27	46,00	1,22 a
10	108	85	78,70*		62	72,94**	23	27,06	2,70 b
Total 5	537	394	73,91	±2,56	266	66,12	±5,30	127	33,59±5,12 2,26

Superscripts down column 4 denote significant difference between survival in different batches of fish ($p < 0,05$),

Superscripts in column 6 denote significant difference with a 1:1 sex ratio ($p < 0,01$),

Superscripts in column 9 denote significant difference between different batches of fish ($p < 0,05$),

The overall sex ratio was 2.26 ± 0.487 males to female. The highest proportion of male obtained was 78.72% and the lowest was 52.02%.

The sex ratios were significantly different $p < 0.05$ in two sets as shown in column 9. Three of the sex ratios were significantly different from the normal 1:1, but were not significantly different from the 3:1 Mendelian ratio. The mean ratio for the three spawns that deviated from normal was 2.7:1.

1.2 'Backcrosses' of F_1 *O. niloticus* x *O. macrochir* treated with 50mg/kg ethynylestradiol

Over a six week period, two females spawned naturally in the tanks. Eight others were prevented from spawning naturally, instead they were captured, stripped and each batch of eggs sub divided into two for fertilization with *O. niloticus* in one case and with *O. macrochir* in the other.

Only four fish remained unspawned at the end of the six week experimental period.

The progeny sex ratios from the two sets are presented in Table 1.2 and 1.3.

F_1 (*O. niloticus* x *O. macrochir*) x *O. niloticus*

The average survival of fish over a nine week period was 72.21 ± 4.01%.

Of the 359 fish sexed, 267 (74.37 ± 1.91%) were male and 82 (25.62 ± 1.76%) were female. The overall sex ratio was 3.14 ± 0.195 males per female. The sex ratio range was 2.33 to 3.77.

Analysis of variance on sex ratio was significantly different ($p < 0.05$). All seven spawns resulted in male proportions that differed significantly from the expected 1:1.

The average survival was 87.07 ± 2.62%. Of the 371 fish sexed, 361 (97.35 ± 0.99%) were male. No females were found. Ten fish (2.70%) could not be positively sexed due to inconspicuous genitalia or degenerative gonads.

There was no significant variation between proportion of males in all the five groups. All the proportions of male differed significantly from the normal.

Table 1.2 Survival and progeny sex ratio of hormone treated O. niloticus x O. macrochir F_1 hybrid with O. niloticus

Pair No	Number of fry Initial	Final	Survival %	Sex Male	%	Female	%	Sex Ratio ma/Fe
1	86	51	59,30a	35	68,63*	16	31,27	3,10b
2	59	45	76,27cd	34	76,07**	11	23,93	2,10a
4	90	62	68,89ab	49	79,03**	13	20,97	3,77bc
5	60	53	88,33d	38	71,70**	15	28,30	2,53a
6	65	40	61,54ab	28	70,00**	12	30,00	2,33a
7	110	76	69,09bc	60	78,94**	16	21,05	3,75bc
3	39	32	82,05d	23	71,88**	9	28,13	3,4b
7	509	359	72,21±4,01	267	74,37±1,91	92	25,63±1,76	3,14±0,11

Superscripts in coloum four denote significant difference between survival in different batches of fish ($p < 0,05$). Superscripts in column six denote significant difference with a 1:1 sex ratio * $p < 0,05$; ** = $p < 0,01$.

Superscripts in column nine denote significant difference between sex ratio in different batches of fish ($p < 0,05$).

Table 1.3 Survival and progeny sex ratio of EE_{SO} treated with F_1 hybrids of *O. niloticus* x *O. macrochir* with normal *O. macrochir*

Pair No	Number of fry Initial	Final	Survival %	Male	%	Female	Unidentified
2	126	115	91,27b	110	95,65***	0	5
3	88	73	82,95ab	73	100 ***	0	0
4	53	42	79,25a	41	97,62***	0	1
6	60	56	93,33b	53	94,64***	0	3
7	96	85	88,54ab	84	98,82***	0	1
	423	371	87,07±2,62	361	97,35±0,99	0	10

Superscripts in column four denote significant difference between survival in different batches of fish ($p < 0,05$).

Superscripts in column six denote significant difference with a 1:1 sex ratio

*** $p < 0,001$.

There was no significant difference between proportion of males in the different batches of fish.

DISCUSSION

Results of the S. galileaus treatment show that overall, larval survival was 73.91 ± 2.56%. Of the five phenotypic females that spawned with normal male, 2 spawns resulted in progeny numbers that were not significantly different from the expected 1:1 ratio. The other three spawns resulted in progeny numbers that differed significantly from the normal. The average male to female sex ratio for the three batches was 2.7:1.

The significant difference from the expected ratio is indicative that functional sex reversal has taken place in some of the Ethynylestradiol treated fish. The progeny obtained from the three spawns that deviated from normal were tested for similarity with a 3:1 Mendelian ratio and found to be not significantly different ($p > 0.05$).

These results are indicative of a female homogametic (XX) sex determining mechanism for S. galileaus.

In similar experiments, Chevassus et al. (1983) treated trout with estrogen and obtained 73 to 83% females. Progeny sex ratios from sex reversed females yielded 76.6% males suggesting an XX-XY sex determination with total viability of YY genotype. Chevassus et al. comfirmed YY viability by mating nine of the males from the progenies with normal females. Four crosses

resulted in all-male offspring. Simco et al (1986) used estrogen sex reversed females and obtained 3:1 male to female ratios in seven of the 13 treated fish that spawned with normal males. their ratios were compatible with a normal homogametic female genotype (XX) and indicated total viability of males with YY genotype.

Although no significant difference was found between the sex ratios obtained in the present experiments and the expected results on the basis of Mendelian ratio, the proportion of males in the present work was generally lower than 3:1 suggesting partial viability of males with the YY genotype.

Survival of progeny from hormone treated F_1 hybrids spawned with normal O. niloticus was 72.21 ± 4.01%. Progeny sex ratios from all seven successfully spawned hormone treated females were significantly different from the normal 1:1 ratio. In spite of significant variation in progeny sex ratios for different pairs of fish (2.33 - 3.77), the overall mean of 3.14:1 males to females did not differ significantly ($p > 0.05$) from the expected Mendelian ratio of 3:1.

This result is indicative of complete heterogamety of the F_1 hybrid, and by inference, the result also indicates heterogamety of the normal male O. niloticus used in the cross.

Fertilization of sub batch eggs from these fish with milt from normal O. macrochir resulted in higher survival (87.07 ± 2.62%). Progeny obtained from the five successfully reared batches yielded 94.64 - 100% males. No females were found but 2.70% of the fish had severely retarded gonads and could not be positively placed either as males or as females (Table 1.3). Since from the previous paragraph it was indicated that the hormone treated hybrids were heterogametic for sex chromosomes, the obtainment of all-males in the present cross with O. machrochir can only mean that the male used is homogametic.

The genotypic forms derived from both backcrosses with O. niloticus and with O. macrochir are in agreement with those obtained by previous workers for these species (see also Jalabert et al, 1974).

No other studies were found in the literature combining use of interspecific hybridization with hormone treatment for sex determination. The wide variation of sex ratios (Table 1.2) makes it difficult to state with certainty whether the YY genotype is totally viable as implied by their mean value.

In direct hormone treatment, one half of the derived monosex group will have female genotype while the other half will have male genotype. In the present state of knowledge, the only way in which individual fish can be

identified as belonging to one class or the other is by determination of sex ratio of their progeny (Thorgaard, 1977; Johnstone et al, 1979).

Liu (1977), quoted by Jensen and Shelton (1979) obtained all-male offspring from a sex reversed female O. aureus and concluded that sex reversal of a genotypic male had taken place. Mair et al. (1986) sex reversed O. aureus using a combination of Methallibure and 17 α-ethynylestradiol. Progeny obtained from these fish yielded 90%male. Jensen et al. (1983) noted that use of a monosex group for hormone treatment would eliminate questionable interpretations of sex ratios as is often the case in bisexual groups. This approach was effectively used in the present work. Hormone treatment of a potentially all-male F_1 hybrid with 17 α-ethynylestradiol at 50 mg.kg yielded 76.33% female. Because the control group yielded all-male, the presence of females in the hormone treated group served as evidence that genotypic males were sex reversed.

In backcrossing with normal parental type males, about 70% of the females tested produced viable offspring. Results from the backcross with O. macrochir indicate that all-male populations may be consistently generated by crossing functionally sex reversed female with normal homogametic males.

The technique used here is limited in its application to homogametic male. Its main advantages are:

1. hormones are not directly administered to fish destined for human consumption

2. provided that the hybrid cross yields monosex broods, all females found after hormone treatment can reasonably be presumed sex reversed.

3. broodstock may benefit from increased heterozygosity and hybrid vigour resulting from a combination of two male genotypes.

CHAPTER 9

Summary and Conclusion

SUMMARY

1. Generally, tilapia species show significant sex linked differences in growth rate, and ultimate size between male and female. These differences have great significance in economically important species used in aquaculture. One aspect of tilapia culture that has attracted most attention in recent years is the possibility of obtaining all-male broods. This possibility has only infrequently been realized. One probable reason for this is that the process of sex differentiation in tilapia is not well understood.

2. In the present investigations, six experiments were conducted to investigate sex determining mechanisms in O. niloticus, O. mossambicus, O. spilurus, O. macrochir, S. galileaus and T. mariae.

3. Histological studies were undertaken to determine onset of gonadal development and of sex differentiation in O. niloticus, O. aureus, O. mossambicus and S. galileaus. Gonadal development and sex differentiation was also investigated histologically in O. niloticus and O. aureus reared at 21.0°C, 27.5°C and 35.0°C.

Initially all larvae were in the undifferentiated state. Primordial gonads appeared in the posterior body cavity immediately ventral to the trunk kidney and gas bladder.

In S. galileaus gonadal morphogenesis started about 12 - 14 days after hatching. Females and males became identifiable about 22 days and 26 days post hatch respectively. In O. mossambicus, gonadal morphogenesis started around 8 - 10 days after hatching. Females and males were discernable from about 22 days and 25 days after hatching respectively. In O. aureus, gonadal morphogenesis was observed about 15 days after hatching; females and males became identifiable as such 25 days and 33 days after hatching respectively. In O. niloticus gonadal development started about 17 days after hatching; females and males became discernable 35 days and 46 days post hatch respectively.

Temperature did not significantly alter onset of gonadal morphogenesis. At 21.0°C the rate of sexual differentiation was markedly retarded.

Results demonstrate that onset of gonadal morphogenesis is age dependent and varies in different species.

Results provide a useful time guide for hormone sex induction experiments in the four tilapia species.

Further studies are required to determine the corresponding point of physiological sex differentiation.

4. Several reports have demonstrated that sex determination in some organisms including several fish is labile and can be influenced by exogenic factors such as environmental temperature. the basis for ESD is that both male and female parents contribute autosomal genes equally to the next generation and that the sex of the offspring will be in direct response to physiological processes resulting from environmental pressures.

O. niloticus and O.aureus, two of the most popular tilapias in aquaculture were used to test the hypothesis. The experiment was undertaken in a constant temperature room at 21.0°C, 27.5°C and 35.0°C using first feeding fry.

In O. aureus, overall survival in 21.0°C was significantly lower than in either 27.5°C or 35.0°C ($p < 0.05$). In O. niloticus survival in 35.0°C was significantly lower than in either 21.05C or 27.5°C ($p < 0.05$).

In both O. aureus and O. niloticus, growth in 27.5°C and 35.0°C did not differ significantly with each other, but differed significantly with growth of fish reared at 21.0°C ($p < 0.001$).

Sex ratio within sib groups was significantly different in one of the three batches of O. aureus, but no significant sex ratio difference was found between the three different temperature ranges ($p > 0.05$).

In O. niloticus, significant sex ratio difference was found between batches receiving the same treatment, but there was no significant sex ratio difference obtaining between the three temperature ranges ($p > 0.05$).

Results demonstrate that within the range of temperature used in the present experiments, sex differentiation of the two species was not significantly influenced.

On the basis of these observations, it is concluded that environmental sex determinism is not important in tilapia.

5. The objective of the single pair spawning experiments was to determine the extent of progeny

sex variation within sib groups and between different pairs of parental fish.

The species used in the experiments were O. mossambicus, O. niloticus and O. spilurus. Progeny sex ratios from single pair crosses of O. niloticus x O. mossambicus and O. mossambicus x O. spilurus were also evaluated.

Survival of progeny in the five crosses did not differ significantly with each other ($p > 0.05$). However, significant difference was found in survival of progeny from different pairs of O. mossambicus and O. mossambicus x O. spilurus.

There was significant difference between progeny sex ratios in sib groups and between different pairs of O. niloticus. The proportion of males in O. niloticus ranged from 40.56% to 59.22% and the mean was 50.04%.

There was significant difference between progeny sex ratios in sib groups but not between pairs of parents within O. mossambicus, O. spilurus, O. massambicus x O. spilurus, and O. niloticus x O. massambicus.

The absence of significant difference in survival between pure species and the hybrids suggests that

inbreeding may not have reached significant proportions in the three pure species used in the experiments.

The results of the single pair spawning experiments demonstrated that sex ratios of progeny from different pairs of parents, and sometimes within sib groups, do commonly differ significantly with each other ($p < 0.05$).

Results of single pair breeding experiments seem to suggest existence of a multiple factor sex determining mechanism in O. spilurus O. niloticus and O. mossambicus.

6. The possibility of obtaining monosex broods for aquaculture without the necessity for feeding steroid hormones, hand sexing, or applying high technology manipulations is the major reason for the growing interest in interspecific hybridization.

Presently, wide fluctuations in progeny sex ratios obtained makes universal application of the technique unfeasible.

The objective of the present hybridization experiments is to investigate sex determining

mechansims in O. niloticus, O. mossambicus, and O. spilurus by analysis of hybrid sex ratios.

Crosses attempted in the experiments for obtainment of F_1 and F_2 progeny were O. niloticus x O. mossambicus; O. mossambicus x O. spilurus; backcrosses obtained in the experiments were (O. niloticus x O. mossambicus) X O. niloticus; and (O. niloticus x O. mossambicus) X O. mossambicus.

Survival of the F_1 hybrids of O. niloticus x O. mossambicus was significantly higher than that of mixed progeny of pure O. mossambicus , and of the backcross involving O.mossambicus males. all other survival rates did not differ significantly with each other ($p > 0.05$).

Within each hybrid cross, sex ratios differed significantly in O.niloticus ♂ x O. mossambicus, F_1; in O. niloticus ♂ x O. mossambicus F_2; in the backcross with O. mossambicus males; and in the F_2 hybrids of O. mossambicus x O. spilurus.

The sex ratios obtained in the 18 different treatments were 4:1, 3:1, 7:4, 3:2, 7:5, 7:6, 1:1, 2:3, 3:5, 5:9 and 1:4 females to males.

Progeny sex ratio in crosses O. niloticus O. mossambicus were significantly biased in favour of females.

Progeny sex ratios in O.mossambicus x O. spilurus were biased in favour of males.

Not all the sex ratios obtained in these crosses can be explained on the basis of the sex chromosome theory proposed by Chen (1969).

Only six of the eleven sex ratios obtained fitted the hypothesis of three gonosomes complemented by one pair of autosomes as proposed by Avtalion and Hammerman (1978).

In the absence of firm evidence for existance of multiple sex chromosomes in tilapia, the present results support the proposal made for intraspecific breeding that the probable sex determining mechanism in tilapia is the polygenic sex determining mechanism.

At hatching tilapia, like many other fishes, are not clearly differentiated into either male or female. Potentially therefore, the sex of most tilapias can be artificially manipulated by influencing the sequence of events controlling the differentiation process.

The purpose of feeding 17 α-Methyltestosterone and 17 α-ethynylestradiol to fish in the present experiment was to investigate the influence of

steroid hormones on sex differentiation. the species used in the experiments were T. mariae, S. galileaus, O. niloticus and O. mossambicus for the androgen treatment, and S. galileaus, O. niloticus and F_1 hybrid O. niloticus x O. macrochir for the oestrogen treatment.

Survival of T. mariae and O. niloticus treated with 17 α-MT at 60 mg/Kg diet was significantly lowered ($p > 0.05$). Survival of S. galileaus and O. mossambicus treated with different dosages of the androgen in the range 30 mg/Kg to 60 mg/Kg was not significantly different from the control.

Survival of S. galileaus and O. niloticus treated with 17α-ethynylestradiol at 65 mg/Kg diet was significantly lower than the respective controls. Survival of S. galileaus, O. niloticus and O. niloticus x O. macrochir hybrids treated with MT50 did not differ significantly ($p > 0.05$) from the controls.

Mean weight of T. mariae treated with 17α-Methyltestosterone was not significantly different ($p > 0.05$). from the control 71 days after hatching, but treated fish were significantly heavier after 120 days.

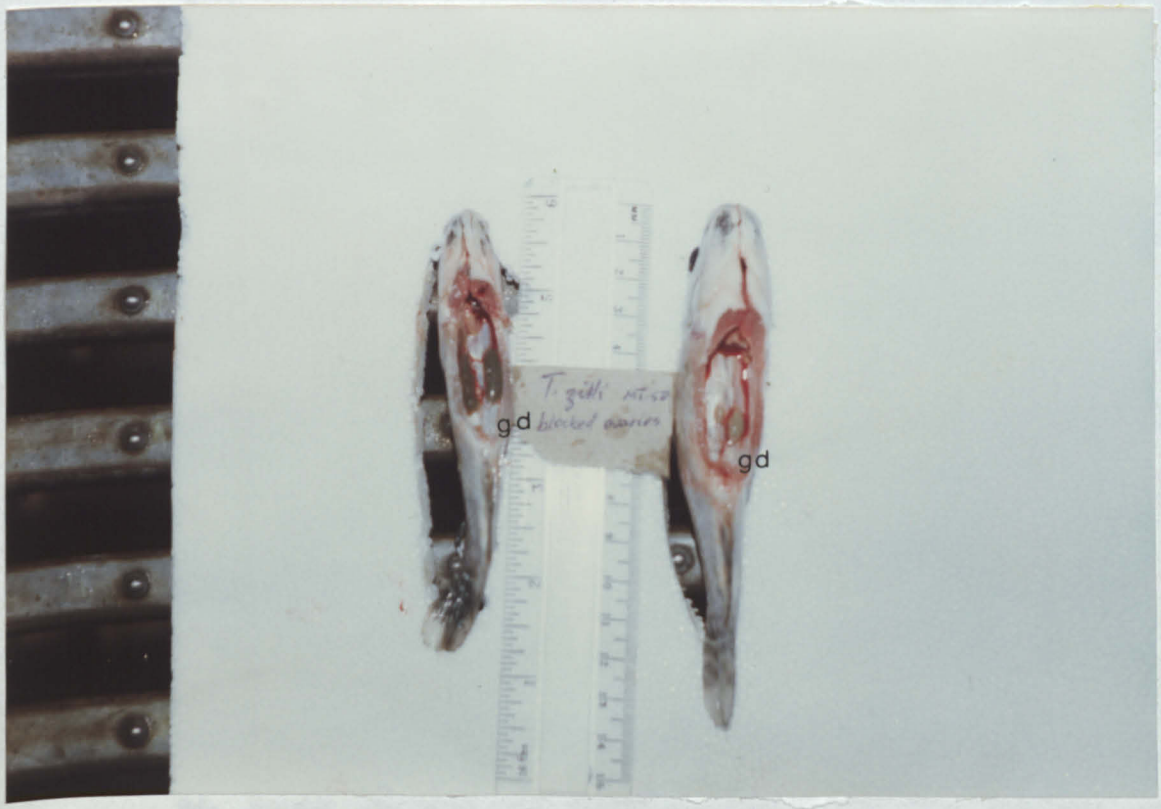


Plate 2.5 Maturing T. zilli female showing poor development of gonal ducts (gd) in the posterior area following treatment with MT50

Mean weight of S. galileaus treated with MT30 and MT45 was significantly higher than that of the controls 89 days after hatching.

Mean weight of O. niloticus treated with different dosages of methyltestosterone was not significantly different from the controls 98 days after hatching.

Mean weight of S. galileaus treated with EE 65 was significantly lower than the control and groups treated with EE35 and EE50, 67 days after hatching ($p < 0.05$). Mean weight of O. niloticus treated at three dose levels of 17α-ethynylestradiol did not differ significantly with each other and with the control 78 days after hatching. Mean weight of O. niloticus O. macrochir treated with EE50 was significantly lower than the control ($p < 0.05$) 76 days post hatch.

Sex ratios of T. mariae treated with MT 45 and MT 60 were significantly different with the control and with batches of fry treated with MT30.

All sex ratios of S. galileaus, O. niloticus and O. mossambicus treated with MT30 or above, differed significantly with the controls.

S. galileaus treated with EE35, EE50 and EE65 yielded 72.57%, 98.77% and 78.89% females respectively.

O. niloticus treated with EE35, EE50 and EE65 yielded 94.97%, 99.52% and 99.39% female respectively.

O. niloticus X O. macrochir F_1 hybrids treated with MT 50 yielded 76.33 ± 2.90 % female.

Results of the hormone treatment experiments indicate that generally, 17 α-Methyltestesterone has positive growth effects when supplied in the range 30 to 50 mg/Kg diet. 17α -ethynylestradiol has no effect on growth at doses up to 50 mg/Kg diet but at 65 mg/Kg growth is generally retarded.

Results also show that the masculinizing effects of MT and the feminizing effects of EE are dose dependant and may vary in different treatments. EE treatments generally result in higher rates of abnormal development of gonads than MT treatments.

8. On the basis of the homo-heteromorphic sex determination theory, progeny sex ratio in S. galileaus indicate female homogamety. Progeny sex ratios in the backcrosses indicate male

heterogamety in O. niloticus and male homogamety in O. macrochir.

9. Overall, the results of the present series of experiments show that multiple spouse single pair spawning experiments in both pure and hybrid combinations, seemingly offer the best chance for selecting brood stock for repeated production of monosex fish in commercial tilapia culture.

CONCLUSION

The generally observed sex linked growth superiority in tilapia probably has a genetic basis, so that pro rata, production of all-male broods would be advantageous over production of all-female or mixed broods.

In the present investigations, interspecific hybridization did not generally enhance growth rates. Treatment of fry with medium doses of Methyltestosterone did on the other hand significantly promote growth in T. mariae and in S. galileaus

Within the size range of the experiments, MT had no significant effect on growth rate in O. niloticus.

Treating fish with the synthetic oestrogen EE in the range 35-50 mg/Kg did not significantly affect growth rate in any of the species treated. However, treatment of fish with 65 mg/Kg resulted in significant retardation in growth of S. galileaus and in the F_1 hybrid O. niloticus x O. macrochir.

The results indicate that whereas MT demonstrated positive growth effect, EE had no effect at dosages up to about 50 mg/Kg. The observed retardation in growth is probably indicative of toxic effects at the higher dose as evidenced by a corresponding increase in mortality in

some treated batches. Observation on growth of hormone treated fish further indicates that the growth promotion effects of MT act additively on genetic factors so that growth advantage is not merely a reflection of proportion of the two sexes. this was clearly demonstrated in T. mariae where treated males grew faster than control males and treated females.

The results clearly demonstrate added growth advantages of using an androgen in production of monosex broods when contrasted with the application of oestrogens.

In the present investigations, it has repeatedly been demonstrated that sex ratios of tilapia offspring vary more commonly than generally acknowledged. This fact is indicative that sex determination in tilapia is influenced by factors whose modus operandi is not uniformly fixed in all individuals of a population; so that the lability of sex factors in each individual frequently vary, as exemplified by differences in response to hormone treatments in the present experiments and occurrance of intersexes in some treated fish (McIntosh et al. 1985). The observations in the foregoing experiments seem at variance with the generally assumed all or non role of the sex chromosomes.

Further, the range of sex ratios obtained in intraspecific and interspecific breeding could not be wholly explained on the basis of the three gonosomes with

two autosomes theory of Avtalion and Hammerman (1975). the theory also fails to contribute a logical explanation for the observed sliding range of sex ratios in hormone treated fish or the frequent occurrance of abnormally developed gonads. this inadequacy is also shared by the multiple sex chromosome theory which assumes a fixed combination of sets of chromosomes in each of the two sexes.

The weaknesses of the foregoing sex determining theories are probably the main strength of the polygenic sex determining mechanism which dwells on individual allotment of sex determining genes.

Generally polygenic sex determination, by its apparently abstract nature, has been difficult to demonstrate. However, observation of trends of data in the literature and from analysis of the present investigations, there seems to be adequate circumstantial evidence to cautiously postulate existance of a polygenic sex determinism for tilapia. As indicated earlier, in this thesis, it is probable that the observed sexualizing tendencies in a species or a population represent a mean value for sexualizing tendencies in individual fish such that the total value for female tendancies approximates that of male tendencies and the net value is a 1:1 sex ratio.

By this theory, some species have stronger masculinizing tendencies while others have stronger feminizing tendencies. In summary each tendency can be represented by a value X/Y and W/Z such that in species with higher male tendencies $Y > X$ and in species with higher female tendencies $Z < W$. The net result from observed facts would be

$$Y > W > Z > X$$

REFERENCES AND FURTHER READING

ADEDIRE C.O. AND ODULEYE S.O. 1983
Stress induced water permeability changes in Oreochromis niloticus.
In L. Fishelson and Z. Yaron, compilers,
Proceedings of International symposium on tilapia in aquaculture, Nazareth, Israel.

AIDA, T 1936
Sexual reversal in Aplocheilus latipes and a new explanation of sex differentiation.
Genetics 21: 136-153 (or in Yamamoto, 1969)

AL-AMOULDI, M.M. L982
Studies on the acclimation of Commercially cultured Sarotherodon species to sea water.
Stirling University, PH.D. Thesis.

ALLANSON, B.R. 1966
A note on histological changes in Tilapia mossambica Peters exposed to low temperatures.
Summaries: The third Ann. Cong. Limnol. Soc. of South Africa. pp 16-19.

ALLANSON, B.R. AND NOBLE, R.G. 1964
The tolerance of Tilapia mossambica (Peters) to high temperature.
Trans. Am. Fish. Soc. 93 (4): 323-332.

ALLANSON, B. R., and NOBLE, R. G., 1971
The influence of exposure to low temperatures on Tilapia mossambica I.
J. Fish. Biol. 3:181-185

ALSTON, R.E. 1967
Cellular continuity and development.
Scott Foresman, Palo Alto.

ANDERSON, C.E. AND SMITHERMAN, R.O. 1978
Production of normal male and androgen sex reversed Tilapia aurea and T.nilotica fed a commercial catfish diet in ponds.
In: Culture of Exotic fishes, W.L. Shelton and J.H. Grover, (eds.) Auburn, Alabama, U.S.A.

ANDRIYASHEVA, M.A. 1970
Heterosis in intraspecific crossings of Carp.
In. V.S. Kirpichnikov (ed.).
Selective breeding of Carp and Intensification Of Fish breeding in Ponds. Kefer press. Jerusalem.

ARAI, R. 1967
Androgenic effects of 11-Ketotestosterone on some sexual characteristics in the teleost, Oryzias latipes.
Annot. Zool. Japan. 40: 1-5.

ARAI, K. 1984
Developmental genetic studies on Salmonids: morphogenesis, isozyme phenotypes and chromosomes in hybrid embryos.
Mem. Fac. Fish. Hokk. Univ., 31(1;2): 1-94.

ARAI, K., ONOZATO, H., and YAMAZAKI, F., 1978
Artificial androgenesis induced with Gamma irradiation in Masu Salmon, Oncorhynchus masou
Bull. Fac. Fish. Hokkaide Univ. 30(3):181-186

ARONSON, L.R. 1951
Factors affecting the spawning frequency in the female Cichlid fish Tilapia macrocephala, Am. Mus. Novit. No. 1948 pl-26

ASHBY, K.R. 1957
The effect of steroid hormones on the brown trout, Salmo trutta L. during the period of gonadal differentiation.
Journal of Embryol. Exptl. Morphology 5: 225-249.

ASHBY, K. R., 1956 (See Yamamoto, 1969)

ATZ, J. W. 1964
Intersexuality in fishes, pl45-232
In C.N. Armstrong and A.J. Marshall (eds.), Intersexuality in vertebrates including man. Academic Press. London and New York.

AVAULT, J.W. AND SHELL, E.W. 1968
Preliminary studies with the hybrid tilapia T. nilotica x T. mossambica. F.A.O. Fish Rep., 44(4): 237-242.

AVTALION, R.R. AND HAMMERMAN, I.S. 1978
Sex-determination in Sarotherodlon (Tilapia).
I. Introduction to a theory of autosomal influence.
Bamidgeh 30(4): 110-115.

BACCI, G. 1965
Sex determination. Pergamon Press Oxford, London, Edinburgh, New York, Toronto, Paris, Frankfurt vii + 306 p.

BAKOS, J., KRASZMAI, Z. , MARIAN, T. 1978
Crossbreeding experiments with carp, tench and Asian phytophagous cyprinids, Aquacultura Hungarica 1: 51-57.

BALARIN, J.D. AND HATTON, J.P. 1979
Tilapia - A guide to their biology and culture in Africa. University of Stirling, Stirling, Scotland, 174 pp.

BARASH, H. 1984
Growth rates of young tilapia fingerings fed on commercial eel and trout diets, Bamidgeh 36(3): 70-79.

BARD, J., 1969
Tilapia hydridization. F.A.O. Fish Culture Bulletin 1:5-6.

BARDACH, J.E., RYTHER, J.H., AND McLARNEY, W.D. 1972
Aquaculture:
The farming and husbandry of freshwater and marine organisms. Wiley - Interscience, New York, 868 pp.

BARLOW, G.W 1975
On the sociobiology of some hermaphroditic Serranid fishes, the hamlets, in Puerto Rico. Marine Biology 33: 295-300.

BARR, W.A. 1963
The endocrine control of the sexual cycle in the plaice, Pleuronectes platessa (Parts I and II). General Comparative Endocrinology 3: 205-225.

BECK, M.L., BIGGERS, C.J. AND DUPREE, H.K. 1980
Karyological analysis of Ctenopharyngodon idella, Aristichthys nobilis, and their F_1 hybrid. Transactions of American Fisheries Society 109: 435-438.

BIDWELL, 1985
Polyploidy induced by heat shock in Channel catfish. Aquaculture, 51: 25-32.

BISHAI, H.M. 1965
Resistance of Tilapia nilotica I. to high temperatures. Hydrobiologia, 25: 473-488.

BRUSLÉ, J. AND BRUSLÉ, S. 1975
Ovarian and testicular intersexuality in two protogynous Mediterranean groupers Epinephelus aeneus and Epinephelus guaza. In: Intersexuality in the Animal Kingdom. R. Reinboth, (ed) pp 222-227. Springer-Verlag, Berlin and New York.

BRUTON, M.N. 1983
Breeding, juvenile biology and growth of Oreochromis mossambicus in its natural range in South East Africa.
In: Intern. Symp. Tilapia in aquaculture. L. Fishelso and Z. Yaron (compilers) Nazareth, Israel.

BULKLEY, R.V. 1972
Diethylstilbestrol in catfish feed.
Trans. Amer. Fish. Soc. 101: 537-539.

BULL, J.J. 1980
Sex determination in reptiles. Quarterly Review of Biology 55: 3-21.

BULL, J.J. 1981a
Evolution of environmental sex determination from Genotypic Sex determination. Heredity 47(2): 173-184.

BULL, J.J. 1983
Evolution of sex determining mechanisms. Menlo Park, Benjamin, Cummings; 316p.

BULL, J.J AND VOGT, R.C. 1979
Temperature-dependent sex determination in Tutles.
Science, 206: 1186-1188.

BULMER, M.G AND BULL, J.J. 1982
Models of polygenic sex determination and sex ratio evolution.
Evolution 36: 13-26.

CAMPOS, H.H. AND HUBBS, C. 1972
Cytomorphology of six species of gambusiine fishes.
Copeia 1971: 566-569.

CAULTON, M.S. 1975
Diurnal movement and temperature selection by juvenile and sub-adult Tilapia rendalli Boulenger.
Transactions of the Rhodesia Science Assoc. 56: 51-56.

CAULTON, M.S. 1976
The importance of pre-digestive food preparation to Tilapia rendalli Boulenger when feeding on aquatic macrophytes.
Trans. Rhod. Sci. Assoc. 57: 22-28.

CAULTON, M.S. 1977
The effect of temperature on routine metabolism in Tilapia rendalli Boulenger. Journal of Fish Biology 11: 549-553.

CHAN, S.T.H. 1970
Natural sex reversal in vertebrates. Philos. Trans. R. Soc. London, Ser. B, 259: 59-71.

CHAN, S.T.H., O.W.S., HUI, S.W.B. 1975
The gonadal and adenohypophysial functions of natural sex reversal. In: Intersexuality in the Animal Kingdom. R. Reinboth (ed) pp 201-221. Springer-Verlag, Berlin and New York.

CHAN, S.T.H. AND YEUNG, W.S.B. 1983
Sex control and sex reversal in fish under natural conditions.
In: Fish Physiology Vol. IX B
W.S. Hoar and D.I. Randall (eds).

CHARNOV, E.L. AND BULL, J.J. 1977
When is sex environmentally determined?
Nature 266: 828-830.

CHEN, F.Y. 1968
Further studies on sex-determining Mechanism of Tilapia hornorum Trewavas.
Annual Rep. Trop. Cult. Inst. Butu Berendam. Mallaca. pp4.

CHEN, F.Y. 1969
Preliminary studies on the sex determining mechanism of Tilapia mossambica Peters and T. hornorum Trewavas.
Verh. Int. Verein. Limnol ., 17: 719-724.

CHEN, T.T., AGELLON, L.B. AND POWERS, D.A. 1986
Genetic engineering in fish: A new frontier.
In: Proceedings of EIFAC/FAO Symposium on Selection, hybridization and Genetic engineering in Aquaculture. Bordeaux (France).

CHERFAS, N.B. 1966
Natural triploidy in the females of the unisexual variety of the silver crucian carp.
Genetika (Moscow) 2: 16-24

CHERFAS, N.B. 1969
Results of a cytological analysis of unisexual and bisexual forms of Silver crucian Carp. Pages 79-90 in B.I. Cherfas (ed). Genetics, Selection and hybridization of fish
(National Technical Information Service, Springfield, Virginia, 1972).

CHERFAS, N.B. 1975
Investigation of radiation induced diploid gynogenesis in the carp (Cyprinus carpio L.). I.
Genetika 9: 78-86.

CHERVINSKI, J. AND HERING, E. 1973
Tilapia zillii and its adaptability to various saline conditions. Aquaculture 2: 23-29.

CHERVINSKI, J. AND YASHOUV, A. 1971
Preliminary experiments on the growth of Tilapia aurea steindacher (Pisces, Cichlidae) in sea water ponds.
Barmidgeh, 23(4): 125-129.

CHEVASSUS, B. 1983
Hybridization in fish.
Aquaculture, 33: 245-262.

CHEW, L.E. AND STANLEY, J.G. 1973
The effects of methyltestosterone on Sex reversal in blue gill sunfish.
Prog. Fish. Cult. 35: 44-47.

CHIMITS, P. 1957
The tilapia and their culture.
F.A.O. Fisheries Bulletin, 10: 1-24.

CHOAT, J.H. AND ROBERTSON, B.R 1975
Protogynous hermaphroditism in fishes of the family Scaridae.
In: Intersexuality in the Animal Kingdom. R.Reinboth (ed.) pp 263-383.
Springer-Verlag, Berlin and New York.

CHOURROUT, D. 1986
Genetic manipulations in fish: Review of Methods.
Proceedings of EIFAC/FAO SYMPOSIUM ON Selection, hybridization and genetic engineering in aquaculture.
Bordeaux, France.

CHOURROUT, D. AND ITSKOVICH, J. 1983
Three manipulations permitted by artificial insemination in tilapia: Induced diploid gynogenesis production of all triploid populations and intergeneric hybridization.
In: Proceedings of Inter. Symp. on Tilapia in aquaculture. L. Fishelson and Z. Yaron (compilers).
Tel Aviv, Israel . pp 246-255.

CHOURROUT, D. AND QUILLET, E. 1982
Induced gynogenesis in the rainbow trout: sex and survival of progenies, production of all triploid populations.
Theoretical Applied Genetics, 63: 201-205.

CIMINO, M.C. 1972
Egg production, polyploidization and evolution in diploid all-female fish of the genus Poeciliopsis. Evolution 26(2): 294-306.

CLARKE, E. 1965
Mating of groupers. New studies detected reversal of stripes in hermaphroditic fish. Natural History, N.Y. 74: 22-25. (Cited by Yamamoto, 1969).

CLEMENS, H.P. AND INSLEE, T. 1968
The production of unisexual broods by Tilapia mossambicus sex-reversed with methyltestosterone. Trans. Amer. Fish. Soc. 97(i): 18-21.

COCHE, A.G. 1976
Fish culture in rice fields. A worldwide synthesis. Hydrobiologia 30: 1-44.

COCHE, A.G. 1982
Cage culture of tilapias. In: R.S.V. Pullin and R.H. Lowe-McConnell (eds), The biology and Culture of tilapias. ICLARM conference proceedings 7, 432p, Manila, Phillipines.

COE, M.J. 1966
The biology of Tilapia grahami in Lake Magadi, Kenya. Acta Tropicana 23: 146-177.

CONOVER, D.O. 1984
Adaptive significance of temperature-dependent sex determination in a fish. The American Naturalist 123(3): 297-313.

CONOVER, D.O. AND KYNARD, B.E. 1981
Environmental sex determination: Interaction of temperature and genotype in a fish. Science 213: 577-579.

COOK, F.A., WILSON, S.W. AND PETER, R.E. 1983
Development and validation of a carp growth hormone radio immunoassay. Gen. Comp. Endocrinol. 50: 335-347.

CRAPON DE CAPRONA, M.D. 1986
Are 'preferences' and 'tolerances' in Cichlid mate choice important for speciation? J. Fish. Biol. 29(A): 151-158.

CRAWFORD, K.W., DUNSETH, D.R., ENGLE, C.R., HOPKINS, M.L., McCOY, E.W. AND SMITHERMAN, R.O. 1978
Marketing tilapia and chinese carps.
In: R.O. Smitherman, W.L. Shelton, J.H. Grover, (eds). Symp. culture of exotic fishes. Auburn, Alabama.

DADZIE, S. 1975
A preliminary report of the use of methallibure in Tilapia culture.
Afr. J. Trop. Hydrobiol. Fish. 4: 127-141.

DADZIE, S. 1982
Species combination in tilapia culture.
Aquaculture, 27: 295-299.

D'ANCONA, U. (see CHAN ET AL 1983) 1959

DAS, P., MUKHOPADHYAY, M.K., DAS, K.M., AND PANDIT, P.K. 1986
Gonadal sex manipulation of Oreochromis mossambicus (Peters).
In: Proceedings of the EIFAC/FAO Symposium on selection, hybridization and genetic engineering in Aquaculture, Bordeaux, France.

DE ALMEIDA TOLEDO, L.F., FORESTI, H. AND DE ALMEIDA TOLEDO FILHO, S. 1984
Complex sex chromosome system in Eigenmannia sp. (Pisces, Gymnotiformes). Genetica 64: 165-169.

DENZER, H.W. 1967
Studies on the physiology of young tilapia.
F.A.O. Fish. Rept. 44(4): 357-366.

DE VLAMING, V.L. 1972a
Environmental control of teleost reproductive cycles: a brief review.
Journal of Fish Biology 4: 31-140.

DONALDSON, E.M., FAGERLUND, U.H.M., HIGGS, D.A., McBRIDE, J.R. 1979
Hormonal enhancement of growth. In: Fish Physiology, Vol. 8: 455-596. (ED. W.S.Hoar, D.J. Randall and J.R. Brett). Academic Press; N.Y., London.

DONALDSON, E.M. AND HUNTER, G.A. 1982
Sex control in fish with particular reference to Salmonids.
Canadian Journal of Fisheries and Aquatic Sciences 39: 99-110.

DONALDSON, E.M. AND HUNTER, G.A. AND DYE, H.M. 1983
Induced Ovulation in Coho Salmon (Oncorhynchus Kisutch).III. Preliminary study on the use of the antiestrogen tamoxifen. Aquaculture.

DORFMAN, R.I. AND SHIPLEY, R.A. 1956
Androgens. John Wiley, New York, 590 pp.
(Quoted by Tayamen and Shelton, 1978).

DUNCAN, D.B. 1955
Multiple range multiple F-tests. Biometrics, 11: 1-42.

EBELING, A.W. AND CHEN, T.R. 1970
Heterogamety in teleostean fishes.
Transactions of the American Fisheries Society 99: 131-138.

ECKSTEIN, B. 1970
Metabolic pathways of steroid biosynthesis in ovarian tissue of a Teleost, Tilapia aurea. Gen. Comp. Endocrinol. 19: 303-312.

ECKSTEIN, B. AND SPIRA, M. 1965
Effects of hormones on gonadal differentiation in a Cichlid, Tilapia aurea, Biological Bulletin, 129: 482.

EGAMI, N. and R. Arai, 1964
Excerpta Medica International Congress Series No. 83, 146 (In Arai, 1967)

FARMER, S.W., PAPKOFF, H., HAYASHIDA, T., BEWLEY, T.A., BERN, H.A., AND LI, C.H. 1976
Purification and properties of teleost growth hormone.
General Comperative Endocrinology 30: 91-100.

FARMER, S. W., HAYASHIDA, T. PAPKOFF, H. and POLENOV, A.L. 1981
Characterization of growth hormone isolated from Sturgeon (Acipenser Guldenstadti) pituitaries. Endocrinol. 108: 377-381.

FERGUSON, M.W.J. AND JOANEN, T. 1982
Temperature of egg incubation determines sex in Alligator mississippiensis.
Nature 296: 850-852.

FERREIRA, J.T. 1986
The Genetic impoverishment in a closely managed population of Oreochromis mossambicus (Peters). In: Proceedings of EIFAC/FAO Symposium on selection, hybridization and genetic engineering in aquaculture. Bordeaux, France.

FISCHER, E.A. 1981
Sexual allocation in a simultaneously hermaphroditic coral reef fish.
The American Naturalist 117(1): 64-82.

FISHELSON, L. 1962
Tilapia hybrids.
Fisherman's Bull. Haifa 4(2)32: 14-19.

FISHELSON, L. 1970
Protogynous sex reversal in the fish, Anthias squaminipis, regulated by the presence or absence of a male fish. Nature 227: 90.

FREEMAN, H.C., UTHE, J.F., AND SANGALANG, C. 1980
The use of steroid hormone metabolism studies in assessing the sublethal effects of marine pollution. Rapp. P.V. Reun., Cons. Int. Explor. Mer. 179: 16-22.

FRICKE, H. AND FRICKE, S. 1977
Monogamy and sex change by aggresive dorminance in coral reef fish.
Nature 266: 830 - 832.

FRYER, G. AND ILES, T.D. 1972
The Cichlid fishes of the Great Lakes of Africa.
T.F.H. Publications, 641p.

FUKAYAMA, S. AND TAKAHASHI, H. 1983
Sex differentiation and Development of the gonad in the Sand Lamprey, Lampetra reissneri.
Bull. Fac. Fish. Hokkaido Univ. 34(4): 279 - 290.

FUKUSHO, K. 1968
The specific difference of Temperature responses among Cichlid Fishes Genus Tilapia
Bull. Jap. Sci. Fish. 34(2): 103-111.

GALMAN, O.R. 1986
Selective breeding and hybridization Studies of red tilapia in the Phillipines.
In: Proceedings of EIFAC/FAO Symposium on Selection, hybridization and genetic engineering in aquaculture. Bordeaux, France.

GALMAN, O.R., AND AVTALION, R.R. 1983
A preliminary investigation of the characteristics of Red Tilapia from the Phillipines and Taiwan. Proc. Inter. Symp. Tilapia Aqua. 291-301.

GARCIA PINTO, L. 1978
Hybridacion entre especies del pez del genero Tilapia (Familia Cichlidae) en Puerto Rico. MSc. University of Puerto Rico, Mayaquez, Puerto Rico. (in Lope Garcia Pinto, 1982).

GARCIA, P.L. 1982
Hybridization between species of Tilapia Transactions of American Fisheries Society 111 (4): 481-484.

GERVAI, J., MARIAN, T., KRASZNAI, Z., NAGY, A., AND CSANYI, V. 1980
Occurance of aneuploidy in radiation gynogenesis of Carp, Cyprinus Carpio L.
Journal of Fish Biology 16: 435-439.

GHISELIN, M.T. 1969
The evolution of hermaphroditism among animals.
Quarterly Review of Biology 44: 189-208.

GOETZ, F.W., DONALDSON, E.M., HUNTER, G.A., AND DYE, H.M. 1979
Effects of estradiol-17β and 17α - Methyltestosterone on gonadal differentiation in the Coho Salmon, Oncorhynchus Kisutch.
Aquaculture 17(4): 267-268.

GOLD, J.R. 1979
Cytogenetics
In: Fish Physiology Vol. VIII
W.S.Hoar and D. Randall (eds).
Acadmic Press, New York. pp 353-405.

GOLDSCHMIDT, R. 1911 (See Schreck, 1974)

GOLOVINSKAYA, K.A. 1968
Genetics and selection of fish and artificial gynogenesis in the carp (Cyprinus Carpio).
In: Proc. World Symp. Warm Water Pond Fish Cult.
Rome, FAO Fish. Rep. 44(4): 215-222.

GOLOVINSKAYA, K.A. 1972
Artificial gynogenesis in Carp.
In: Genetics, Selection and hybridization of fish.
B.I. Cherfas (ed). Israel programme for Scientific
Translation. Jerusalem. pp 74-78.

GORDON, M. 1947
Genetics of Platypoecilus maculatus IV. The
sex determining mechanism in two wild populations
of the Mexican platyfish.
Genetics 32: 8-17.

GORDON, M. 1954
Two opposing sex determining mechanisms, one XX -
XY and the other WY - YY in different natural
populations of the platyfish. Xiphophorus
maculatus.
Proc. 9th Int. Cong. Gen. 2: 960-964.

GUERRERO, R.D. 1975
Use of androgens for the production of all-male
Tilapia aurea.
Transactions of American Fisheries Society 1021:
342-348.

GUERRERO, R.D., III, 1975
Culture of monosex male Tilapia mossambica and
Ophiocephalus striatus in fertilized ponds with
supplementary feeding.
Phillipine Journal of Fisheries, 12: 64-74.

GUERRERO, R.D. 1976b
Tilapia mossambica and Tilapia zillii treated with
ethynyltestosterone for sex reversal.
Kalikasan 5: 187-192.

GUERRERO, R.D. 1977
Production of tilapia fry in floating net enclosures.
FAO Aquaculture Bull. 8(3-4): 4.

GOURDIE, C.A., REDNER, B.D., SIMCO, B.A., AND DAVIS, K.B.
1983
Feminization in Channel catfish with oral
administration of sex steroid hormones.
Trans. Am. Fish. Soc. 112: 670-672.

HAMILTON, W.D. 1967
Extraordinary sex ratios.
Science, 156: 477-488.

HAMMERMAN, I.S. AND AVTALION, R.R. 1979
Sex determination in Sarotherodon (Tilapia). Part 2: The sex ratio as a tool for the determination of genotype - A model of autosomal and gonosomal influence.
Theoretical Applied Genetics 55: 177-187.

HANSON, T.R., SMITHERMAN, R.O., SHELTON, W.L. AND DUNHAM, R.A. 1983
Growth comparisons of monosex tilapia produced by separation of sexes, hybridization, and sex reversal.
In: L. Fishelson and Z. Yaron, (compilers) International Symposium on Tilapia in Aquaculture.
Tel Aviv University, Tel Aviv, Israel.

HARDISTY, M.W. 1965
Sex defferentiation and gonadogenesis in Lampreys (Parts I and II) Journal of Zoology 146: 305-387.

HARRINGTON, R.W.Jr. 1957
Sexual photoperiodicity of the cyprinid fish, Notropis bifrenatus (Cope), in relation to the phases of its annual reproductive cycle.
Journal of Experimental Zoology 135: 529-555.

HARRINGTON, R.W. 1967
Environmentally controlled induction of primary male gonochorists from eggs of the self-fertilizing hermaphroditic fish, Rivulus marmoratus Poey.
Biological Bulletin, 132(2): 174-199.

HARRINGTON, R.W. 1968
Delimitation of the thermolabile phenocritical period of sex determination and differentiation in the ontogeny of the normally hermaphroditic fish, Rivulus marmoratus Poey.
Physiological Zoology 41(4): 447-460.

HARRINGTON, R.W. 1974
Sex determination and differentiation in fishes
In: Schreck (ed). Control of sex in fishes.

HENDERSON, N.E. 1963
Influence of light and temperature on the reproductive cycle of the eastern brook trout, Salvelinus fontinalis (Mitchell). J. Fish. Res. Board Can. 20: 859-897.

HEUT, M. 1975
Textbook of Fish Culture: Breeding and cultivation of fish.
Fishing News Books Ltd., West Byfleet, England.

HICKLING, C.F. 1960
The Malacca Tilapia hybrids. Journal of Genetics 57(1): 1-10.

HICKLING, C.F. 1968
Fish hybridization.
F.A.O. Fish. Rep., 44(4): 1-11.

HIKITA, T. AND HASHIMOTO, S. 1978
A hermaphroditic chum salmon Onchorhynchus keta, from the Chitose river, with an example of its self-fertilization. Sci. Rep. Hikkaido Salmon Hatchary 32: 61-64 (in Japanese).

HOFSTEDE, A.E, AND BOTKE, F. 1950
Tilapia mossambica as a factor in malaria control in the vicinity of Djakarta.
Landbouw 22: 453-468 (In H.S.Swingle).

HONMA, Y. 1980
A synchronous hermaphroditism in a Chum salmon, Oncorhynchus keta, from the sea of Japan. Ann. Rep. Sado Mar. Biol. Stn., Niigata Univ., No 10: 17-22.

HOORNBECK, F.K., KLEIN-MacPHEE, G., AND MOROZ, L. 1986
Intergeneric flounder hybridization.
(Abstract) In: Symp. Selection, hybridization and genetic engineering in aquaculture. Bordeaux, France.

HOPKINS, K.D. 1977
Sex reversal of Genotypic male Sarotherodon aureus (Cichlidae).
M.Sc Thesis abstract, Dept. of Fisheries and Allied Aquacultures, Auburn University.

HOPKINS, K.D., SHELTON, W.L., AND ENGLE, C.R. 1979
Estrogen sex reversal of Tilapia aurea.
Aquaculture 18: 263-268.

HSIAO, S.M. 1980
Hybridization of Tilapia mossambica, T. nilotica, T. aurea and T. zillii.
China Fisheries Monthly Taipei 332: 3-13 (Abstract).

HUBBS, C.L. 1955
Hybridization between fish species in nature.
Systematic Zoology 4: 1-20.

HUBBS, C.L., AND HUBBS, L.C. 1932
Apparent parthenogenesis in nature of fish of hybrid origin.
Science 76: 628-630.

HULATA, G.I., ROTHBARD, S. AND WOHLFARTH, G.W. 1981
Genetic approach to the production of all-male progeny of tilapia.
Europ. Mari. Soc., Spec. Publ. 6: 181-190.

HUNTER, G.A., SOLAR, I.I., BAKER, I.J., AND DONALDSON, E.M. 1986
Feminization of Coho Salmon (Oncorhynchus Kisutch) and Chinook Salmon (Oncorhynchus Tshawytscha) by Immersion of Alevins in a solution of estradiol-17B.
Aquaculture, 53: 295-302.

IBRAHIM, K.H. 1976
Progress and present status of aquaculture in Tanzania. FAO/CIFA Symp. on Aquaculture in Africa.
Accra, Ghana.

IDLER, D.R., BITNERS, I.I., AND SCHMIDT, P.J. 1961
Il-Ketotestosterone: an androgen for sockeye salmon.
Can. J. Biochem. Physiol. 39: 1737-1742.

IDLER, D.R., SCHMIDT, P.J., AND BIELY, J. 1961
The androgenic activity of 11-ketotestosterone: a steroid in Salmon plasma.
Can. J. Biochem. Physiol. 39: 317-320.

ILES, T.D. 1973
Dwarfing or stunting in the genus Tilapia (Cichlidae); a possibly unique recruitment mechanism.
Rapp. R.V- Reun. Cons. Perm. Int. Explor. Mer. 164: 247-254.

JALABERT, B., KAMMACHER, P. AND LESSENT, P. 1971
Determonisme du sexe chez les hybrides entre Tilapia macrochir et Tilapia nilotica, Etude de la sex-ration dans les recrousements des hybrides de premiere generation par les especes parentes.
Ann. Biol. Anim. Biochem. Biophys., 11: 155-165.

JALABERT, B., MOREAU, J., PLANQUETTE, D., AND BILLAR, R. 1974
Determinisme du sexe chez Tilapia macrochir et Tilapia nilotica: action de la methyltestosterone dans l'alimentation des alevins sur la differenciation sexuelle; proportion des sexes dans la descendance des males 'inverse'.
Ann. Biol. Anim. Biochem., Biophys. 14(4-B): 629-739.

JALABERT, B., BILLARD, R. AND CHEVASSUS, B., 1975
Preliminary experiments on sex control in trout: production of sterile fishes and simultaneous self-fertilizable hermaphrodites.
Ann. Biol. Anim. Biochem. Biophys., 15: 19-28.

JENSEN, G.L. 1976
The effects of several naturally occurring estrogens of Sarotherodon arueus and their potential application to yield monosex genetic males
In: Shelton et al, 1978

JENSEN, G.L. AND SHELTON, W.L. 1979
Effects of estrogens on Tilapia aurea: Implications for production of Monosex genetic male tilapia.
Aquaculture 16: 233-242.

JENSEN, G.L., SHELTON, W.L., YANG, S. AND WILKEN, L.O. 1983
Sex reversal of gynogenetic grass carp by implantation of Methyltestosterone.
Transactions of American Fisheries Society, 112: 79.

JOBLING, M. AND WANDSVIK, A. 1983
Effect of social interactions on growth rates and conversion efficiency of Arctic Charr, Salvelinus alpinus L. J. Fish. Biol. 22: 577-584.

JOHN, G. AND REDDY, P.V.G.K. 1986
A note on the Labeo rohita (Ham.)X Cyprinus carpio L. hybrid.
In: Sympo. on Selection, hybridization and genetic engineering in aquaculture. Bordeaux, France.

JOHNSTONE, R. 1985
Induction of triploidy in Atlantic Salmon by heat shock.
Aquaculture, 49: 133-139.

JOHNSTONE, R. SIMPSON, T.H. AND YOUNGSON, A.F. 1977
Sex reversal in Salmonid Culture.
Aquaculture 13: 115.

JOHNSTONE, R., SIMPSON, T.H., AND WALKER, A.F. 1979
Sex reversal in Salmonid Culture. Part 3. The production and performance of all-female populations of Brook trout.
Aquaculture 18: 241.

JOHNSTONE, R., MacINTOSH, D.J., AND WRIGHT, R.S. 1983
Elimination of orally administered 17α - Methyltestosterone by O. mossambicus (Tilapia) and Salmo gairdneri (Rainbow trout) juveniles.
Aquaculture 35: 249-257.

JONES, P., JACKSON, H., AND WHITING, M.H.S. 1975
Parthenogenetic development after chemical treatment of Xenopus laevis spermatozoa.
J. Exp. Zool. 192: 73-83.

KALLMAN, K.D. 1964
Homozygosity in a gynogenetic fish.
Genetics 50: 260-261.

KALLMAN, K.D. 1965
Genetics and geography of sex determination in the Poeciliid fish, Xiphophorus maculatus. Zoologica 50: 151-190.

KALLMAN, K.D. 1968
Evidence for the existence of transfomer genes for sex in the teleost Xiphophorus maculatus.
Genetics 60: 811-828.

KALLMAN, K.D. 1982
Genetic control of sexual maturation in fishes of the genus Xiphophorus (poeciliidae).
Proc. 9th Symp. Comp. Endocrinol.

KALLMAN, K.D. 1984
Sex ratio and the genetics of sex determination in sword tails Xiphophorus poeciliidae.
Genetics 107 (3 pt 2).

KATZ, Y., ABRAHAM, M., AND ECKSTEIN, B. 1976
Effects of adrenosterone on gonadal and body growth in Tilapia nilotica (Teleostei, Cichlidae).
General Comparative Endocrinology 29: 414-418.

KAYA, C.M., AND HASLER, A.D. 1972
Photoperiods and temperature effects on the gonads of green sunfish, Lepomis cyanellus, during the quiscent winter phase of its annual sexual cycle.
Trans. Am. Fish. Soc . 101: 270-275.

KERBY, J.H. 1979
Meristic characters of two Morone hybrids.
Copeia, 3: 513-518.

KINCAID, H.L. 1986
Development of strain registry for trout species used to manage United States Fisheries. Proce. EIFAC/FAO. Symp. selection, hybridization and genetic engineering in aquaculture, Bordeaux, France.

KIRK, R.G. 1972
A review of recent developments in tilapia culture with special reference to fish farming in the heated effluents of power stations.
Aquaculture 1: 45-60.

KIPICHNIKOV, V.S. 1970
Methods of progeny testing spawners in carp fish farms.
In V.S.Kirpichnikov (ed).
Selective breeding of carp and intensification of fish breeding in ponds. Keter Press. Jerusalem.

KIRPICHNIKOV, V.A. 1970
Selective breeding of carp and intensification of fish breeding in ponds.
Izv. Gos. Nauchn. I. Inst. Kho. Leningrad.

KIRPICHNIKOV, V.S. 1981
Genetic bases of fish selection (Translation).
Springer-Verlag, Berlin and New York, 410p.

KOSSWIG, C. 1935 (See Yamamoto, 1969)

KOSSWIG, C. 1964
Polygenic sex determination.
Experientia 20: 190-199.

KOWTAL, G.V. 1986
Hybridization amongst Indegenous and exotic carps in India.
In: Proceedings of EIFAC/FAO symposium on selection, hybridization and genetic engineering in aquaculture, Bordeaux, France.

KOWTAL, G.V., AND CHERR, G. 1986
Preliminary experiments in induction of polyploidy, gynogenesis and andrcgenesis in the white sturgeon, Acipenser transmontanus Richardson.
In: Symp. on Selection, hybridization and genetic engineering in aquaculture. Bordeaux, France.

KRASZNAI, Z.L. 1986
Interspecific hybridization of warm water finfish.
In: Proceedings of EIFAC/FAO symposium on Selection, hybridization and genetic engineering in aquaculture. Bordeaux, France.

KRONERT, U., HÖRSTGEN-SCHWARK AND LANGHOLZ, J. 1986
Investigations on the possibility to select for late maturity in Tilapia.
In: EIFAC/FAO symp. on Selection, hybridization and genetic engineering in aquaculture, Bordeaux, France.

KUO, H. 1969
Notes on hybridization of tilapia.
Taiwan Fish. Ser. 8: 116-117.

LAGLER, K.F., BARDACH, J.E., MILLER, R.R., AND PASSINO, D.M. 1962
Ichthyology (2nd edn). John Wiley, New York.

LAM, T.J. 1982
Applications of endocrinology to Fish Culture.
Canadian Journal of Fisheries and Aquatic Science 39: 111.

LANGHOLZ, H.J., AND HÖRSTGEN-SCHWARK, G. 1986
Family selection in Rainbow trout.
In: Proceedings of EIFAC/FAO Symposium on Selection, hybridization and genetic engineering in aquaculture, Bordeaux, France.

LEE, J.C. 1979
Reproduction and hybridization of three Cichlid fishes Tilapia aurea (Steindachner), Tilapia hornorum (Trewavas) and T. nilotica (Linnaeus) in aquaria and plastic

pools.
Ph.D. Dissertation (Abstract); Alabama, 94p.

LESSENT, P. 1968
(See Wohlfarth and Hulata 1981).

LILEY, N.R., AND STACEY, N.E. 1983
Hormones, Pheromones, and Reproductive Behaviour in Fish. pp 1-64.
In: Fish Physiology vol. IX B.
W.S.Hoar and D.J. Randall (eds).
Academic Press, New York, London.

LINCOLN, R.F. 1981
The growth of female diploid and triploid plaice (Pleuronectes platessa) x flounder (Platichthys flessus) hybrids over one spawning season.
Aquaculture, 25: 259-268.

LI SIFA, LU WEIMIN, PENG CHANGDIE, ZHAO PINRU 1986
A study on the growth performance and their genetic and environmental causes of silver carp from Changjiang river and Zhujiang river.
In: Proceedings of EIFAC/FAO symposium on Selection, hybridization and genetic engineering in aquaculture, Bordeaux, France.

LIU, C.Y. 1977
Aspects of reproduction and progeny testing in Sarotherodon aureus (Steindachner).
M.Sc. Thesis, abstract, Auburn University, Alabama.

LODI, E. 1967a
Sex reversal of Cobitis taenia L. (Osteichthyes, fam. Cobitidae).
Experientia 23: 446-447.

LODI, E. 1980
Sex inversion in domesticated strains of the swordtail, Xiphophorus helleri Heckel (Pisces, Osteichthyes).
Bull. Zool. 47: 1-8.

LOPE GARCIA PINTO 1982
Hybridization between species of Tilapia, Transactions of the American Fisheries Society 111: 481-484.

LOVSHIN, L.L. 1982
Tilapia hybridization. In Pullin and Lowe-McConnek (eds), pp. 279-308.

LOVSHIN, L.L., AND DA SILVA, L.L. 1976
Culture of monosex and hybrid tilapias.
FAO/CIFA/75/SR9 p 16.

LOWE-McCONNELL, R.H. 1955
The fecundity of Tilapia species.
East African Agriculture Journal, 21: 45-52.

LOWE, R.H. 1958
Observations on the biology of Tilapia nilotica in East African waters.
Review of Zool. Bot. Africa, 57(1-2): 129-170.

LOWE McCONNELL, 1959
Breeding behaviour patterns and ecological differences between tilapia species and their significance for evolution within the genus Tilapia (Pisces: Cichlidae).
Proc. Zool. Soc. Lond. 132: 1-30

LUCAS, G.A. 1968
Factors affecting sex determination in Betta Splendens.
Genetics 60: 199-200.

MacINTOSH, D.J., VARGHESE, T.J., AND RAO, G.P.S. 1985
Hormonal sex-reversal of wild spawned tilapia in India.
Journal of Fish Biology, 26: 87-94.

MacLEAN, N., PENMAN, D., AND ZHU, Z. 1987
Introduction of novel genes into fish.
Biotec. Magazine (in Press).

MAGEE, S.M., AND PHILLIPP, D.P. 1982
Biochemical genetic analysis of the grass carp (F) x big head carp (M) F_1 hybrid and the parental species. Trans. Am. Fish. Soc. 111: 593-602.

MAIR, G.C., PENMAN, D.J., SCOTT, A., SKIBINSKI, D.O.F., BEARDMORE, J.A. 1986
Hormonal sex-reversal and the mechanism of sex determination in Oreochromis.
In: Proceedings of EIFAC/FAO symposium on Selection, hybridization and genetic engineering in aquaculture, Bordeaux, France.

MAJUMDAR, C.K. 1984
Genetic studies in Tilapias.
Ph.D. Thesis; University of Stirling.

MAJUMDAR, K.C., AND McANDREW, B.J. 1983
Sex ratios from interspecific crosses within the tilapias.
In: L. Fishelson and Z.Yaron, compilers, Proceedings of International symposium on tilapia in aquaculture, Nazareth, Israel.

MAJUMDAR, K.C., AND McANDREW, B.J. 1987
Growth performance in interspecific hybrids of the genus Oreochromis.
In press - Aquaculture.

MAKEYEVA, A.P. 1975
The phenomenon of hybrid gynogenesis in fishes.
Journal of Ichthyology 15: 72-81.

MARIAN, T., AND KRASZNAI, Z. 1978
Karyological investigations on Ctenopharyngodon idella and Hypophthalmichthys nobilis and their cross-breed.
Aquaculture Hungarica I: 44-50.

MARIAN, T., KRASZNAI, Z., OLAH, J., AND BAKOS, J. 1986
Morphology and biochemical markers of bighead carps, silver carp and their reciprocal hybrids.
Proceedings of a symp. on Selection, hybridization and genetic engineering in aquaculture. Bordeaux, France.

MARUYAMA, T. 1958
An observation on Tilapia mossambica in pond referring to diurnal movement with temperature change.
Bull. Freshwat. Fish. Res. Lab. Tokyo 8: 25-32.

MARUYAMA, T. 1975
On the possibility of acclimatization of Tilapia nilotica to Low Water Temperature condition.
Abstract: Bull. Freshwater Res. Labs. Tokyo. 25(1)13.

MATTY, A.J. 1985
Fish endocrinology.
Groom Helm, London and New York.

McANDREW, B.J., AND MAJUMDAR, K.C. 1983
Tilapia stock identification using electrophoretic markers.
Aquaculture 30: 249-261.

McANDREW, B.J., AND MAJUMDAR, K.C. 1984
Evolutionary relationships within three Tilapiine genera (Pisces: Cichlidae).
Zool. Journ. Linn. Soc. 80: 421-435.

McBAY, L.G. 1961
The biology of Tilapia nilotica (Linnaeus) Proc. Annual Conf. Southeast Association Game Fish Comm. 15: 208-218.

McBRIDE, J.R. AND FAGERLUND, U.H.M. 1973
The use of 17∝- Meth. T. for promoting weight increase in juvenile Pacific Salmon. J. Fish. Res. Board. Can. 30: 1099-1104.

MIRES, D. 1974
On the high percent of Tilapia males encountered in captive spawnings and the effect of temperature on this phenomenon.
Bamidgeh 26: 3-11.

MIRES, D. 1977
Theoretical and practical aspects of the production of all-male tilapia hybrids.
Bamidgeh, 29(3): 94-101.

MIRES, D. 1983
Current techniques for the mass production of tilapia hybrids as practiced at Ein Hamifrats Hatchery. Bamidgeh 35(1) 3-8.

MITTWOCH, U. 1965
Genetics of sex differentiation.
In: Sex determination; G. Bacci, (ed), Academic Press, New York.

MITTWOCH, U. 1969
Do genes determine sex?
Nature 221 (5179): 446-448.

MITTWOCH, U. 1971
Sex determination in birds and mammals.
Nature 231 (5303): 432-434.

NAEVDAL, G., AND DALPADADO, P. 1986
Interspecific hybridization - Cold water species.
In: Proceedings of EIFAC/FAO symposium on Selection, hybridization and genetic engineering in aquaculture, Bordeaux, France.

NAGY, A. 1986
Genetic manipulations, performance of warm water fish. EIFAC/FAO symposium on Selection, hybridization and genetic engineering in aquaculture, Bordeaux, France.

NAGY, A., RAJKI, K., BAKOS, J., AND CSANYI, V. 1979
Genetic analysis in carp (Cyprinus Carpio) using gynogenesis. Heredity 43: 35-40.

NAGY, A., RAJKI, K., HORVATH, L., AND CSANYI, V. 1978
Investigation on Carp Cyprinus carpio L. gynogenesis.
Journal of Fish Biology 13: 215-224.

NAKAMURA, M., AND TAKAHASHI, H. 1973
Gonadal sex differentiation in Tilapia mossambica with special regard to time of oestrogen treatment effective in inducing complete feminization of genetic males.
Bull. Fac. Fish. Hokk. Univ. 24(1): 1-13.

NAKAMURA, M. 1975
Dosage-dependent changes in the effect of oral administration of Methyltestosterone on gonadal sex differentiation in Tilapia mossambica. Bull, Fac. Fish., Hokkaido Univ. 26(2): 99-108.

NAKAMURA, M. 1981a
Effects of 11-Ketotestosterone on gonadal sex differentiation in Tilapia mossambica. Bull. Fac. Fish., Hokkaido Univ. 47: 1323-1327.

NAYUDU, P.L. 1979
Genetic studies of Melanic color patterns and aatypical sex determination in the Guppy, Poecilia reticulata.
Copeia 9(2): 225-231.

NIJJHAR, B., NATEG, C.K., AND AMEDJO, S.D. 1983
Chromosome studies on Sarotherodon niloticus, S. multifasciatus and Tilapia busumana (Cichlidae, Pisces). Proc. Int. Symp. Tilapia Aqua. 256-260.

NOGUSA, S. 1955
Chromosome studies in Pisces IV.
The Chromosome of Mogrunda obscura (Gobiidae), with evidence of male heterogamety.
Cytologia 20: 11-18.
(Tokyo).

NYAMBI, T.A.J. 1982
The effects of water temperature, pH and dissolved oxygen concentration on the survival and growth of first feeding stage Oreochromis mossambicus fry.
M.Sc. Thesis, Univ. of Stirling, Stirling, Scotland.

OHNO, S. 1967
A comparative study of the chromosomes of fishes with particular considerations on taxonomy and evolution.
Mem. Hyodo. Univ. Agric 3: 1-62.

OKADA, H., MATUMOTO, H. and YAMAZAKI, F., 1979
Functional masculinization of genetic females in rainbow trout. Bull. Jap. Soc. Sci. Fish. 45(4): 413-419

PADOA, E. 1939 (See Yamamoto, 1969)

PANKHURST, N.W., STACEY, N.E., AND PETER, R.E. 1986
An evaluation of techniques for the administration of 17β- estradiol to Teleosts.
Aquaculture 52: 145-155.

PARK, E.H., AND GRIMM, H. 1981
Distribution of C-band heterochromatin in the ZW sex chromosomes of European and American eels (Anguillidae, Teleostomi). Cytogenet. Cell Genet. 31: 167-174.

PARSONS, J. E. and THONGAARD, G. H. 1984
J. Exp. Zool. 231: 407-412

PAYNE, R.H., CHILD, A.R., AND FORREST, A. 1972
The existence of natural hybrids between the European trout and the Atlantic Salmon.
Journal of Fish Biology 4: 233-236.

PENMAN, D.J., SHAH, M.S., BEARDMORE, J.A., AND SKIBINSKI, D.O.F. 1986
Sex ratios of gynogenetic and triploid tilapia.
Proc. EIFAC Symp. on Selection, hybridization and genetic engineering in aquaculture, Bordeaux, France.

PERSEV, G.M. 1975
The period of gonadal differentiation in fishes. In sex differentiation in fishes. 148p. cited by Donaldson and Hunter, 1982.

PRICE, D.J. 1984
Genetics of sex determination in fishes

In: Fish reproduction: strategies and tactics; G.W. Potts and R.J. Wooton (eds).
Academic Press, pp 77-79.

PRUGININ, Y. 1967
Report to the Government of Uganda 1965-1966.
Rep. FAO/UNDP (TA), (2446). 16p.

PRUGININ, Y., ROTHBARD, S., WOHLFARTH, G., HALEVY, A., MOAV, R., AND HULATA, G. 1975
All-male broods of Tilapia nilotica x T. aurea hybrids.
Aquaculture 6: 11-21.

PULLIN, R.S.V. 1983
Choice of tilapia species for aquaculture.
Proceedings: Intern. Symp. on Tilapia in Aquaculture.
Tel Aviv, Israel.

PURDOM, C.E. 1969
Radiation induced gynogenesis and androgenesis in fish.
Heredity 24: 431-444.

PURDOM, C.E. 1972
Induced polyploidy in plaice Pleuronectes platessa and its hybrid with the flounder Platichthys flesus.
Heredity, 29: 11-24.

PURDOM, C.E., AND LINCOLN, R.F. 1973
Chromosome manipulation in fish.
In: J.H. Schroder, (ed). Genetics and Mutagenesis of fish p. 83-90.
New York, Springer-Verlag.

PURDOM, C.E. 1983
Genetic engineering by the manipulation of chromosomes.
Aquaculutre 33: 287-300.

REFSTIE, T., STOSS, J., AND DONALDSON, E. 1982
Production of all-female Coho Salmon (Onchorhynchus kisutch) by diploid gynogenesis using irradiated sperm and cold shock.
Aquaculture 29: 67-82.

REINBOTH, R. 1970
Hormones and the environment.
Memoirs Soc. Endocrinology No 18: 515-543.

REINBOTH, R. 1975b
Intersexuality in the Animal Kingdom.
Springer-Verlag, Berlin, Heidelberg, New York.
pp 449.

RIFAI, S.A. 1980
Control of reproduction of Tilapia nilotica using cage culture. **Aquaculture 20:177-185**

ROBERTSON, D.R. 1972
Social control of sex reversal in a coral-reef fish. Science 177. 1007-1009.

SACLAUSO, C.A. 1985
Interaction of growth with social behaviour in Tilapia zillii raised in three different temperatures. J. Fish. Biol. 26: 331-337.

SANICO, A.F. 1975
Effects of 17α-Ethynyltestosterone and Estrone on sex ratio and growth of Tilapia aurea (Steindachner). M.Sc. Thesis abstract, Dept. of Fisheries and Allied Aquacultures, Auburn University.

SCHRECK, C.B. 1974
Control of sex in fishes.
Extension division, Virginia Polytechnical Institute and State University, Blacksburg, Virginia.

SCHULTZ, R.J. 1961
Reproductive mechanisms of unisexual and bisexual strains of the viviparous fish Poeciliopsis. Evolution 15: 302-325.

SCHULTZ, R.J. 1967
Gynogenesis and triploidy in the viviparous fish Poeciliopsis.
Science 157: 1564-1567.

SCHULTZ, R.J. 1971
Special adaptive problems associated with unisexuality in fish.
Amer. Zool. 11: 351-360.

SHAFLAND, P.L., AND PESTRAK, J.M. 1982
Lower lethal temperatures for fourteen non-native fishes in Florida.
Env. Biol. Fish. Vol. 7(2): 149-156.

SHAH, M.S., AND BEARDMORE, J.A. 1986
Production of Triploid tilapia (Oreochromis niloticus) and their comparative growth performance with normal diploids.
In: Proceedings of EIFAC/FAO symposium on Selection, hybridization and genetic engineering in aquaculture. Bordeaux, France.

SHAPIRO, D. Y., 1977
Social structure and sex reversal in the coral reef fish Anthias squamipinnis.
Ph.D. Thesis, University of Cambridge (Reinboth, 1980)

SHELL, E.W. 1967
Mono-sex culture of male Tilapia nilotica Linnaeus in ponds stocked at three rates. Proc. FAO World Symposium on warm-water pond fish culture. FAO Fish. Rept. 44(4): 353-356.

SHELTON, W.L., HOPKINS, K.D., AND JENSEN, G.L. 1978
Use of hormones to produce monosex tilapia for aquaculture.
In R.O. Smitherman, W.L. Shelton, J.H. Glover (eds). Culture of exotic fishes symposium proceedings. American Fisheries Society, Auburn, Alabama.

SHELTON, W.L., D. RODRIGUEZ-GUERRERO, AND J. TOPEZ-MACIAS. 1980
Factors affecting androgen sex reversal of Tilapia aurea.
Aquaculture 25: 59-65.

SHELTON, W.L., MERIWETHER, F.H., SEMMENS, K.J., AND CALHOUN, W.E. 1983
Progeny sex ratios from intraspecific pair spawnings of Tilapia aurea and T. nilotica.
In L. Fishelson and Z. Yaron, compilers, Proceedings of International symposium on tilapia in aquaculutre, Nazareth, Israel.

SICK, K., FRYDENBERG, O., AND TONNES-NIELSEN, J. 1963
Haemoglobin pattern of plaice and flounder and their natural and artificial hybrids. Nature, 198: 411-412.

SMITH, C.L. 1965
The patterns of sexuality and the classification of serranid fishes.
Am. Museum Novitates 2207: 1-20.

SMITH, C.L. 1975
The evolution of hermaphroditism in fishes.
In: Intersexuality in the Animal Kingdom. R. Reinboth (ed). Springer Verlag, Berlin, Heidelberg, New York. pp. 295-310.

SMITHERMAN, R.O., WOHLFARTH, G., SHELTON, W.L., DUNHAM, R.A., HULATA, G., AND ROTHBARD, S. 1984
Selective breeding of farmed fish.
United States - Israel Binational Research Report.

STANLEY, J.G. 1976
Production of hybrid, androgenetic, and gynogenetic grass carp.
Transactions of the American Fisheries Society 105: 10-16.

STANLEY, J.G. 1979
Control of sex in fishes, with special reference to the grass carp. p201-242 In: J.V. Shireman (ed) Proc. of the grass carp conference. Univ. Florida, Gainesville,FL. Cited by Thorgaard, G.H. 1983.

STANLEY, J.G., AND JONES, J.B. 1976
Morphology of androgenetic and gynogenetic grass carp, Ctenopharyngodon idella.
Journal of Fish Biology 9: 523-528.

STANLEY, J.G., AND SNEED, K.E. 1973
Artificial gynogenesis and its application in genetics and selective breeding of fishes.
In: The early life history of fish, J.H.S. Blaxter (ed).
Springer-Verlag, pp. 527-536.

STANLEY, J.G., AND THOMAS, A.E. 1978
Absence of sex reversal in unisex grass carp fed methyltestosterone.
In: Culture of Exotic fishes symposium proceedings. Auburn, Alabama, U.S.A.

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.

SUNDARARAJ, B.I., AND VASAL, S. 1976
Photoperiod and temperature control in the regulation of reproduction in the female catfish Heteropneustes fossilis.
Journal of Fisheries Research Board of Canada 33: 959-973.

SUFFERN, J.S., ADAMS, S.M., BLAYLOCK, B.G., COUTANT, C.C., GUTHRIE, C.A. 1978
Growth of monosex hybrid tilapia in the laboratory and sewage oxidation ponds. In: symp. on Culture of Exotic Fishes. Atlanta, Georgia. p. 65-81.

SUN RU-YUNG AND ZHANG YU-SHU 1983
The influence of water temperature on the growth of Tilapias.
Abstract: Bulletin of Marine Science 33(3).

SUZUKI, R., AND KATO, T. 1966
Hybridization in nature between Salmonid fishes Salvelinus pluvius x Salvelinus fontinalis.
Bull. Freshwater Fish. Res, 16: 83-89.

SWINGLE, H.S. 1968
Estimation of standing crops and rates of feeding fish in ponds.
FAO Fisheries Reports 44: 416-423.

SWINNEY, G.N., AND COLES, T.F. 1982
Description of two hybrids involving silver bream, Blicca bjoerkna (L) from British waters.
Journal of Fish Biology 20: 121-129.

TANIGUCHI, N., KIJIMA, A., TAMURA, T., AND TAKEGAMI, K. 1985
Colour growth and maturation in ploidy manipulated fancy carp.
In: Second Intern. Symp. on genetics in aquaculture, Abstract, pp 34.

TAVE, D., AND SMITHERMAN, R.O. 1980
Predicted response to selection for early growth in Tilapia nilotica.
Transactions of the American Fisheries Society 109: 439-445.

TAYAMEN, M.M., AND SHELTON, W.L. 1978
Inducement of sex reversal in Sarotherodon nilotica (Linnaeus).
Aquaculture, 14: 349-354.

THORGAARD, G.H. 1978
Sex chromosomes in the sockeye Salmon: AY-autosome fusion.
Canadian Journal of genetics and Cytology 20: 349-354.

THORGAARD, G.H. 1983
Chromosome set manipulation and sex control in fish.
In: Fish Physiology Vol. IXB 405-434 W.S.Hoar, D.J.Randall and E.M. Donaldson (eds).

TREWAVAS, E. 1981
Nomenclature of the tilapias of Southern Africa
Journal of Limnology Society of South Africa 7: p.42.

TREWAVAS, E. 1983
Tilapiine fishes of the genera Sarotherodon, Oreochromis and Danakilia.
British Meseum; Natural History p. 583.

TSOI, R.M. 1969
The effect of nitrosomethyl urea and dimethylsulphate on sperm of the rainbow trout (Salmo irideus Gibb) and the peled (Coregonus peled Gmel.) Dokl. Biol. Sci. USSR 189: 849-852. Cited by Donaldson and Hunter 1982.

UFODIKE, E.B.C., AND MADU, C.T. 1986
Effects of Methyltestosterone on food utilization and growth of Sarotherodon niloticus fry.
Bulletin of the Japanese Society of Scientific Fisheries. 52(11): 1919-1922.

UTTER, F.M., JOHNSON, O.W., THORGAARD, G.H., AND RABINOVITCH, P.S. 1983
Measurement and potential applications of induced triploids in Pacific Salmon.
Aquaculture 35: 125-135.

UWA, H. 1965
Gynogenetic haploid embryos of the Medaka (Oryzias latipes). Embryologia 9: 40-48

UYENO, T., AND MILLER, R.R. 1971
Multiple sex chromosomes in a Mexican cyprinodontid fish.
Nature 231: 452-453.

VAN DEN HURK, R., AND LAMBERT, J.G.D. 1982
Temperature and Steroid effects on gonadal sex differentiation in rainbow trout.
Proc. Int. Symp. Reprod. Physiol. Fish, pp 69-72.

Van DOORN, W. A., 1962
Geschlachtsbeinvloeding door temperatur.
Het. Aquarium, 32(9): 208-209 (in Harrington, 1967)

VALLOWE,H.H. 1957
Sexual differentiation in the teleost fish Xiphophorus hellerii, as modified by experimental treatment.
Biological Bulletin 111: 422-429.

VICTOROVSKII, R.M. 1970
Morphological traits of hybrids of Carp, (Cyprinus Carpio L) and Tench (Tinca tinca L). In V.S.Kipichnikov 1S

VILLALUZ, A.C., AND UNGGUI, A. 1983
Effects of temperature on behaviour, growth, development and survival in young milkfish, Chanos chanos (Forskal).
Aquaculture, 35: 321-330.

WARNER, R.R., ROBERTSON, D.R., AND LEIGH, E.G. 1975
Sex change and sexual selection.
Science 190: 633-638.

WELCOMME R. L. 1963
Notes on the present distribution and habits of the non-endemic species of Tilapia which have been introduced into Lake Victoria.
East African freshwater fisheries research organisation Annual Report p36-41.

WELCOMME, R.L. 1964a
The habits and habitat preferences of the young of the Lake Victoria Tilapia (Pisces, Cichlidae).
Rev. Zool. Bot. Afr. 70: 1-28.

WELCOMME, R.L. 1966
Recent changes in the stocks of Tilapia in Lake Victoria.
Nature 212: 52-54.

WELCOMME, R.L. 1967
Observations on the biology of introduced species of Tilapia in Lake Victoria.
Rev. Zool. Bot. Africa, 76: 249-279.

WHEELER, A., AND EASTON, K. 1978
Hybrids of Chub and Roach (Leuciscus cephalus and Rutilus rutilus) in English rivers.
Journal of Fish Biology 12: 167-171.

WHITEHEAD, P.J. 1960
Interspecific hybrids of Tilapia: T. nigra x T. zillii. Nature 187: 898.

WHITEHEAD, P.J.P. 1962
The relationship between Tilapia nigra and T. mossambica Peters in the eastern rivers of Kenya.
Proc. Zool. Soc. London. 138: 605-637.

WIBERG, U.H. 1983
Sex determination in the European eel Anguilla anguilla a hypothesis based on cyto-genetic results correlated with the findings of skewed sex ratios in eel culture ponds.
Cytogenet. Cell. Genet. 36(4): 589-598.

WINGE, O. 1922
A peculiar mode of inheritance and its cytological explanation. J. Genetics 12: 137-144

WINGE, O. 1932
The nature of sex chromosomes.
Proc. 6th Int. Congr. Genet. 1: 343-355.

WINGE, O. 1934
The experimental alteration of sex chromosomes into autosomes and vice versa, as illustrated by Lebistes.
Compt. Rend. Lab, Carlsberg. Ser. Physiol, 21: 1-49.

WITHLER, R. 1986
Genetic variation in flesh pigmentation of Chinook Salmon (Oncorhynchus Tshawytscha).
In: Proceedings of EIFAC/FAO symposium on Selection, hybridization and genetic engineering in aquaculture. Bordeaux, France.

WOHLFARTH, G.W., AND HULATA, G. 1981
Applied genetics of tilapias.
ICLARM Studies and review 6, Manila, Phillipines, 26 pp.

WOHLFARTH, G., HULATA, G., ROTHBARD, S., ITZKOWICH, J., AND HALEVY, A. 1983
Comparisons between interspecific tilapia hybrids for some production traits.
In L. Fishelson and Z. Yaron, (compilers). International symposium on Tilapia in Aquaculture.
Tel Aviv University, Tel Aviv, Israel.

WOIWODE, J.G. 1977
Sex reversal of Tilapia zilli by injestion of methyltestosterone.
Tech. Pap. Ser. Bur. Fish. Aquat. Resour. Phillipines. 1(3): 1-5.

YAMAMOTO, T. 1953
Artificially induced sex in genotypic males of the Medaka (Oryzias Latipes).
Journal of Experimental Zoology 123: 571-594.

YAMAMOTO, T. 1958
Artificial induction of functional sex-reversal in genotypic females of the Medaka (Oryzias latipes). J. Exp. Zool. 137: 227-262.

YAMAMOTO, T. 1959
The effects of estrone dosage level upon the percentage of sex-reversals in genetic male (XY) of the medaka Oryzias latipes. J. Exp. Zool. 141: 133-153.

YAMAMOTO, T. 1961
Progenies of sex-reversal females mated with sex-reversal males in the medaka, Oryzias latipes. Journal of Experimental Zoology, 146: 163-179.

YAMAMOTO, T. 1962
Hormonic factors affecting gonadal sex differentiation in fish.
Gen. Comp. Endocrin., Supple., 1: 341-345.

YAMAMOTO, T., AND KAJISHIMA, T. 1968
Sex hormone induction of sex reversal in the goldfish and evidence for male heterogamety.
Journal of Experimental Zoology 168: 215-222.

YAMAMOTO, T. 1969
Sex differentiation.
p117-175. In W.S.Hoar and D.J. Randall (eds).
Fish Physiology. Vol. 3. Academic Press. New York.

YAMAZAKI, F. 1976
Application of hormones in fish culture.
Journal of Fisheries Research Board of Canada.
33: 948-958.

YAMAZAKI, F. 1983
Sex control and manipulation in fish.
Aquaculture, 33: 329-354.

YASHOUV, A., AND CHERVINSKY, J. 1959
Hybrids of Tilapia nilotica x T. galilea.
Nature 184: 1739.

YASHOUV, A., AND ECKSTEIN, B. 1965
Regulation and control of spawning in Tilapia.
Bamidgeh 17: 66-67.

YOSHIKAWA, H., AND OGURI, M. 1978
Sex differentiation in a Cichlid, Tilapia zillii.
Bulletin of the Jap. Soc. Sci. Fish 44(4): 313-318.

APPENDIX 1

Procedure for histological processing of tissue

Treatment	Duration (Hrs)
70% Methylated Spirits	1
80% Methylated Spirits	2
8% Phenol in Methylated spirits I	3
8% " " II	2
8% " " III	2
Absolute Alcohol I	2
Absolute Alcohol II	1
Chloroform I	1
Wax I	2
Wax II	2
Wax III	1
	20 Hrs.

APPENDIX II

Procedure for staining with Haematoxylin and Eosin (H + E).

Treatment	Duration
Xylene	5 minutes
Absolute alcohol	2 "
Methylated spirits	1½ "
wash in tap water	1 minutes
Mayer's Haematoxylim	5 minutes
wash in tap water	1 minute
1% Acid alcohol	3 quick dips
Wash in tap water	1 minute
Scott's Tap Water	30 seconds
Wash with tap water	1 minute
Eosin	5 minute
Wash with tap water	1 minute
Methylated spirits	30 second
Absolute alcohol I	2 minutes
Absolute alcohol II	1½ minutes
Xylene	5 minutes

Mount with appropriate mounting fluid.

Appendix 3.

Sample list of species with detected sex chromosome hetermorphism.

1. Male heterogamety (XY system)

X

	reference
Bathylagus species	Chen 1969
Carassius auratus	Yamamoto 1969
Galaxias Platei	Campos 1972
Morgunda obscura	Nogusa 1955
Oryzias latipes	Yamamoto 1961
Orchor hynchus kisutch	Hunter et al 1982
Onchorhynchus nerka	Thorgaard 1977
Oreochramis niloticus	Jalabert et al 1971
Oreochromis mossambicus	Chen 1969
Poecilia reticulta	Winge 1922
Salmo gairdneri	Thorgoard 1977
Xiphophorus variatus	Kosswig 1935
O. Spilurous X	
O. Nigra	

ii Female heterogamety (ZW system)

Anguilla anquilla	Park & Grimm, 1981
Anguilla rostrata	Park & Grimm, 1981
Gambusia affaris	Ebeiling and Chen 1970
Oreochromis hornorum	Chen 1969
Oreochromis macrochir	Jalabert et al 1971
O. aureus	
O. variabilis	

iii. Female heterogamety (ZO system)

Colisa lalius	Price 1984
Sternoptyx disphana	Chen 1969
Lampanyctus ritteri	Chen 1969
Porvitux ingens	Chen 1969

Xiphophorus maculatus is the only recorded species with both male and female heterogamety (Kalliman, 1965).