Genetic and environmental factors affecting growth and sex ratio in the Nile tilapia :Oreochromis niloticus L.

Abucay, Jose S

GENETIC AND ENVIRONMENTAL FACTORS AFFECTING GROWTH AND SEX RATIO IN THE NILE TILAPIA, *Oreochromis niloticus* L.

JOSE S. ABUCAY

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DECEMBER 1997

SCHOOL OF BIOLOGICAL SCIENCES
UNIVERSITY OF WALES SWANSEA
WALES, UK

DECLARATION

This work has not previously been accepted for any degree and is not being concurrently submitted in candidature for any degree.

Signed _____ (Candidate)

Date 17 DECEMBER 1997

STATEMENT 1

This thesis is the result of my own investigations, except where otherwise stated.

Other sources are acknowledged by explicit references. A reference list is appended.

Signed _____ (Candidate)

Date 17 DECEMBER 1997

STATEMENT 2

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organizations.

Signed _____ (Candidate)

Date 17 DECEMBER 1997

ACKNOWLEDGEMENTS

The chance to pursue and complete a Ph.D. will never be possible without the support and assistance from a number of people. I should like to use this opportunity to say...

"THANK YOU VERY MUCH"

To the British Department for International Development (formerly Overseas Development Administration) through the University of Wales Swansea, for its research grants (R 6058 and R 6938) to Dr. Graham C. Mair and Professor David O.F. Skibinski, for providing my financial support.

To Professors David O.F. Skibinski and John A. Beardmore, my very kind supervisors, for their confidence in giving me the opportunity to do Ph.D.

To Dr. Graham C. Mair, my local supervisor and my "mentor", I can say if not because of him I may not be what and where I am now.

To Drs. Tereso A. Abella and Ruben C. Sevilleja, my teacher and my guidance, for their untiring support and encouragement.

To the technical staff and student of UWS; Eric Roderick, for his unselfish assistance and for making us feel like at home while at Swansea; Cath Gallager and Dr. McConnell, for their patience in assisting me while trying to send and unzipping files; Farhad, for the interesting discussion on politics (not genetics), Dr. Quesada, Dot, Chris, Jean, Edson and all the others I just know by face but in one way or another have extended me some assistance.

To my fellow staff (former and present) of the GMIT project; Dr. Josie C. Mair, Dr. Maryann G. Abiado, Lolit, Almira, Leah, Leo, Arnel, Jack, Zaldy, Anna, Ed T., Ed C., Elito, Aurelio, Dong-Dong, Mang Iko, Mang Dong, Mang Remy, Nick, Alex R., Alex P., Balong and Boy, for extending me extra ordinary assistance during the conduct of the studies presented in this thesis.

To the faculty and staff of CIF/FAC: Drs. Shen Cagauan, Med Bolivar and Ed Lopez; Professors Tony Circa, Dory Bartolome, Pol Yambot, Danny Monje and Bongbee Vera Cruz; Precy Monje, Janet Saturno, Gina, Ruben and Mang Caloy.

To Drs. A. Toguyeni and J.-F. Baroiller, for the two sets of thermoregulator which were instrumental in the conduct of some of the experiments described in Chapters three and six.

To my external examiner, Dr. Jean-Francois Baroiller and internal examiner, Dr. Richard Tilney-Bassett, for their constructive criticism and for making my oral examination very enjoyable.

To my loving wife, Aida and our lovely kids, for their patience and spiritual support while I am away.

Above all, Our Almighty, for all the graces and blessings.

ABSTRACT

The experiments described in this thesis were performed to study and evaluate a number of relevant issues associated with the YY male technology. YY males are expected to produce all-male progeny. However, a small proportion of females have usually been observed in the progeny of XX♀ x YY♂ crosses. A series of experiments evaluating the effect of temperature and salinity on sex ratio in sexually undifferentiated putative all-female, all-male and YY males were conducted. High temperature (36°C) was observed to affect the sex ratio towards male and towards female in different circumstances. Purebred YY males (Egypt-Swansea strain) were found to be sensitive to high temperature. No effect of salinity on sex ratio was observed.

Improving the growth of genetically male tilapia (GMT) through crossbreeding has been initiated. An initial study showed the hybrids had better growth than purebred progeny (Abucay, 1996). A follow up study evaluating the growth and sex ratio of progeny in a 2 x 2 diallele cross of Egypt-Swansea and Egypt-ICLARM strains using YY males as broodstock was performed. A parallel study was also conducted to determine the capacity of YY males in the Egypt-Swansea strain to sire all male progeny in crosses with females from a number of strains in *O. niloticus*. In the diallele cross, the reciprocal hybrids had a higher mean growth that the smaller parent and parental mean but not higher than the larger parent. In the parallel study, YY males were observed to be capable of siring high proportion of male progeny (93.12 to 100%) in the inter strain crosses.

The growth of GMT, genetically female tilapia (GFT) and mixed-sex tilapia (MST) was evaluated; (i) during a period of androgen hormone treatment designed to alter the phenotypic sex, (ii) during the post-treatment period, and (iii) during the late life stages in monosex and mixed-sex culture environments. GMT was observed to exhibit faster growth at the early ages. No anabolic effect of the hormone was observed during the period of hormone treatment and post-treatment. The sex-reversed genetic females appeared to grow better than the genetic males during the late life stages. The culture of all females appears to have great potential.

A series of experiments were performed to evaluate factors affecting sex reversal. The effect of not changing the water and increasing the temperature during hormone treatment was evaluated. The possible leaching out of hormone from hormone-treated feed was also investigated. Some of the sexually undifferentiated fry reared in water previously used for hormone treatment were sex-reversed indicating that hormone residues had accumulated during previous hormone treatment. Increasing the temperature during hormone treatment was observed to improve the efficiency of sex reversal, particularly feminisation. Results show that hormone leaches from hormone-treated feed suggesting that the observed accumulation of hormone residues could be partly due to leacheates.

TABLE OF CONTENTS

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Biology and Distribution

Among cultured fish species, the tilapias are probably the most widely distributed geographically. Tilapia are native exclusively to the African continent (excluding Madagascar) and from the Jordan valley and coastal rivers of Palestine (Philippart and Ruwet, 1982), but through human intervention they have been distributed far outside their natural zones of distribution. The earliest known international transfers of these species were with *Oreochromis mossambicus* (Peters) (Philippart and Ruwet, 1982; Welcomme, 1988). Since its appearance in Java in the 1930s, possibly through the escape of aquarium fish, the species has spread through deliberate introduction, diffusion or escape of pond fish and has now achieved pan-tropical status, spread worldwide to over 100 countries (Balarin and Hatton, 1979) and is cultured in over 30 developing countries (Guerrero, 1985).

Tilapias are warmwater fishes which are widely cultured for food in many countries of the Asia-Pacific region. At present, there are approximately 80 species of tilapia known to exist (Schoenen, 1982). Originally, the tilapias were regarded as members of a single genus, *Tilapiine*, within the family Cichlidae (Trewavas, 1966). However, after a much more thorough investigation, the genus *Tilapiine* was reclassified into three distinct genera based on their mode of reproduction (Table 1.1). Initially, the tilapias were only divided into two genera, *Tilapia* and *Sarotherodon*, the former group being substrate spawners and the latter group being mouthbrooders, but later Trewavas (1982) suggested that the

mouthbrooder group should be further reclassified; *Sarotherodon* for the paternal mouthbrooders and *Oreochromis* for the maternal mouthbrooders. However, the original name tilapia has been retained as the common name for all of these species.

Table 1.1. Reproductive characteristics of the *Tilapiine* genera and the main species of importance to aquaculture (table was adopted from Macintosh and Little, 1995).

Genus	Mode of reproduction	Important species in aquaculture
Tilapia	Substrate-spawners (guarded nests)	*T. zilli* *T. rendalli*
Sarotherodon	Paternal or bi-parental mouthbrooders	*S. galilaeus*
Oreochromis	Maternal mouthbrooders	*O. niloticus* *O. mossambicus* *O. aureus* *O. urolepsis-hornorum* *O. andersoni* *O. macrochir* *O. spilurus*

In the Philippines, the culture of tilapia began with the introduction of the Mozambique tilapia, *O. mossambicus*, from Thailand in 1950 (Guerrero, 1985). However, due to its undesirable characteristics such as slow growth, early sexual maturation and rapid reproduction, the species which was once considered as a "wonder fish" lost its appeal to the fish farmers. To refresh the interest in the culture of tilapia, in the 1970s further introductions of other species were made. Overall, there were four different species of tilapia introduced into the Philippines (see Table 1.2). However, the introduction of the Nile tilapia, *O. niloticus,* which was known as the "giant tilapia" renewed the interest of the fish farmers. Since 1970s several strains of this species were introduced and more recently the implementation of the Genetic Manipulations for Improved Tilapia (GMIT) and the Genetic Improvement of Farmed Tilapia (GIFT) projects have paved the way for introductions of more strains from different sources and origins including several sub-species of *O. niloticus* (Table 1.3).

Table 1.2. Physical description of tilapia species in the Philippines (table was adopted from Guerrero, 1987).

Species	Body coloration	Fin appearance
Blue tilapia (*Tilapia aurea*) (*Oreochromis aureus*)	Bluish; male with dark throat when actively breeding	White spots on caudal, dorsal and anal fins
Mozambique tilapia (*Tilapia mossambica*) (*Oreochromis mossambicus*)	Black to gray; male with yellow or white throat when actively breeding	Bright red margin of dorsal and caudal fins
Nile tilapia (*Tilapia nilotica*) (*Oreochromis niloticus*)	White to bluish; male with reddish throat when actively breeding	Prominent bars on caudal fin; white stripes on dorsal and anal fins
Zill's tilapia (*Tilapia zilli*)	Yellowish-green male with reddish breast when actively breeding	Adults have prominent dark spots at dorsal fins; yellowish-green spots on caudal fins

Note: The old and new scientific name of the blue, mozambique and nile tilapia were given here to guide readers who are still using the old classification.

Table 1.3. Strains of *O. niloticus* introduced into the Philippines (Bolivar, 1993; Capili, 1995).

Strain (common name)	Original country of origin	Date introduced	Agency
Israel	Uganda	1972	LLDA [a]
Thailand	Egypt	1972	BFAR [b]
Israel	Ghana	1977	CLSU [c]
Singapore	Ghana	1977	BFAR [b]
Singapore	Ghana	1979	SEAFDEC [d]
Israel	Ghana	1979	CLSU/ICLARM [e]
Israel	Ghana	1982	PS [f]
Egypt	Egypt	1988	ICLARM [e]
Ghana	Ghana	1988	ICLARM [e]
Senegal	Senegal	1988	ICLARM [e]
Egypt	Egypt	1989	ICLARM [e]
Kenya	Kenya	1989	ICLARM [e]
Swansea	Egypt	1989	CLSU/GMIT [g]
Baringo	Kenya (Baobab Farm)	1990	CLSU/GMIT [g]
AIT	Egypt	1991	CLSU/GMIT [g]
Baobab	Kenya (Baobab Farm)	1993	CLSU/GMIT [g]
Stirling	Egypt (Lake Manzalla)	1993	CLSU/GMIT [g]
Vulcani	Kenya (Crater Lake A)	1993	CLSU/GMIT [g]

[a] Laguna Lake Development Authority
[b] Bureau of Fisheries and Aquatic Resources
[c] Central Luzon State University
[d] Southeast Asian Fisheries Development Center
[e] International center for Living Aquatic Resources Management
[f] Private Sector
[g] Genetic Manipulation for Improved Tilapia Project

1.2 The Status of Tilapia in Aquaculture

Among the many cultured fish, particularly freshwater species, the tilapias are rapidly emerging as one of the most important. Aside from being one of the main sources of animal protein for people in the poor and developing countries, particularly those in Asia and Africa, it is also now being cultured in developed countries such as the United States. In 1993, the world production for tilapia was estimated at 550,600 metric tons and valued at 772.3 million US Dollars (FAO, 1995). Although the quantity is still much lower than the estimated world production for carps (7,638,001 metric tons), the popularity of tilapia among producers and consumers is steadily increasing as evidenced by the increasing trend in production. From 1984 to 1993, the world tilapia production increased by 195.27% (see Figure 1.1).

In the United States, where it once criticized as "trash fish" and never expected to sell, in 1992, tilapia confounded its critics when imports doubled and there was an increase in domestic production (Anonymous, 1993). The results of a survey by the American Tilapia Association (ATA) in 1992 revealed a domestic production of 4090 metric tons of live fish and imports estimated at 5000-7000 metric tons (Anonymous, 1993).

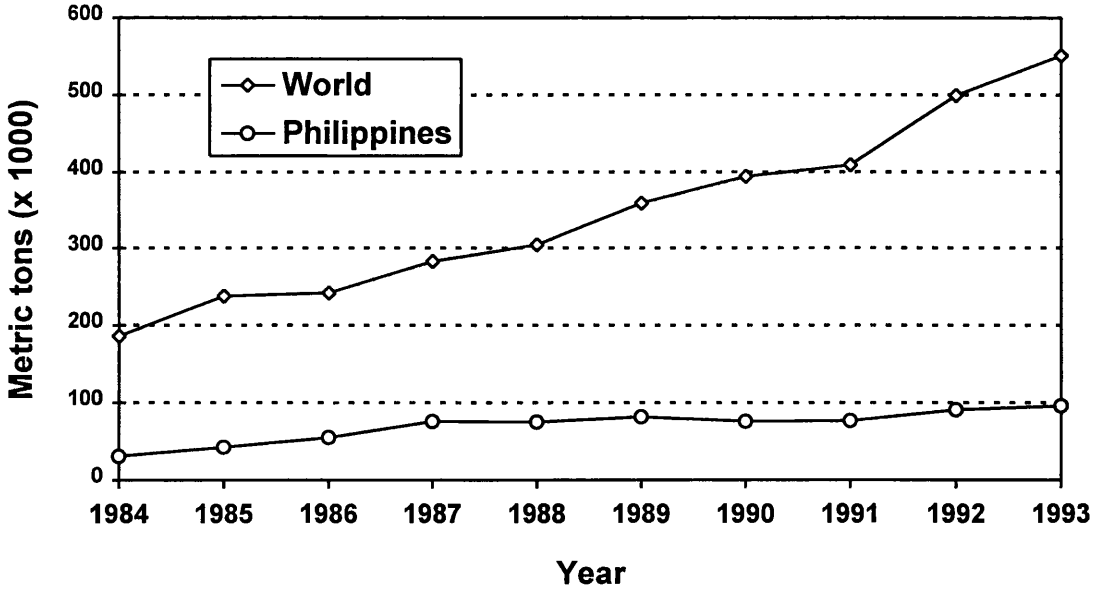


Figure 1.1. Annual World and Philippine tilapia production for the last ten years: 1984-93 (FAO, 1995).

In the Philippines, the popularity of tilapia increased tremendously over the last decade where inland aquaculture was almost completely devoted to tilapia farming. The culture of tilapia has already evolved into a very lucrative investment. In terms of production the Tilapias followed closely the Milkfish which has been the traditional cultured species. In 1993, the annual production of tilapia in the country was estimated at 96,339 metric tons (FAO, 1995). From 1984 to 1993, the total annual tilapia production increased by 211% (see Figure 1.1). Among the countries producing tilapia, the Philippines has the second

highest production after China, and the highest production per capita. The estimated annual production is likely to further increase in the coming years not least because of the continuously increasing demand. The increased attractiveness of tilapia to consumers is due to a number of factors: they can be sold very fresh, even alive; they are well suited to *Filipino* cooking styles (frying and broiling) and are competitively priced compared to marine fish prices which have increased as the catch has leveled off and the operating costs increased (Smith and Pullin, 1984). Likewise the increasing consumer acceptance of tilapia as a food fish is attributed to its relatively cheaper price compared to other fish and animal protein sources (Bimbao and Ahmed, 1990).

1.3 Prevailing Problems in Tilapia Culture

The widespread culture of tilapia is primarily due to their many excellent features such as fast growth, excellent taste, tolerance to extreme culture environments, ability to thrive on low cost feeds and ease of breeding in captivity (Bardach *et al.*, 1972; Philippart and Ruwet, 1982). Tilapia can be grown in different culture systems such as ponds (Hepher and Pruginin, 1982), cages (Coche, 1982) and tanks (Balarin and Haller, 1983). Because they are easy to grow, the culture of tilapia has been promoted in areas where simple systems of culturing fish are needed for inexperienced rural producers such as those in rural Latin America (Lovshin and Pretto, 1983). Likewise, the tilapias have been one of the subjects for integration with other related agricultural and aquacultural

culture systems. For example, the tilapias were found to be effective in clearing irrigation ponds of aquatic weeds and at the same time providing high quality protein (Bondari *et al.*, 1983). It has also been one of the species used for different integrated culture systems such as rice and fish (Cruz, 1980; Little and Muir, 1987), pig and fish (Cruz and Shehadeh, 1980; Little and Muir, 1987), and duck and fish (Cruz and Shehadeh, 1980; Little and Muir, 1987). Also because of their simple and efficient feeding habit the tilapias can be raised together with other cultured aquatic species such as carps (Sarig and Arieli, 1980; Sarig, 1983), catfish (Behrends *et al.*, 1986; Morison *et al.*, 1995), grouper (Manzano, 1989), freshwater prawn (Behrends *et al.*, 1986) and tiger shrimp (Samonte, 1991). Because of these culture flexibilities the tilapias have been regarded as the "everyman's fish" or the "aquatic chicken" (Pullin, 1985).

However, despite their versatility there are also problems constraining their culture which if not checked or prevented cause a substantial reduction in yield potential. The potential problems are outlined next.

1.3.1 Early maturity and overcrowding

Tilapia have the capacity to breed easily in captivity, maturing early and breeding at a small size which results in overcrowding especially in culture conditions conducive to spawning. Also, breeding at a small size diverts energy from growth into reproduction and the recruits compete with the stocked fish for

available space and food which results in harvests that include 10 to 20% of small fish with little or no marketable value (Mair and Little, 1991). Further, some species particularly those within the genus *Oreochromis* have a very distinct sexual dimorphism where the males grow faster and bigger than the females. Dunham (1990) noted that male *O. niloticus* grow 2.2 and 2.5 times faster than females when grown in cages separately and communally, respectively. This dimorphism increases further the problem due to the reduction in marketability of the stocked fish because of size variability.

1.3.2 Inbreeding

Another problem facing the culture of tilapia is the possible reduction in growth due to inbreeding. Inbreeding is the mating of related individuals which results in reduction in genetic variability and a corresponding increase in genetic homozygosity. It has long been known from studies with domestic animals that the loss of genetic variation generally has harmful effects on development, survival and growth rate (Falconer and Mackay, 1996). A reduction in growth rate as a result of inbreeding has been observed in tilapia (Kincaid, 1983; Hulata *et al.*, 1988; Ch'ang, 1971 cited in Tave, 1988).

The reduction of genetic variability through inbreeding seems to be common in hatcheries where the life cycle of the broodstock used are either closed within the hatchery and/or the effective population size (N_e) is small. A

study comparing a hatchery stock founded 16 years ago to present day wild stock of a lake dwelling brown trout, *Salmo trutta*, using starch gel electrophoresis, Vuorinen (1984) found that the overall heterozygosity of the hatchery stock was reduced by a third from 6.2 to 4.2%. Similar observations were made by Sbordoni *et al.* (1986) in a comparison of a founder stock and five subsequent hatchery generations of *Penaeus japonicus*. The authors noted that there is a constant reduction in levels of allozyme polymorphism and the average heterozygosity decreased from 0.102 to 0.039 over 5 generations.

Similar phenomena might also be occurring in some tilapia hatcheries in the Philippines. For example, in a survey of tilapia hatcheries located in Laguna and Rizal Provinces in the Philippines, Yater and Smith (1985) found that the majority of the hatchery operators interviewed obtained their initial broodstock from the Bureau of Fisheries and Aquatic Resources (BFAR) or other private hatcheries, however, 75% used their own fingerlings as replacement for their old broodstock.

Although the surveys were done only in selected locations, it may well be the case that the same process is also happening in hatcheries in other locations. With these kind of practices the deterioration of broodstock and consequently their progeny, which will be stocked for grow-out, is expected to continue if no action is taken to correct the problem. These problems are likely to happen unconsciously in hatcheries where the operator does not have enough knowledge or understanding on the nature of fish breeding and subsequently a

lack of any good method of monitoring and maintaining an effective number of broodstock (Tave, 1984; Smitherman and Tave, 1987).

1.3.3 Introgressive contamination

The growth performance of cultured species could be also affected by introgression with genes from a less desirable species. The capacity of tilapia species to interbreed easily also poses a serious problem. This problem is likely to arise in hatcheries in areas with significant feral populations and intrusions from the wild, which are common, especially if it has no facilities for preventing their entrance. For example, the results of a genetic survey of tilapia samples (all assumed to be *O. niloticus*) collected from government and private farms in different locations in the Philippines, using gel electrophoresis, showed that the Nile tilapia were introgressed with the less desirable *O. mossambicus* (Taniguchi *et al.*, 1985; Macaranas *et al.*, 1986)

On the other hand, genetic introgression could be also beneficial to the cultured species if already undergoing inbreeding and there is a need for the introduction of new genes. Likewise, it is also of benefit if the new genes come from a species or strain with a better growth performance than the cultured species.

1.4 Approaches to Solve the Problems in Tilapia Culture

1.4.1 Production of all-male fish

The elimination of the problem of early breeding and overcrowding by stocking monosex fish, particularly all-male, ensures higher yield due to the combined benefits of controlling overpopulation and exploiting the faster growth of males (Mair and Little, 1991). There are many ways in which monosex can be produced but their application has certain limitations (see Mair and Little, 1991). The different approaches are discussed briefly.

1.4.1.1 Manual sexing

Manual sexing involves the separation of sexes by ocular inspection of their genital papillae and retaining the sex of interest. This method is the cheapest and simplest to use because it does not require any special facility although it requires the expertise to do it. However, the application of this method might be appropriate only for small-scale culture which requires only a few hundred fish for stocking but may no longer be appropriate for commercial culture involving the stocking of thousands of fish. Its limitation is mainly due to its being laborious and the obvious wastage of the unselected females. Also, it needs an expert technician to do the work to ensure accurate sexing. However,

even an expert technician can usually achieve only 80 to 90% accuracy (Bardach *et al.*, 1972).

1.4.1.2 Interspecific hybridisation

Monosex fish can be also produced by crossing different species of tilapia which are known to produce all or nearly all-male progeny. In fish, there are nine known systems of sex determination (Tave, 1986). Among these systems, those with homogametic (XX) female and heterogametic (XY) male (e.g. *O. niloticus*, *O. mossambicus*), and those with heterogametic (WZ) female and homogametic (ZZ) male (e.g. *0. aureus*, *O. hornorum*) are the most common. In XX and XY system the presence of the Y chromosome determine the male sex while in WZ and ZZ the presence of the W chromosome determine the female sex. However, when crossing an XX♀ and a ZZ♂, the presence of the Z chromosome determine the male sex so that the F_1 progenies are expected to have the XZ genotype and be males. A good example is the cross of female *O. niloticus* and male *O. aureus* which is the commonly used species-cross combination for this system (Wohlfarth, 1994).

The potential of interspecific hybridisation to produce all-male progeny was accidentally discovered by Hickling (1960) when he attempted to produce fast growth sterile "mule" tilapia to solve the problem of overcrowding. However, he found out later that the fish he produced were not sterile as he wanted but

mostly fertile males. In this particular case, Chen (1969) concluded that Hickling had actually crossed tilapia species with XX/XY and WZ/ZZ sex determining mechanisms. His experience initiated the widespread use of hybridisation to produce all-male progeny for grow-out.

In the Philippines, hybridisation has been also tried with the introduction of *O. aureus* and the results from experimental evaluation were promising (Bautista *et al.*, 1988). However, the use of this approach has not been well exploited for commercial use due to problems associated with differences in the adaptability and breeding of the species involved which results in low production of hybrid progeny (Wohlfarth, 1994; Hulata *et al.*, 1985). Also, *O. aureus* is more adapted to cooler temperatures while *O. niloticus* is best suited for Philippine conditions.

In Israel, the commercial-scale hybridisation to produce monosex progeny has been implemented although its potential has not been fully exploited yet. Wohlfarth (1994) reviewed the major reasons for the slow adoption of the method. Among the reasons are; the inconsistency of the sex ratio of the hybrid progeny, slow growth, low fecundity and poorer consumer acceptance due to unappealing coloration.

1.4.1.3 Sex reversal

Monosex progeny can also be produced by manipulating the sex of the fish prior to sexual differentiation. In most animals, the genetic sex is already

determined on fertilisation and usually the phenotypic sex is already developed at birth. However, in the case of the tilapia the differentiation of gonads either into ovaries or into testes becomes histologically discernible only at about 20 days of age from hatching (Nakamura and Takahashi, 1973). During this particular stage, the sex of the fish can be directed to the sex of interest by the application of exogenous hormones (Nakamura and Takahashi, 1973).

The effectiveness of hormones, most particularly the use of androgens to produce all-male progeny has been proven in a number of studies (Guerrero, 1975; Guerrero, 1976; Buddle, 1984b; Varadaraj and Pandian, 1987; Lone and Ridha, 1993; Watanabe *et al.*, 1993). Likewise, the use of androgen is not only capable of directing sex but is also observed to enhance growth in *O. mossambicus* (Kuwaye *et al.*, 1993) although the same phenomenon was not observed in *O. niloticus* in a similar study (Green and Teichert-Coddington, 1994).

The commercial production of sex-reversed all-male progeny using simple facilities have been proven feasible (Guerrero and Guerrero, 1988; Popma and Green, 1990; Buddle, 1984a). Despite these facts, the use of exogenous hormones is not yet a universal technique for producing all-male progeny although has been adopted and practiced in some countries. The aim of producing 100% monosex has not been consistently achieved and this single reason has slowed its adoption for commercial use. The success of sex reversal depends on many factors which are required to be controlled during the hormone application. Among these factors are: age and size of the fry, quantity and

quality of the hormone and feed use, and the optimum treatment environment (Mair and Little, 1991). The failure to implement correct procedures will result in a low percentage of sex-reversed fish. Likewise, there is the social issue concerning the possible adverse effect of eating sex-reversed fish on human health. Although there were already studies conducted which show that treated fish were already cleared of hormone a few days after termination of treatment (Johnstone *et al.*, 1983; Rothbard *et al.*, 1990; Curtis *et al.*, 1991) there is still a potential risk to the persons carrying out the hormone treatment. In commercial production of sex-reversed fish where the hormone-treatment is being done in suspended nets in earthen ponds (Popma and Green, 1990), it may be possible that hormone or hormone metabolites are dissolved in the surrounding water or absorbed by the nets. In this conditions, the workers is likely of being exposed to hormone.

1.4.1.4 Production of sterile fish

Recently the potential of producing sterile fish for stocking has been given more attention. Sterilisation is one alternative to prevent unwanted reproduction in ponds. This can potentially be accomplished through surgery, immunology, radiation, chemical treatments, hybridisation or ploidy manipulation (Dunham, 1990). Dunham (1990) mentions that the culture of sterile fish can be advantageous for several reasons such as the potential increase in growth, increased carcass yield, and increased flesh quality in addition to its desirability

for species such as tilapia where excess reproduction may occur in production ponds.

In tilapia, a great deal of experimental work has been done on the production of triploid fish (Mair, 1988; Mair, 1993; Hussain, 1992; Garcia-Abiado, 1995). Triploids are most commonly produced directly by the induction of second polar body retention during the second meiotic division of newly fertilised eggs or might be generated indirectly by crossing of tetraploid and diploid individuals. Triploid individuals are expected to be sterile because of a failure of homologous chromosomes to synapse correctly during the first meiotic division (Hussain, 1992). Such sterility has the potential for increasing fish production because metabolic energy for gonad development could be used for increasing growth and life expectancy instead (Hussain, 1992).

However, the potential of using triploidy in tilapia has yet to be realised. Although the production of sterile triploids has been proven possible under laboratory conditions using pressure and heat shock (Hussain, 1992; Garcia-Abiado, 1995), this may not yet be practical for commercial culture. The necessity to induce triploidy following artificial fertilisation would prevent the production of great numbers of triploids at any one time for stocking. Mass production of triploids may be achieved by crossing tetraploid with diploid individuals giving all-triploid progeny. However, there are still technical problems facing this technique (Mair, 1993; Purdom, 1986) and the perfection of the production of viable tetraploids has yet to be achieved. Don and Avtalion (1990) were able to produce two 4n females but these were observed to be smaller than

their diploid siblings and did not show any sexual behaviour after 18 months of age. Similarly, because of possible difference in developmental rate among the fry during cold or heat treatment to produce triploids some may still be diploid and are likely to be reproductively viable (Dr. Baroiller, pers. com.).

1.4.1.5 Genetic manipulation of sex ratio

On the assumption that the species of interest exhibits a monofactorial sex determining mechanism it is possible to produce a monosex, particularly all-male progeny, by combining sex reversal and breeding. There are two ways in which this objective can be achieve depending on the nature of the sex determining mechanism. The two approaches are discussed briefly.

Production of ZZ females. In species which have a WZ/ZZ sex determining mechanism wherein the females have WZ while the males have ZZ sex chromosomes, the development of females with ZZ sex genotype could lead to all-ZZ progeny which are males when ZZ females crossed to normal males with similar ZZ sex chromosomes (Dunham, 1990). The development of ZZ females (pseudofemales) can be achieved by treating normal mixed-sex progeny with estrogen following the usual method of sex reversal (Jensen and Shelton, 1979; Melard, 1995). The "pseudofemales" can then be identified through progeny testing by mating several females from the treated group (hoping to include potential ZZ females) to normal ZZ males. Those females that produce

nearly all- or all-male progeny are ZZ females. The identified ZZ females are then crossed again to normal males and their progeny which are genetically all ZZ are treated with estrogen to mass produce F_2 ZZ females.

In Israel, this technique has been adopted to produce all-male progeny in *O. aureus.* Although there are techniques to produce all-male progeny such as sex reversal and hybridisation, the culture of pure all-male *O. aureus* is preferred during winter months because of the their higher cold tolerance. The results of an experiment to determine the potential of this technique show that ZZ females are capable of producing 98 to 100% male populations in commercial production trials (Lahav, 1993).

However, this system is not yet feasible on a commercial scale for *O. aureus* because of the small numbers of functionally sex-reversed ZZ individuals - most of the estrogen-treated ZZ males were only partially sex-reversed and developed abnormal genitalia and/or ovotestes (Dunham,1990). Desprez *et al.* (1995) also observed that the percentage of non-spawners was higher in ZZ females (40%) compared to the normal females (20%). In a commercial production of all male progeny, the apparent decrease in the spawning capacity of the ZZ females could affect production.

Similarly, the sex ratio of the progeny from potential ZZ females is not always 100% as expected. In large scale production of high percentage male progeny using F_2 ZZ females, Desprez *et al.* (1995) observed a sex ratio ranging from 90.7 to 91.0%. The success of the approach to produce all male progeny

seems to depend very much on the quality and purity of the broodstock being used. In intrastrain matings in *O. aureus*, Mair *et al.* (1991b) observed a highly heterogeneous sex ratio with an overall deficit of males (41.4%). The deviation from the expected 1:1 ratio could be the main reason for the failure to produce the expected sex ratio of progeny from ZZ females.

Production of YY males. Recently, a great deal of research work has focused on the elucidation of sex determining mechanism in fishes. In the University of Wales Swansea, the mechanism of sex determination in tilapia has been studied extensively. In these studies, Penman *et al.* (1987), Shah (1988) and Mair *et al.* (1991a) observed that the strain of *O. niloticus* which they used in most of their experiments exhibited a predominantly monofactorial sex determination mechanism with homogametic female (XX) and heterogametic male (XY). During their attempt to further investigate the sex determining mechanism in tilapia, Mair (1988) and Scott *et al.* (1989) were able to produce and identify a few YY males. Based from this finding a model (Figure 1.2) for the large-scale production of YY males was proposed (Mair, 1988, Scott *et al.*, 1989, Mair and Little, 1991). In tilapia, it was found that those males with a YY genotype are viable and have the capacity to sire all-male progeny (Mair, 1988; Scott *et al.*, 1989; Varadaraj and Pandian, 1989a).

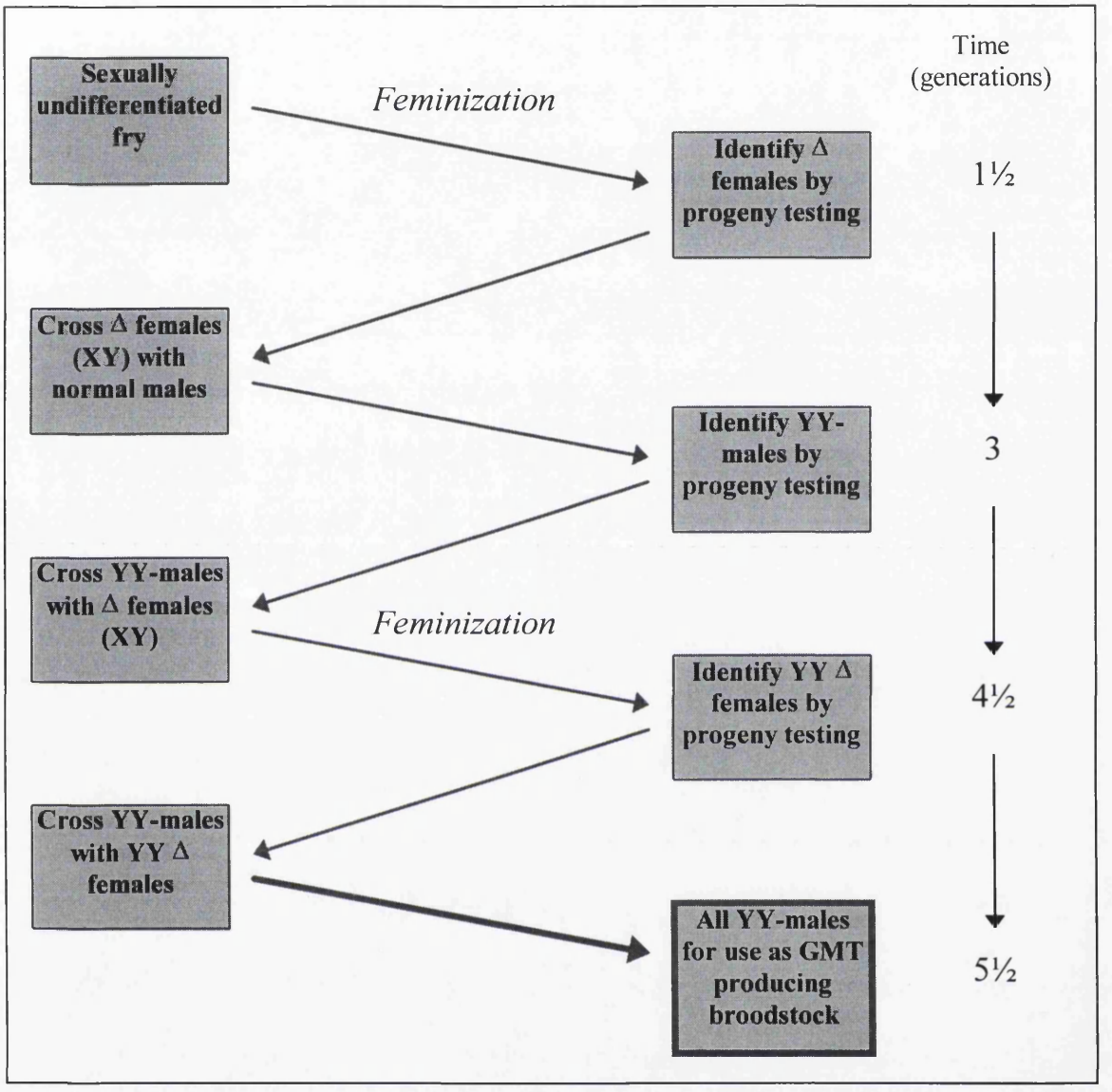


Figure 1.2. Schematic diagram depicting the model for large scale production of monosex male tilapia (figure was adopted from Mair and Little, 1991).

The development of this technology involves a series of stages of feminization and progeny testing (see Mair *et al.*, 1997a). These different stages are discussed briefly as follows;

Stage 1 - The first stage involves the feminisation of progenies from the crossing of normal males and females, with the objective of feminising some or all of the genetically males (i.e. those with XY genotypes that were destined to develop as males). The method of feminisation is similar to that used for androgen sex reversal to male, except that a female hormone (oestrogen) is used. To check for the success of feminisation, part of the broods to be treated should be set aside to serve as control prior to hormone treatment. After the hormone treatment, the control and treated fish are then grown to breeder size. If the percentage of females in the treated group is higher than in control, it means that there are feminised genetic males (Δ♀ - XY) among the treated fish.

Stage 2 - This stage involves the crossing of the previously identified Δ♀ (XY) with a normal XY male (effectively crossing two genetic males). However, to avoid inbreeding it is recommended that each of the two fish genotypes (XY♀ and XY♂) should come from different family. The expected sex ratio of the progeny from this cross is 3 males : 1 female. One third of these males are expected to have a YY genotype and the remaining two thirds should be normal XY males. YY males cannot be distinguished from normal XY males so the identification of males with a YY genotype must be done by progeny

testing. A number of males are crossed to normal females (XX). The sex ratio of the progeny of the normal males with XY genotype should be close to 1:1 while the males with a YY genotype will produce all- or nearly all-male progeny.

Stage 3 - This stage involves the crossing of YY males with Δ♀♀ (XY) (see earlier recommendation). The progeny from these crosses should be all-male, and one half of them are expected to have the YY genotype. At this stage, a second step feminisation is done to the progeny of these XYΔ♀ x YY♂ crosses with the objective of feminising males with YY genotype to produce YY females. The procedure for progeny testing to identify YY females is similar to that used for identifying XYΔ♀. The sex ratio of the progeny of sex reversed YY females (Δ♀♀ YY) crossed to Δ♂ (XX) should be all- or nearly all-males whilst sex reversed XY-females (Δ♀♀ XY) should produce a progeny with a sex ratio close to 1:1.

Stage 4 - Once YY males and YY females have been identified, large-scale production of YY males can now be done. YY males and YY females are crossed to produce all YY male progeny. The YY males can then be used in crosses with normal females to mass produce all- or nearly all-male progeny (known as genetically male tilapia - GMT) for grow-out. YY males can be perpetuated by occasionally feminising some YY progeny to produce the next generation of YY females.

There are advantages of the YY male technology over the other techniques for producing all-male fish. First, it does not require the direct use of hormone to the progeny to be used for grow-out and, second, the results of the on-station (Mair *et al.*, 1995) and on-farm (Mair *et al.*, 1997b) trials show that the growth performance of GMT during grow-out was better than that of sex-reversed tilapia (SRT) and mixed-sex tilapia (MST) by as much as 30 and 50%, respectively, in within or interstrain comparisons.

This method of producing all-male progeny, is however, not a new technique. Xia and Wu (1993) stated that research on the development of YY males in *O. mossambicus* was initiated in the late 1970s in China and was used to produce six hundred supermales (this term is commonly used for males with the YY genotype). Similarly in India, Varadaraj and Pandian (1989) reported the first supermale tilapia in *O. mossambicus* produced by integrating endocrine sex reversal with gynogenetic techniques. However, none of these attempts has advanced to a stage of mass production of YY males and commercial production of monosex fish for consumption. In the Philippines, the development of this technique is being carried out at the Freshwater Aquaculture Centre, Central Luzon State University, Nueva Ecija. Based on the extensive progeny testing being done, it has been confirmed that the model developed is feasible and that YY males can be mass produced and have the capacity to sire all- or nearly all-male progeny (Mair *et al.*, 1992; Mair *et al.*, 1997a).

1.4.2 Production of genetically improved tilapia

In the case of growth deterioration of existing broodstock caused by inbreeding depression or stock contamination, the development of a better strain through selective breeding (Gjedrem, 1985; Jhingran and Gupta, 1989) to replace existing broodstock or the identification and crossbreeding of genetically compatible lines or strains to exploit heterosis and/or to increase heterozygosity (Gjedrem, 1985) are usually employed.

1.4.2.1 Selective breeding

In domesticated farm animals and plants, the use of selective breeding has been the traditional tool for improving growth performance. In fishes, even as early as 1400-1600 AD there was already the pressure to produce faster growing and heavier fish which led to a process of selective breeding (Pillay, 1990). However, the early attempts of selection were not properly documented although it resulted in the formation of distinct strains of carps (Pillay, 1990). In common carp, which is considered as one of the earliest and most domesticated fish species, the first documented genetic improvement in this species was started in the 1950s in Israel (Hulata, 1995b). In tilapia, It was only during the conference on the biology and culture of tilapias held in Bellagio, Italy in 1980 that the lack of attention to tilapia genetics as one of the major research gaps was identified (ICLARM, 1994). Since then much research has been done

attempting to improve the growth performance of tilapia through selection and crossbreeding.

In the Philippines, in the wake of the observed deterioration of existing cultured stocks of tilapia (Macaranas *et al.*, 1986; Taniguchi *et al.*, 1985) as a result of inbreeding and/or introgression of genes from other species, genetic improvement projects were initiated. The main objective of these were to develop strains which are superior over the existing stocks and have wider genetic variability. In 1988, the Genetic Improvement of Farmed Tilapia (GIFT) project was established and implemented by the International Center for Living Aquatic Resources Management (ICLARM) in collaboration with other institutions such as the Freshwater Aquaculture Centre (FAC), Bureau of Fisheries and Aquatic Resources (BFAR), University of the Philippines Marine Sciences Institute (UPMSI) and the Institute of Aquaculture Research of Norway (AKVAFORSK) (ICLARM, 1994).

In the GIFT project, a genetically improved strain known as the "GIFT fish" is being developed by the application of selective breeding from a base population consisting of eight diverse strains gathered locally and from Africa. Bolivar (1993) cited that even before selection began, the established broad genetic base population already has a mean growth rate of at least 20 to 30% higher than the most widely cultured commercial strain of tilapia in the Philippines. After one generation of a combined family and within family selection for growth in the synthetic breed, the selected fish were reported to

grow 26% faster than the previous generation and 75% faster than the most commonly farmed strain in on-station trials (ICLARM, 1994; Tayamen, 1992).

A similar project known as the IDRC-CLSU/FAC fish genetics project, funded by the International Development Research Centre (IDRC) of Canada in its support to the Aquaculture Genetics network in Asia (AGNA) has also been initiated. The approach of this project combines ideas developed in AGNA over the past five years with those of classical animal breeding (Tayamen, 1992). The approach of the project involves the use of within family selection for growth after 16 weeks of growing in concrete tanks. After two generations of selection, the selected fish gave a higher growth rate than the control in both tanks and cages (Abella, 1990) while after eight generations, the selected fish were 8 to 37% heavier than the control line (Bolivar, 1993).

Similar attempts have been also made elsewhere. Hulata *et al.* (1986) performed a mass selection for rapid growth in juvenile and adult *O. niloticus*. After two generations of selection for growth it was observed that no improvement over the original base population was achieved. A similar result was observed in the study of Huang and Liao (1990) using a bi-directional mass selection for high (H) and low (L) body weight and total length in *O. niloticus*. After one generation of selection it was observed that there was little response to mass selection for growth rate.

The results of the first two projects indicate that selection can be used to improve culture performance although the latter study shows that selection does

not always give positive results. Further, these selection programmes does not address the problem of overcrowding in culture ponds due to unwanted reproduction. To harness fully the potential of selective breeding consideration should be given in combining it with other techniques such as sex reversal or the YY male technology.

1.4.2.2 Crossbreeding

When selection is not applicable due to low genetic variability of the population or breed of interest and consequently low response to selection, crossbreeding can be used. The main purpose of this is to exploit additive and non-additive genetic variation between breeds for different traits (Cunningham and Connolly, 1989; Falconer and Mackay, 1996) and subsequently the potential of exploiting a heterosis effect (measurement of the superiority or inferiority of a hybrid compared to the parental stock). It is also considered as one of the most effective methods of increasing and preserving genetic variability or heterosygosity (Kincaid, 1977; Moav *et al.*, 1978).

The use of crossbreeding to improve growth in fish has been tried in a number of cultured species such as carp (Basavaraju *et al*, 1990; Wohlfarth, 1993; Hulata, 1995a,b; Bakos and Gorda, 1995), trout (Horstgen-Schwark *et al.*, 1986; Gjerde, 1988; Subba Rao and Chandrasekaran, 1978); catfish (Yant, *et*

al., 1975), bass (Wolters and DeMay, 1996) and tilapia (Avault and Shell, 1968; Jayaprakas *et al.* 1988; Villegas, 1990; Tave, 1990; Abucay, 1996).

In carp, particularly the common carp, although its history of culture dates back 4000 years, there has been relatively little genetic improvement (Wohlfarth, 1993). In Israel, genetic improvement of pond fish started in the 1950s by selecting for growth rate. However, there was very little response to selection for increase in weight (Wohlfarth, 1993). In subsequent experiments, the effect of inbreeding and crossbreeding on growth and viability were estimated and the growth of crossbreds was 10 to 20% higher than that of inbreds (Hulata, 1995a). In Hungary, the crossbreeding approach for genetic improvement in common carp has also been practiced (Bakos and Gorda, 1995) with similar results. However, the increase in growth of the F_1 progeny was not observed in interstrain crossbreeding of carps (Basavaraju, *et al.*, 1990).

In trout, the results of crossbreeding studies are variable. In a complete diallele cross between six inbred groups of rainbow trout, *Oncorhynchus mykiss*, Gjerde (1988) observed a very low genetic gain. The average heterosis and inbreeding effects were low and quite similar for a given trait. Similar results were also observed in a diallele cross with four strains of spring-spawning rainbow trout (*Oncorhynchus mykiss*) (Horstgen-Schwark *et al.* 1986). It was observed that the mean growth performance of all crossbreds was not significantly different from that of the purebreds. On the other hand, in a hybridisation experiment crossing females of common rainbow trout with the males of albino rainbow trout, the survival rates in eggs, hatchlings, fry and

yearling stages, and the annual growth rate of the crossbreds was higher than either parent (Subba Rao and Chandrasekaran, 1978).

In tilapia, there are only a few studies on crossbreeding which consider its effect on growth rate. Most studies were focused on the production of all-male progenies (Wohlfarth and Hulata, 1983) although the studies which had looked into the potential of using crossbreeding to improved growth rate showed positive results (Jayaprakas *et al.*, 1988; Abucay, 1996).

The advantage of crossbreeding over selection is that the potential increase in growth can be achieved over a short period. Once a potential combination has been identified the potential lines can be maintained separately and crossed to produce F_1 hybrid for grow-out. However, the disadvantage of this approach is that the increased in growth of the hybrids is the highest that can be achieved. Moreover, by selecting and combining the best lines or strains which exhibit fast growth the F_1 progeny can be further improve. Also, the identification of potential strain combinations requires an understanding of the source and identity of the species/breed of interest. It should be borne in mind that the selection for potential breeds should not only be compatible in terms of producing progenies with fast growth but also the breeds should have good fertility and survival. The review of Chevassus (1979) on the results and prospect of crossbreeding/hybridisation in salmonids summarises the factors to be considered when choosing potential combinations.

1.5 Prevailing Problems and Issues Needing Investigation

In a British Overseas Development Administration's report (Final Report, 1994) on renewable natural resources research strategy for the next ten years (1995-2005) it was stated that fish genetics is one of the key technologies which can contribute to sustainable increase in yield of fish protein. In the report, it was emphasized that poor species selection as a constraint and better selection and genetic improvement is a solution. Likewise the manipulation of sexual phenotype, particularly for tilapia, was also given high priority. The realization of these priorities are clearly what tilapia culture requires to further improve yield.

The YY male technology has been initiated in pursuit of the latter mentioned priority. Although there are already available approaches to produce monosex progeny, each has its own limitation and none has yet been exploited for universal use. The recently developed YY male technology is now being considered as an alternative for sex reversal in the production of all-male tilapia (Mair and Little, 1991), and one of the ODA priorities is about to be realized.

However, in addition to the development of appropriate dissemination techniques and assessment of its social and economic impact, the technology has to be further refined and likewise there is a need to develop the technology in a variety of strains in *O. niloticus.* As a basis for this there is a need to further investigate and understand the foundation of the technology - the sex determining mechanism of the strain/species being used. In earlier studies on the sex determination of the Egypt-Swansea strain where the YY male

technology was developed, Scott *et al.* (1989) and Mair *et al.* (1991a) observed that this strain exhibits monofactorial, genotypic sex determination with male heterogamety with some occasional rare autosomal or environmental sex-modifying factors. However, the incidence of exceptional sex ratios can be high. The observed sex ratios from YY males in within strain crosses ranged from 71.6 to 100% (Mair *et al.*, 1992) and from 14.0 to 100% male in between strain crosses (Capili, 1995). These sex ratios cannot be fully explained on the basis of Mendelian inheritance of a single determining factor. Also, the results of the study of Mair *et al.* (1990) and Baroiller *et al.* (1996) showed that a significant deviation of sex ratios from the expected was observed in some progeny reared at high temperature (mean of 36°C) compared to the control reared at ambient temperature during the period encompassing sexual differentiation. In the light of these unexpected observations, it was felt that there is a need to further investigate and understand the underlying factors affecting sex determination and determine the extent of their effect on sex ratio, particularly in relation to attempts to produce monosex fish.

Likewise, with the objective of further improving the growth of GMT it is very important to determine and evaluate potential methods to achieve this. It is possible that the potential growth of GMT produced in the Egypt-Swansea strain has not been fully exploited because the strain is known to have passed through a number of genetic bottlenecks and is considered to be inbred (Mair *et al.*, 1995). The selection for growth rate is not likely to produce any positive result due to low genetic variability of this strain where the YY male technology was

developed. However, it is possible to select for other lines or strains compatible with the original YY male line that produce fast growing all-male progeny. For example, in the study comparing the growth of three crossbred to purebred progenies from Egypt-Swansea YY males, Abucay (1996) observed that all the crossbreds had a better growth and two of the crossbreds had a significantly faster growth than the purebred. The study shows that the outcrossing of YY males could improve the growth of the progeny. However, despite the significant improvement in growth of crossbred over purebred progeny it is not known whether it was due to heterosis or simply an additive effect of the parental strains since there was no reciprocal cross made.

However, despite the likely negative effects due to inbreeding, it was observed that the growth of genetically male tilapia (GMT) was still better than sex-reversed tilapia (SRT) in within strain comparisons (Mair *et al.* 1995). Theoretically, there should be no difference between the growth of GMT and SRT considering that both are all-male or near to all-male. In fact, the growth of the SRT might expected to be better due to a possible anabolic effect of the hormone used. However, the result of the above study was different from the expected. The lower growth of the SRT could be due to a lower percentage of males although in the first trial the percentage of males in GMT and SRT was almost the same. It is possible the lower growth of the SRT is due to the fact that even if it is 100% male 50% of them are still genetically female while in the GMT all of them are genetically male. However, this hypothesis has yet to be investigated.

The development of a better and more effective approach to achieve complete sex reversal should also be made. Despite the many research works on sex reversal, the inconsistency of results and the failure to achieve 100% reversal is still one of the main problems in using this technology. Although with the development of the YY male technology it is now possible to produce all-male progeny without the direct use of hormone, there is still a need to apply hormone on a routine basis to produce YY females for crossing to YY males to mass produce YY males. Similarly, there is a need to produce more XX males for the mass production of XX females for crossing to YY males to mass produce all-male progeny for grow-out. Also an effective and repeatable method of sex reversal is important in the light of the observed difficulty in feminisation of YY males (Abucay and Mair, in press b).

1.6 Objectives and Structure of the Research Undertaken

With all the stated problems and issues, the research presented in this thesis was directed to the ultimate objective of gaining a better understanding of the genetics of sex determination, and ultimately to improve the YY male technology. The experiment approaches used should be considered in the context of available equipment and resources at FAC, CLSU where the research was conducted. Techniques were largely limited to breeding and growth trial experiments.

The research comprises four separate studies. The first study evaluated the effect of extreme environments such as low (mean of 26°C) and high (mean of 36°C) temperature and salinity (max. of 30 ppt) on the sex ratio in *O. niloticus*. Significant effect of temperature on sex ratio had been reported in *O. mossambicus* and *0. aureus* by Mair *et al.* (1990)and in *O. niloticus* by Baroiller *et al.* (1995, 1996). The occurrence of females in the progeny of YY males is hypothesized to be partly due to environment. It is the aim of this study to throw further light on this phenomenon and to assess the relative effects of genetic environmental components. The study consisted of a series of experiments which involved exposing different genotypes (putative all-female, all-male and all YY male) of purebred and crossbred fish to different environments during the stage of sexual differentiation. The results are presented and discussed in Chapter three.

One of the objectives of the YY male technology is to improve the growth performance of GMT. The use of the crossbreeding approach by outcrossing the Egypt-Swansea YY males to females from other strains had been initially evaluated by Abucay (1996). A follow up study was carried out (presented in Chapter four), evaluating the growth of GMT produced from a 2 x 2 diallele cross using novel YY males developed in Egypt-Swansea and Egypt-ICLARM strains. The aim of the experiment was to determine the factors that influenced the observed improvement in growth of the crossbred progeny of YY males. Likewise, a further evaluation on the sex ratio of the progeny of YY males in the Egypt-Swansea strain in crosses with females from other strains of *O. niloticus*

were also done aimed at determining the extent of these males to sire all-male progeny and to gain further understanding of the mechanism of sex determination in *O. niloticus*.

Chapter five presents the results of a study aimed at throwing light on the results of Mair *et al.* (1995) where the GMT was observed to have a faster growth than the SRT in within strain comparisons. The growth of androgen hormone treated putative all XX female, all XY male and mixed-sex (1m:1f) progeny and their controls were monitored during hormone treatment, post treatment and subsequently in all-male and mixed-sex culture environments. The aim was to determine whether androgens, specially the 17α-methyltestosterone, has an anabolic effect and if there is a differential growth of the hormone-treated genetic male and genetic female fish. It was also hoped to find out whether the hormone promotes higher masculinity making the hormone treated males more aggressive especially in the presence of females thus affecting their growth.

The final study evaluates approaches to improve the efficiency of sex reversal. Gomelsky *et al.* (1994) and Abucay and Mair (in press a) observed that the controls receiving water or reared in the same water as with the treated fish during hormone treatment were also sex-reversed. The authors discussed the possibility that hormone metabolite (s) either from the uneaten hormone treated food and/or excreted by the hormone treated fish accumulates in the system until it reach a level at which the untreated fish are also affected. In another study on sex reversal of common carp, Nagy *et al.* (1981) produced high rate and more complete sex reversal when treatment was done at 25°C that at 20°C in which

intersex appeared frequently. Similarly, in sex reversal of *O. mossambicus*, Varadaraj *et al.* (1994) indicated that the production of sex-reversed males was dependent more on the level of temperature during hormone treatment than the hormone dose. The study aim to investigate further the cause of the phenomenon of incidental sex determination and also to evaluate the effect of increasing the temperature during hormone treatment. The study also determine the possibility of exploiting the potential of "hormone metabolites" and manipulation of temperature to improve the efficiency in sex reversal. The results are presented and discussed in Chapter six.

The present research tackled topics that have been previously studied but in one way or another have failed to produce conclusive results or which clearly require further investigation. It is hoped that the present research will contribute to the further improvement of the YY male technology in particular and to aquaculture in general.

CHAPTER TWO

GENERAL MATERIALS AND METHODS

2.1 Introduction

This chapter presents and describes the different activities, directly or indirectly undertaken during the execution of the studies presented in this thesis. Some of the activities were indirectly undertaken in the context of this thesis as they were routinely performed as part of the core activities of the Genetic Manipulations for Improved Tilapia (GMIT) project, particularly the production of broodstock and monosex fry used in some of the experiments. In order to present a more comprehensive treatment, it was thought worthwhile to include those activities in the description of the different procedures that have been carried out, specifically in relation to this thesis work.

2.2 Experimental Fish

2.2.1 Origin and history of the strains used in these studies

The different studies presented in this thesis have utilised a number of strains of *Oreochromis niloticus* including Egypt-Swansea, Egypt-ICLARM, Egypt-Stirling, Egypt-AIT, Egypt-BFAR, Kenya-Turkana, Kenya-Baringo, Kenya-Baobab, Ghana-BFAR and two selected strains; GIFT and IDRC fish. The nomenclature for each of the above strains was designated following the suggestion of Dr. G.C. Mair (pers. comm.) in which the country of origin should

be given as the first part of the name followed by its immediate source when first obtained by the project. This method has been suggested to avoid confusion as several of the strains have already been introduced to many localities and citing only the original source may be misleading. The majority of the above strains are held at the Freshwater Aquaculture Centre (FAC) of Central Luzon State University (CLSU) in the Philippines as part of the broodstock maintenance programme of the GMIT project. The origin and histories of these strains are summarised briefly as follows;

Egypt-Swansea - One of the initial activities of the GMIT project was to collect different strains of *O. niloticus* to be used for various experimental purposes. Since 1988, several hundred fish, representing more than 10 families were transferred to the GMIT project from the School of Biological Sciences, University of Wales Swansea (Mair *et al.*, 1997a). Originally, this stock was collected from Lake Manzala in Egypt in 1979 and transferred to University of Stirling, Scotland. In the years of 1982, 1983 and 1985, a number of fish were transferred from the University of Stirling to the University of Wales Swansea (Mair, 1988).

This strain is considered to be somewhat inbred due to having been through a number of genetic bottlenecks (Mair *et al.*, 1995). At the University of Stirling and University of Wales Swansea this strain has been maintained as laboratory populations and possibly with a small number of broodstock. Studies on sex determining mechanisms in tilapia by hormonal sex manipulation and gynogenesis conducted at the University of Wales Swansea have used this strain (Mair, 1988). A few of the estrogen-treated individual females were transferred

to the GMIT project in the Philippines which eventually were used as a founder stock for the development of YY males (Mair *et al.*, 1997a).

Egypt-Stirling - This strain is closely related to Egypt-Swansea, both being obtained from University of Stirling. Although both came from the same source, this strain is being treated as distinct and has been maintained separately since it was transferred to the Philippines directly from the University of Stirling in 1992. Since the strains were already isolated from each other for a decade it could be likely that they have evolved differently in this time.

Egypt-ICLARM - This was obtained from the Genetic Improvement of Farmed Tilapia (GIFT) Project of International Centre for Living Aquatic Resources Management (ICLARM) in 1989. This was one of a several strains collected from Africa to form the base population for the GIFT synthetic fish. This strain, which was collected in creeks along desert road to Port Said, Egypt (Eknath *et al.*, 1993), could also be considered to be somewhat inbred as the fish held by the GMIT Project are known to be descended from a single pair that survived from the original introduction (Capili, 1995). In a comprehensive evaluation of eight strains of *O. niloticus* in different Philippine farm environments, this strain exhibited the best growth performance among the tested strains (Eknath *et al.*, 1993).

Egypt-AIT - This was obtained from the Asian Institute of Technology (AIT) in Bangkok, Thailand. This strain was said to have originated from Egypt and introduced to Japan in 1962 (Welcomme, 1981). In 1965, 50 individuals of this

strain were introduced to Thailand, but the number that survived to breed (the founder stock) is unclear (Pongsri, 1994). As the stock has been maintained in the Royal Jitralada Palace and kept well-isolated from other tilapias, this stock is also known as the Jitralada strain. In March 1966, after successfully breeding the founder stocks, His Majesty the King of Thailand gave 10,000 fingerlings to the Thai Department of Fisheries and the strain was subsequently introduced throughout Thailand (Pongsri, 1994). The individual fish introduced at AIT were already reproductively isolated for several years and were bred in cages for at least 10 generations (Tuan, 1997). Having been already isolated and undergone several breeding generations, this strain probably has evolve some differences from the Jitralada strain commonly cultured in Thailand.

In 1991, Dr. G.C. Mair, the GMIT Project Leader, brought a small number of fry from AIT, Thailand. The fry were carefully grown to sexual maturity and formed the founder stock for the strain called Egypt-AIT.

Egypt-BFAR - This strain was introduced to the Philippines from Thailand in the years 1972 (Bolivar, 1993) and 1987 (Capili, 1995), through the Philippine Bureau of Fisheries and Aquatic Resources (BFAR). The origin and history of this and was similar to that of the Egypt-AIT (or the Jitralada strain), both being from Thailand. In 1989 the GMIT project obtained a number of fish of this strain from BFAR, which was named Egypt-BFAR, to form part of the stock maintenance of the project.

Kenya-Turkana - This strain was originated from Kenya in Africa. In 1991, twenty post fingerlings were collected from Crater Lake A in Kenya and transferred to the School of Biological Sciences at the University of Wales Swansea (E.E. Roderick, pers. comm.). In 1993, a number of fish were transferred to the GMIT project in the Philippines.

Kenya-Baringo - The strain was transferred to the School of Biological Sciences at the University of Wales Swansea (UWS), from Baobab farm in Kenya in 1987 (Abban, 1988). Originally, the stock was descended from two batches collected from Lake Baringo, the first in 1975 which consisted of nine males and eight females and the second in 1982, consisting of 20 males and 14 females (Capili, 1995). In 1990, a number of fish were transferred from UWS to the GMIT project in the Philippines.

Kenya-Baobab - This strain originated from the Baobab Fish Farm in Kenya and was transferred to the GMIT project in 1992 from the University of Wales Swansea. This strain was a product of interstrain hybridisation of *O. niloticus* originating from various sources in Kenya, including Lakes Turkana, Victoria, Baringo and an introduced Egyptian strain of *O. niloticus* (Capili, 1995).

Ghana-BFAR - Presently this is the most widely cultured strain in the Philippines (Tayamen, 1992). From Israel, the strain was introduced to the Freshwater Aquaculture Centre of the Central Luzon State University (FAC-CLSU) in 1977 (Bolivar, 1993) and again in 1979 (Pullin and Capili, 1988; Bolivar, 1993). Originally, this strain was collected from Lake Volta in Ghana in 1974 and

transferred to Israel (Hulata *et al.*, 1993). In 1989, a number of fish were transferred to the GMIT project from the National Freshwater Fisheries Training and research Center of the Philippine Bureau of Fisheries and Aquatic Resources (NFFTRC-BFAR) which had held the strain and dispersed it widely in the Philippines since its introduction.

GIFT selected tilapia - The GIFT fish is a selected synthetic strain formed from a base population consisting of eight diverse tilapia germplasms from Africa and Asia (Bolivar, 1993). The synthetic strain is known to have a good growth performance. Eknath *et al.* (1991) reported that that the mean growth of the base population alone was at least 20-30% higher than the most widely commercially cultured strain of tilapia in the Philippines. Starting from the synthetic base population a combination of family and within family selection for growth has been performed over several generation. The GIFT fish used for the experiment described in Chapter four were part of the fish used in an experiment conducted at the FAC of CLSU which said to be an unselected progeny of second generation selected fish.

IDRC selected tilapia - This selected strain is a product of a long running within family selection for fast growth programme (funded by the International Development Research Council of Canada). The fish were reportedly progeny from the eighth generation selected broodstock (Zaldy Bartolome, pers. comm.). The selected strain is descended from a foundation stock consisting of an indeterminate mix of four strains of *O. niloticus* with a history of domestication in

the Philippines. These original strains included Ghana-Singapore (maintained at FAC and BFAR), Ghana-Israel (BFAR) and a Taiwanese strain imported by International Center for Living Aquatic Resources Management (ICLARM) in 1984 (Abella, 1994). In an evaluation of growth performance, the eight selected generation fish were observed to be 8 to 37% heavier than the second generation random-bred control (Bolivar, 1993).

Table 2.1. Summary of the description of the different strains of *O. niloticus*.

Strain	Place of origin	Year collected	Number of fish collected	Year transferred to the Philippines	Location where the strain was obtained	Year obtained by the GMIT project	Number of fish obtained
Egypt-Swansea	Egypt	1979	No exact number but was able to bred 70 of the founder stocks	1989	University of Wales Swansea, Wales	1989	Several hundreds representing 10 families
Egypt-Stirling	Egypt	1979	No exact number but was able to bred 70 of the founder stocks	1993	Institute of Aquaculture, University of Stirling, Scotland	1992	NIA
Egypt-ICLARM	Egypt	1988	No exact number but a single pair was known to only have survived during the first generation	1988	ICLARM, Philippines	1989	NIA
Egypt-AIT	Egypt	1962	200 individuals. 50 fish were transferred to Thailand	1991	Asian Institute of Technology (AIT), Thailand	1991	≈150 fry
Egypt-BFAR	Egypt	1962	200 individuals. 50 fish were transferred to Thailand	1987	NFFTRC/BFAR, Nueva Ecija, Philippines	1989	NIA
Kenya-Turkana	Kenya	1991	20 post-fingerlings	1993	University of Wales Swansea, Wales	1993	NIA
Kenya-Baringo	Kenya	1975, 1982	20 males and 14 females	1990	University of Wales Swansea, Wales	1990	NIA
Kenya-Baobab	Kenya	1988	NIA	1993	University of Wales Swansea, Wales	1993	NIA
Ghana-BFAR	Ghana	1974	50 individual	1979	NFFTRC/BFAR, Nueva Ecija, Philippines	1989	NIA
GIFT fish	NA	NA	NA	NA	FAC-CLSU	1995	25 females
IDRC fish	NA	NA	NA	NA	FAC-IDRC Project	1995	25 females

NA = not applicable, NIA = no information available

2.2.2 Maintenance of broodstock

The different strains and genotypes of *O. niloticus* maintained by the GMIT project are kept separately in rectangular concrete tanks (0.85m x 4.65m x 1.25m) to minimise the chances of contamination or loss of strain. Once a week the tanks are drained and cleaned to remove accumulated waste and algae that have grown on the wall of the tank. A continuous flow of fresh and clean water is maintained in all tanks. The fish are fed (approx. 0.5% of fish biomass daily - sufficient for sustenance but not enough for the fish to grow fast) twice a day with a commercial brand feed. Occasional inventory is carried out to monitor the number and condition of the fish, especially those with PIT tags. Manipulated genotypes (YY males, YY females and XX males) are routinely tagged using Passive Integrated Transponder (PIT) tags.

The effective life span of broodstock is expected to be at least 18 months. After this period, the maintained stocks are replaced with batches of fingerlings from the next generation. The replacement for the different strains were generated by producing at least 20 families from different male and female parents from each strain which were then grown separately up to when approaching sexual maturation. When approximately 20-30g average weight, a minimum of 5 females and 3 males (free of any physical deformities) were randomly selected from each of the 20 families, and pooled together in the maintenance in tanks. Another similar (or sometimes larger) number of broodstock were also selected and stocked in cages as back up. Other remaining fish were used for experimental purposes when required. Broodstock

was maintained in this way to try to minimise on inbreeding within the limitation of available resources.

2.3 Production of Experimental Fish

2.3.1 Production of broodstock

YY males and YY females - The breeding programme for producing YY males and YY females was described in passing in Chapter one and in more detail by Mair *et al.* (1997a). After producing YY males and YY females, these can be crossed to reproduce more YY males. YY females can be produced by application of estrogen to some of the sexually undifferentiated YY males. When producing the next generation of YY males and YY females, the potential parents were first progeny tested to confirm their genotype. This was usually carried out by crossing YY males to normal females (XX genotype) and YY females to XX males. Those that produced all-male progeny (see Table 2.2 for the predicted sex ratio) were selected and used as parents for the next generation of YY broodstock.

Table 2.2. Expected sex ratios from crosses of different genotypes. The prediction is based on the assumption that sex chromosomes are the only determinants of sex. The delta (Δ) prefix denotes sex-reversed genotypes that do not normally occur in nature (Mair *et al.*, 1987).

	Male genotype					
	Δ X X		**X Y**		**Y Y**	
Female genotype						
X	*XX*	*XX*	*XX*	*XY*	*XY*	*XY*
X	*XX*	*XX*	*XX*	*XY*	*XY*	*XY*
sex ratios	**0♂ : 1♀**		**1♂ : 1♀**		**1♂ : 0♀**	
Δ X	*XX*	*XX*	*XX*	*XY*	*XY*	*XY*
Y	*XY*	*XY*	*XY*	*YY*	*YY*	*YY*
sex ratios	**1♂ : 1♀**		**3♂ : 1♀**		**1♂ : 0♀**	
Δ Y	*XY*	*XY*	*XY*	*YY*	*YY*	*YY*
Y	*XY*	*XY*	*XY*	*YY*	*YY*	*YY*
sex ratios	**1♂ : 0♀**		**1♂ : 0♀**		**1♂ : 0♀**	

Note: Table was adopted from Abucay, 1997

The YY male and YY female broodstock that were used in the studies presented were all progeny tested and confirmed to have a YY genotype. The exception were those broodstock that were used for the routine production of monosex progeny used in the experiments described in Chapter six. These broodstock were assumed to be YY males considering the YY genotypes of both their parents.

XX males - The production of males with XX genotype is less complicated than to the production of YY males (see Figure 2.1). The procedure for the production of XX males was described for *O. mossambicus* by Pandian and Varadaraj (1990) and for *O. niloticus* by Abucay (1997). Initially, the production of XX males involves the application of androgen to sexually undifferentiated mixed-sex progeny of normal female (XX) and male (XY) parents to alter the phenotypic sex to male. If the masculinisation treatment is 100% successful it is expected that 50% of the treated progeny would be males with an XX genotype (see Table 2.2). The two genotypes can only be distinguished by means of progeny testing. The androgen-treated males were crossed to normal females and those that yield all-female progeny were XX males. It may also be possible to the distinguish the two genotypes by careful examination of the morphological appearance including body colour, shape of belly, dorsal profile of head and size at a certain age (Patil and Varghese, 1994), but this technique is not 100% reliable, although if combined with progeny testing it may increase the chances of identifying a higher proportion of XX males.

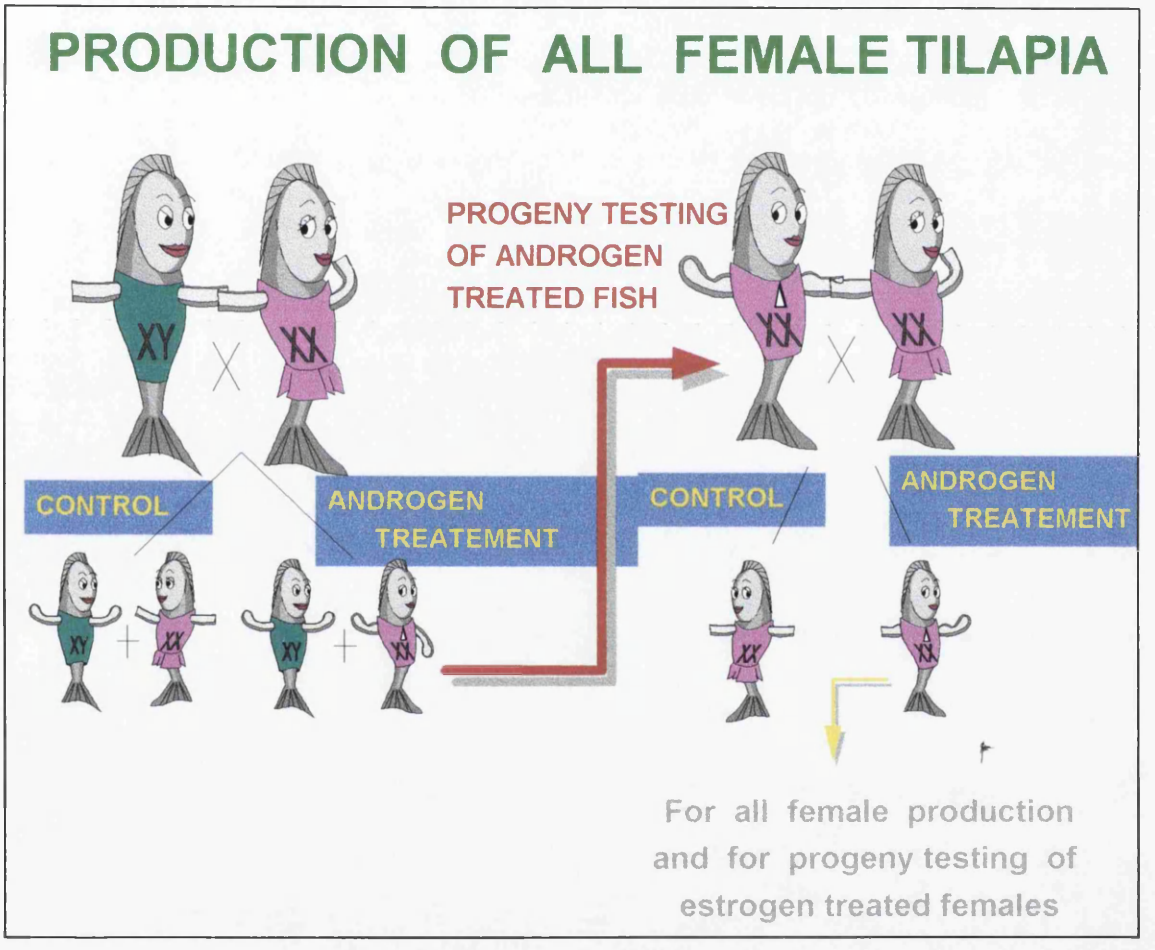


Figure 2.1. A Model for the mass production of normal female tilapia, XX genotype (figure was adopted from Mair *et al.*, unpublished).

To produce larger numbers of XX males, those identified as XX males were crossed to normal females, which were then expected to produce all-female progeny. The sexually undifferentiated progeny was divided into two; half were treated with androgen hormone while the other half served as untreated controls. If the control were all-female then the males in the hormone-treated progeny may be safely assumed to be XX males, without the need of progeny testing. However, when producing the next generation of XX males it was preferred to use those XX males confirmed by progeny testing and preferably to use males which produced small percentages of males in their progeny.

2.3.2 Production of fry

All-male - Depending on the desired genotype, the production of all-male progeny normally involved the use of YY males as broodstock. When desired to produce all XY male (genetic males - GMT), YY males were crossed to normal females. The breeding method could be either in cages-in-ponds or directly in earthen ponds (see fry production systems in section 2.4 of this chapter). Before stocking the broodstock, it was checked that no other fish were inside the ponds or cages to prevent contamination. It should be noted that XX♀ x YY♂ crosses did not always produce 100% male progeny. The sex ratio from individual YY males commonly ranged from 71.6 to 100% male with a mean of approximately 97% male (Mair *et al.*, 1992).

When desired to produce males with a YY genotype (YY males), YY males were crossed to YY females. In this cross 100% male progeny was expected. Unlike in XX x YY cross, no females have yet been found in the progeny produced from the YY x YY crosses in an on-going within strain selection for sex ratio programme (Mair and Skibinski, 1997).

All-female - All-female progeny were produced by crossing XX males with normal XX females. XX males have already been developed in three *O. niloticus* strains (Mair and Skibinski, 1997) and these XX males have been used in the experiments described in Chapter three. The breeding was carried out as described in fry production systems (section 2.4 of this chapter). The XX males used were either first progeny tested or were produced from parents with confirmed XX genotype and having all-female normal siblings.

2.4 Fry Production Systems

In the GMIT project the production of fry or fingerlings for research or commercial purposes is routinely being carried out either in cages-in-ponds or in earthen ponds. Some of the fish used in some of the studies presented were produced independently from the routine activities of the project but the procedures used were relatively similar. The different systems are described briefly.

2.4.1 Breeding in cages

The principle of breeding in cages is very much similar to that in earthen ponds; the only difference is that the broodstock are stocked in net cages instead of being stocked directly in pond. The major reason for using this technique is the ease in management and the lesser chances of potential contamination (Morales, 1997). Whilst the studies presented in this thesis were being conducted, several cage designs and modifications were evaluated for maximum breeding efficiency. Although the routine production of fry for research and commercial purposes, including those fry used for the studies presented here, were done mostly using the original cage design and dimension (5m x 2m x 1m).

The cages were installed in earthen ponds (ranging from 200 to 1000 m^2) with each of the corners tied to wooden stakes (see Photo 2.6). Usually, the cages occupied a maximum of 60% of the total surface area of the pond to allow enough space for easier access around each cage and for the water to circulate for maximum oxygenation. Before breeding, the pond where the cages were installed was first prepared following standard preparation methods which include draining, poisoning to kill unwanted fish, drying, water filling and initial fertilisation. Organic (chicken manure) and inorganic (16-20-0, N:P:K) fertilisers at a standard rates of 3000 $kg.ha.month^{-1}$ and 100 $kg.ha.month^{-1}$, respectively, were applied to induce primary productivity. The monthly rate was apportioned on a weekly basis for greater efficiency.

Cage breeding involved two stages; breeding and conditioning. During breeding, the stocking rate was 2 fish per m^2 of the cage surface area and at a ratio of 3♀ : 1♂. Commercial feed at a rate of 2-3% biomass daily was given to the fish twice a day (0800 and 1600 hr). When the females were mouthbrooding the feeding rate was normally reduced. Depending on whether eggs or fry were collected, the duration between stocking and egg collection was for minimum period of 7 days. During egg collection, the cage was lifted slowly to prevent the incubating females from prematurely spitting out of eggs. Each female was individually checked for the presence of eggs in her buccal cavity. The collected eggs were then cleared of dirt and debris and placed in artificial incubators.

After egg collection, the male and female broodstock were separated and stocked in conditioning cages. Due to limitation of male breeders (XX and YY males) they usually remained in spawning cages and were paired for the next batch of females. Although, occasional conditioning of the male broodstock was carried out when poor fertilization rates were observed. The spent females were conditioned for at least 7-10 days (in mesh #17 net cages at 10 fish per m^2 of the cage surface area) and were given supplemental feeds at a rate of 3% biomass daily after which they were again ready for spawning.

For the fry collection method (as opposed to egg collection) the breeding duration was usually up to 14 days. In this method, the female broodstock were allowed to incubate their eggs and collect the fry only when hatched. The swim-up fry were collected by carefully scooping them with a fine mesh scoop net. Individual inspection of females was still carried out to check for eggs in the

mouth. Due to asynchronous spawning sometimes there were females which were still mouth brooding after this period.

2.4.2 Breeding in ponds

The breeding in ponds was done in 200 m^2 (10 m x 20 m) ponds with a water depth of not more than one meter (see Bartolome, 1997). Prior to stocking the broodstock, the breeding ponds were prepared following a standard method as described earlier. Standard rates of organic and inorganic fertilisers were applied to accelerate the growth of natural food. Broodstock at a ratio of 3♀ : 1♂ were stocked at a density of 2 fish per m^2 of pond surface area. Supplemental feed was given at a rate of 2.5 to 3% fish biomass daily (the rate was slowly increased when females started releasing their fry to avoid cannibalism) using commercial pelleted feed.

Beginning from the second week after stocking, regular inspection at the edge of the pond during early hour in the morning was carried out to check for the presence of swim-up fry. When schools of fry were observed, chicken manure was applied at a rate of 20 $kg.pond^{-1}$ to bring down the dissolved oxygen level thus forcing the incubating females to release their fry. Collection of fry commenced by running a big round scoop net (3 to 4 feet diameter) around the perimeter of the pond. The collected fry were transferred into a nursery pond (one nursery pond for every 3-4 breeding pond). The fry collection period usually lasts from five to seven days after which the ponds were drained and prepared

for the next spawning cycle. The male and female breeders were separated and conditioned while the breeding ponds were being prepared for the next spawning cycle.

2.4.3 Artificial incubation

The collected eggs from hapa-based breeding were artificially incubated in an upwelling incubators made from two liter plastic bottles with round bottom (Photo 2.1). The incubators were supplied with water from a plastic hose with the tip almost touching the bottom creating an upwelling movement of water as the name implies. The upwelling water caused the eggs to rotate around in the base of the incubator simulating the natural movement of eggs in the mother's mouth during incubation. Depending on the volume of eggs being placed in an incubator, the volume of water was regulated to prevent the eggs from excessive movement, which might result in damage to the egg and subsequent mortality. The incubated eggs were cleaned daily removing any dead eggs which might have caused fungal infections. When hatched, the swim-up fry were then transferred to rearing trays.

Photo 2.1. An upwelling incubators made of plastic bottles.

2.4.4 Rearing of swim-up fry

One or two days after hatching, the hatchling were then transferred into an aluminum tray and further nursed until the yolk sac was fully absorbed and ready for stocking. The aluminum trays were installed in an adjacent area outside the incubation facility. Each tray was provided with holes on both sides for water outlets (also for maintaining a 3cm water depth) but covered with fine mesh net to prevent the fry from escaping. Fresh and clean water from an overhead tank was supplied to each tray through a PVC pipe which was installed in such a way that the incoming water caused the hatchling to move in a slow circular motion simulating the natural movement in the buccal cavity of the mother during mouth incubation. The trays were inspected regularly to check the condition of the hatchling and to remove dead fry which might clog the holes of the trays. Normally, the fry would stay in the nursing trays for an average of five days after which they were stocked for nursing and subsequent grow-out.

2.5 Tagging and Marking Methods

2.5.1 Fin clipping

The fish used for the growth evaluation studies were all marked by fin clipping for group identification. This was carried out by cutting either the left or right pectoral or pelvic fin (Photo 2.2). To avoid regeneration, the fin was clipped

close to the base. Even clipping fins in this way, some of the clipped fins still regenerated but usually were deformed so they could still be distinguished from a normal fin. When clipping small fish it was sometimes very difficult to cut close enough to the base of the fin and some would then regenerate normally. To avoid this situation, the clipped fish were not immediately stocked into the grow out pond or cage but only after a minimum of two weeks, after which the fish were checked for fin regeneration and any regenerating fins were reclipped.

Photo 2.2. A typical fish marked by fin clipping.

This technique had been found suitable for marking in carp (Murugesan and Selvaraj, 1990; Roy *et al.*, 1991). The growth and survival of the species tested in the above studies were found not to be significantly affected by fin clipping. The fish used in the growth evaluation experiments described in Chapters four and five were fin clipped when they were already approximately 10 or 30g average weight, respectively, and the cut fins were allowed to heal completely before stocked to minimise the possibility of bias due to clipping effect. Furthermore, for the growth evaluation experiments described in Chapter four, the fish in each of the three replicates were clipped in different locations (Table 2.3) to further minimise the effect of any bias due to the location of the fin clipped.

Table 2.3. Locations of the different fin clips of the fish used for the growth evaluation experiment described in Chapter four.

Strain crosses (XX♀ x YY♂)	Replicates		
	1	2	3
E.-Swansea x E.-Swansea	left pectoral	right pectoral	left pelvic
E.-ICLARM x E.-Swansea	right pectoral	left pectoral	right pelvic
E.-ICLARM x E.-ICLARM	left pelvic	right pelvic	left pectoral
E.-Swansea x E.-ICLARM	Right pelvic	left pelvic	right pectoral

NB. E = Egypt

2.5.2 Passive implantable transponder tag (PIT tag)

For important fish such as YY males, YY females and XX males for which it was required to identify each individual fish, passive implantable transponder (PIT) tags were used. The PIT tag is a microchip encased in a 10mm x 4mm glass bead. The standard technique for the application of this tag is by surgical implantation by cutting a small opening and slipped the glass bead through into the peritoneal cavity (see manual by Brzeski *et al.*, 1989). Quartararo and Bell (1992) evaluated the effect of this tagging method on juvenile snapper, *Pagrus auratus* (Bloch and Schneider) and found no significant effect on growth and survival.

However, in tilapia, the tag had been found more stable and effective when applied in the muscle between the base of the dorsal fin and the lateral line, approximately below the third or fourth dorsal spines. When the tags were applied in the peritoneal cavity, the tag was found to move and harder to find (personal observation) The tags were implanted using a syringe applicator by inserting the tip of the needle under a scale and slowly pushing in the tag to avoid hitting a bone. While tagging the fish was covered with a wet towel to prevent it from struggling. After inserting the tag the fish was put in clean, well aerated water and allowed to recover. Sometimes, even a newly tagged big fish died because of stress and shock, especially if stocked immediately in tanks or cages with poor oxygenation. In a study measuring the oxygen consumption in *O. niloticus*, a pronounce increase in oxygen consumption from 150 to 300% was observed following a stressful handling (Ross and Ross, 1983).

Although found effective, this tagging technique also has some limitations. For a small scale research operation this tagging technique may be found too expensive with the tags costing more than £3 each. It requires an electronic PIT tag reader to identify the fish which is also expensive. Furthermore, the fish should be a large size (at least 50g) before it can be safely tagged. However, for maintenance of broodstock this technique was found to be very suitable. Moreover, the tags can be easily recovered and can be used again for tagging other fish.

2.6 Progeny Testing

Progeny testing is a method of determining the genotype of an individual fish by examining the phenotype (or sex ratio as in the case of the studies presented in this thesis) of its progeny when crossed to a male or female with a known genotype. Progeny testing has been the basis for identifying functionally sex-reversed tilapia for their use in breeding programmes to produce monosex populations (Jensen and Shelton, 1979). The YY males and YY females used for the experiments described in Chapter three were all progeny tested to confirm their genotype. For the experiments described in Chapter four, progeny testing was performed by crossing Egypt-Swansea YY males to females from a number of strains to determine the extent of the capacity to sire all-male progeny.

The breeding was done either in circular concrete tanks (approx. 900-l capacity) or in fine mesh cages installed in earthen ponds (1 m^3 or 1.5m x 1.5m x 1m). Before stocking the fish to be tested they were first marked with PIT tags for identification. Each tank or cage was provided with a label bearing the identity (strain, generation, PIT tag number) of the fish being tested. Feeding was done twice a day (0800 and 1600 hr) at a rate of 1-2% fish biomass daily. Egg collection was done once a week. At the same time the tanks or cages were cleaned or washed to removed accumulated waste.

The progeny testing of potential YY males and XX males was done by pairing each male to a minimum of 3 females. The reason for pairing at least three females to one male was due to the instinctive aggressiveness of the latter. When there were more than one female, the attention of the male was not focused only to one female thus minimizing the chance of aggression which might result in mortality. Furthermore, when there were more females it ensured a higher chances of spawning. Once a female had spawned, the male was removed and another male was paired.

In tanks, fish were inspected daily as female carrying eggs in its mouth could be easily distinguished by the enlarged buccal cavity. However, the eggs were not immediately collected as it had been observed that survival was higher when the mother was allowed to incubate its eggs for at least three or four days before collection. The collected eggs were then artificially incubated in hatching jars.

The sex ratio of the progeny would depend on the predicted genotype of the fish to be tested. For example, when progeny testing for YY males from a group consisting of XY and YY males, the expected sex ratio that may be observed from crossing with normal XX females would be either 1:1 MF (sex ratios are always presented as M:F) or 1:0, respectively (see Table 2.1). When progeny testing for confirmation of YY male genotypes from a group predicted to consist of all YY male, such those progeny of YY x YY cross, the expected sex ratio of all the progeny would be all- or nearly all-male.

For the progeny testing for XX males, when testing for XX males from a previously androgen treated mixed-sex progeny, there are two possible sex ratios that may be observed in crosses with normal XX females; all-female (0:1) and mixed-sex (1:1) progeny. Those males that produced nearly all- or all-female progeny were XX males. When progeny testing for XX males from a group predicted to consist of all XX male, such as androgen-treated progeny of XX x XX cross, the expected sex ratio of all the progeny would be all- or nearly all-female.

For the progeny testing of potential YY females, a maximum of five females were paired with one XX male. Initially, the progeny testing of potential YY females involved single pair mating but aggression by male and sometime between females (probably because they were still genetically males) was usually common and led in mortality. By pairing more females to a single XX male aggression was reduced. After egg collection those females that have already spawned were stocked in separate tanks or cages. New potential YY

females were then added to the breeding tank or cage as replacement for the spawned females.

Initially, XY males were used as male breeders when progeny testing for YY females. However, it had been found much more easier to establish the genotype of a female being tested when crossed to XX males. When progeny testing for YY females from a group consisting of XY and YY females the possible sex ratio that maybe observed if use XX males would be mixed-sex (1:1) and all-male (1:0) progeny. When progeny testing for YY females from group predicted to consist all YY female, such as estrogen-treated progeny of YY x YY crosses, the expected sex ratio of the progeny would be all- or nearly all-male.

2.7 Sex Reversal

2.7.1 Preparation of hormone feed

The hormone feed used for the experiments described in Chapters five and six was prepared following the alcohol evaporation method described by Guerrero (1975). Diethylstilbestrol (DES) and methyltestosterone (MT) hormones (Sigma brand) were incorporated in finely sieved commercial brand fry mash (approximately 30% crude protein). A minimum of 100g was usually prepared at any one time. The required hormone was weighed in a Metler

analytical balance to the nearest milligram (Metler H80; minimum capacity, 0.0001g and maximum capacity, 160g) and dissolved in 50ml (500 $ml.kg^{-1}$ diet) technical grade (95% proof) ethyl alcohol. The alcohol with hormone was then stirred thoroughly to homogeneously mix the hormone and then sprayed evenly over the feeds. The feeds were then mixed thoroughly and dried at room temperature. While drying the hormone-treated feed was occasionally mixed to prevent it from clumping. The control feed was prepared in the same manner without hormone. When preparing DES incorporated feed, all safety precautions were employed including wearing of gloves, breathing mask, laboratory gown and allowing only males to do the job. DES hormone has been known to cause cancer in human, particularly in women.

2.7.2 Hormone treatment in concrete tanks

The hormone treatment of the fish used for the experiments described in Chapter five was carried out in concrete tanks. The fry were stocked in small fine mesh cages (approx. 15cm x 15cm x 18cm) installed in outdoor circular concrete tanks (approx. 900 l capacity) at a stocking density of 150 fry per cage (approx. 54 $fry.l^{-1}$) (see Abucay and Mair, in press a). All treatments were triplicated and the treated and control fry were stocked in separate tanks to avoid contamination (Abucay and Mair, in press a). A flow of fresh and clean water at a rate of one $l.min^{-1}$ was maintained except during occasional power failures. Water flow was

usually resumed within one hour as the Project had a standby diesel-run water pump.

Prior to initiation of hormone treatment, the fry were sampled for initial size. The fish were fed *ad libitum* four times daily (0800, 1000, 1400 and 1600 hr). A feeding ring made of plastic hose was provided for each cage to concentrate the feeds at the center to prevent it from scattering. To monitor the size and number of the fish, weekly sampling was carried out. At the same time the cages and tanks were also cleaned of accumulated waste and algae, and the tanks were filled with fresh and clean water.

At the end of hormone treatment, all the fish in each cage were counted, weighed and measured. The fish were weighed by slowly pouring a plastic basin containing the fish into a plastic strainer with round bottom allowing the water to drain off. The bottom of the strainer was then wiped with a towel to absorbed any excess water. The fish were then poured into a plastic bowl containing a small volume of water mounted on a digital weighing scale (Sartorius brand; minimum capacity, 0.1g) with the weight adjusted to zero. The average weight of each fish was then estimated by dividing the total weight of all the fish in each cage with the total number of fish surviving. The total length (measured from the tip of the head to the tip of the caudal fin) of each of ten randomly sampled fry from each replicate were measured to the nearest millimeter (mm) using a caliper. Only ten samples from each replicate were measured to prevent the fish from being subjected to too much stress. All the fish were then stocked and grown for sexing (see section 2.8 of this chapter).

2.7.3 Hormone treatment in aquaria

The hormone treatments of the fish used for the experiments described in Chapter six were conducted indoors in glass aquaria (approx. 195- and 264-l capacity). In each aquarium three small fine mesh cages (approx. 20- and 30-l capacity for 195- and 264-l aquarium, respectively) were installed for replication (Photo 2.3). To stretch the cage for maximise use of the available space and to retain the shape, a welded square metal frame was tied at the bottom of each cage. Each cage was provided with a feeding ring made of plastic hose fixed in the middle to prevent the food from scattering. A plastic bowl fixed at the bottom of each cage was also provided to prevent food easily passing through the bottom. Occasional cleaning was carried out by carefully lifting the bowl and washing it to remove accumulated waste. If necessary, the cages were also washed to removed particles that clogged the holes, to ensure efficient water recirculation and oxygenation. The water was not changed throughout each experiment but siphoning of accumulated waste at the bottom of the aquarium was occasionally carried out if needed. Fresh, clean water was usually added to replenished lost water due to evaporation and seepage (if any) to maintain the desired level of water. All aquaria were aerated with diffused air from a centralized electric air blower (0.25 horse power).

Photo 2.3. An indoor aquarium set used for the experiment described in Chapters three and six.

For the sex reversal experiments described in Chapter six and those experiments described in Chapter three which were performed under high temperature, the set up of the aquaria used were similar as those described above except that a controlled heating was provided. The desired temperature was maintained using a 14 to 38°C range Biotherm-2000 thermoregulator and Nimrod thermostatic heater. Since the heater was installed in one corner only, a motorized pump filter was installed in each aquarium to maintain uniform temperature by continuously circulating the water (see Photo 2.3). Similarly, the filter also prevent the accumulation of waste products. The temperature was monitored daily using a Jenway digital thermometer (Model 9070). The control fish were stocked in a similar set up without heating.

During treatment, the fish were fed four times a day (0800, 1000, 1400 and 1600 hr). At the end of treatment, all the surviving fish were counted after which they were stocked and grown for sexing.

2.8 Sexing of Fish

2.8.1 Growing of fish for sexing

The growing of fry to fingerling size for sexing was done in fine mesh cages (1 m^3) installed in earthen ponds. The cages were tied to wooden stakes with at least 60% (60 cm) of the total cage depth submerged leaving a 40% (40 cm) freeboard as allowance for the fluctuating water level. Each cage was provided with label bearing the identity of the stocked fish. Feeding was done twice a day (0800 and 1600 hr); initially with commercial brand fry mash and later with crumbled feeds, when the fish were approximately 1g in weight, at a feeding rate of 2-3% biomass daily. To supplement the feeding, the pond was fertilized following the standard rate.

Occasionally, the cages were lifted to check the condition of the fry (see Photo 2.4). At the same time, the cages were also washed and scrubbed to remove any particles and algae that had clogged the net holes and which would prevent the exchange of water resulting in poor oxygenation. Before washing the cage, the fish were carefully scooped out from the cage and put in a plastic bucket. After cleaning, the fish were put back into the cage. The nursing of fish prior to sexing usually took from two to four months depending on the stocking density, climate and feeding.

Photo 2.4. Inspection of fry stocked in 1 m^3 fine mesh hapa suspended in earthen ponds.

2.8.2 Gonad squash

When the fish were only intended for the determination of sex ratio of progeny such as in the progeny testing of YY males, YY females and XX males or in sex reversal experiments, the fish were sexed by gonad squash as described by Guerrero and Shelton (1974). This technique involved the removal of the gonads, placing them on a slide and applying a drop of aceto-carmine

stain solution (Photo 2.5). The gonad was then gently squashed beneath a cover slip and examined under a microscope (100X). If the gonads were already fully developed, a clearly defined oogonia or oocytes could be seen in females while in males a granular appearance indicative of presence of spematocytes (see Mair, 1988).

The aceto-carmine solution was prepared by dissolving 0.5g carmine powder in 100ml (milliliter) 45% acetic acid (45ml pure acetic acid and 55ml distilled water). The solution was stirred thoroughly until mixed homogeneously and boiled for 3-4 minutes after which was cooled down and stored in sealed bottle.

Usually the fish were sexed when they were already at least 60 days old or an average size of 2.5g. Fish were killed by overdosed in water containing high concentration of amino-benzocaine solution (solution was prepared by dissolving 0.5g amino-benzocaine powder in 100ml technical grade ethyl alcohol). The ratio of water and amino-benzocaine solution was usually determined by putting the fish in a plastic basin with water and add drops of the solution until the fish died. The gonad was removed following the procedures described by Mair (1988). A posterior-anterior cut was made along the belly and a further dorso-ventral cut made to the rear of the gill operculi (below the lateral line). The belly flaps were opened and the viscera were lifted or removed and the gonad, which was to be found to be enclosed in a thin peritonium in contact with the ventral portion of the swim bladder, then removed using a forceps and mounted on a glass slide.

Photo 2.5. A typical view during sexing of fish by gonad squash, a regular activity of the project staff.

2.8.3 Manual sexing

The sex of fish that were not to be killed was determined manually by carefully examining the urino-genital papillae. A female has round distinct genital papilla with two openings; one is a slit across the papilla where eggs pass through and another small opening for the passage of urine. A male has a protruding long genital papilla with a single urino-genital pore at the tip.

This manual sexing technique is very simple and easy to do, although one must have adequate experience to be able to distinguish the males and females. Fish to be sexed should be already large enough (>25g) to clearly differentiate the urino-genital papillae. To ensure 100% accuracy during the manual sexing of some of the fish used in the presented studies, a method was devised by using a soft but firm weed stem (@1mm diameter). The tip of the stem was inserted into the genital papillae, if could not be insert into the pore the fish was most likely to be a male. However, this technique was only used when there was doubt with the sex.

2.9 Growth Evaluation

The evaluation of growth of different genotypes or groups of fish was performed in earthen ponds or cages installed in earthen ponds, using separate or communal rearing. The different culture environments and rearing approaches are described.

2.9.1 Initial growing of fish for growth evaluation

The rearing of fry to fingerling size for growth evaluation was carried out in bigger cages (5m x 2m x 1m) because of the higher number of fish in each group. Initially, the fry were stocked in fine mesh cages until they were big

enough (>1g) to be marked by fin clipping after which they were transferred into cages made with bigger mesh net cages (mesh #24). During the rearing in fine mesh cages, sometimes it would be necessary to reduce the stocking density by splitting the fish and stocked in two separate cages at identical densities. To prevent or at least minimize differential growth, all the fish from all treatments were treated in the same way.

Initially, the fish were fed with fry mash and later with crumbled feeds at 3-5% biomass daily. The cages were occasionally lifted to check the condition of the fish and for cleaning.

2.9.2 Growth evaluation in ponds

A component of the growth evaluation experiments described in Chapters four and five was performed in earthen ponds under communal rearing. The use of communal rearing has many advantages (see Wohlfarth and Moav, 1985). When comparing the growth of different genotypes or groups of fish, the fish should be grown under homogeneous conditions to avoid or minimize biases due to differences in the environment. However, when growing fish separately in ponds (or cages), ensuring homogeneous conditions for all the fish has often not been realistic due to possible physico-chemical differences between ponds (or differences in location between cages). By stocking the different groups of fish to be evaluated in one pond all the fish are exposed to the same conditions and are thus treated equally. Similarly, communal rearing may be the more appropriate

method when there is a limited facilities. To ensure reliable results, the fish to be stocked should, as far as possible be of the same size and age. Normally individual fish interact and compete with each other, particularly for food and space (McGinty, 1985). When the sizes are variable, the bigger fish may be more dominant than the smaller fish thus may have a competitive advantage and grow faster than the smaller fish.

The growth evaluations were performed in 200 m^2 earthen ponds (Photo 2.6). Prior to stocking, the ponds were prepared following standard method as described earlier. A one meter average water depth was maintained to all ponds and fresh and clean water from a deep-well pump was regularly added to maintain the desired depth. To prevent the accidental intrusion of wild fish into the grow-out pond, the water outlets and inlets were screened. The grass growing on the dike, particularly on the slope, was regularly cut to provide maximum space for the fish and also to prevent the grass from shading the water.

Before stocking, the average initial weight of the fish in each group was first determined. At least 50% of the total fish in each group were individually weighed (Sartorius brand digital weighing balance). To monitor growth and adjust feeding rate, the stocked fish were sampled every three weeks beginning from stocking. The sampling was carried out by a partial seining of the fish using a net specially made for this purpose. At least 20 fish in each group were randomly picked out and individually weighed. Seining was performed for a maximum of three times in each pond if insufficient fish were caught. This was

necessary to prevent the fish from too much stress and to minimise disturbance to the pond bottom which can affect water turbidity. The different fish groups were sorted according to the location of clipped fin.

Photo 2.6. Aerial view of the 200 m^2 earthen ponds used the growth evaluation experiments.

For the growth evaluation described in Chapter four, the experiment was performed in three ponds which served as replicates. All fish groups were stocked and equally represented in each pond. The fish were grown following the method of Green (1992) but the fertilization rate was modified. For the first 60 days the fish were not given artificial food but organic and inorganic fertilizers were applied following standard rates as described earlier. The fertilizers were apportioned on a weekly basis for more efficient results. After 60 days, the application of fertilizers was discontinued and the fish were fed commercial pelleted feeds at a rate of 3% biomass daily which was given twice a day (0800 and 1600 hr). This method was adopted because when the fish are still small the natural food present in the water may still be sufficient for them to attain adequate rates of growth and giving supplemental feeds may just be an additional cost without any significant effect on growth (see Green, 1992).

For the growth evaluation experiments described in Chapter five, the ponds used were not replicated due to limited facilities and experimental fish. As the fish were already approximately 30g in weight at the start of the growth evaluation, they were given commercial pelleted feeds at a rate of 3% biomass daily, given twice a day.

At harvest, partial harvesting was first carried out by seining, to reduce the number of fish in the pond prior to draining. When draining pond without initial partial harvesting, the bulk of the fish are concentrated in the deepest and muddy portion of the pond. If the fish are not collected immediately, their gills are

clogged with mud and die easily. The number, individual weight (to the nearest 0.1g) and sex of the surviving fish were noted.

2.9.3 Growth evaluation in cages

Separate rearing - The evaluation of post treatment growth component of the experiments described in Chapter five was performed in net cages (mesh #24) using separate stocking. The cages, which measured 1.5m x 1.5m x 1m were installed in 200 m^2 earthen ponds. The cages were tied to wooden stakes with 60% (60cm) of the total cage depth submerged into the water and the remaining 40% (40cm) as freeboard for the fluctuating water level. The position of the cages were arranged in such a way to provide enough space for easy access around each cage and for the water to circulate for maximum oxygenation. Fresh and clean water was added to the pond daily to maintain a one meter average pond water depth.

Before stocking the average initial weight of the fish was noted by individually weighing 50 random samples in each cage. To monitor the growth of the fish, sampling was carried out every 14 days by individually weighing a random sample of 50 fish from each cage. The stocked fish were fed with a commercial brand crumble feed at a rate of 10% of the fish biomass daily. Standard rates of organic and inorganic fertilizer (100 $kg.ha.month^{-1}$ 16-20-0 NPK) were also applied to induce and maintain primary productivity.

Communal rearing - The cage component of the growth evaluation experiments described in Chapters four and five utilised communal rearing. The advantage of communal stocking was described earlier. The fish were stocked in 3m x 3m x 1.5m net cages (mesh #17) installed in a 1000 m^2 earthen pond. Artificial feeding at a rate of 5% biomass daily was done throughout the duration of the growth evaluation. Initially, the fish were fed a commercial brand crumble feed and later with a commercial brand pelleted feed, when the fish were already 20-30g in weight. Standard rates of organic and inorganic fertilizers were also applied to induce and maintain primary productivity

Before stocking the average initial weight of the fish in each group was first determined by individually weighing all the fish in each group. To monitor the growth and adjust the feeding rate, the stocked fish were sampled every three weeks beginning from stocking by individually weighing all the fish in each group.

2.10 Determination of Gonadosomatic Index (GSI) and Percentages of Dress-out and Fillet Yield

The total weight may be a sufficient basis for determining the growth of fish. However, presenting the body weight alone does not show the net proportion of edible flesh that we can get from the fish. It may be possible that an individual fish could be big or heavy but a differential proportion of this weight gain may be due to inedible parts such as the head, skeleton, scales, fins and

viscera. In the Philippines, before cooking, tilapia are normally prepared or dressed by removing the scales, fins, gills and viscera. For export purposes, fish are normally processed to only include the edible flesh (fillet).

Initially, GSI has been primarily used to determine the stage of maturity and fecundity of individual fish at a certain stage (Peters, 1971; Babiker and Ibrahim, 1979). However, recently, Abucay (1996) observed that in *O. niloticus* males the GSI has an indirect relationship to dress-out and fillet weight. Those fish with a higher GSI tend to have a relatively lower percentage of dress-out and fillet weight.

The inclusion of the determination of GSI and percentages of dress out and fillet weight in any growth evaluation aid in the interpretation of the results with regard to priorities of the consumer.

2.10.1 Gonadosomatic index (GSI)

GSI is the percentage of the gonad weight relative to total body weight. GSI was determined by dividing the whole gonad weight with the body weight and multiplying this by 100 ($\frac{gonad-weight}{total-weight} \times 100\%$). The gonad weight of individual fish was determined by carefully removing the whole gonad and weighing it. The removal of the gonad was carried following the procedure described in section 2.8.2 of this chapter. Both ends of the gonad were cut and removed using

forceps and weighed using Metler analytical weighing balance (H80). The total weight of the fish was first noted before removing the gonad.

2.10.2 Percentage of dress-out weight

After removing the gonad, the viscera, fins, scales, and gills were carefully removed, after which the fish was weighed (Sartorius weighing balance) to get the dress-out weight. The percentage of dress out weight relative to total body weight was determined by dividing the dress out weight with the total weight and multiplied by 100% ($\frac{dressout-weight}{total-weight} \times 100\%$).

2.10.3 Percentage fillet weight (fillet yield)

The edible flesh of the dressed fish was then removed to get the fillet weight. The process was done by removing the flesh starting immediately below the base of the dorsal fin. Using a sharp pointed knife, the muscle or flesh was carefully cut down and peeled off leaving only the bones. The flesh was then weighed (Sartorius weighing balance) to get the fillet weight and determined the percentage of fillet weight relative to body weight by dividing the fillet weight with the total weight multiplied by 100% ($\frac{fillet-weight}{total-weight} \times 100\%$).

CHAPTER THREE

ENVIRONMENTAL SEX DETERMINATION:

THE EFFECTS OF TEMPERATURE AND SALINITY

ON SEX RATIO

3.1 Introduction

One factor in the study of sex determination in tilapia is the theoretical assumption of the presence of sex chromosomes and that these chromosomes are the major factors which control the sex. There are two systems of what is usually termed chromosomal sex determination; one with homogametic female (XX) and heterogametic male (XY) and one with heterogametic female (WZ) and homogametic male (ZZ). In the XX/XY system, the female sex determining gene or genes are located on the X chromosome while the male gene or genes are on the Y chromosome. The Y chromosome factor override more on the X chromosome so that those individuals with a Y chromosome will develop into males (XY) while those without will develop into females (XX). On the other hand, in the WZ/ZZ system, the female gene or genes are located on the W chromosome while the male gene or genes are on the Z chromosome. However, unlike the XX/XY system, the W chromosome factor override more on the Z chromosome so that those individuals that carry the W chromosome will develop into females (WZ) while those that do not will develop as males (ZZ). The sex chromosomes segregate and combine in a Mendelian manner so that under normal conditions the ratio of male and female in the progeny is expected to approximate to 1:1.

Studies on the mechanism of sex determination indicates that the chromosomal systems are found in tilapia (see review of Hunter and Donaldson, 1983). Studies on *Oreochromis niloticus* show that this species exhibits male

heterogamety and female homogamety (Penman *et al.*, 1987; Shah, 1988; Mair *et al.*, 1991a; Muller-Belecke and Horstgen-Schwark, 1995). In accordance with this system it is expected that a cross of XX♀ x XX♂, XX♀ x YY♂ and YY♀ x YY♂ should give all XX female, XY male and YY male progeny, respectively.

However, some of the actual sex ratios observed from intrastrain crosses of *O. niloticus* deviate from the predicted ratios. For example, in crosses of normal males and females (XX ♀ x XY ♂), Shelton *et al.* (1983) observed male ratios ranging from 31 to 83% with a mean of 54.7% males. The mean is still within the expected range, although, the sex ratios of 21% of the 71 progeny groups tested had sex ratios significantly outside the expectation of 1:1, with 18% being skewed towards a male preponderance. In single pair matings with sibling sex-reversed genetic females (XX♀ x XX♂), of 35 crosses tested, Baroiller (1996) observed all-female progenies in only five crosses, the others having female ratios ranging from 65 to 99%. In another study, Calhoun and Shelton (1983) observed that the sex ratio of the progeny of sex-reversed genetic females varies depending on the source of the females used. The percentage of female progeny from mothers that were half and full siblings of the sex-reversed genetic female brooders (99.7%) was significantly higher than that in the progeny of randomly selected females (94.7%). Theoretically, based on simple monofactorial sex determination, the progeny of sex-reversed genetic females should be all-female and there should be no difference between the two female groups. Similarly, in a series of progeny tests in crosses of XX♀ x YY♂ (33 families), Mair *et al.* (1992) observed ratios ranging from 71.6 to 100% male.

The occasional deviations of observed sex ratio from the predicted may be due to rare autosomal or environmental sex-modifying factors as suggested by Mair *et al.* (1991a).

Environmental sex determination (ESD) occurs when the sex of an individual is determined by the environment encountered rather than being fixed by genotype at conception (Charnov and Bull, 1977). In some reptiles the temperature at which the eggs are incubated determines the sex of the hatchling (see reviews by Bull, 1980). For example, in the American alligator, *Alligator mississippiensis*, when the eggs are incubated at 30°C the young are all female but at 33°C all the hatchling are male (Deeming and Ferguson, 1989). On the other hand, in turtles, all-male progeny are produced at low temperatures (22 to 28°C) and all-female at high temperatures (30 to 35°C), whilst in a narrow range of temperature around 29°C both sexes are found (Bull *et al.*, 1982). The reptiles and turtles which exhibit environmental sex determination lack heteromorphic sex chromosomes as seen in mammals and other animals which exhibit chromosomal sex determination (Bull, 1980).

Whilst the determination of sex by environmental factors after conception is a relatively rare phenomenon among gonochoristic species (those having separate sexes), there are experimental studies which show this to occur also among poikilothermic vertebrates (Conover and Kynard, 1981). The occurrence of unexpected sex ratios in studies on sex determination was thought to be due to the effect of the environment. Winge (1934) noted that, in *Lebistes reticulatus*, relatively high proportions of males appear during the early part of the summer

while the percentage of females increases considerably outside this season and in some instances the offspring during winter was made up of females alone. Romer and Beisenherz (1996) reported that in *Apistogramma* (Teleostei, Cichlidae), when the temperature during incubation period changed from 26°C, which yielded a balanced sex ratio, to a warmer (29°C) or cooler (23°C) temperatures, the percentage male in the offspring increased and decreased, respectively, by about the same proportion. The sex ratio in the Atlantic silverside (*Menidia menidia*) was also found to be sensitive to temperature during a specific stage, late in larval development, just before completion of metamorphosis (Conover and Kynard, 1981). At a warm fluctuating temperature, (17 to 25°C) the sex ratio of the progeny (male : female) was either 1:0, or nearly so, or 1:1. At a cold fluctuating temperature (11 to 19°C) the progeny had a consistently higher proportion of females. Similarly, other environmental extremes, such as low pH, have also been observed to affect sex ratio in Cichlids and Poecillids (Rubin, 1985).

Whilst the results of the aforementioned studies were observed in controlled conditions in laboratories, some observed sex ratios of fish in the natural environment also appears to have a relationship with temperature. Beamish (1993) reported that the male ratios of 20 populations of southern brook lamprey larvae, *Ichthyomyzon gagei*, collected from throughout much of their range in the southeastern United States varied between 9 and 49% males. The variation was suggested to be due to environmental sex determination since differential mortality was an unlikely factor because the sex ratio was similar

among age groups within each population. Lagomarsino and Conover (1993) examined the sex ratios of wild populations of *Menidia menidia* in Nova Scotia (NS) for which no evidence of ESD and in South Carolina (SC) where the level of ESD has previously been shown to be among the highest recorded for this species. The sex ratios of the NS fish were heterogeneous but fall into distinct classes like that expected from Mendelian segregation, while for the SC fish the proportion of females was near to zero. The sex determination in *M. menidia* is suggested to be controlled by an interaction between major genetic, polygenic and temperature factors and that the relative importance of each component differs with latitude.

However, unlike reptiles, the tilapias are known to exhibit chromosomal sex determination, and yet, the observed sex ratio can be affected by temperature as mentioned earlier. In an initial study on the effect of temperature on the sex ratio in *Oreochromis* species (Mair *et al.*, 1990), it was observed that at a relatively cold temperature (19°C) the sex ratio in *O. mossambicus* was skewed to male and at a warm temperature (32°C) the sex ratio in *O. aureus* was skewed to female. In another study on *O. niloticus*, Baroiller *et al.* (1996) observed the proportion of males in the fish reared at temperatures ≥32°C was increased from 0 to 90% as compared to the controls.

The aims of the works presented in this chapter are to;

1. Evaluate the effect of extreme temperatures and salinity on sex ratio in *O. niloticus*,

2. Evaluate the response in sex ratio of different fish genotypes under extreme environmental conditions,

3. Discuss the implication of the results in relation to the validity of the hypothesis of the theory of an underlying predominantly monofactorial genetic sex determining mechanism.

3.2 Materials and Methods

To determine the effect of environment on sex ratio, a series of experiments were performed indoors, in glass aquaria (following a similar set up used for the sex reversal experiments as described in section 2.7 in Chapter two). Sexually undifferentiated progeny of the same size (8-10mg) and age (first feeding) were reared (treated) in extreme conditions, which the fish would not frequently encounter in nature, for a minimum duration of 21 days during the period of sex differentiation. The fish were fed four times a day *ad libitum* (at 0800, 1000, 1400 and 1600 hr) with normal food (hormone-free food) using a commercial brand fry mash. After termination of treatments, the number of fish surviving was noted to determine the percentage survival, after which they were grown on for later sexing by gonadal squash to determine the sex ratio. The different experiments and the statistical analysis used are described as follows;

Experiment 1. Purebred and crossbred putative all-female (XX♀ x XX♂) and all-male (XX♀ x YY♂) progeny were reared at elevated (36°C) and at ambient temperatures. The progeny were produced from XX males and YY males in the Egypt-Swansea, Egypt-ICLARM and Ghana-BFAR strains in crosses with females in the Egypt-Swansea, Egypt-ICLARM, Kenya-Turkana and Ghana-BFAR strains. The progeny of each family from each strain cross was divided into two. Half were reared at high temperature and the other half were reared at ambient temperature to serve as control.

Experiment 2. Sexually undifferentiated putative all-female (XX♀ x XX♂), all-male (XX♀ x YY♂) and all YY male (YY♀ x YY♂) progeny in the Egypt-Swansea strain were reared in triplicates at three temperatures (Treatments); low (26°C), normal (30°C) and elevated (36°C), at a density of 100 fish per replicate. This experiment was conducted during cooler months (January to February) to enable a "low" temperature regime at ambient temperatures. The temperature in treatments under "normal" and "high" temperature levels were then increased and maintained using a thermostatically controlled heater.

Experiment 3. With the observation in experiment 2 that YY males in the Egypt-Swansea strain were sensitive in sexual development, to high temperature, a follow up experiment to determine the response of purebred and crossbred YY males to high temperature was performed. Purebred YY males from crosses of YY♀ and YY♂ (Egypt-Swansea) and crossbred YY males from crosses of Egypt-Swansea YY♀ x Egypt-ICLARM YY♂ were reared at elevated (36°C) and

ambient temperatures. The progeny of each family from a strain cross was divided into two. Half were reared at high temperature and the other half at ambient temperature, serving as a control. No survival data was collected for the control of purebred YY-male progeny. After splitting each family into two, the control fish were stocked directly in cages (instead of rearing in aquaria) and sexed manually when they were approximately ≥10g in weight (the control progeny for the pure strain YY males are left as part of an on going selection for sex ratio programme where it is hoped to give more light on the cause of the observed sensitivity to high temperature).

Experiment 4. Putative all-female progeny from within strain cross of XX♀ x XX♂ in the Egypt-Swansea (ES) and Egypt-ICLARM (EI) strains were reared in triplicates at three salinity levels (Treatment); 0 (control), 10, 20 and 25 ppt, at a density of 250 (ES) and 280 (EI) per replicate. The fish in each replicate were stocked separately in 40-l capacity indoor glass aquaria. After stocking the fry, the salinity level was slowly increased (approximately 2-3 ppt for every application; once in the morning and once in the afternoon or 4-6 ppt per day) by dissolving a commercial grade unionized salt (crystallized sodium chloride) at a rate of 2.5 gram.l^{-1} of water or a total of 100g per aquarium. A maximum of four to five days was required before reaching the desired salinity level, particularly for those treatments at higher levels. The control fish were reared using normal ground water (zero salinity). The salinity level was monitored daily using a refractometer.

Statistical analysis. For experiments 1 and 3, the significance of difference between the survival rate of the treated and control progeny was tested by a Paired-Samples T-Test. The deviation of sex ratio of the treated fish from that of the control was measured using a 2 x 2 contingency χ^2 test. The percentage decrease of males or females in the treated progeny was determined by dividing the difference between the sex ratio of the treated and control fish with that of the control and multiplying this by 100. The mean sex ratios of the progenies of different strains of XX males were compared using a one-way analysis of variance (ANOVA). The presence of interactions between XX males and females (strain effect) on sex ratio was determined by a two-way ANOVA.

For experiments 2 and 4, the difference between percentage survival and sex ratio of the different treatments was tested by a one-way ANOVA. Before the analysis, all mean values in percentage form were first arcsine transformed. The comparison among treatment means was performed using the Bonferroni multiple range test.

3.3 Results

3.3.1 Effect of high temperature on sex ratio in purebred and crossbred putative all-female and all-male progeny

Table 3.1 summarises the survival and sex ratio of the treated and control purebred and the crossbred putative all-female progeny. The stocking density for the different replicate batches was variable. Initially, a stocking density of 100 fish.cage^{-1} was used. However, it was necessary to increase the stocking density for those experiments that were performed during periods of colder months to give allowance for higher mortality rate. Overall, the result of a Paired-Samples T-Test shows the treated fish had a significantly (P=0.038) lower mean survival (62.72%) than the control (71.64%). Although, if we examine the survival of individual replicates, there were a few cases where the controls have a lower survival rate than the respective treated fish. It should be noted that the control fish were not provided with controlled heating. During cold months, ambient temperatures would normally drop below optimum levels (28-30°C) which may have affected survival rates.

The sex ratios of the controls were not all the expected 100% female. A proportion of males which ranged from 0 to 36.84% was observed in 21 of the 30 control replicate families. High temperatures caused a remarkable decrease in the proportion of females in the treated compared to the control progeny with differences ranging up to 88.62% (see Figure 3.1). The percentage of females

was lower in the treated progeny than in controls in 28 of the 30 replicates tested, with the difference being significant ($P<0.01$) in 18 of these.

To determine the difference in proportional decrease of females in the treated progeny compared to controls in the different strains of XX males and females, a two-way ANOVA was performed. The strain effect of two XX males (Egypt-Swansea and Egypt-ICLARM) and the three maternal sources (Egypt-Swansea, Egypt-ICLARM and Kenya-Turkana) on sex sensitivity to high temperature was determined. It was not possible to include the Ghana-BFAR XX males in the two-way ANOVA as they were crossed to only one of the females used in the analysis. The treated progeny of the Egypt-Swansea XX males had a significantly ($P=0.003$) greater decrease in the proportion of females (55.77%) compared to that of the treated progeny of the Egypt-ICLARM XX males (20.42%). The results indicate that the sex ratio of the progeny of Egypt-Swansea XX males tend to be more easily affected in sexual development by high temperature than the of Egypt-ICLARM XX males.

There is no significant effect of female source on the observed difference in sensitivity to high temperature between the progeny of the two strains (Figure 3.2). When comparing the three groups of XX males (regardless of female source), the mean percentage decrease of females in the treated progeny of Egypt-Swansea XX males (55.77%) is significantly higher than that of the Egypt-ICLARM (20.42%) and Ghana-BFAR males (14.77%); the latter two were not significantly different.

Table 3.1. Summary of the percentage of females and survival rates of purebred and crossbred progeny from XX♀ x XX♂ crosses reared at elevated (36°C) and at ambient temperatures during sexual differentiation stage. Survival is the percentage of fish surviving after a minimum of 21 day temperature treatment. Replicate denotes the number of batches or families tested from each strain cross.

Strain cross (XX♀ : XX♂)	Replicate	Stocking density (fish.cage^{-1})	Treated (36°C)			Control (ambient)			Decrease in females (%)	2 x 2 χ^2 value
			Actual temp.	Survival (%)	Females (%)	Actual temp.	Survival (%)	Females (%)		
ES x ES	1	100	36.23 (± 0.15)	33.67	50.60	27.54 (± 1.11)	89.33	97.04	47.86	77.36 ***
	2	100	36.47 (± 0.18)	80.67	48.0	27.54 (± 1.11)	79.67	96.15	50.08	52.95 ***
	3	100	37.01 (± 0.23)	82.67	20.42	29.44 (± 0.51)	66.98	80.0	74.48	102.90 ***
	4	100	36.89 (± 0.53)	71.0	36.92	29.16 (± 0.64)	80.67	73.88	50.03	35.04 ***
KT x ES	1	100	36.45 (± 0.32)	72.33	57.14	27.24 (± 0.77)	94.0	78.60	27.30	17.69 ***
	2	100	36.22 (± 0.36)	85.0	10.42	26.93 (± 0.76)	92.33	91.54	88.62	222.90 ***
	3	100	37.14 (± 0.45)	84.67	22.14	30.15 (± 0.81)	64.33	63.16	64.95	33.98 ***
EI x ES	1	100	36.12 (± 0.36)	73.81	29.17	27.56 (± 0.61)	92.38	96.77	69.86	137.40 ***
	2	100	36.88 (± 0.17)	79.33	71.27	30.05 (± 0.87)	79.67	95.76	25.57	26.21 ***
	3	250	36.52 (± 0.27)	31.60	28.57	28.91 (± 0.76)	9.67	100.0	71.43	5.77 ***
EI x EI	1	100	36.88 (± 1.28)	61.33	80.50	29.88 (± 0.58)	78.0	94.47	14.79	15.33 ***
	2	100	36.99 (± 0.21)	35.0	83.33	29.88 (± 0.58)	78.0	97.86	14.85	14.07 ***
	3	100	36.78 (± 0.51)	74.33	100.0	29.90 (± 0.55)	74.0	100.0	0	0 ^{ns}
	4	100	37.47 (± 0.07)	64.0	82.01	25.00 (± 0.52)	92.33	79.19	-3.56	3.74 ^{ns}
ES x EI	1	100	35.99 (± 1.39)	53.0	60.0	27.10 (± 0.73)	54.67	93.98	36.16	28.47 ***
	2	100	36.09 (± 0.48)	61.06	96.77	26.62 (±0.98)	87.61	100.0	3.23	0.02 ^{ns}
	3	175	36.08 (± 0.58)	42.86	83.87	26.62 (±0.98)	n/a	100.0	16.13	3.75 ^{ns}
	4	300	36.09 (± 0.60)	46.67	91.18	26.62 (±0.98)	n/a	100.0	8.82	1.39 ^{ns}
	5	300	36.09 (± 0.48)	48.0	97.73	26.62 (±0.98)	23.0	100.0	2.27	0.32 ^{ns}
KT x EI	1	100	36.81 (± 0.51)	85.0	18.18	29.59 (± 0.54)	83.0	63.25	71.26	48.34 ***
	2	100	36.76 (± 0.24)	74.33	77.37	29.38 (± 0.53)	83.33	92.42	16.28	10.65 **
GB x GB	1	100	37.32 (± 0.48)	78.33	77.62	30.10 (± 0.60)	96.67	96.03	19.17	20.50 ***
	2	100	36.72 (± 0.50)	n/a	80.0	28.44 (± 0.77)	n/a	97.75	18.16	10.64 **
	3	100	36.06 (± 0.58)	36.36	87.50	26.62 (± 0.98)	n/a	96.67	9.49	0.33 ^{ns}
EI x GB	1	100	36.97 (± 0.47)	73.50	88.78	29.60 (± 0.96)	99.50	98.48	9.85	8.21 **
	2	100	36.13 (± 0.58)	35.56	83.33	26.77 (± 0.86)	55.11	97.96	14.93	0.42 ^{ns}
	3	300	36.94 (± 0.42)	14.0	67.67	26.62 (±0.98)	7.33	100.0	32.33	2.00 ^{ns}
	4	200	36.09 (± 0.48)	53.19	87.50	26.62 (±0.98)	57.98	100.0	12.50	3.08 ^{ns}
	5	300	36.06 (± 0.58)	27.33	84.91	26.62 (±0.98)	n/a	93.10	8.80	0.54 ^{ns}
	6	300	36.06 (± 0.58)	27.33	92.31	26.62 (±0.98)	n/a	100.0	7.69	1.18 ^{ns}

ES = Egypt-Swansea, EI = Egypt-ICLARM, KT = Kenya-Turkana, GB = Ghana-BFAR

χ^2 value - represents the deviation of sex ratio of the treated progeny from that of the control (^{ns}= not significantly different; ** = P<0.01; *** = P<0.001)

n/a = not available

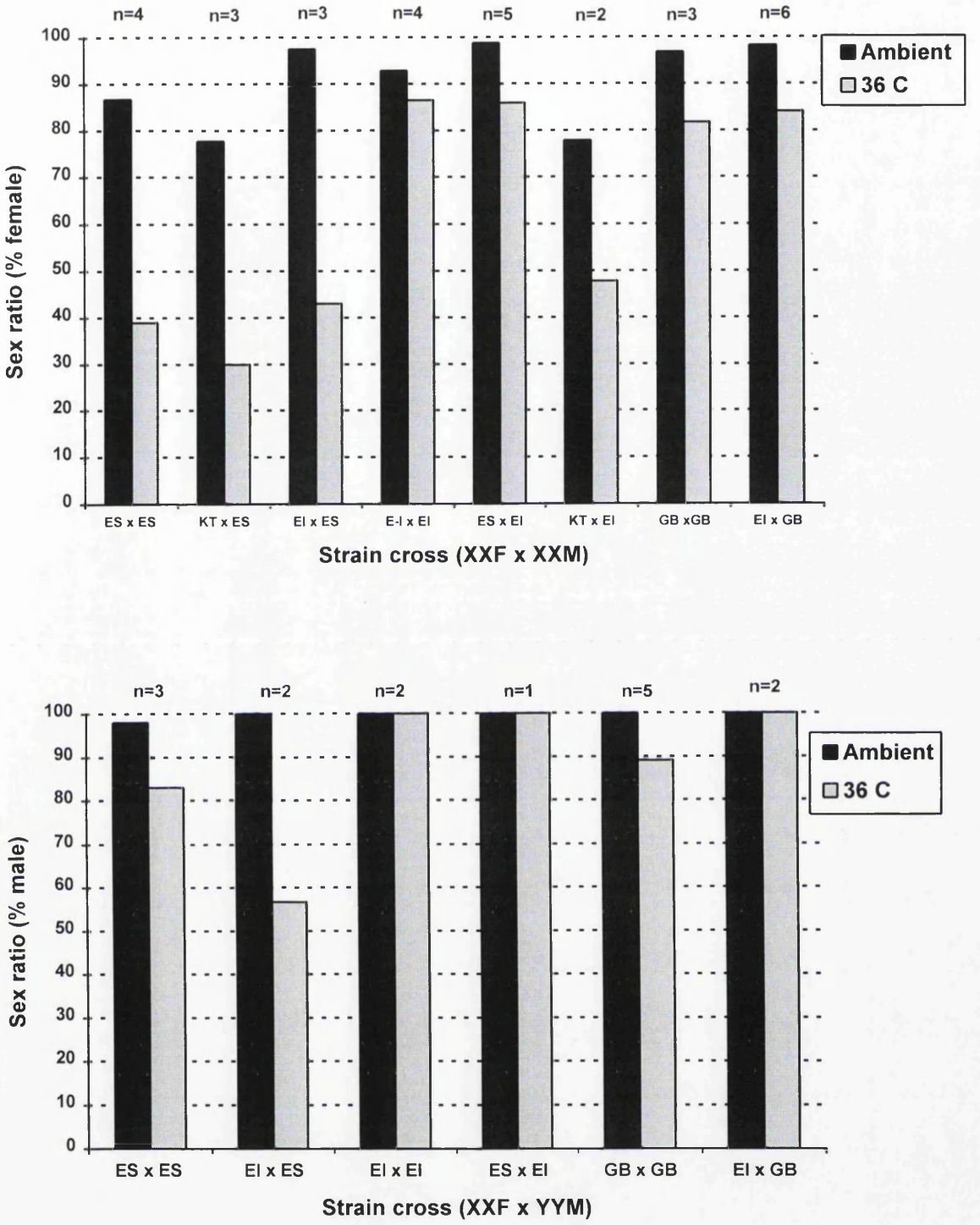


Figure 3.1. Histograms showing the mean percentage of females of the putative all-female (upper chart) and percentage of males of putative all-male progeny (lower chart) from different strain crosses reared at elevated (36°C) and at ambient temperatures during the period of sex differentiation (n = number of replicate batches families for each strain cross). ES = Egypt-Swansea, EI = Egypt-ICLARM, KT = Kenya-Turkana, GB = Ghana-BFAR.

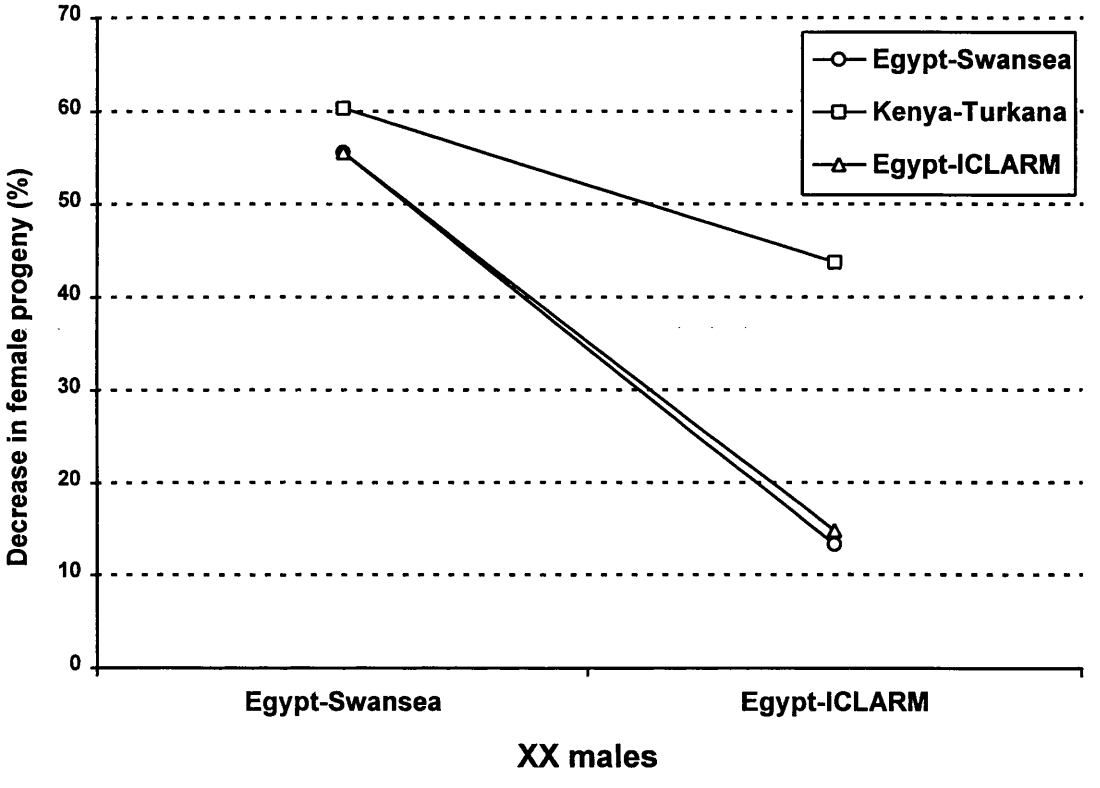


Figure 3.2. Graph showing the comparative percentage decrease of the proportion of females in the treated progeny of XX males in Egypt-Swansea and Egypt-ICLARM strain crossed with females in Egypt-Swansea, Kenya-Turkana and Egypt-ICLARM females.

For the purebred and crossbred putative all-male progeny, the survival and sex ratios are presented in Table 3.2. The treated progeny had a higher mean survival (59.52%) than the control (43.39%), although a Paired-Samples T-Test shows that the difference is not significant. This result is somewhat opposite to that of the treated all-female progeny which had a lower mean survival (62.72%) than the control (71.64%) (P=0.038). It appears that genetic females may be more sensitive to high temperature than genetic males.

Except for two replicates, all the control crosses had 100% male progeny. High temperature also has an effect on sex ratio of genetically male progeny as indicated by a significant decrease in percentage males of the treated progeny in six replicates, although the majority did not show any response (see lower graph in Figure 3.1). The mean percentage decrease of males in treated progeny of Egypt-Swansea YY males (26.61%) was higher than those sired by YY males in the Ghana-BFAR strain (7.79%). The treated progeny of Egypt-ICLARM YY males did not respond to high temperature treatments. However, a one-way ANOVA shows the difference between the mean percentage decrease of males in the treated progeny of the three strains of YY males is not significant even after transformation, probably due to the high within group variability.

Table 3.2. Summary of the percentage of males and survival rates of purebred and crossbred progeny from XX♀ x YY♂ crosses reared at elevated (36°C) and at ambient temperatures during the sexual differentiation stage. Survival is the percentage of fish surviving after a minimum of 21 day temperature treatment. Replicate denotes the number of batches or families tested from each strain cross.

Strain cross	Replicate	Stocking density	Treated (36°C)			Control (ambient)			Decrease	2 x 2
(XX♀ : YY♂)		(fish.cage^{-1})	Actual temp.	Survival (%)	Males (%)	Actual temp	Survival (%)	Males (%)	in males (%)	χ^2 value
ES x ES	1	100	36.83 (± 0.12)	60.0	88.80	29.73 (± 1.14)	96.67	96.05	7.55	6.52 *
	2	100	37.00 (± 0.12)	60.67	61.17	29.83 (± 0.94)	9.67	98.06	37.62	63.35 ***
	3	100	36.90 (± 0.15)	48.33	98.89	29.06 (± 1.50)	92.33	100.0	1.11	6.0 *
EI x ES	1	300	36.34 (± 0.58)	40.33	80.95	26.62 (± 0.98)	n/a	100.0	19.05	7.64 **
	2	300	36.34 (± 0.58)	69.33	32.26	26.62 (± 0.98)	n/a	100.0	67.74	30.06 ***
EI x EI	1	250	36.44 (± 0.31)	63.03	100.0	28.53 (± 0.60)	21.01	100.0	0	0 ^{ns}
	2	300	36.59 (± 0.58)	90.0	100.0	28.03 (± 0.63)	24.0	100.0	0	0 ^{ns}
ES x EI	1	300	36.25 (± 0.59)	63.67	100.0	26.62 (± 0.98)	n/a	100.0	0	0 ^{ns}
GB x GB	1	150	36.55 (± 0.64)	62.76	100.0	27.69 (± 0.77)	17.24	100.0	0	0 ^{ns}
	2	125	36.44 (± 0.71)	76.0	45.45	27.27 (± 0.91)	44.80	100.0	54.55	14.60 ***
	3	100	36.40 (± 0.66)	74.44	100.0	27.05 (± 0.91)	52.22	100.0	0	0 ^{ns}
	4	300	36.77 (± 0.64)	23.33	100.0	27.00 (± 0.95)	6.67	100.0	0	0 ^{ns}
	5	300	36.77 (± 0.64)	36.67	100.0	27.00 (± 0.95)	69.33	100.0	0	0 ^{ns}
EI x GB	1	250	36.25 (± 0.59)	53.60	100.0	26.62 (± 0.98)	n/a	100.0	0	0 ^{ns}
	2	110	36.33 (± 0.56)	83.64	100.0	26.62 (± 0.98)	n/a	100.0	0	0 ^{ns}

ES = Egypt-Swansea, EI = Egypt-ICLARM, KT = Kenya-Turkana, GB = Ghana-BFAR

χ^2 value - represents the deviation of sex ratio of the treated progeny from that of the control

^{ns} = not significantly different; * = P<0.05; ** = P<0.01; *** = P<0.001

n/a = not available

3.3.2 Effect of three temperature levels on sex ratio of putative all-female, all-male and all YY male progeny in Egypt-Swansea strain

The results of the experiment aimed at determining the effect of low, normal and high temperatures on the sex ratio of putative all-female (GFT), all-male (GMT) and all YY male (YY) progeny in the Egypt-Swansea strain are presented in Table 3.3. A one-way ANOVA shows a significant variance in the survival of the three genotypes under the three temperature regimes. Except for the GFT, where, inexplicably, the lowest survival was observed at normal temperature, the trend for both the GMT and YY was similar; with the lowest survival at high temperature followed by low temperature and then normal temperature. For the sex ratio, at low temperature, a response was seen only in the GFT with a 39.47% decrease in the percentage of males compared to the normal temperature control but this difference was not significant. At high temperature, the percentage males of the YY male progeny deviated significantly ($P<0.001$) from those reared at normal temperature with a difference of 92.38% (see Figure 3.3). For the GFT and GMT, the percentage of males at high temperature was respectively higher and lower than the percentage of males at normal temperature with differences of 17.08% and 13.68%, respectively, but these differences were again not significant. Contrary to expectation, the YY males which had been thought to be the most resistant to temperature effects and to be consistently all-male, were the most affected by high temperature.

Table 3.3. Summary of the results of an experiment aimed at determining the effect of three levels of temperature on sex ratio of putative all-female (GFT), all-male (GMT) and all YY progenies in the Egypt-Swansea strain. The mean values in each genotype were compared using a one-way ANOVA.

Genotype	Level of temperature	Actual temp. (°C) (± sd)	Survival [1,2] (%)	Male	Female	Intersex	Males [2] (%)
GFT	Low	25.51 (±1.18)	89.0 [a]	11	79	0	12.22 [a]
GFT	Normal	29.57 (±0.23)	58.33 [b]	21	83	0	20.19 [ab]
GFT	High	37.48 (±0.86)	75.00 [c]	41	69	0	37.27 [b]
Probability			P=0.001				P=0.023
GMT	Low	25.88 (±1.29)	84.00 [ab]	69	0	0	100.0 [a]
GMT	Normal	30.07 (±0.27)	90.67 [a]	98	0	0	100.0 [a]
GMT	High	37.12 (±0.83)	70.33 [b]	82	13	0	86.32 [a]
Probability			P=0.029				P=0.137
YY	Low	25.96 (±1.14)	84.33 [a]	110	0	0	100.0 [a]
YY	Normal	29.68 (±0.18)	90.67 [a]	119	0	0	100.0 [a]
YY	High	36.51 (±1.54)	53.00 [b]	8	97	0	7.62 [b]
Probability			P=0.001				P<0.001

[1] - Survival values were based on counts of fry at the time of removal from aquaria at the end of the treatment

[2] - Mean values with different letter subscript are significantly different (P<0.05)

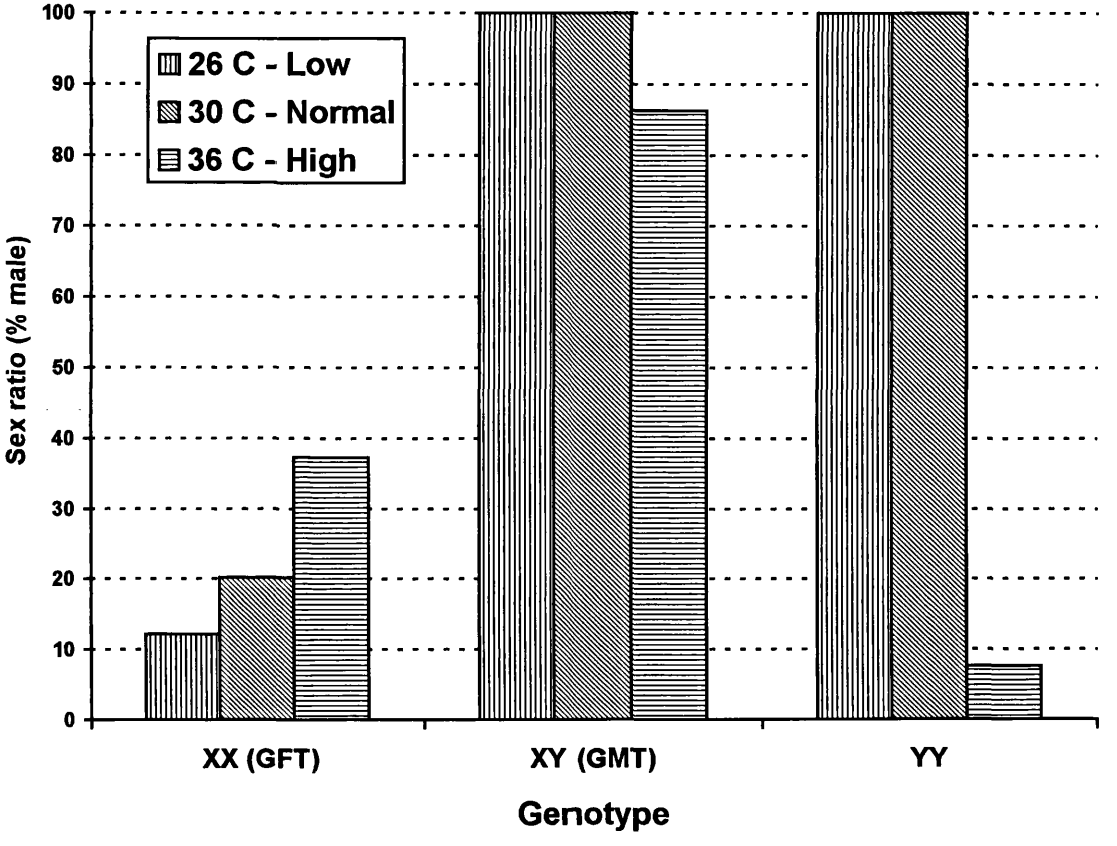


Figure 3.3. Histogram showing the mean percentage of males of putative all-female (GFT - produced using XX males), all-male (GMT - produced using YY males) and all YY males (produced from YY x YY) progeny in the Egypt-Swansea strain reared at three temperature regimes during the period of sex differentiation. The mean represents the average of three replicates.

3.3.3 Effect of high temperature on sex ratio in purebred and crossbred YY males

The results of the experiment aimed at determining the effect of high temperature on sex ratios of purebred and crossbred YY males are presented in Table 3.4. No data are available on the survival for the purebred YY males. For the crossbred YY males, the result of a Paired-Samples T-Test shows the mean survival for the treated (72.38%) and for the control progeny (66.08%) are not significantly different. No unexpected females were observed in all the control progeny including those of the crossbred (see Figure 3.4). In the treated purebred YY males, females were observed in significant proportions (4.35 to 94.44%) in 12 of the replicate families and only one replicate gave no response to the temperature treatment. For the crossbred YY males, significant proportions of females were also observed in two treated replicates but the percentages were smaller than for the purebreds (11.54% and 2.7%).

Table 3.4. Summary of the percentage of males and survival rates of the purebred and crossbred putative all YY male progeny from YY♀ x YY♂ crosses reared at elevated (36°C) and at ambient temperatures during sex differentiation stage. Replicate denotes the number of batches or families tested in each strain cross. The survival is the percentage of fish surviving after a minimum of 21 days temperature treatment.

Strain cross (YY♀ x YY♂)	Replicate	Stocking density (fish.cage^{-1})	Treated (36°C)			Control (ambient)			2 x 2 χ^2 value
			Actual temp.	Survival (%)	Males (%)	Actual temp.	Survival (%)	Males (%)	
ES x ES	1	150	36.61 (± 0.51)	33.3	40.54	n/a	n/a	100.0	37.50 ***
	2	100	36.40 (± 0.47)	61.0	46.67	n/a	n/a	100.0	29.95 ***
	3	100	36.45 (± 0.41)	72.0	71.43	n/a	n/a	100.0	18.16 ***
	4	100	36.52 (± 0.33)	50.0	25.0	n/a	n/a	100.0	39.97 ***
	5	75	36.35 (± 0.27)	44.0	5.56	n/a	n/a	100.0	54.10 ***
	6	100	36.35 (± 0.27)	52.0	100.0	n/a	n/a	100.0	0 ns
	7	100	36.42 (± 0.39)	48.0	47.22	n/a	n/a	100.0	36.75 ***
	8	100	36.42 (± 0.39)	50.0	42.86	n/a	n/a	100.0	22.87 ***
	9	100	36.45 (± 0.71)	85.0	95.65	n/a	n/a	100.0	5.84 *
	10	100	36.45 (± 0.71)	79.0	53.85	n/a	n/a	100.0	18.50 ***
	11	150	36.35 (± 0.27)	66.0	76.92	n/a	n/a	100.0	14.90 ***
	12	60	36.60 (± 0.67)	90.0	25.81	n/a	n/a	100.0	49.0 ***
	13	150	36.65 (± 0.51)	37.67	28.57	n/a	n/a	100.0	142.91 ***
ES x EI	1	300	36.57 (± 0.50)	67.33	100.0	28.69 (± 0.83)	70.0	100.0	0 ^{ns}
	2	195	36.44 (± 0.38)	87.18	100.0	28.75 (± 0.54)	78.46	100.0	0 ^{ns}
	3	280	36.57 (± 0.50)	45.94	100.0	28.69 (± 0.83)	48.76	100.0	0 ^{ns}
	4	240	36.65 (± 0.50)	90.83	100.0	29.80 (± 0.69)	49.17	100.0	5.70 *
	5	150	36.41 (± 0.68)	48.67	88.46	27.10 (± 0.93)	n/a	100.0	0 ^{ns}
	6	150	36.61 (± 0.50)	75.33	100.0	28.94 (± 0.88)	83.61	100.0	0 ^{ns}
	7	110	36.61 (± 0.50)	55.56	100.0	28.94 (± 0.88)	75.45	100.0	0 ^{ns}
	8	240	36.57 (± 0.50)	84.49	97.30	28.69 (± 0.83)	57.14	100.0	4.16 *
	9	150	36.59 (± 0.52)	68.67	100.0	28.94 (± 0.88)	n/a	100.0	0 ^{ns}
	10	150	36.59 (± 0.52)	51.33	100.0	28.94 (± 0.88)	n/a	100.0	0 ^{ns}

ES = Egypt-Swansea; EI = Egypt-ICLARM
n/a = not available
χ^2 value - represents the deviation of sex ratio of the treated progeny from that of the control
^{ns} = not significantly different; * P<0.05; *** P<0.001

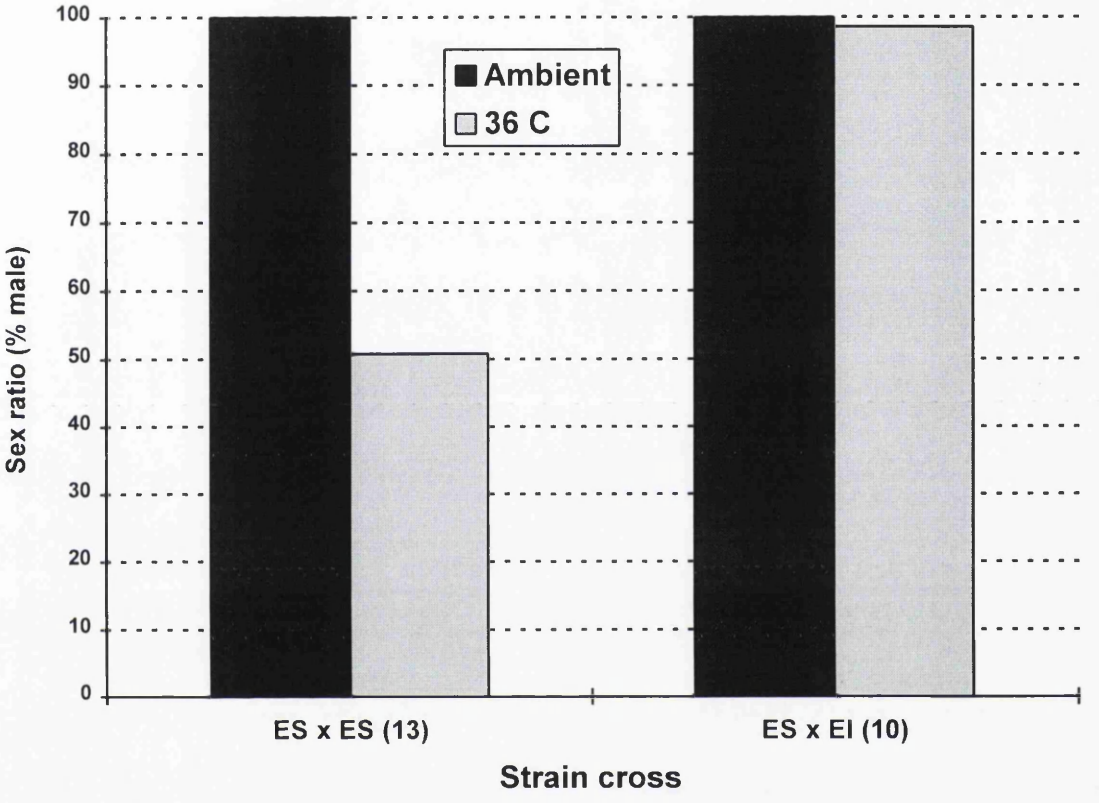


Figure 3.4. Histogram showing the mean percentage of males of purebred (YY♀ x YY♂ - Egypt-Swansea) and crossbred (Egypt-Swansea YY♀ x Egypt-ICLARM YY♂) YY male progeny reared at elevated (36°C) and at ambient temperatures during the period of sex differentiation. The values in parenthesis denote the number of replicate batches for each strain cross.

3.3.4 Effect of salinity on sex ratio in putative all-female progeny in Egypt-Swansea and Egypt-ICLARM strains

For determining the effect of salinity on sex ratio, two separate experiments were performed using putative all-female progeny from Egypt-Swansea and Egypt-ICLARM strains. The results of the two experiments are presented in Table 3.5. The survival rates of the progeny of Egypt-Swansea under the different salinity levels were not significantly different but a significant difference was observed for the progeny of Egypt-ICLARM strain (P=0.002). The control groups of Egypt-ICLARM suffered high mortality caused by bacterial infection during the treatment period. No significant effect of salinity on sex ratio was observed in either experiment.

Table 3.5. Summary of the results of the experiment aimed at determining the effect of different salinities on sex ratio of putative all-female progeny from XX♀ x XX♂ crosses in the Egypt-Swansea and Egypt-ICLARM strains. The mean values for each strain were compared using a one-way ANOVA.

Strain	Salinity level (± sd)	Survival [1, 2] (%)	Male	Female	Intersex	Females [2] (%)
E.-Swansea	12.75 (±0.80)	65.73 [a]	4	204	0	98.08 [a]
	19.87 (±0.91)	78.13 [a]	14	247	0	94.64 [a]
	26.75 (±0.13)	79.60 [a]	6	120	0	95.24 [a]
	0	62.40 [a]	23	179	0	88.61 [a]
Probability		P=0.055				P=0.220
E.-ICLARM	11.30 (±0.26)	66.19 [a]	51	181	0	78.02 [a]
	18.34 (±0.45)	87.74 [a]	45	147	0	76.56 [a]
	24.34 (±0.88)	71.07 [a]	38	172	0	81.90 [a]
	0	26.31 [b]	26	107	0	80.45 [a]
Probability		P=0.002				P=0.649

[1] - Survival rates were based on counts of fry at the time of removal from aquaria at the end of treatment
[2] - Mean values marked with different letter subscript are significantly different (P<0.05)

3.4 Discussion

The results presented here provide evidence of a significant effect of high temperature on sex ratio in tilapia, confirming results obtained in previous studies (Mair *et al.*, 1990; Baroiller *et al.*, 1995; Baroiller *et al.*,1996). Not all the replicate crosses tested were affected by the increase in temperature, supporting the observation of Baroiller *et al.* (1995, 1996) and Conover and Kynard (1981) that different genotypes have different sensitivity to environmental effects on sex ratio. But unlike previous studies where only putative all-female (Baroiller *et al.*, 1995, 1996) or mixed-sex fish (Mair *et al.*, 1990; Baroiller *et al.*, 1995) were used, the present study shows that high temperature can influence sex ratio not only towards male but also towards female.

The observed sensitivity to high temperature of the fish used in the study could be related to an effect during sexual differentiation. An environmental shock, such as high temperature, might disrupt the normal processes during sex differentiation causing the switch to male for the genetic females and to female for the genetic males. There are numerous observations in the literature on tilapia of sex ratios deviating from the norm of a 1:1 or from predicted ratios of the progeny of sex reversed fish (Shelton *et al.*, 1983; Majumdar and McAndrew, 1983; Calhoun and Shelton, 1983; Mair *et al.*, 1991a,b). These phenomena are commonly explained on the basis of autosomal or polyfactorial gene effects. As most of the progenies examined were raised in either controlled or partially controlled conditions with minimal temperature fluctuations, caution must be used

in attributing these observed deviations to temperature effects. However, based on the present results, it is possible to suggest that the environment may enhance the expression of genetic sex factors. Winge (1934) proposed, based on the results of his studies in *Lebistes reticulatus*, a model for explaining the occurrence of unexpected sex ratios, which could not be accounted for by the chromosomal sex determination model. This model was based on the hypothesis that major male and female sex determining genes are located in the sex chromosomes but that minor sex-modifying genes are scattered in the autosomes which occasionally interact and override the sex chromosomes resulting in the occurrence of unexpected sex ratios. In the present study, the same model has also been adopted to explain the unexpected sex ratio of the control progeny and the observed sensitivity to high temperature.

In Table 3.1, it can be seen that the majority of the control replicates for the putative all-female progeny contained some males and those replicates that had a high percentage of males tend to have a higher percentage males in the respective treated group (see upper diagram in Figure 3.5). A Bivariate Pearsons Correlation Analysis shows that the percentage of males in the treated and control groups were significantly positively correlated ($n=30$, $r=0.653$, $P<0.001$). For the all-male progeny of YY males (Table 3.2), the percentage males of the treated progeny in six replicates also decreased, particularly those sired by YY males of the Egypt-Swansea strain. Although, the result of a Bivariate Pearsons Correlation Analysis shows the percentage of males for the

treated and control groups are not significantly correlated (n=15, r=0.126, P=0.653) (see lower diagram in Figure 3.5).

The presence of unexpected sex ratios in the control replicates could suggest the presence of a substantial influence of autosomal sex modifying factors in the progeny. During the labile period of sex differentiation, a change from normal to extreme environmental conditions, such as high or low temperatures, those individuals carrying more of an autosomal male or female modifying genes may have the tendency to develop into female or male (Beamish, 1993).

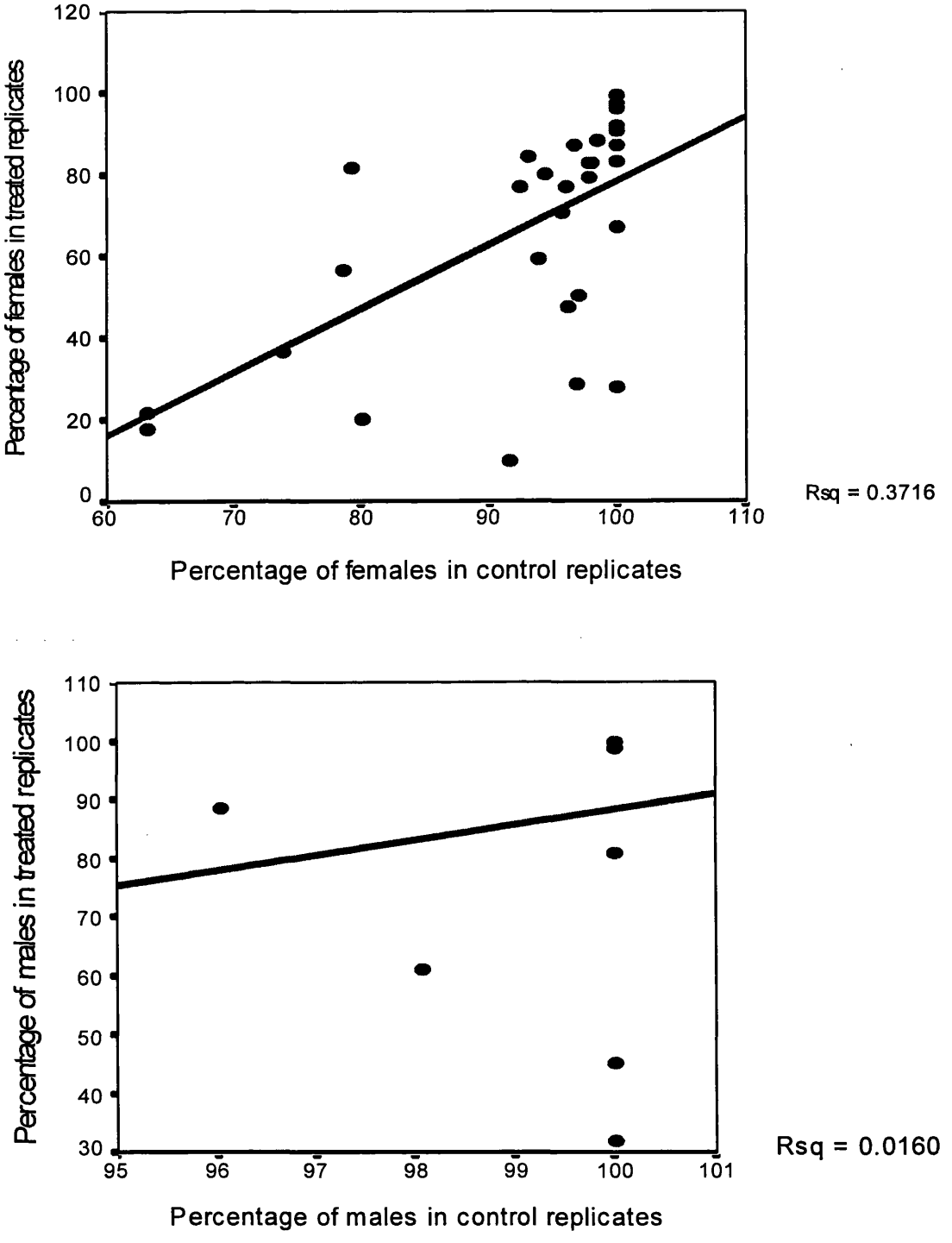


Figure 3.5. Scatter diagrams showing the percentage females for the treated and control putative all-female progeny (upper diagram) and percentage males for the treated and control putative all-male progeny (lower diagram).

The production of all-female progeny involved the use of XX males as broodstock. There is the possibility of selection for male determining genes during the production of these fish. Baroiller (1996) observed that, in a "Bouake strain" of *O. niloticus*, the majority of the sex-reversed genetic females tested have significant proportions of unexpected males in their progeny. During hormonal masculinisation treatment to produce XX males, it is possible that those genetically female progeny that are carrying higher loading of autosomal male modifying genes would tend to be sex-reversed to male more easily compared to other progeny which carry more female autosomal genes. Rothbard *et al.* (1987) demonstrated that newly hatched fry are carrying high level of endogenous hormones and the level differ among individual fish. It may be possible that those fry with a higher loading of male autosomal genes may have higher level of endogenous androgens. During hormone treatment for sex reversal, the administered exogenous hormone combined with the higher level of endogenous androgen make the fish to be masculinised more easily. If these sex-reversed fish, which are carrying more male modifying genes are then used as broodstock, the number of male genes will tend to increase in the next generation and along with this the sensitivity of the progeny to extreme conditions.

For the YY males, the same theory could also explain the presence of females in the treated replicates, particularly the purebreds (Table 3.3 and 3.4). It is possible that female autosomal genes are also accumulating in the progeny. During the hormonal feminisation treatment of sexually undifferentiated

genetically YY male to produce YY females, those progeny that are carrying more female determining genes might tend to be sex-reversed more easily compared to the other progeny which carry more male genes.

In the case of the all-male progeny, the accumulation of autosomal male genes in females and autosomal female genes in YY male lines could result in a more balanced ratio of the sex determining genes in the progeny when the two lines (XX♀ x YY♂) are crossed. This may be the reason for the seemingly lower response of the all-male progeny to high temperature (Tables 3.2 and 3.3).

The presence of autosomal sex modifying genes could be one of the reasons for the exhibition of a continuous variation of the observed sex ratios in intrastrain crosses in *O. niloticus* (see data of Shelton *et al.*, 1983, Tuan *et al.*, 1997, and Chapter four of the present thesis) which cannot be explained by the chromosomal sex determination model. Lester *et al.* (1989) suggested, on the basis of the observed heritability of sex, that *O. niloticus* appears to have a multifactorial or polygenic sex determination system which yields an underlying genetic variability for sex that is more continuous than discrete. However, in the present study the majority of the control progeny have exhibited the expected sex ratio based on the theory of chromosomal sex determining mechanism. Thus, it is more likely that the sex ratio in *O. niloticus* is governed by sex chromosomes with occasional influence of environment, as consequence of the unbalance ratio of autosomal sex modifying genes (Baroiller *et al.*, 1995). In Atlantic silverside, *Menidia menidia*, Conover and Kynard (1981) presented data

demonstrating that the skewing of sex ratio by temperatures outside the normal range is under genetic and environmental control.

In the case of the observed higher response to high temperature of the purebred YY males compared to the crossbred, it is believed that the level of inbreeding of the population (Egypt-Swansea strain) that was used in the production of YY males may have affected fitness and developmental stability. The possible loss of developmental stability coupled with increase of an autosomal sex-modifying genes could have made sex differentiation more sensitive to change by the environment. Price (1984) cited work which demonstrated the existence of autosomal genes affecting sex in an inbred line of platyfish. When XX males were outcrossed to normal females the progenies were all-female as expected, however when interbred, both sexes were produced in extremely variable proportions.

Purdom (1993) proposed an alternative hypothesis for the observed atypical sex ratio in inbred gynogenetic progeny. This is that sexual homeostasis is disrupted by inbreeding and that the greater level of inbreeding implicit in mitotic gynogens causes greater deviation from expected sex ratio. The loss of genetic variation in inbreeding reduces the ability of an individual to adapt to different conditions, whilst those individuals with a higher level of heterozygosity have relatively higher levels of individual homeostasis, i.e. stability and consistency (Mitton and Grant, 1984). If we consider the results in Table 3.4, there seem to be a strong indication that inbreeding might have an effect on sex ratio, as has been suggested earlier. The Egypt-Swansea strain is already

considered inbred for having passed through a number of genetic bottlenecks (Mair *et al.*, 1995). Likewise, the level of inbreeding in the progeny used in that particular study may have been further intensified by being produced from a cross of YY♀ x YY♂ since the YY parents are very closely related. On the other hand, the progeny from crosses of Egypt-Swansea YY♀ x E.-ICLARM YY♂ are likely to have a higher genetic variability considering that the two strains have no known close genetic relationship. Thus the evidently higher sensitivity of the pure strain YY males as compared to the hybrid YY males may have been due to their being relatively more inbred.

However, this has not been the case for the putative all-female progeny as even the crossbreds had significant proportions of male progeny at high temperatures. It may be possible that both the strains used were loaded with a relatively high number of male sex modifying genes.

3.5 Conclusion

The study presents new data on the effect of temperature on sex differentiation in this commercially important tilapia species. High temperature can have profound effects on sex ratio which are clearly seen in putative monosex progeny. Temperature change can influence sex ratio both towards male and towards female and there appears to be a genetic basis to the

susceptibility to temperature effect on sex ratio. Further research is required to determine whether normal seasonal and diurnal temperature fluctuations can affect sex ratio but environmental condition should be considered when interpreting data from sex determination studies in tilapia.

CHAPTER FOUR

GROWTH AND SEX RATIO IN CROSSBRED GMT

4.1 Introduction

4.1.1 Growth

Fast growth is probably one of the most important traits which fish farmers value in the strain or species they are culturing. Over the years, fish farmers and researchers have tried to improve the growth in domesticated fish. Generally, the exhibition of fast growth may be influenced by three factors; genotype, environment and that related to the interaction of genotype and the environment. The growth performance of a population may be altered by a change in genotype or the environment (Mather and Jinks, 1977). For example, the average growth of a population may be improved by careful and judicious selection of potential parents that carry the important traits, which can combined to produce the desired phenotype in their progeny. Similarly, improvement in husbandry such as lower stocking density, better quality food and optimal culture environment may also lead to faster growth of cultured fish.

In recent years, the exploitation of genetic factors to improve yield in cultured species has been given much consideration (see reviews by Dunham, 1986 and Hulata, 1995a,b). Genetic factors have two major components; additive and dominance variance. Additive variance is a genetic component that is due to the additive effects of all genes in the genome. As it is a function of the genes, the effect of additive variance is heritable, i.e. may be transmitted from parents to offspring in a predictable and reliable manner, it can be exploited by

means of a selection programme. If the percentage of phenotypic variance that is due to additive genetic variance, commonly termed heritability, is known, a farmer can predict the amount of improvement that can be made from a particular intensity of selection. On the other hand, dominance variance is a genetic component that is due to interaction of the alleles at each locus. Since both alleles contribute to the effect, dominance genetic variance is disrupted during meiosis and is recreated in new and different combinations in every generation. Because dominance genetic variance is not heritable but a function of the mating, hybridisation or crossbreeding is the breeding programme that is use to exploit this component of variance and to improve traits in the population.

Of the two genetic components, the exploitation of additive variance has been considered the better approach for growth improvement in some species such as the rainbow trout (Gjedrem, 1992). However, the response to selection depends on the genetic variability present in the strain/species of interest. Generally, the response to selection tends to correlate with the level of genetic variability. The response to selection for fast growth in an inbred population may be very low or zero, as has been observed in common carp (Hulata, 1995b). In tilapia, the estimated heritability for weight and length in an apparently inbred population of *O. niloticus* is close to zero (Tave and Smitherman, 1980)

When the selective approach is not feasible crossbreeding may be the alternative. When heritability is moderate to high (>0.2) selection programme is usually effective but when is less than 0.2 crossbreeding is usually more effective (Smitherman and Dunham, 1985). Crossbreeding allows the exploitation of both

additive and non-additive genetic effects by combining the desirable qualities of the diverse parental strain/species in the offspring (Gjedrem, 1988; Basavaraju *et al.*, 1990). Moreover, one of the many practical use of crossbreeding is in the improvement of productivity by utilising the phenomenon of hybrid vigour or heterosis (Clayton and Price, 1994). Heterosis reflected in the difference between the average growth of the crossbred and the parental means (Falconer and Mackay, 1996) (but in applied breeding it is best represented as the difference between the F_1 and the better parental mean). Through crossbreeding it is also possible, in some cases, to produce sterile hybrids; limiting both the economically unfavorable characteristics of sexual maturation as well as the genetic impact of escaped fish on wild fish stocked Chevassus (1983). Crossbreeding has been suggested as a solution to genetic improvement of wild populations (Moav *et al.*, 1978).

The exploitation of the potential of crossbreeding to improve growth has been most notable in common carp. Following the very low response of five-generations of selection for growth rate in common carp in Israel (Hulata, 1995b), efforts have been shifted to the use of interstrain crossbreeding as an alternative approach. The growth rate of crossbreds was found to be 10 to 20% faster than that of the inbreds (Hulata, 1995b). In Hungary, a similar result has also been observed (Bakos and Gorda, 1995); the classical method of mass or family selection to improve the productivity in common carp was found to produce poor results. Through crossbreeding, a number of hybrids have shown 20 to 25%

higher productivity than the traditional races of common carp (Bakos and Gorda, 1995).

The potential of crossbreeding has also been exploited for the improvement of important traits in other species. In catfish, growth (Yant, *et al.*, 1975), catchability (Dunham *et al.*, 1986), low oxygen concentration tolerance (Dunham *et al.*, 1983) and reproductive performance (Dunham *et al.*, 1983b) have been improved through crossbreeding. In a preliminary report on hybridisation experiments involving females of common rainbow trout and males of albino rainbow trout, Subba Rao and Chandrasekaran (1978) noted the higher survival rates of the hybrids during egg, hatchling, fry and yearling stages, and faster annual growth compared to either parental strains. Similarly, the hybridisation with domesticated rainbow trout reduced seasonal variation in seawater adaptability in the anadromous steelhead trout (Johnsson *et al.* 1994). In contrast, Hortsgen-Schwark *et al.* (1986) reported that the mean growth performance of crossbred progeny of four strains of spring-spawning rainbow trout (*Oncorhynchus mykiss*) was not significantly different from the purebred parents.

In tilapia, early work on crossbreeding mostly involved interstrain hybridisation to produce all-male progeny (see reviews by Wohlfarth, 1983; Hulata, 1995b). Whilst the interest had been focused on the production of all-male progeny, the results of some studies on crossbreeding to improve growth looked promising. In preliminary studies with the hybrid *O. niloticus* x *O. mossambicus*, Avault and Shell (1968) reported that the reciprocal hybrids grew

faster and had a better food conversion efficiency than the parental species. In interstrain crossbreeding in *O. niloticus*, Khater (1985) observed heterosis for the Egypt x Ghana, Egypt x Ivory coast and Ghana x Ivory coast F_1 hybrids which were 11.6, 3.0 and 5.8% better than the parental mean, respectively, after 47 day growth trials in plastic pools. Jayaprakas *et al.* (1988) also observed heterosis for length and weight in F_1 (9.5 and 28.3%), F_2 (11.8 and 36.6%) and backcrosses (11.3 and 32.1%) of Egyptian and Ivory coast strains of *O. niloticus* held at Auburn University, following 60 day yield trials in hapas. Shackell (1991) observed that the growth of the reciprocal hybrids of Egypt-NIFI (Thailand) and Ghana-Israel strains of *O. niloticus* was higher than the parental mean. In contrast, Uraiwan and Phanitchai (1986) cited by Tave (1988) found a -28.6% heterosis for growth in the hybrid of an Egyptian and an Israeli strain of *O. niloticus*.

Recently, an attempt to improve the growth of genetically male tilapia (GMT) using a crossbreeding approach has been initiated. YY males of the Egypt-Swansea strain were outcrossed to females of the Egypt-Stirling, Egypt-AIT and Kenya-Turkana strains. The crossbreds were observed to grow 6.67 to 25% faster compared to purebred Egypt-Swansea progeny (Abucay, 1996). However, in that study the increase in growth could not be attributed to heterosis as it could simply have been due to the additive effect of the parental strains.

The general objective of this study was to analyse the growth of GMT in a 2 x 2 diallele cross of Egypt-Swansea and Egypt-ICLARM strains using YY males as broodstock. Specifically, the objectives were as follows;

1. To evaluate the growth of two reciprocal hybrids in pond and cage culture environments in comparison with the parental strains,

2. To determine whether any observed improvement in growth of the crossbred GMT is due to heterosis,

3. To determine if, through crossbreeding, other important traits including percentage of dress-out and percentage of fillet weight can be improved.

4.1.2 Sex determination

The interest in the understanding of the mechanism of sex determination in tilapia started when Hickling (1960) accidentally observed all-male progeny from a cross of Malayan and an allied but distinct form of tilapia from Africa. Chen (1969) described the Malayan and African tilapia used by Hickling (as suggested to him by Kosswig and Traut), as *O. mossambicus* and *O. hornorum* species. Based on the results of Hickling (1960) and from his own study, Chen (1969) proposed, following the earlier work of Gordon (1947), the existence of dual systems of sex determining mechanisms in tilapia (see introduction of Chapter three).

This model has been found to generally apply and fit to the observed sex ratio in later works (Pruginin *et al.*, 1975, Hulata *et al.*, 1983; Shah, 1988; Mair *et*

al., 1991a,b; Lahav, 1993; Muller-Belecke and Hortsgen Schwark, 1995; Desprez *et al.*, 1995). However, whilst the average of the observed sex ratio usually approaches the predicted sex ratio, the highly variable sex ratios of individual families from interspecific (Pruginin *et al.*, 1975; Majumdar and McAndrew, 1983) and intraspecific crosses (Shelton *et al.*, 1983; Mair *et al.*, 1991b; Baroiller, 1996) could not all be accounted for by Chen's model.

Several attempts have been made to explain the observed irregularities of sex ratio. Avtalion and Hammerman (1978), and Hammerman and Avtalion (1979) proposed a model of autosomal and gonosomal influence to explain the observed variable sex ratio from interspecific crosses. In the model, the presence of three gonosomes (W, X, Y), following the earlier model of Gordon (1947), and two different autosomal alleles (A and a) were assumed. The sex was hypothesized to be determined by a combination of the two autosome alleles at one locus, which would act together with two of a possible three sex chromosomes to produce six possible gamete types (AX, AY, AW, aX, aY, aW) and consequently the combination of these gametes would result in 18 possible genotypes. The determination of sex would depend on the additive effect of the sex determining genes present in the autosomes and chromosomes. With each pure species the pair of autosomes were thought to be identical so that it give a consistently 1:1 sex ratio.

Whilst the model with an autosomal sex determining locus was able to explain most of the sex ratios observed by Chen (1969), it failed to explain the highly variable sex ratios from the interspecific and intraspecific crosses

produced by Majumdar and McAndrew (1983). Majumdar and McAndrew suggested that the mechanism responsible for sex determination in *Oreochromis* may involve a number of different alleles of differing "strength", i.e. a polygenic system of sex determination. Mair *et al.* (1991b) also suggested, based on the highly variable interspecific and intraspecific sex ratio in their study, a multifactorial mechanism of sex determination in *0. aureus* but with an underlying primary mechanism of male homogamety.

Studies on the sex determining mechanism in *O. niloticus* have indicated a monofactorial, genotypic sex determination mechanism, with male heterogamety although rare autosomal or environmental sex-modifying factors may cause occasional deviations from expected sex ratios (Penman *et al.*, 1987; Shah, 1988; Mair *et al.*, 1991a; Muller-Belecke and Horstgen-Schwark, 1995). Lester *et al.* (1989), however, had suggested that the observed inheritance of sex in *Oreochromis* is consistent with a polygenic model of sex determination in which sex may be determined by multiple loci not linked on a sex chromosome. The results presented by Wohlfarth and Wedekind (1991) further support the theory of polyfactorial sex determination in *Oreochromis* species. They presented evidence for the possibility of selection for a change in sex ratio in *O. niloticus.*

Whilst observed variable sex ratios have been accounted for mostly by a difference in sex determining mechanisms, genetic introgression could also be another possible reason. Wohlfarth (1994) remarked that increasing proportions of females in hybrid progenies could be due to the infiltration of parental broodstock by individuals of different genotypes. In a review on sex

determination in tilapia, Trombka and Avtalion (1993) also mentioned the infiltration of "xenogeneic" genes into the parental stock as a cause of the lack of homogamety of the parental species.

Lastly, whilst most of the observed variation in sex ratio may be largely due to genotype, environmental effects could also have an influence on the sex ratio. Relevant information on this subject is presented in Chapter three.

Recently, a model for the production of YY males has been proposed (Mair *et al.*, 1988; Scott *et al.*, 1989). This model is based on the theory of monofactorial mechanism of sex determination with an XX and XY system. A male with YY sex chromosomes when crossed to normal XX females would be expected to produce all XY male progeny. The development of YY males has been reported in *O. mossambicus* (Yang *et al.*, 1980; Varadaraj and Pandian, 1989) and in *O. niloticus* (Mair *et al.*, 1988; Baroiller and Jalabert, 1989; Scott *et al.*, 1989; Mair *et al.*, 1992; Mair et al, 1997a). The feasibility of mass production of YY males has also been demonstrated in *O. niloticus* (Mair *et al.*, 1997a).

In the aforementioned studies, the sex ratio of YY males was determined in within strain crosses. However, crossbreeding of YY males may be necessary to exploit heterosis and further improve the growth of the GMT progeny. Hence, this study aimed to determine the extent of the capacity of YY males to sire all-male progeny. Specifically, the objectives were as follows;

1. To determine the sex ratio of the progeny of YY males in the Egypt-Swansea and Egypt-ICLARM strain in a 2 x 2 diallele cross,

2. To determine the sex ratio of the progeny of YY males in the Egypt-Swansea strain in crosses with females from other strains of *O. niloticus*.

4.2 Materials and Methods

Evaluation of growth - The growth of Egypt-Swansea (ES x ES) and Egypt-ICLARM (EI x EI) strains and their reciprocal hybrids (EI x ES and ES x EI) was evaluated in ponds and in cages. The evaluation was performed by communal stocking in three 200 m^2 earthen ponds and three 3 m x 3 m x 1.5 m (L x W x H) net cages (mesh #17) suspended in a 1000 m^2 earthen pond. The three ponds and three cages served as replicates (see lay out in Table 4.1). During grow-out, the stocked fish were periodically sampled to monitor growth. The growth evaluation (pond and cage-1) was over a period of 147 days after which the fish were harvested and the number, individual weight and sex of the fish surviving were noted. The GSI and percentages of dress-out and fillet weight of 10 random samples (males only) from each replicate were determined. However, in cages, the size of the fish was still small after this period so that the growth evaluation was extended for a further 105 days (cage-2). The stocking density was reduced to 30 fish per genotype in each cage for this second phase of grow-out. The remaining fish from each replicate were sampled for GSI, dress-out and fillet weight.

Table 4.1. Experimental layout showing the design of the growth evaluation experiments. All the strain crosses were communally stocked and equally represented in each pond or cage unit. After 147 days of culture, the growth evaluation of the fish in Cage-1 was extended for another 105 days (Cage-2). ES = Egypt-Swansea, EI = Egypt-ICLARM.

Culture environment	Replicate 1	Replicate 2	Replicate 3
Pond	pond-1 ES x ES EI x EI EI x ES ES x EI	pond-2 ES x ES EI x EI EI x ES ES x EI	pond-3 ES x ES EI x EI EI x ES ES x EI
Cage - 1	cage-11 ES x ES EI x EI EI x ES ES x EI	cage-12 ES x ES EI x EI EI x ES ES x EI	cage-13 ES x ES EI x EI EI x ES ES x EI
Cage - 2	cage-21 ES x ES EI x EI EI x ES ES x EI	cage-22 ES x ES EI x EI EI x ES ES x EI	cage-23 ES x ES EI x EI EI x ES ES x EI

Evaluation of sex ratio - The sex ratios of YY male progeny in a diallele cross of Egypt-Swansea and Egypt-ICLARM strains were determined by crossing YY males to females within and between strains. At the same time, YY males in the Egypt-Swansea strain were crossed with females from different strains *of O. niloticus* to determine the extent of the capacity of YY males to sire all-male progeny. Where possible, each of the families produced in each strain cross was produced using a different female. However, it was not possible to use different YY male for each of the families produced in each strain cross because of their limited number available, particularly for the Egypt-ICLARM strain, where only three YY males were available at the time of study.

Statistical analysis - The difference between mean values (Table 4.2) of the four strain crosses, which were termed "genotypes", was determined using a one-way analysis of variance (ANOVA). For each treatment (genotype) there were three mean values corresponding to the replicates per treatment (see Table 4.1). Prior to analysis, all mean values in percentage form were first arcsine transformed. A separate analysis was performed for each of the three culture environments (pond, cage-1 and cage-2). In an environment where the mean values were found to be significantly different, a comparison among treatment means was performed using Tukey multiple range test.

For the analysis of gain in growth, the results of an initial analysis (data not shown) using replicate means as source of error showed no significant difference among the strain crosses, probably because of a significant difference

between replicate means within each culture environment (see Table 4.4). Because of this, it was decided to use the weight gain of individual fish as replicates and perform a separate one-way ANOVA in each pond or cage unit. To determine all other possible interactions, a nested ANOVA was performed on all the data (weight gain only).

To correct for possible effects of the difference in initial weight on final weight (Wohlfarth and Moav, 1985; Wohlfarth, 1992; Kulikovsky *et al.*, 1994), the mean gain in weight was determined by subtracting the initial replicate mean weight from final weight of individual fish. Similarly, the final mean weights were adjusted following the method used by the GIFT project in the Philippines (A.V. Circa and M. Palada-de Vera, pers. comm.). A correction factor was generated by dividing the initial grand mean weight of all the replicate groups within each culture unit (pond or cage) by the initial mean weight of each replicate. The computed correction factor was then multiplied by the final weight of individual fish in each culture unit. This multiplicative correction factor adjusts the final weights relative to the initial weights. Both the weight gain and corrected final weight are presented in the tables for comparison, although, to avoid confusion the weight gain will be use mostly in the presentation of data in the text.

To present a more comprehensive comparison for gain in weight and percentages of dress-out and weight fillet, the percentage difference of the hybrids over the larger parent ($\frac{hybrid - larger parent}{larger parent} \times 100\%$), percentage difference of the hybrids over the parental mean ($\frac{hybrid - parentalmean}{parentalmean} \times 100\%$), and percentage difference

of the hybrids over the smaller parent ($\frac{hybrid-smallerparent}{smallerparent} \times 100\%$) was determined. Parental mean refers to the average growth of the parental strains.

The relationship between two variables (i.e. GSI, dress-out weight, fillet weight) was determined by a Bivariate Pearsons correlation analysis.

4.3 Results and Discussion

4.3.1 Growth

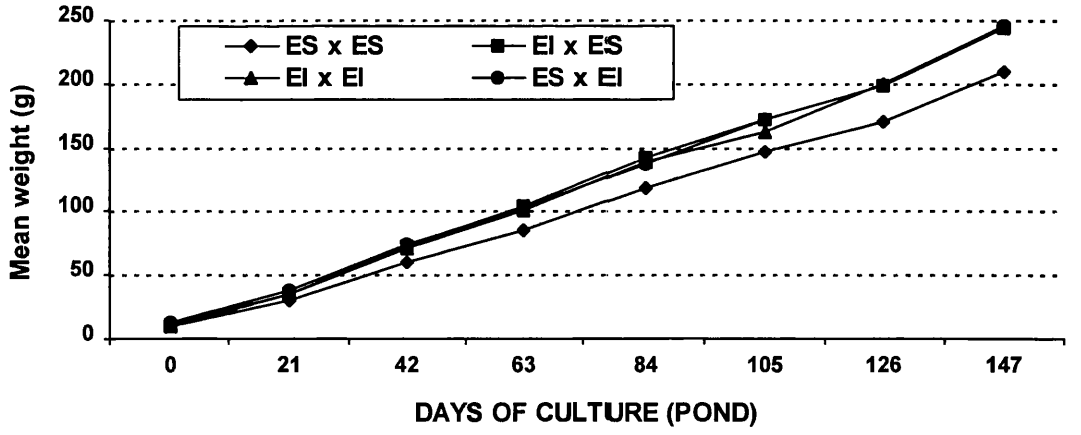
The initial data for growth evaluation of the different strain crosses are presented in Table 4.2. The Initial mean weights of the four genotypes in the pond and cage-2 culture environments were not significantly different but the initial weights in cage-1 were significantly variable (P=0.003). The coefficient of variance (CV) for initial weights in the ponds were significantly variable (P=0.023). In cage-1 and cage-2 the CV values for initial weight were not significantly different. The difference in mean weight and CV may be due to the early exhibition of fast growth in some of the strain combination. In a study comparing the growth of hybrids and parental strains, Jayaprakas *et al.* (1988) noted the faster growth of some groups even as early as 15 days of age.

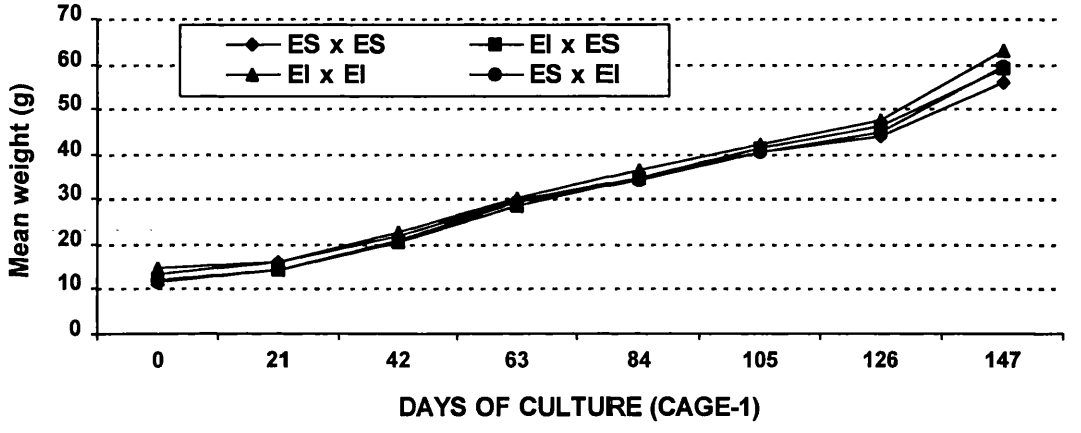
The results of the periodic sampling (every 21 days) are presented graphically in Figure 4.1. In the pond culture environment, it can be seen that there is an obvious faster growth of EI x ES, EI x EI and ES x EI compared to ES x ES even at an early stage and this trend was maintained up to harvest. Although the initial weights of the former three genotypes were higher than ES x ES it is unlikely that this is the cause of the faster in growth. The initial size of EI x ES was lower than that of ES x EI but it had a higher gain in weight.

Table 4.2. Initial and final data from the growth evaluation of Egypt-Swansea (ES) and Egypt-ICLARM (EI) strains and their reciprocal hybrids using novel YY males as broodstock. The difference between mean values, which represent the average of three replicates, were determined using a one-way ANOVA.

Strain cross (XX♀ x YY♂)	Stocking density (fish.replicate^{-1})	Mean survival (%) (± sd)	Initial mean weight	Mean CV for initial weight	Mean CV for final weight
P - ES x ES	60	91.11 ^{a} (± 8.22)	9.29 ^{a}	24.52 ^{ab}	21.57 ^{a}
P - EI x EI	60	86.67 ^{a} (± 5.00)	11.00 ^{a}	27.69 ^{ab}	14.72 ^{b}
P - EI x ES	60	93.89 ^{a} (± 3.85)	10.05 ^{a}	18.71 ^{a}	14.19 ^{b}
P - ES x EI	60	95.55 ^{a} (± 4.81)	12.11 ^{a}	29.66 ^{b}	16.75 ^{ab}
Mean		91.80	10.61	25.14	16.81
Probability		P=0.294	P=0.107	P=0.023	P=0.016
C1 - ES x ES	45	88.15 ^{a} (± 10.96)	13.23 ^{ab}	18.88 ^{a}	12.53 ^{a}
C1 - EI x EI	45	85.19 ^{a} (± 21.92)	14.75 ^{a}	16.28 ^{a}	13.21 ^{a}
C1 - EI x ES	45	87.41 ^{a} (± 14.80)	11.91 ^{b}	15.46 ^{a}	13.47 ^{a}
C1 - ES x EI	45	87.41 ^{a} (± 14.29)	11.54 ^{b}	16.02 ^{a}	13.51 ^{a}
Mean		87.04	12.86	16.66	13.18
Probability		P=0.997	P=0.003	P=0.494	P=0.977
C2 - ES x ES	30	88.89 ^{a} (± 13.88)	56.34 ^{a}	11.87 ^{a}	12.82 ^{a}
C2 - EI x EI	30	86.67 ^{a} (± 8.82)	62.89 ^{a}	13.76 ^{a}	15.39 ^{a}
C2 - EI x ES	30	80.00 ^{a} (± 23.09)	59.30 ^{a}	12.42 ^{a}	12.73 ^{a}
C2 - ES x EI	30	96.67 ^{a} (± 3.33)	58.87 ^{a}	14.03 ^{a}	16.64 ^{a}
Mean		88.06	59.35	13.02	14.39
Probability		P=0.471	P=0.281	P=0.802	P=0.500

P = Pond; C1 = Cage-1; C2 = Cage-2
Means in column within culture environment with different letter subscripts are significantly different (P<0.05)





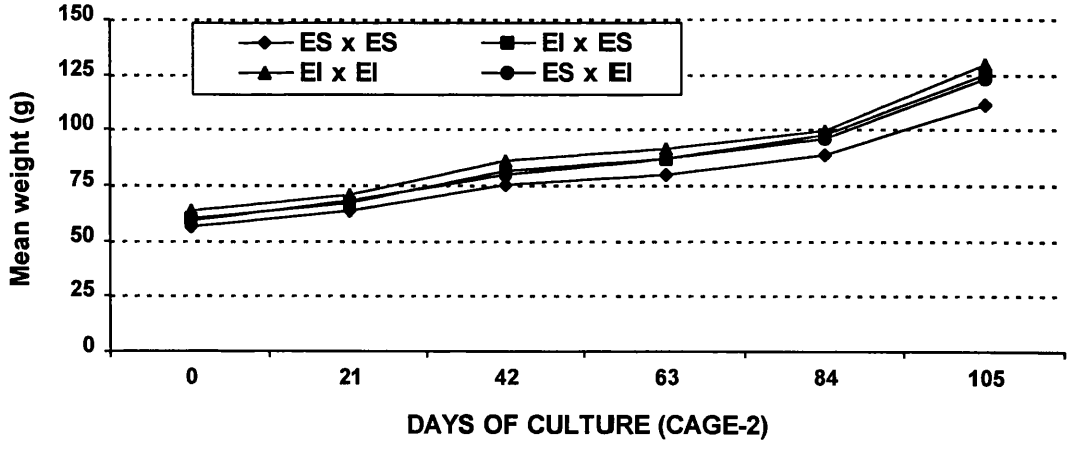


Figure 4.1. Growth curves for the mean weights of Egypt-Swansea and Egypt-ICLARM strains and their reciprocal hybrids in the three culture environments with periodic sampling every 21 days from the time of first stocking.

In cage-1, the growth of the four genotypes were more similar than in ponds and the differences in weight between the strains widened only during the last three weeks of culture. ES x EI had the lowest initial weight but finished with the second highest gain in weight at harvest. The result is contrary to the observed strong positive association of initial weight and weight gain in carps (Wohlfarth and Moav, 1993). In cage-2, the growth of the four genotypes were more variable than that shown during the first phase (cage-1) and the differences tended to correlate with the initial weight. This could be due to the fact that the fish were already over 50g (56.34 to 62.89g) when stocked for this second phase. According to Palada-de Vera and Eknath (1993), the predictability of final weight in communal rearing of *O. niloticus* is highest when weights of males and females are greater than 33 and 25g respectively. The initial weights for cage-2 were higher than these values. Kulikovsky *et al.* (1994) did not find any positive association between the initial and final weight, during communal rearing of *O. niloticus* and *0. aureus*, when the difference in size of the fish were between 3.9 to 8.3g but they did find positive association when the difference was larger than 20g.

A summary of the growth of the four strain crosses is presented in Table 4.3. The result of a one-way ANOVA performed for each culture unit shows that the mean gains in weight of the four genotypes are significantly variable, except for the mean values in cage 23 which were not significantly different. Generally, the ranking of the four genotypes was similar in all culture environments. The hybrids grew faster than the Egypt-Swansea parental strain and their growth was very similar to but not higher than that of the Egypt-ICLARM parental strain (Figure 4.2). In some culture units, either or both hybrids were not significantly faster growing than the Egypt-Swansea parental strain. This maybe be due to the fact that the condition of the culture environments used in the present study is not be considered optimal, particularly in cages. In a growth evaluation of seven strains of *O. niloticus,* Reyes and Eknath (1991) observed that the growth of all fish was severely depressed in some environments. They detected little difference between the strains when grown in these poor environmental conditions (cages installed in reservoirs and in poorly managed earthen ponds). Differences in growth only occurred when the fish were transferred to optimal culture conditions. The authors noted that the evaluation of growth performance in extremely poor environments may be difficult because of poor expression of genetic potential in such environment.

Table 4.3. Mean weight gains (A) and corrected final weights (B) of progeny from a 2 x 2 diallele cross of Egypt-Swansea (ES) and Egypt-ICLARM (EI) strains using novel YY males as broodstock. Mean values represent the averages of individual fish in each strain cross in each culture unit. Values in parenthesis represent the standard error of the mean.

Strain cross (XX♀ YY♂)		Culture environment Pond - 1	Pond - 2	Pond - 3	Overall mean
ES x ES	A	210.28 ^{a} (±6.17)	173.84 ^{a} (±5.78)	216.70 ^{a} (±6.53)	201.62 (±3.85)
EI x EI	A	258.95 ^{b} (±5.42)	209.79 ^{b} (±4.70)	234.50 ^{ab} (±5.03)	235.36 (±3.33)
EI x ES	A	249.98 ^{b} (±5.25)	202.87 ^{b} (±3.09)	249.57 ^{b} (±5.81)	234.50 (±3.29)
ES x EI	A	260.16 ^{b} (±5.20)	214.15 ^{b} (±4.49)	226.37 ^{a} (±6.74)	233.77 (±3.49)
Probability		P<0.001	P<0.001	P=0.001	P<0.001
ES x ES	B	261.66 ^{a} (±7.35)	191.98 ^{a} (±6.07)	268.90 ^{a} (±7.77)	243.36 (±4.91)
EI x EI	B	262.63 ^{a} (±5.26)	217.14 ^{b} (±4.65)	231.38 ^{b} (±4.73)	237.93 (±3.21)
EI x ES	B	278.88 ^{a} (±5.62)	214.30 ^{b} (±3.12)	280.53 ^{a} (±6.28)	258.44 (±3.83)
ES x EI	B	229.98 ^{b} (±4.37)	214.98 ^{b} (±4.31)	203.39 ^{c} (±5.73)	216.48 (±2.87)
Probability		P<0.001	P<0.001	P<0.001	P<0.001
		Cage - 11	**Cage - 12**	**Cage - 13**	
ES x ES	A	37.41 ^{a} (±1.00)	44.64 ^{a} (±1.11)	45.94 ^{a} (±1.26)	42.49 (±0.74)
EI x EI	A	47.84 ^{c} (±1.32)	52.70 ^{c} (±1.32)	44.50 ^{a} (±1.44)	47.73 (±0.85)
EI x ES	A	42.07 ^{b} (±1.03)	47.32 ^{ab} (±1.64)	52.21 ^{b} (±1.25)	47.01 (±0.83)
ES x EI	A	44.98 ^{bc} (±0.88)	51.97 ^{bc} (±1.24)	46.98 ^{ab} (±1.78)	47.59 (±0.82)
Probability		P<0.001	P<0.001	P=0.002	P<0.001
ES x ES	B	47.10 ^{a} (±0.92)	56.97 ^{a} (±1.10)	59.53 ^{a} (±1.28)	54.30 (±0.82)
EI x EI	B	56.20 ^{c} (±1.20)	60.68 ^{a} (±1.20)	49.28 ^{c} (±1.16)	54.67 (±0.81)
EI x ES	B	59.38 ^{bc} (±1.14)	61.41 ^{a} (±1.69)	70.61 ^{b} (±1.38)	63.83 (±0.91)
ES x EI	B	61.67 ^{b} (±0.96)	69.33 ^{b} (±1.35)	67.69 ^{b} (±2.06)	65.89 (±0.94)
Probability		P<0.001	P<0.001	P<0.001	P<0.001
		Cage - 21	**Cage - 22**	**Cage - 23**	
ES x ES	A	49.80 ^{a} (±2.47)	53.74 ^{a} (±2.93)	59.97 ^{a} (±3.02)	54.84 (±1.71)
EI x EI	A	60.44 ^{ab} (±4.69)	69.85 ^{b} (±3.73)	69.01 ^{a} (±3.54)	66.77 (±2.30)
EI x ES	A	60.84 ^{ab} (±4.12)	63.74 ^{ab} (±2.97)	72.09 ^{a} (±3.10)	66.34 (±1.96)
ES x EI	A	63.64 ^{b} (±3.17)	66.60 ^{b} (±2.86)	64.19 ^{a} (±4.81)	64.85 (±2.08)
Probability		P=0.043	P=0.003	P=0.100	P<0.001
ES x ES	B	109.75 ^{a} (±2.65)	119.42 ^{a} (±3.14)	121.89 ^{a} (±3.11)	117.63 (±1.83)
EI x EI	B	111.71 ^{a} (±4.31)	126.98 ^{a} (±3.47)	127.42 ^{a} (±3.47)	122.63 (±2.27)
EI x ES	B	118.35 ^{a} (±4.20)	128.66 ^{a} (±3.12)	127.85 ^{a} (±2.91)	126.05 (±1.94)
ES x EI	B	120.30 ^{a} (±3.17)	126.41 ^{a} (±2.78)	128.84 ^{a} (±5.14)	125.11 (±2.17)
Probability		P=0.104	P=0.162	P=0.552	P=0.020

Means in a column within culture environment with different letter subscripts are significantly different (P<0.05)

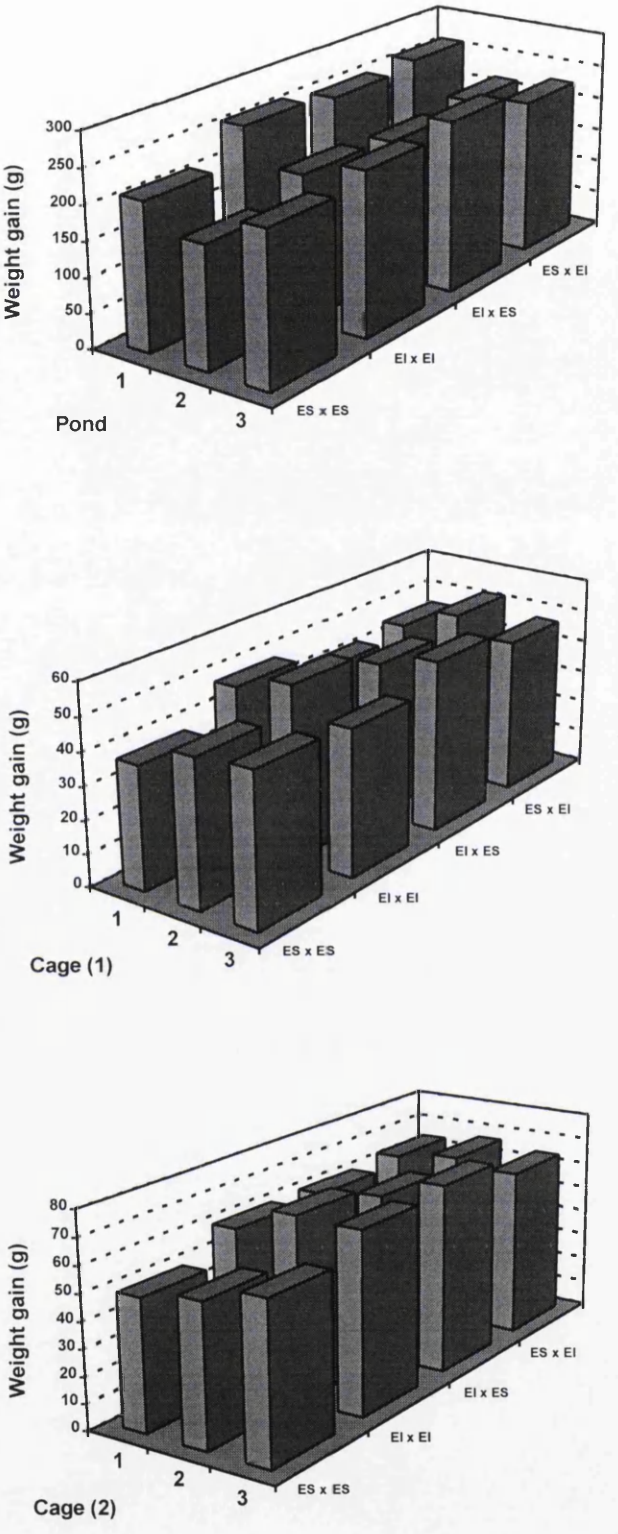


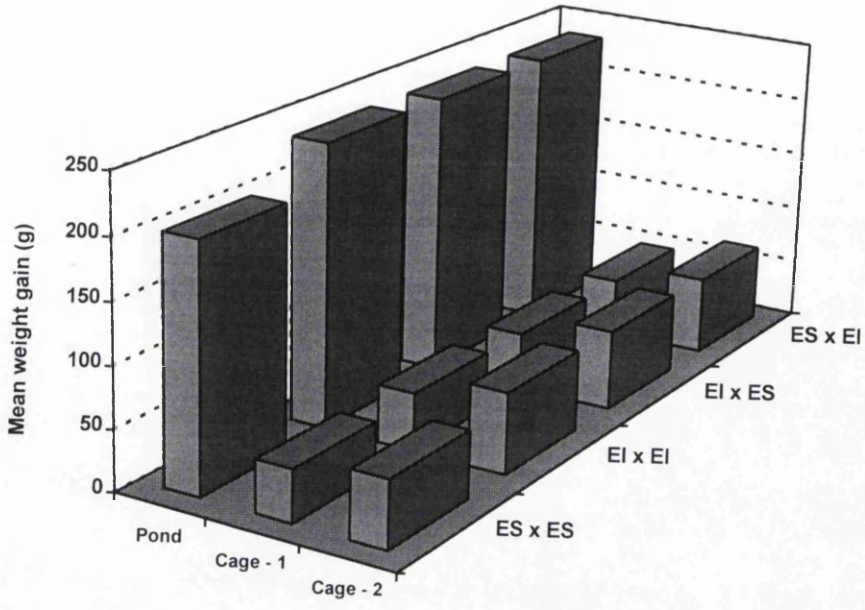
Figure 4.2. Bar charts showing the comparative mean weight gain of individual fish in each of the four genotypes in pond or cage culture units.

The result of a nested ANOVA are presented in Table 4.4. A significant difference ($P<0.001$) between the weight gain of the four strain crosses is observed. This is opposite to the result of an earlier analysis using replicate means as source of error (data not shown) where no significant difference between the genotype was observed and, similarly, no significant genotype x culture environment interaction. The significant difference could be due to the fact the weights of individual fish were used as replicates in the nested ANOVA. Because of the larger number of the replicates, a normal distribution was exhibited which is a requirement for ANOVA. There is also a significant genotype x culture environment interaction for weight gain ($P<0.001$). Although the overall means were different, the weights of individual fish in each strain cross were not categorically different from those of the other cross resulting in interaction. However, there is an obvious difference between the growth of the four genotypes and their ranking are relatively similar in all the three culture environments (see upper graph in Figure 4.3).

Table 4.4. Results of a nested analysis of variance performed on weight gain of individual fish of the four strain crosses.

Source of Variation (SV)	Degrees of Freedom (DF)	Sum of Squares (SS)	Mean of Squares (MS)	F Value [a]	F Value [b]	Level of Probability [a]	Level of Probability [b]
Strain cross (A)	3	68367	22789	24.859	9.106	P<0.001	P<0.001
Replicate (B)	8	11004320	1375540	1500.498	549.640	P<0.001	P<0.001
Culture environment (C)	2	10820222	5410111	5901.583	2161.782	P<0.001	P<0.001
Between B within C	6	184098	30683	33.470	12.260	P<0.001	P<0.001
A x B	24	90286.92	3761.955	4.104	1.503	P<0.001	P>0.05
A x B within C	18	45047.088	2502.616	2.729		P<0.001	P<0.001
A x C	6	45239.832	7539.972	8.225	3.013	P<0.001	P<0.05
Groups	35	11162973.92	318942.112	347.916	127.443	P<0.001	P<0.001
Residual	1411	1293494.75	916.722				
Total	1446	12456468.67	8614.432				

[a] Used the mean of squares of residual as source of error
[b] Used the mean of squares of A x B within C as source or error



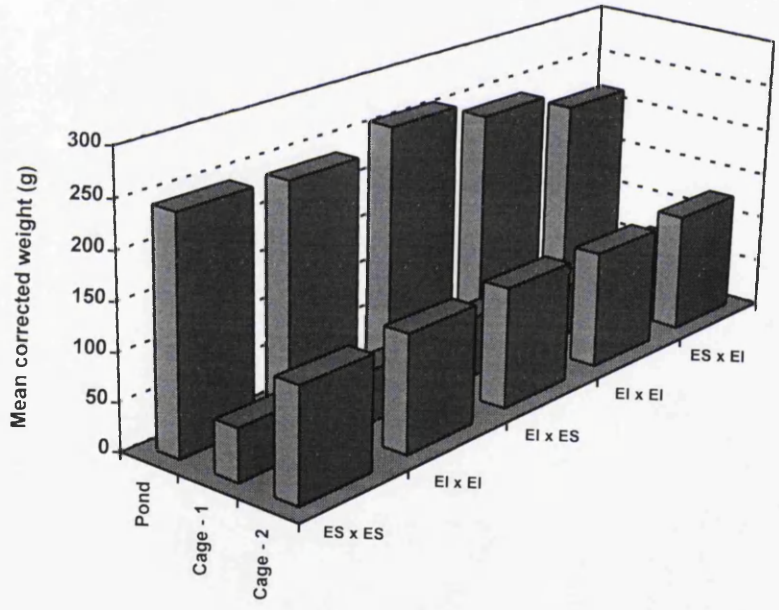


Figure 4.3. Bar charts showing the comparative overall mean weight gain and corrected final weight of the four genotypes in the three culture environment.

The results of the analysis of corrected final weights are also presented in Table 4.3. The corrected mean weights of the four genotypes in ponds and cage-1 were significantly variable but corrected weights in cage-2 did not differ significantly. The correction for final weight relative to initial weight may be important for the evaluation of growth performance in other species such in carps (Wohlfarth and Moav, 1993) which show a strong positive relationship between the initial and final weight but may not be that important in tilapia, as in the case of the present study. The ranking of the four genotypes, based on the corrected final mean weight, was relatively different from that of the gain in weight (see lower graph in Figure 4.3). It was observed that even a small difference in initial weight, which normally does not significantly affect the gain in weight, would result in a large difference when a correction factor was applied on the final weights. In a growth evaluation of eight strains of *O. niloticus* in different farm environments, Eknath *et al.* (1993) noted that the effect of differences in initial size and age on subsequent growth performance of fingerlings under communal stocking was found to be of limited importance. The apparent lesser effect of initial weight on final weight justified the use of gain in weight in the analysis and satisfactorily represented the actual growth of the fish.

The percentage difference of the hybrids from the larger parent (heterosis), parental mean and smaller parents for gain in weight are presented in Table 4.5. On average, both hybrids had a lower growth than the larger parent (-0.31 to -2.87%) but had a higher mean growth than the parental mean (+3.80 to +8.44%) and the smaller parent (+11.93 to +20.33).

Table 4.5. Percentage difference of hybrid over the larger parent - "heterosis" (A), percentage difference of hybrid over the parental mean (B) and percentage difference of hybrid over the smaller parent (C) for gain in weight. ES = Egypt-Swansea and EI = Egypt-ICLARM.

Culture environment	Hybrid - 1 (EI♀ x ES♂)			Hybrid - 2 (ES♀ x EI♂)		
	A	B	C	A	B	C
Pond - 1	-3.46	+6.55	+18.88	+0.47	+10.89	+23.72
Pond - 2	-3.30	+5.76	+16.70	+2.08	+11.64	+23.19
Pond - 3	+6.43	+10.63	+15.17	-3.47	+0.34	+4.46
Mean	-0.11	+7.65	+16.92	-0.31	+7.62	+17.12
Cage - 11	-12.06	-1.30	+12.46	-5.98	+5.52	+20.24
Cage - 12	-10.21	-.2.77	+6.00	-1.39	+6.78	+16.42
Cage - 13	+13.65	+15.46	+17.33	+2.26	+3.89	+5.57
Mean	-2.87	+3.80	+11.93	-1.70	+5.40	+14.08
Cage - 22	+0.66	+10.38	+22.17	+5.29	+15.46	+27.79
Cage - 22	-8.75	+3.15	+18.61	-4.65	+7.78	+23.93
Cage - 23	+4.46	+11.78	+20.21	-6.98	-0.47	+7.04
Mean	-1.65	+8.44	+20.33	-2.11	+7.59	+19.59

The CV for final weight in ponds was significantly variable (P=0.016) whilst the CV values in cage-1 and cage-2 were not significantly different from each other (Table 4.2). In ponds, ES x ES had the highest CV for final weight. This result is similar to that obtained by Abucay (1996) where the same strain also had the highest CV for final weight compared to a range of hybrids. In that study it was noted that the crossbreds had a more uniform size than the purebred progeny. In cages, the distribution of size of the fish among strain crosses was relatively similar. This could be due to the higher stocking density and the likely greater competition for food and space, which might limited the growth of fish, particularly those potentially fast growers, thus relatively have a similar size. Similarly, it is possible that because of the higher stocking density the fish were prevented from exhibiting either sexual or territorial behaviour thus a more uniform size among the fish was observed.

The mean survival of the four genotypes in all the three culture environments were not significantly different (Table 4.2). The relatively similar survival may imply that crossbreeding does not improve survival during growth evaluation. Similar result was also observed by Tave *et al.* (1990) in crossbreeding of an Egypt and Ivory Coast strains; the viability of the hybrids was intermediate between the parental strains. However, in some studies purebred progeny have tended to have a lower survival rate than crossbreds (Subba Rao and Chandrasekaran, 1978; Ayles and Baker, 1983). It may be possible that the lower percentage survival of purebred fish such as Egypt-Swansea strain is already expressed at an early age (Holsinger, 1988).

The gonadosomatic index and percentages of dress-out and fillet weight of the four strain crosses are presented in Table 4.6. The mean GSI in pond and cage-1 culture environments were significantly variable (P=0.007 and P=0.009, respectively). In both culture environments, EI x EI had the lowest GSI whilst ES x EI had the highest GSI in pond and ES x ES had the highest in cage-1. The mean GSI values in cage-2 were not significantly different.

The mean dress-out and fillet weight in all culture environments were not significantly different. The results of a correlation analysis show that in ponds and cage-1 the percentage of dress-out (r=-0.079, P=0.392 and r=-0.031, P=0.751, respectively) and fillet weights (r=-0.379, P<0.001 and r=-0.259, P=0.006, respectively) are negatively correlated with GSI. In cage-2 the percentage of dress-out (r=0.039, P=0.670) and fillet weight (r=0.212, P=0.020) are positively correlated with GSI.

Table 4.6. Mean gonadosomatic index (GSI) and percentages of dress-out and fillet weight in Egypt-Swansea (ES) and Egypt-ICLARM (EI) strains and reciprocal hybrids. The difference between mean values was determined using a one-way ANOVA.

Strain cross (XX♀ x YY♂)	GSI (± sd)	Dress-out (± sd)	Fillet (± sd)
P - ES x ES	0.41 ^{ab} (± 0.13)	79.89 ^{a} (± 0.71)	34.52 ^{a} (± 0.29)
P - EI x EI	0.19 ^{a} (± 0.02)	80.97 ^{a} (± 1.12)	35.26 ^{a} (± 0.67)
P - EI x ES	0.24 ^{ab} (± 0.12)	80.32 ^{a} (± 0.63)	35.52 ^{a} (± 0.35)
P - ES x EI	0.46 ^{b} (± 0.08)	79.94 ^{a} (± 1.00)	33.97 ^{a} (± 0.35)
Mean	0.32	80.28	34.82
Probability	P=0.007	P=0.169	P=0.355
C1 - ES x ES	0.63 ^{a} (± 0.10)	77.24 ^{a} (± 0.49)	28.39 ^{a} (± 0.76)
C1 - EI x EI	0.13 ^{b} (± 0.00)	76.79 ^{a} (± 0.22)	29.06 ^{a} (± 0.16)
C1 - EI x ES	0.31 ^{a} (± 0.12)	76.78 ^{a} (± 0.38)	30.33 ^{a} (± 0.11)
C1 - ES x EI	0.27 ^{b} (± 0.25)	76.17 ^{a} (± 0.30)	28.24 ^{a} (± 0.61)
Mean	0.33	76.74	29.00
Probability	P=0.009	P=0.815	P=0.184
C2 - ES x ES	0.23 ^{a} (± 0.04)	79.77 ^{a} (± 0.26)	31.05 ^{a} (± 0.75)
C2 - EI x EI	0.20 ^{a} (± 0.09)	77.75 ^{a} (± 0.25)	30.32 ^{a} (± 0.84)
C2 - EI x ES	0.33 ^{a} (± 0.06)	78.74 ^{a} (± 3.69)	31.71 ^{a} (± 1.00)
C2 - ES x EI	0.30 ^{a} (± 0.14)	78.86 ^{a} (± 0.90)	31.37 ^{a} (± 0.47)
Mean	0.26	78.78	31.11
Probability	P=0.249	P=0.360	P=0.474

P = Pond, C1 = Cage-1, C2 = Cage-2

Means in column within a culture environment with different letter subscripts are significantly different (P<0.05)

The negative correlation of dress-out and fillet weight with GSI agrees with the earlier results of Abucay (1996) and the results presented in Chapter five. Those fish with a higher GSI tend to have a lower dress-out and fillet yield. It has been known that tilapia have complex social hierarchies which arise as a result of competition for food, space and territory (McGinty, 1985). The increase in GSI was thought to be due to the increase in reproductive activity and aggressiveness (discussed in more detail in Chapter five). An effect of increase in aggressiveness could be a reduction in dress-out and fillet yield as a result of the extra energy spent on reproductive activities and aggression. Similarly, the tendency of the more dominant and aggressive fish to grow bigger gonad will have a negative effect on dress-out yield since a large proportion of the total fish weight is due to gonad weight.

The percentage difference of the hybrids over the larger parent (heterosis), parental mean and smaller parent for dress-out and fillet weight are presented in Table 4.7. On average, both hybrids had lower percent dress-out weight than the larger parent (-0.90 and -1.27%) and parental mean (-0.16 and -0.53%) but slightly higher than the smaller parent (+0.60 and +0.23%).

For the percentage of fillet weight, hybrid-1 had a higher fillet weight than the larger parent (+2.41%), parental mean (+3.58%) and smaller parent (+4.77%). Hybrid-2, had a lower percent fillet weight than the larger parent (-1.82%) and parental mean (-0.71%) but slightly higher than the smaller parents (+0.45%).

Table 4.7. Percentage difference of hybrids over the larger parent - "heterosis" (A), percentage difference of hybrid over the parental mean (B) and percentage difference of hybrid over the smaller parent (C) for dress-out and fillet weights. ES = Egypt-Swansea and EI = Egypt-ICLARM.

Culture Environment	Hybrid - 1 (EI♀ x ES♂)			Hybrid - 2 (ES♀ x EI♂)		
	A	B	C	A	B	C
P - dress-out	-0.80	-0.14	+0.54	-1.27	-0.61	+0.06
C1 - dress-out	-0.60	-0.31	-0.01	-1.39	-1.10	-0.81
C2 - dress-out	-1.29	-0.03	+1.27	-1.14	+0.13	+1.43
Mean	-0.90	-0.16	+0.60	-1.27	-0.53	+0.23
P - fillet	+0.74	+1.81	+2.90	-3.66	-2.66	-1.59
C1 - fillet	+4.37	+5.59	+6.83	-2.82	-1.69	-0.53
C2 - fillet	+2.13	+3.34	+4.58	+1.03	+2.23	+3.46
Mean	+2.41	+3.58	+4.77	-1.82	-0.71	+0.45

P = Pond; C1 = Cage-1; C2 = Cage-2

Although the hybrids have a mean gain in weight similar to that of the larger parent, they tend to have a lower mean dress-out yield. This shows that a great proportion of their weight could be due to non-edible parts. It should be noted than in an earlier result; the GSI was shown to be negatively correlated with dress-out and fillet yield. Similarly, they may have also produced more adipose fat, although this has been noted during the conduct of the study. For the fillet yield, hybrid-1 had a heterotic yield but not for hybrid-2. Although, the mean heterosis value was relatively small, the difference between the two hybrids could be due to a maternal effect; hybrid-1 being produced from females of the Egypt-ICLARM strain which have been tested to be a fast growing strain (Eknath *et al.*, 1993).

4.3.2 Sex ratio

A summary of the sex ratios of novel YY males in a diallele cross of Egypt-Swansea and Egypt-ICLARM strains is presented in Table 4.8. The total number of families tested in each strain cross ranged 14 to 57 and was highly variable. At the time of the study there were only three Egypt-ICLARM YY males available; one YY male was used for each pure and hybrid cross whilst the third YY male had been alternately used for both crosses to improve spawning efficiency. Moreover, the Egypt-ICLARM females were observed to have a lower spawning frequency, even with the hybrid cross as indicated by the lower number of families tested in EI x ES and EI x EI compared to the other strain cross. The small number of available YY males and the difficulty of spawning limited the ability to produce more families using the Egypt-ICLARM strain.

The average number (39.43 to 56.3) of fingerlings sexed in each family was fairly low. All the families tested were grown in 1 m^3 fine mesh hapas in earthen ponds. The hapas were only occasionally inspected to check for the size of the fish. When the hapas were lifted to get the fish for sexing, carnivorous gobies were often found inside the cages. The intrusion of this predatory species in suspended net cages was also observed by Bolivar *et al.* (1994). Similarly, it is possible that birds had fed on the fish since the hapas used were not covered.

Table 4.8. Summary of the sex ratios from a diallele cross of Egypt-Swansea (ES) and Egypt-ICLARM (EI) strains using novel YY males. The χ^2 values were determined using heterogeneity chi-square analysis.

Strain cross (XX♀ x YY♂)	Number of families tested	Number of progeny tested	Mean number of progeny tested	Mean sex ratio (% males) (± se)	Range of sex ratio (% males)	χ^2 value	Families with ≥95% males (%)
ES x ES	41	2334	56.3	98.89 (± 0.39)	86.76 - 100.0	5.45 ^{ns}	40 (97.56)
EI x EI	14	552	39.43	99.29 (± 0.71)	90.0 - 100.0	0.39 ^{ns}	13 (92.86)
EI x ES	24	1268	52.83	98.42 (± 1.93)	53.85 - 100.0	22.31 ^{ns}	23 (95.83)
ES x EI	57	2453	43.04	98.26 (± 0.73)	63.64 - 100.0	10.97 ^{ns}	54 (94.74)

^{ns} = not significantly different

The mean proportion of males of the four cross combinations, were all very high ranging from 98.26 to 99.9%. The sex ratios of individual families tested within each cross combination were also very high, the majority within the ≥95% male category (see Figure 4.4). The majority of families clustered near the 100% mark with the exception of two families, one for each hybrid cross, which had extremely low percentage male values. However, the result of a heterogeneity χ^2 analysis performed to determined the significance of the variability of the sex ratios within each strain cross show values ranging from 0.30 to 22.31, all not statistically significant.

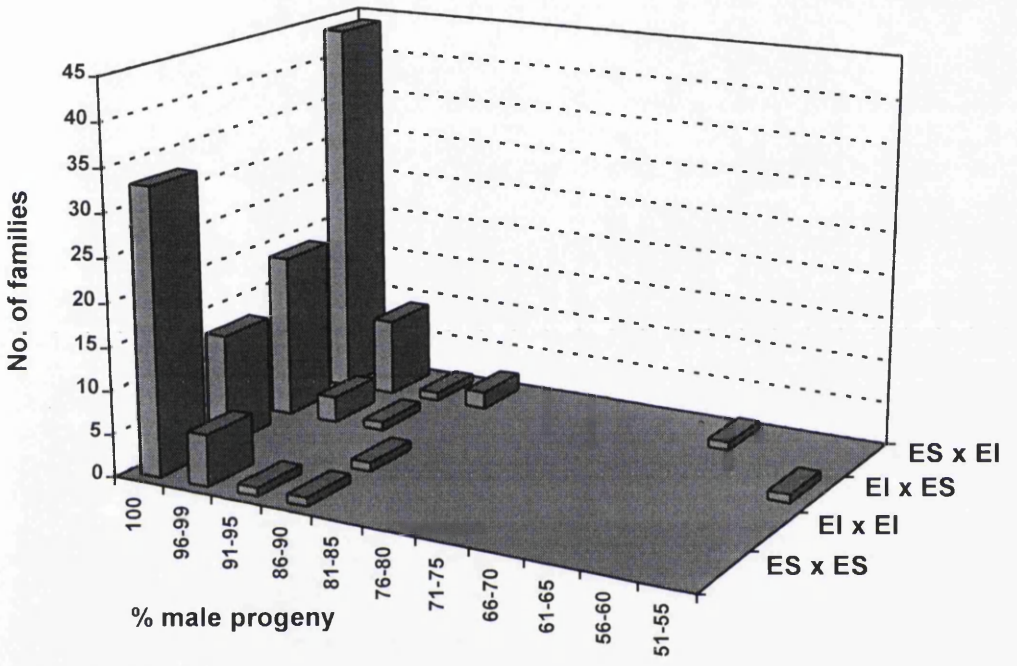


Figure 4.4. Histogram showing the frequency of sex ratios (% males) of purebred and crossbred progeny of novel YY males in a diallele cross of Egypt-Swansea (ES) and Egypt-ICLARM (ES).

The sex ratios of novel YY males of the Egypt-Swansea strain in crosses with females from different strains are presented in Table 4.9. The sex ratios for ES x ES and EI x ES in Table 4.9 were the same data as those presented in Table 4.8 and are tabulated for comparison. There were ten female sources consisting of five Egyptian strains, three Kenyan strains and two selected strains. The mean proportion of males of the different strain crosses were all high ranging from 93.12 to 100.0%.. Egypt-AIT had the lowest mean percentage males (93.12%) whilst Kenya-Baringo had the highest (100%). The result of a heterogeneity χ^2 analysis showed that the sex ratios in each strain cross were statistically homogeneous with the exception of those sex ratios (54.84 to 100% males) in crosses involving females of the Egypt-AIT which were highly heterogeneous ($P<0.001$). In all the other strain crosses with statistically homogeneous sex ratios, there were also cases of sex ratios with extremely low percentage males, particularly those in Egypt-Stirling, Kenya-Baobab, Egypt-ICLARM and IDRC strains, but the frequencies of such families were very low (see Figure 4.5). The majority of the families tested within each strain cross were within the ≥95% category which indicates the capacity of the YY males in Egypt-Swansea strain to sire a high percentage of male progeny in crosses involving females from other strains.

Table 4.9. Summary of the sex ratios of progeny from crosses of novel YY males of the Egypt-Swansea strain with females from different strains in *O. niloticus*. The χ^2 values were determined using heterogeneity chi-square analysis.

Strains (Source of females)	Number of families tested	Number of progeny tested	Mean number of progeny tested	Mean Sex ratio (% males) (± se)	Range of sex ratio (% males)	χ^2 value	Families with ≥95% males (%)
E.-Swansea	41	2334	56.93	98.89 (± 0.39)	86.67 - 100.0	5.45 ^{ns}	40 (97.56)
E.-ICLARM	24	1268	52.83	98.42 (± 1.93)	53.85 - 100.0	22.31 ^{ns}	23 (95.83)
E.-Stirling	34	1623	47.74	95.63 (± 1.59)	64.86 - 100.0	43.95 ^{ns}	28 (82.35)
E.-AIT	41	2311	56.37	93.12 (± 1.67)	54.84 - 100.0	96.52 ***	31 (75.61)
E.-BFAR	9	306	34.0	98.69 (± 1.17)	89.66 - 100.0	1.17 ^{ns}	8 (88.89)
K.-Turkana	27	1216	45.04	98.93 (± 0.64)	83.72 - 100.0	5.03 ^{ns}	26 (96.30)
K.-Baringo	5	202	40.40	100.0 (± 0.0)	all 100.0	0.0 ^{ns}	5 (100.0)
K.-Baobab	9	540	60.0	93.33 (± 4.88)	56.52 - 100.0	19.25 ^{ns}	7 (77.78)
GIFT fish	14	696	49.71	97.13 (± 1.86)	81.40-100.0	9.36 ^{ns}	11 (78.57)
IDRC fish	16	940	58.75	97.45 (± 2.23)	65.71 - 100.0	16.75 ^{ns}	14 (87.50)

E = Egypt; **K** = Kenya
ns = not significantly different; *** = P<0.001

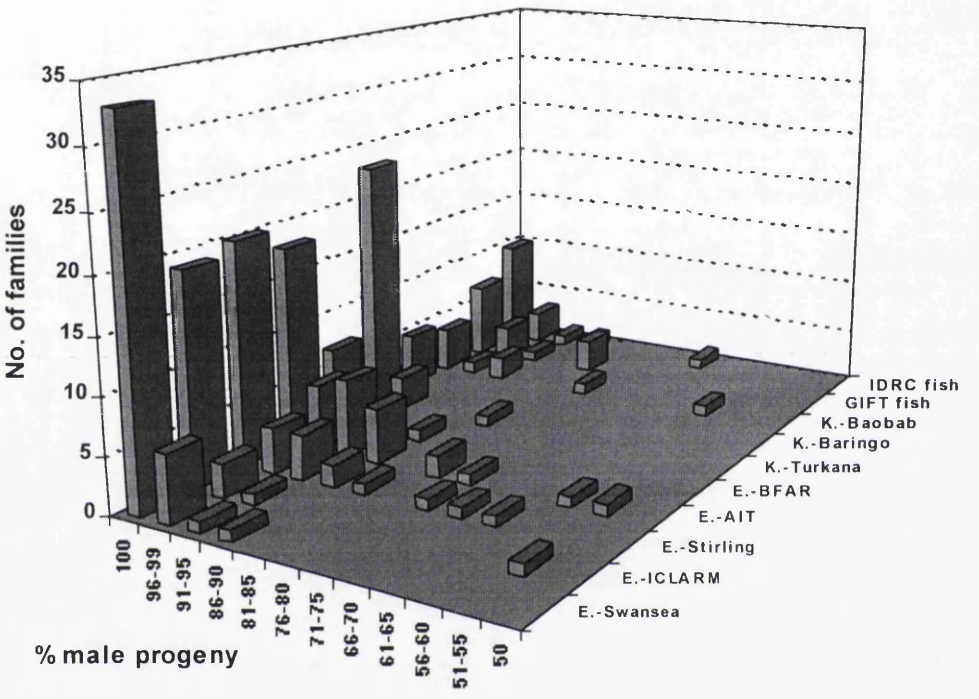


Figure 4.5. Histogram showing the frequency distributions of family sex ratios (% males) of novel YY males of the Egypt-Swansea strain in crosses with females from different strains of *O. niloticus*.

4.4 Discussion

4.4.1 Growth in crossbred GMT

The hybrids show a mean growth higher than the parental mean and the smaller parent (which in most cases was the Egypt-Swansea strain). The results conforms to the earlier results of a study by Abucay (1996) where hybrid GMT grew faster than the purebred Egypt-Swansea GMT. The mean growth of the hybrids was higher than that of the purebred progeny but this author was not able to establish whether it was due to heterosis since no reciprocal crosses were made. However, in the present study neither hybrids were better than the bigger parental strain (which in most cases was the Egypt-ICLARM strain). A similar results were obtained in a study by Khater (1985), in which hybrids between Ghanaian, Ivorian and Egyptian strains of *O. niloticus* all exhibited better growth than the parental mean, but none grew better than the larger parental strain which was the one originating from Egypt.

The exhibition of overdominant growth of the hybrid over the larger parent or heterosis appears to be rare in fish. In crossbreeding of common carp heterotic growth has been demonstrated in hybrids (Hulata, 1995a; Bakos and Gorda, 1995). However, there appears to be some inconsistency in the literature with regard to the definition of heterosis. For example, Bakos and Gorda (1995), compared the traits of the hybrids with those of the parental mean. In a review of heterosis for growth in common carp (Wohlfarth, 1993), in which heterosis was

defined as the amount by which the F_1 hybrid exceeds the mean of the bigger parent, half of the hybrids tested did not have heterotic growth. The heterotic growth in some of the hybrids tested may be attributed to the fact that the strains or lines that were crossed have been maintained separately in isolated farms (Wohlfarth, 1993; Hulata, 1995a) and might have undergone inbreeding at some stage.

Inbreeding, which is known to occur when closely related individuals are mated, is the reduction of the mean phenotypic value shown by characters connected with reproductive capacity or physiological efficiency known as "inbreeding depression" (Falconer and Mackay, 1996). When inbred lines are crossed, the progeny commonly show an increase in those traits that have suffered a reduction as a result of inbreeding, a phenomenon known as "hybrid vigor or heterosis" (Falconer and Mackay, 1996). The original population mean may be restored in the hybrids and their growth tends to be higher than the parental mean of inbred lines due to reduction of the growth of the latter as a consequence of inbreeding depression. Thus in these circumstances, it would appear that hybrids have overdominance with respect to growth. The concept of developing inbred lines is commonly based on the hypothesis that crossing inbred lines could produce heterotic progeny. This has been demonstrated in plants, such as the maize, where heterosis has been successfully exploited (Gjerde, 1988). This may be due to the fact that selfing is a natural phenomenon and inbreeding depression appears to be rare with few effects on growth.

The crossing of two inbred lines may also result in the restoration of lost genetic heterozygosity in the hybrid. High levels of heterozygosity are known to be closely associated with characters related to fitness such as reproductive rates, growth and developmental stability (Smith and Chesser, 1981). Tayamen (1992) reported that the GIFT base population, which has a very broad genetic variability, grew about 60% faster than the most widely cultured "Israel" strain across a range of environments tested. In the present study, the Egypt-Swansea strain is considered to be somewhat inbred as a result of having been through a number of genetic bottlenecks (Mair *et al.*, 1995). The higher growth of the hybrids compared to purebred Egypt-Swansea may be due to the increase in genetic variability.

However, assuming that the better growth of the hybrids over the parent Egypt-Swansea strain is due to a dominance effect, it appears that the ability of a dominant parent to "pull up" the growth of the hybrids seem to be limited only up to its own average growth. In the present study, assuming that Egypt-ICLARM was the more dominant parent in terms of growth, the average growth of the hybrids was similar to but not higher than the growth of the former. To produce larger hybrids than Egypt-ICLARM, the latter should be crossed to a more dominant and faster growing strain. Gjerde (1988) noted in his discussion that unless inbred lines are derived from different base populations, inbreeding and crossing alone cannot produce any improvement; unless selection for genetically faster growing parents has been made at some stage. This may be the reason for the apparent lack of heterosis in crossbreeding of common carp involving a

Dor-70 carp strain, a product of a five year selection program. The growth of the hybrids were either similar or lower but rarely higher than Dor-70 (Wohlfarth, 1993).

The difference in the extent of domestication of two strains might also have some effect on growth. Dunham and Smitherman (1983) reported that in catfish the domestic x domestic crosses were found to be more likely to exhibit heterotic rates of growth than domestic x wild crosses. Similar results were also observed in rainbow trout (Dunham, 1986). The Egypt-ICLARM strain was only introduced to the Philippines from the wild almost a decade ago and has not been through a rigorous domestication as has the Egypt-Swansea strain (≈two decades).

Nevertheless, although heterosis may not be always expected from hybrids, crossbreeding provides opportunities for compromising species with distinct traits; e.g. between fragile, fast growing species and more hardy-slow growing species to produce a desired progeny (Chevassus, 1979). For example, crossbreeding may be the best approach for a development of fast growing saline tolerant progeny by crossing *O. mossambicus* x *O. niloticus* (Villegas, 1990) or cold-tolerant population of red tilapia by crossing *0. aureus*♀ x red tilapia♂ (Behrends and Smitherman, 1984). Crossbreeding has been suggested as a means to rehabilitate the declining growth in wild populations by crossing with "tailor made" selected breeds (Moav *et al.*, 1978).

4.4.2 Sex ratio in YY males

Previous research involving the use of YY males have demonstrated their capacity to sire near all- or all-male progeny in within strain crosses. Scott *et al.* (1989) observed all-male progeny from a single YY male crossed to ten females. Mair *et al.* (1992) observed sex ratios ranging from 71.6 to 100% male and a mean of 97.3% from a total of 33 YY males identified. Varadaraj and Pandian (1989a) observed no females among the progeny of eight YY males in *O. mossambicus.*

In the present study, the high percentage of families (75.61-100%) with male ratios ≥95% in all the strain crosses indicate the ability of YY males to sire a high percentage of male progeny not only in within strain crosses but also in between strain crosses. This result implies that the "Y" chromosome acts as the major determinant of sex, which is an inherent characteristic of a monofactorial sex determining mechanism with heterogametic males (Rosentein, 1990). However, the presence of significant numbers of females in some of the crosses indicate the possible existence of minor sex-modifying factors other than the sex chromosomes (Winge, 1934; Lester *et al.*, 1989; Mair *et al.*, 1991a; Wohlfarth and Wedekind, 1991).

Based on his work on *Lebistes reticulatus*, Winge (1934) proposed a model to explain variation in sex ratio which could not be explained by the chromosomal theory alone (see Chapter three). The number and potency of the male and female determining factors may be different between strains or species

(Kallman 1984). The difference in potency of the combinations of minor sex determining genes could arise due to crossing-over between sex-determining loci located on sex chromosomes (Wohlfarth and Wedekind, 1991) or crossing-over of genes in the autosomes (Winge, 1934) and also in the crossing of strains or species (Mair *et al.*, 1997a).

In the present study the variability of the observed sex ratio in within and between strain cross could be an indication of the difference in the number and potencies of the autosomal sex genes in some of the strains used. The variability of the observed sex ratio may be due to the influence of both parental genotypes, although it may be more likely that the females have exerted more influence considering the more homogeneous sex ratio in the pure Egypt-Swansea strain crosses. Tuan *et al.* (1997) observed sex ratios ranging from 15.5 to 100% male progeny from 97 intrastrain crosses in the Egypt-AIT strain. In the present study, that observed variability of sex ratio, which was evidence of a more polyfactorial mechanism of sex determination, was also partly reflected in the greater sex ratio variability in the inter-strain crosses of Egypt-AIT females and Egypt-Swansea YY males (54.84-100% males). The same might also be the reason for the observed variable sex ratios in some of the other strain crosses, the female parents which produced excess females in the progeny might be carrying more female autosomal genes.

The findings of the present study are in accordance with the results of Capili (1995) which indicated that the minor deviations from the predicted all-male progeny of YY males have a genetic basis (maternal and paternal

influence). However, the scope of the present study was limited only to determination of the effect of maternal source (i.e. strain).

Variation in temperature outside the normal range for the species has also been observed to affect sex ratio in fish (see Chapter three). Unlike in other fish species, particularly the live-bearers, in which phenotypic sex is already differentiates at birth, the sex in tilapias, only differentiates between 10-33 days posthatching (Nakamura and Takahashi, 1973; Alvendia-Casauay and Cariño, 1988). During this stage, the gonads are in labile condition and the predicted sex maybe altered by environmental factors such as rearing at high temperatures environment. Since it has been assumed that the observed variation in sex ratio in the present study is due to autosomal sex-modifying factors, it is possible that this has been partly triggered by the environmental effect.

4.5 Conclusion

The growth performance of GMT progeny of Egypt-Swansea YY males may be improved by crossbreeding, although, the finding that hybrids are better than the larger parent seems to be a rare phenomenon and significant heterosis was not observed in this study. Egypt-ICLARM ♀ x Egypt-Swansea ♂ crossbred GMT appears to be a better strain combination in terms of fillet yield because of the higher value of the hybrid progeny. The YY males in Egypt-Swansea and Egypt-ICLARM strains were found to be both equally capable of siring all-male

progeny. The YY males in Egypt-Swansea were also found capable of siring a high percentage of male progeny even in interstrain crosses. However, the capability to produce consistent sex ratios depends on the genetic quality of the female source.

CHAPTER FIVE

GROWTH OF METHYLTESTOSTERONE-TREATED MALES AND NORMAL FEMALES

5.1 Introduction

In recent years, the use of hormones in domesticated animals has become more common and widespread. The interest in the use of hormones in fish, particularly the synthetic androgens, is usually linked to their property to induce sex reversal and apparent anabolic effects. The effect of anabolic agents as defined by Donaldson *et al.* (1979) is an increase in nitrogen retention where the increase can be due to an increase in food intake or to improved food utilisation.

In aquaculture the application of hormones has been used primarily for inversion of sex to produce monosex for culture (Hunter and Donaldson, 1983) and, in research, for the elucidation of the mechanism of sex determination and sex differentiation (Eckstein and Spira, 1965; Mair *et al.*, 1991a,b; Bongers *et al.*, 1991; Cuisset *et al.*, 1991). However, more recently, because of the apparent positive anabolic effects on commercially important traits, the interest in the use of synthetic androgens has been focused on the practical application during grow out for the purpose of increasing yield. The results of initial trials had shown that the addition of small amounts of androgen hormone in the diet could enhance growth. For example, Matty and Cheema (1978) fed two synthetic androgens; dimethazine and norethandrolone, to rainbow trout (*Oncorhynchus mykiss*) and observed a significant increase in weight gain when both hormones were given at low concentrations (2.5 and 5.0 $mg.kg^{-1}$ diet). Higher concentrations (10 and 20 $mg.kg^{-1}$ diet) produced an initial increase in weight gain but it was not

significantly different from the untreated control. Basavaraja *et al.* (1989) reported that the feeding of testosterone at a concentration of 2.5 $mg.kg^{-1}$ diet was found to increase growth significantly in common carp, *Cyprinus carpio,* although at the higher concentrations of 5.0 and 10.0 $mg.kg^{-1}$ the growth was decreased compared to the control. Deb and Varghese (1988) also reported that growth in *Catla catla*, *Labeo rohita*, *Cirrhinus mrigal* and *Cyprinus carpio* was increased by 40.33, 50.15, 87.46 and 29.77%, respectively, compared to the controls, when 17α-methyltestosterone at a concentration of 1.0 $mg.kg^{-1}$ was added to the diet. At the higher concentrations of 3.0 and 5.0 $mg.kg^{-1}$ the growth was decreased compared to that at the lower hormone concentration although still higher than the controls. Piferrer *et al.* (1991) fed 17α-methyltestosterone to one year old juvenile sea bass, *Dicentrarchus labrax* L., but the growth was only slightly increased at 10.0 $mg.kg^{-1}$ diet whilst at 30.0 $mg.kg^{-1}$ the growth was lower than the untreated control.

The feeding of small amounts of androgen hormone has also been observed to increase growth in tilapia. The feeding of hormone is normally applied at an early age to reverse the sex which usually enhances growth due to the normal sexual dimorphism in growth where males grow faster and bigger than females. Extending the feeding of hormone beyond the normal period of sex reversal has been shown to increase growth further. Kuwaye *et al.* (1993) reported a 30 to 50% additional increase in growth, over early treated fish, when the feeding of 17α-methyltestosterone to *O. niloticus* fry was extended beyond the normal sex labile period, despite the fact that both treatments produced

nearly all-male results (96 to 100%). Whilst the faster growth of androgen-treated fish is normally attributed to their being all- or mostly males, Howerton *et al.* (1992) demonstrated, in *O. mossambicus*, that the application of 17α-methyltestosterone during grow out (i.e. when the sex was already differentiated) can bring about a significant enhancement in growth. Ufodike and Madu (1986) also reported that *O. niloticus* fed with methyltestosterone-treated diet had faster growth compared to fish fed with an untreated diet.

In the aforementioned studies, the application of androgen hormone was purposely applied to enhance growth. When applied early in the life cycle during the normal period of sex differentiation, the hormone alters the phenotypic sex. However, in several studies it was demonstrated that the application of hormone did not only alter the sex but also enhanced growth of the treated fish. Guerrero (1975) reported a significantly higher mean gain in weight in *O. aureus* fry treated with androgens; 1-dehydrotestosterone acetate, 17α-ethynyltestosterone and 17α-methyltestosterone, compared to the untreated controls. In a similar study in *O. niloticus*, Macintosh *et al.* (1988) noted that the methyltestosterone-treated fry were larger, on average, than the control after 40 days hatchery period. Jo *et al.* (1988) fed *0. aureus* with 17α-methyltestosterone at a concentrations of 1.0, 10.0, and 60.0 $mg.kg^{-1}$ diet for 30 days beginning from first feeding stage and found the average growth increased significantly as the hormone concentration increases.

In contrast, Phelps *et al.* (1992) reported that the mean weights of *O. niloticus* fry treated with the androgens; fluoxymesterone (0.2, 5.0 and 25 $mg.kg^{-1}$

diet) and methyltestosterone (60 mg.kg^{-1} diet), were not different from the control after 28 days of treatment. Also Green and Teichert-Coddington (1994) found that slopes of the growth curves for methyltestosterone-treated (60 mg.kg^{-1} diet) and untreated controls in *O. niloticus* were not significantly different, with growth averaging 0.1 g.fry^{-1} after 28 days treatment. Improved growth of hormone treated fish would clearly be an added benefit when culturing sex-reversed fish. However, the contrasting reports on the anabolic effect of the hormone indicate the inconsistency of this effect.

The enhanced growth of androgen hormone treated fish has been observed not only during the treatment period but also during subsequent grow-out when the hormone treatment was already finished. Guerrero (1976) observed higher mean weights of ethynyltestosterone-treated *O. mossambicus* and *Tilapia zilli* grown in earthen ponds when compared to the control, although the comparison of weight of *O. mossambicus* with the control was considered invalid due to differences in stocking density. In a study comparing the growth of six groups consisting of either or combined genetic males, sex-reversed genetic females and genetic females, Hanson *et al.* (1983) found that the methyltestosterone-treated groups; genetic males + sex-reversed genetic females and sex-reversed genetic females, showed the fastest growth. McAndrew and Majumdar (1989) also observed a significantly faster growth of the methyltestosterone-treated *O. niloticus*, *O. mossambicus* and *O. aureus* when compared to their respective untreated controls.

The improved growth of hormone treated fish observed in the previously mentioned studies contrasts with those from the other studies. Meyer (1990) found that the growth of methyltestosterone-treated *O. niloticus* males and all-male hybrids (*O. niloticus* ♀ x *O. hornorum* ♂) were not significantly different to the untreated male *O. niloticus* and all-male hybrids after 180 culture days. Green and Teichert-Coddington (1994) also found that during the grow-out of *O. niloticus*, the growth of methyltestosterone-treated males and control males were not significantly different.

Whilst there is little doubt that, in some conditions, synthetic androgen hormones in the diet can induce faster growth, the observed faster growth of hormone-treated tilapia may not be due to direct effects of the hormone alone. The application of hormones, especially during early stages of development, has two possible effects on the treated fish; the androgenic (ability to alter the phenotypic sex to male during stage of sex differentiation) and anabolic (ability to enhance growth). Both effects have the ability to induce faster growth in the treated fish and it is difficult to determine the relative influence of these effects.

The general objectives of the studies presented in this chapter were to evaluate the effect of a synthetic androgen 17α-methyltestosterone during hormone treatment and post-treatment period, and evaluate the growth of the hormone-treated fish in monosex and mixed-sex culture environments to determine the effect of the presence and absence of females.

Specifically the aims were to;

1. Evaluate the growth of different genotypes: genetically male (XY - GMT), genetically female (XX - GFT), and mixed-sex (XY and XX - MST) progeny, during the period of hormone treatment for the reversal of sex,

2. Evaluate the post-treatment growth of MT-treated GMT, GFT, MST and untreated controls to determine any residual effect of the previously administered hormone,

3. Evaluate the growth of methyltestosterone-treated genetic males (GMT-MT), methyltestosterone-treated genetic females (GFT-MT), methyltestosterone-treated mixed-sex (MST-MT) and untreated males (GMT-CT and MST-CT) as control (CT) in all-male and mixed-sex culture environments,

4. Evaluate the late growth of three female (FEM) genotypes (GFT-FEM, MST-FEM and GMT-FEM) in all-female and mixed-sex culture environments to determine the potential of growing all-female populations.

5.2 Materials and Methods

The effect of 17α-methyltestosterone on the growth of genetically male GMT), genetically female (GFT) and mixed-sex (MST) progeny was determined during a period of hormone treatment. After termination of treatment, the treated and untreated fish were further evaluated to determine the effect of the previously administered hormone on early growth. When the fish approached maturity stage or breeder size, the males and females from each treated and untreated group were separated and the growth was evaluated in monosex and mixed-sex culture environments. The details of the series of growth evaluations that were conducted are summarised in Table 5.1 and are described briefly as follows:

Growth during hormone treatment - The treatment was performed in triplicate cages suspended in two outdoor concrete tanks. The treated and control fish groups were stocked separately in each tank. The treated fish were fed four times a day *ad libitum* with methyltestosterone-treated feed at a concentration of 40 $mg.kg^{-1}$ diet whilst the control fish were fed with the same quality food without hormone. The growth of the fish was monitored by weekly sampling.

Table 5.1. Summary of the different management procedures employed during the conduct of the series of growth evaluation.

Experiment	Genotype	Culture environment	Stocking method	Stocking density	Feeding rate	Sampling period	Culture duration
A	All 6 genotypes	Cage in tank	separate stocking	150.cage^{-1}	ad libitum	Weekly	28 days
B	All 6 genotypes	Cage in pond	separate stocking	85. cage^{-1}	10% biomass	Bi-weekly	126 days
C	All 5 male genotypes	Pond (all-male)	Communal stocking	80.genotype^{-1}	3% biomass	Tri-weekly	126 days
D	All 5 male genotypes	Cage (all-male)	Communal stocking	30.genotype^{-1}	3% biomass	Tri-weekly	189 days
E	All 5 male genotypes	Pond (mixed-sex)	Communal stocking	40.genotype^{-1}	3% biomass	Tri-weekly	126 days
E	GFT-FEM	Pond (mixed-sex)	Communal stocking	86.genotype^{-1}	3% biomass	Tri-weekly	126 days
E	MST-FEM	Pond (mixed-sex)	Communal stocking	86.genotype^{-1}	3% biomass	Tri-weekly	126 days
E	GMT-FEM	Pond (mixed-sex)	Communal stocking	28.genotype^{-1}	3% biomass	Tri-weekly	126 days
F	GFT-FEM	Pond (all-female)	Communal stocking	230.genotype^{-1}	3% biomass	Tri-weekly	126 days
F	MST-FEM	Pond (all-female)	Communal stocking	144.genotype^{-1}	3% biomass	Tri-weekly	126 days
F	GMT-FEM	Pond (all-female)	Communal stocking	28.genotype^{-1}	3% biomass	Tri-weekly	126 days

[A] Evaluation of growth of the treated and untreated fish during hormone treatment using 17α-methyltestosterone

[B] Evaluation of growth of the treated and untreated fish following termination of hormone treatment

[C] Evaluation of growth of the treated and untreated males in all-male culture environment in ponds

[D] Evaluation of growth of the treated and untreated males in all-male culture environment in cages

[E] Evaluation of growth of the treated and untreated males, and untreated females in mixed-sex culture environment in ponds

[F] Evaluation of growth of untreated females in all-female culture environment in ponds

Post-treatment growth - After termination of hormone treatment, the treated and untreated fish were transferred to 1 m^3 fine mesh cages suspended in earthen pond and fed with normal food. When they reached >1 g in size the fish were transferred to 1.5m x 1.5m x 1.0m net cages (mesh #24) suspended in a 200 m^2 earthen pond. The growth was monitored by performing a bi-weekly sampling. The fish were fed twice a day with a commercial brand crumbled feed at a rate of 10% of biomass daily.

Growth of males and females in monosex and mixed-sex culture environments - When the fish approached maturity or breeder size (≈28g), the males and females in each group were manually separated. In one pond the males from five groups (the males from GFT-CT were not included because the proportion of males was very small) were communally stocked in a 200 m^2 earthen pond and in a 3m x 3m x 1.5m net cage (mesh #17). In another similar pond, males from similar groups were also stocked communally with the females from three control groups creating a mixed-sex culture environment. The remaining females were stocked communally in another similar pond as an all-female culture environment. The males and females were marked by clipping either the left or right pelvic and pectoral fins for identification.

During the growth evaluation the fish were fed twice a day with a commercial brand pelleted feed at a rate of 3% biomass daily. The growth was monitored by sampling the fish every three weeks. At the end of the growth evaluation all the fish were counted and weighed. A minimum of 30 fish (or all the fish if the number was small) were randomly picked from each male and

female group and sampled for the estimation of gonadosomatic index, percentage of dress-out weight and percentage of fillet weight.

Statistical analysis - The treatment means were compared using a one-way analysis of variance (ANOVA). For each treatment, there were three mean values corresponding to the three replicates per treatment. All values in percentage form were first arcsine transformed before performing an ANOVA. The comparison of among treatment means was performed using the Tukey's multiple range test. The presence of genotype x culture environment interaction was determined using a two-way ANOVA, where genotypes refer to the different groups of fish tested and culture environments refer to monosex and mixed-sex culture environments. The correlation of two variables was performed using a Bivariate Pearsons correlation analysis.

For the evaluation of males and females in monosex and mixed-sex culture environments, as the culture environment was not replicated due to limited facilities and experimental fish, the weight of individual fish were used as the source of error in the statistical analysis for growth. To correct for the effect of the difference in initial size on final weight, the weight gain of individual fish was determined by subtracting the initial group mean weight from the final weight of individual fish in that group. Similarly, the final weights were corrected following the method described in Chapter four. Both the weight gain and corrected final weight are presented in the tables for comparison, although, to avoid confusion the weight gain will be use mostly in the presentation of data in the text.

5.3 Results

5.3.1 Growth during hormone treatment period

The initial and final data for the 28 days hormone treatment are presented in Table 5.2. The difference between the initial mean weights of the six groups, which ranged from 8.0 to 10.0mg, are just statistically significant (P=0.046), but the comparison among treatment means revealed no significant differences between any two means. The initial mean lengths, which ranged from 8.35 to 9.18mm (total length), are significantly variable (P=0.002).

The growth curves constructed from the weekly sampling data are shown in Figure 5.1. This illustrates that the genetically male tilapia (GMT-MT and GMT-CT) outgrew the other groups from the start until the end of the treatment. In these two groups, the GMT-MT has slightly faster growth but the difference is small and consistent until the end of the treatment, indicating that the difference may be due to the initial difference in size and not due to the anabolic effect of the hormone. The growth curves of the other four groups (GFT-MT, GFT-CT, MST-MT and MST-CT) are similar throughout the treatment period, which confirms further the absence of any detectable anabolic effect of the hormone in the present study.

Table 5.2. Initial and final weights and lengths of hormone-treated fry of three genotypes and their untreated controls held in outdoor concrete tanks. Means were compared by one-way ANOVA. The values in parenthesis represent the estimated standard deviation.

Treatment (genotype)	Initial mean weight (mg)	Initial mean length (mm)	Final mean weight (mg)	Final mean length (mm)	Mean survival (%)
GMT-MT	10.00 ^{a}	9.18 ^{a}	88.06 ^{a} (± 4.61)	17.00 ^{ab} (± 0.38)	64.00 ^{a} (± 4.62)
GMT-CT	9.00 ^{a}	8.96 ^{ab}	83.90 ^{a} (± 1.68)	17.49 ^{b} (± 0.42)	66.00 ^{a} (± 5.81)
GFT-MT	8.00 ^{a}	8.35 ^{c}	55.37 ^{b} (± 5.46)	15.03 ^{c} (± 0.41)	62.22 ^{a} (± 21.55)
GFT-CT	8.00 ^{a}	8.51 ^{bcd}	56.13 ^{b} (± 4.07)	15.51 ^{ac} (± 0.34)	83.33 ^{a} (± 3.01)
MST-MT	10.00 ^{a}	8.85 ^{ac}	57.54 ^{b} (± 2.32)	15.10 ^{c} (± 0.99)	71.78 ^{a} (± 6.05)
MST-CT	9.00 ^{a}	9.03 ^{ad}	54.66 ^{b} (± 2.09)	14.88 ^{c} (± 0.22)	85.33 ^{a} (± 4.16)
Overall Probability	P=0.046	P=0.002	P<0.001	P<0.001	P=0.038

Means within a column with different letter subscripts are significantly different (P<0.05)

The data on final weight and length are also presented in Table 5.2 and summarised graphically in Figure 5.2. The final weights and final lengths of the six groups are both significantly variable (both at $P<0.001$). The final lengths, which ranged from 14.88 to 17.49mm, were above the upper limit for length at the estimated stage of sexual differentiation in *O. niloticus*, 9 to 12mm at 30 to 33 days posthatching (Alvendia-Casauay and Cariño, 1988). It was assumed that sex had already differentiated after the 28 days hormone treatment (approximately 35 days posthatching). The comparison among the final weights shows that the GMT-MT and GMT-CT are not significantly different from each other but are significantly different from the other groups ($P<0.05$), while the other four groups do not differ significantly from each other. The trend for the comparison among the final mean lengths was similar to that for final mean weights.

The mean percentage survival of the six groups is significantly variable ($P=0.038$) but the comparison of means shows no differences between the individual means. Numerically, the mean percentages of survival for all the methyltestosterone-treated groups are lower than their respective controls.

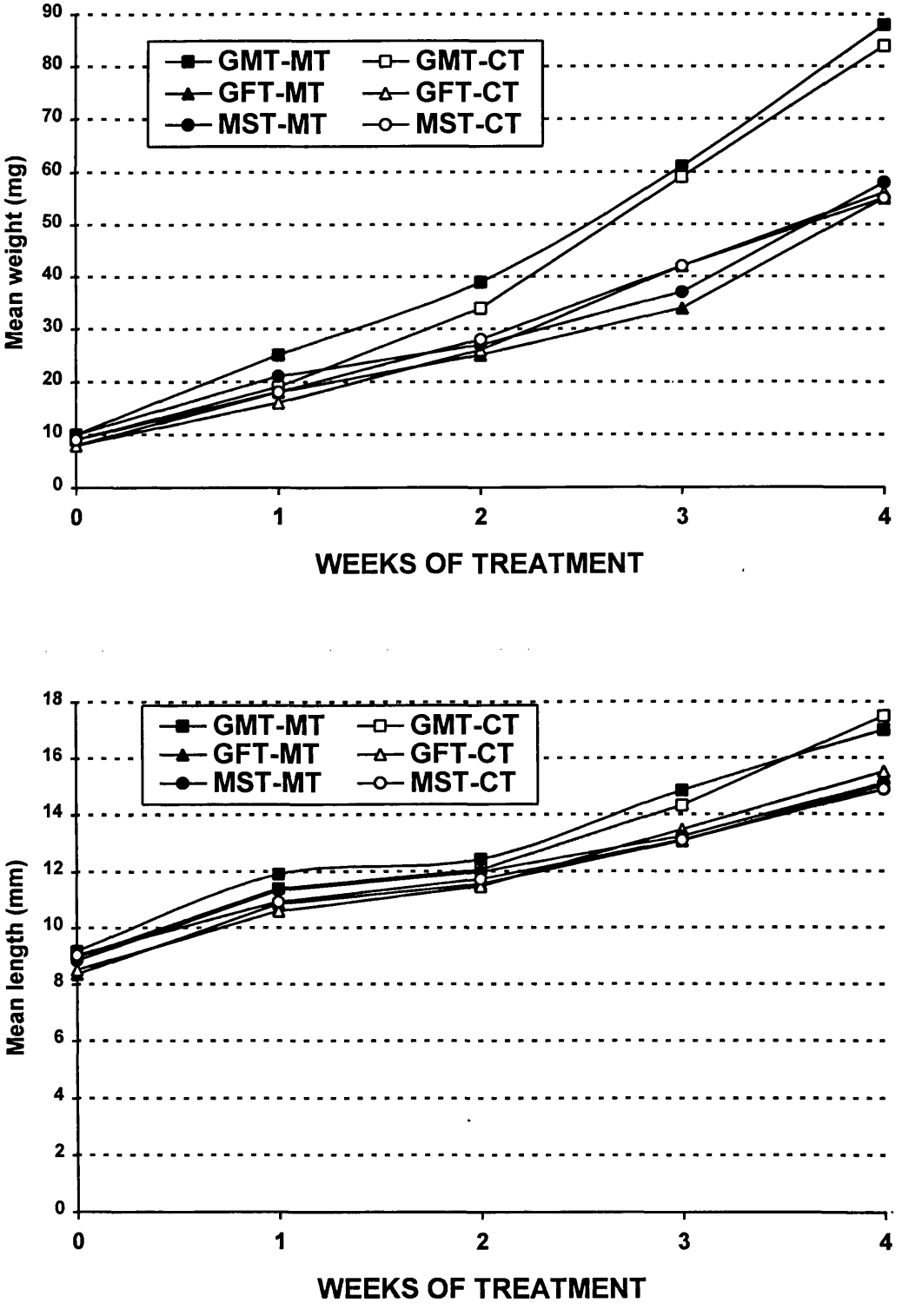


Figure 5.1. Growth curves for the mean weights and lengths of three replicates of the MT-treated and control GMT, GFT, and MST during the 28 day hormone treatment period.

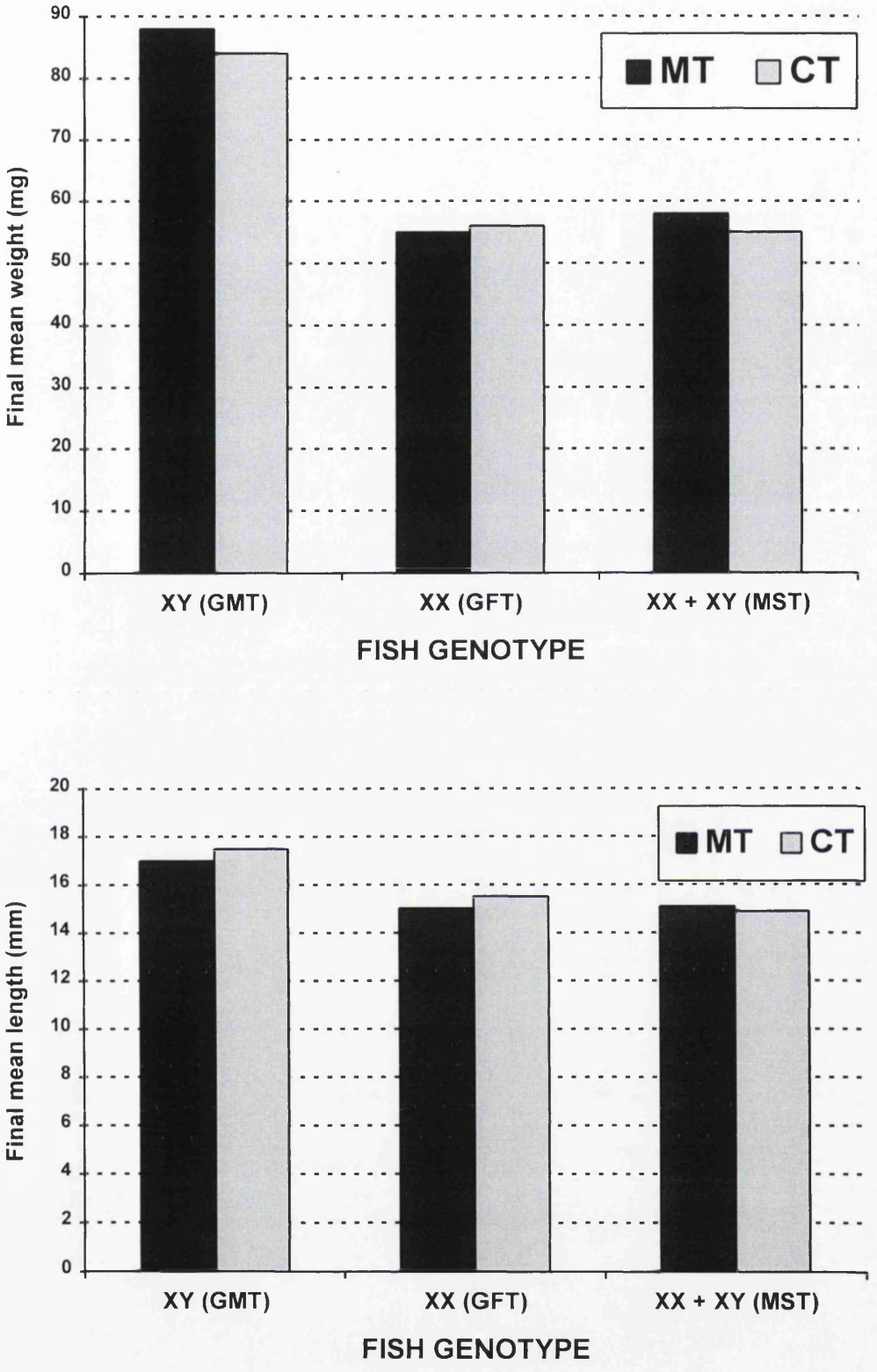


Figure 5.2. Histograms showing the final mean weights and lengths of three replicates of the methyltestosterone-treated and untreated control GMT, GFT and MST after 28 days of hormone treatment.

5.3.2 Growth during post-treatment period

The results of the evaluation for post-treatment growth are presented in Table 5.3. The initial mean weights, which ranged from 0.85 to 1.65g, are significantly variable (P<0.001). The significant difference is due to the lower mean weight of GFT-CT and MST-CT. The coefficients of variation (CV) for initial weights are not statistically different for all groups.

Table 5.3. Summary of the results of growth evaluation of three methyltestosterone-treated genotypes and their untreated controls after 126 culture days in replicated 1.5m x 1.5m x 1.0m net cages suspended in a 200 m^2 earthen pond. Means were compared by one-way ANOVA.

Treatment (genotype)	Initial mean weight (g)	C.V. for initial weight	Mean gain in weight (g)	Mean gain in weight (g) (males only)	C.V. for final weight	Mean % males	Mean survival (%)
GMT-MT	1.65 ^{a}	40.74 ^{a}	30.54 ^{a}	30.54 ^{a}	42.44 ^{a}	100.0 ^{a}	88.23 ^{ab}
GMT-CT	1.37 ^{ab}	45.18 ^{a}	29.15 ^{ab}	30.93 ^{a}	37.42 ^{ab}	86.27 ^{b}	93.33 ^{a}
GFT-MT	1.26 ^{b}	40.70 ^{a}	29.32 ^{ab}	29.71 ^{a}	41.33 ^{a}	95.49 ^{ab}	85.49 ^{ab}
GFT-CT	0.90 ^{c}	31.12 ^{a}	23.05 ^{b}	27.07 ^{a}	26.55 ^{c}	8.21 ^{c}	89.02 ^{ab}
MST-MT	1.14 ^{bc}	37.95 ^{a}	25.86 ^{ab}	27.02 ^{a}	44.19 ^{a}	91.93 ^{ab}	90.98 ^{ab}
MST-CT	0.85 ^{c}	34.81 ^{a}	24.44 ^{ab}	27.21 ^{a}	8.73 ^{bc}	25.97 ^{c}	83.53 ^{b}
Overall probability	P<0.001	P=0.160	P=0.019	P=0.569	P<0.001	P<0.001	P=0.049

Means within a column with different letter subscripts are significantly different (P<0.05)

As there were significant differences in initial mean weights, the mean gain in weight of the fish in each group was used in the analysis of final data. The weight gained by the six groups after 126 culture days was relatively low despite provision of sufficient food (10% biomass). The cage culture environment used for this growth evaluation was different from that of commercial grow-out where the cages would be larger, installed in wider and deeper bodies of water and stocked fish at a lower density. As McGinty (1991) observed, the rate of weight gain in caged fish tends to increase with the cage size.

The mean weight gains for the methyltestosterone-treated and control groups, which ranged from 23.05 to 30.54g, are significantly variable (P=0.019). The comparison among group means shows that the GMT-MT has the highest gain in weight and GFT-CT the lowest and that these are significantly different from each other but not from any of the other groups (see Figure 5.3). The lower gain in weight of GFT-CT and MST-CT could be due to the low proportion of males; 8.21 and 23.97% respectively, although the gain in weight for MST-MT was also low despite having a high percentage of males (91.93%). The ANOVA result on weight gain data for males only (ranged 27.2-30.93g) was not significant. In addition, the mean weight gain of GMT-MT is lower than that of the GMT-CT (see Figure 5.3). This result indicates that the growth of the of hormone treated males, at least up to this stage, was not positively affected by the preceding hormone treatment. In contrast, when ANOVA was performed on weight gain data for females only (data not shown in table), the mean gain in

weights of the females in methyltestosterone-treated groups was lower than the females in the control; GFT-MT (19.67g) < GFT-CT (22.84g) and MST-MT (13.83g) < MST-CT (23.41g), the latter being significantly different ($P<0.05$).

The mean CVs for final weights are significantly variable ($P<0.001$). In Table 5.3, it can be noticed that the CV for final weight of the methyltestosterone-treated groups had increased relative to their CV for initial weight whilst that of the controls had decreased. This result indicates that the size of the methyltestosterone-treated fish was more variable than the control despite being mostly males.

The mean percentage survival of the six groups is just significantly variable ($P=0.049$) and the only significant difference is that between the GMT-CT (93.3%) and the MST-CT (83.5%). Overall, there was little difference between the overall survival of the MT-treated fish (88.23%) and the untreated controls (88.62%).

The hormone treatment successfully induced a high percentage of males although not 100%. There was a small proportion of unexpected females in GMT-CT (13.73%) and males in GFT-CT (8.21%), while the proportion of males in MST-CT was surprisingly low (25.97%). It may be possible that the male parents taken from the broodstock of the GMIT project were contaminated with XX males resulting in a higher proportion of females in the progeny which was significantly different from 1:1 ratio ($\chi^2= 23.09$, $P>0.001$).

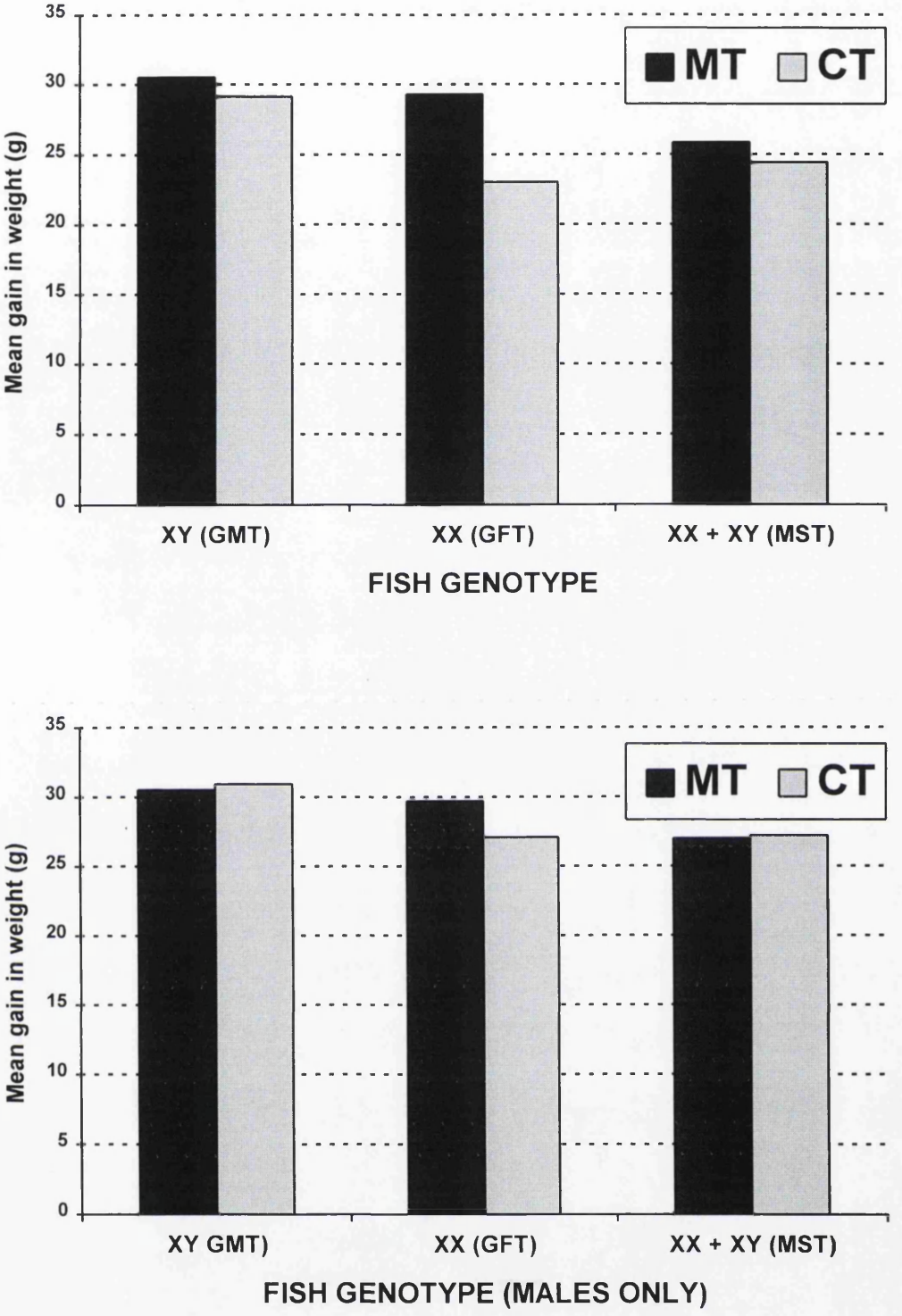


Figure 5.3. Histograms showing separately the mean gain in weight (for all fish and for males only) of the methyltestosterone-treated and untreated control GMT, GFT and MST reared in 1.5m x 1.5m x 1.0m net cages suspended in a 200 m^2 earthen pond, for 126 days.

5.3.3 Growth of males in all-male and mixed-sex culture environments

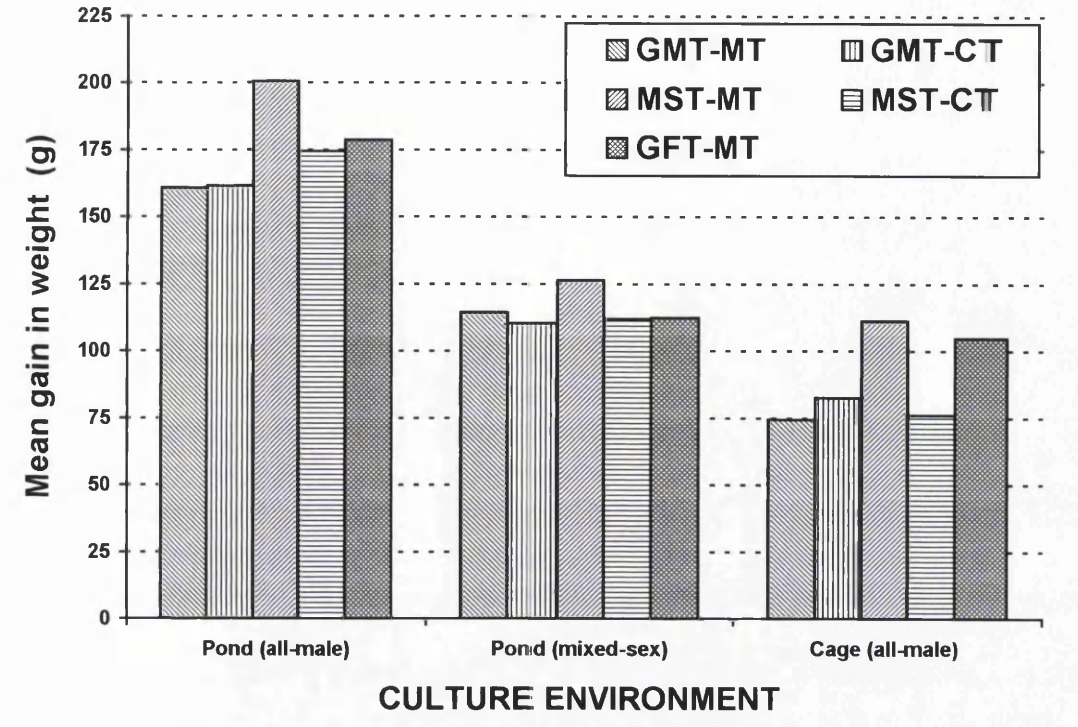
The results of the growth evaluation of hormone-treated males and untreated normal males in an all-male culture environment in pond and cage, and a mixed-sex culture environment in pond are presented in Table 5.4. The initial weights among the methyltestosterone-treated and control genotypes in all the culture environments are significantly different ($P<0.001$). Part of the reason for the difference in initial weight is the low proportion of males in MST-CT (25.97%) which required the addition of normal males from a back up stock to complete the required number of fish for stocking. The added fish were bigger than the original stock resulting in a higher initial mean weight.

The ANOVA analysis for the gain in weight and corrected final weight for the five groups gave all significant results in all cases (Table 5.4). In all the culture environments, the MST-MT showed the highest growth followed by GFT-MT, except for the corrected weight in all-male culture environment in cage where the GFT-MT had the highest growth followed by MST-MT (see Figure 5.4). The results appear to indicate that sex-reversed genetic females (which constituted a great proportion of the males in GFT-MT and MST-MT) would grow faster than genetic males (GMT-MT, GFT-CT, MST-CT) during the late stages in life cycle. This contrasts with the observation that MST-MT had the lowest growth (males only) during the early life stages (evaluation of post-treatment growth).

Table 5.4. Summary of the results of growth evaluation of three hormone-treated genotypes and their untreated controls in all-male and mixed-sex culture environments in ponds for a period of 126 days, and an all-male culture environment in cages for 189 days. The means within a culture environment were compared by a one-way ANOVA. The values in parenthesis represent the standard deviation of the actual weight of individual fish.

Treatment (genotype)	Culture environment	Initial mean weight (g)	Mean gain in weight (g)	Corrected final mean weight (g)	Mean survival (%)
GMT-MT	Pond (all-male)	44.93 ^{ab} (± 31.21)	160.82 ^{a} (± 23.79)	220.15 ^{a}	100.0
GMT-CT	Pond (all-male)	42.91 ^{ab} (± 25.28)	161.63 ^{a} (± 17.36)	231.13 ^{a}	91.25
GFT-MT	Pond (all-male)	46.26 ^{b} (± 17.88)	178.69 ^{a} (± 16.96)	233.95 ^{a}	100.0
MST-MT	Pond (all-male)	38.47 ^{a} (± 31.55)	200.65 ^{b} (± 22.50)	301.30 ^{b}	90.0
MST-CT	Pond (all-male)	68.89 ^{c} (± 27.97)	174.59 ^{a} (± 15.58)	170.43 ^{a}	90.0
Mean		48.29	175.28	231.39	94.25
Probability		P<0.001	P<0.001	P<0.001	
GMT-MT	Pond (Mixed sex)	39.30 ^{a} (± 42.08)	114.45 ^{ab} (± 16.94)	161.44 ^{a}	100.0
GMT-CT	Pond (Mixed sex)	40.35 ^{a} (± 29.48)	110.45 ^{b} (± 15.09)	153.82 ^{a}	95.0
GFT-MT	Pond (Mixed sex)	37.49 ^{a} (± 38.57)	112.52 ^{ab} (± 15.14)	165.01 ^{a}	100.0
MST-MT	Pond (Mixed sex)	36.71 ^{a} (± 33.93)	126.27 ^{a} (± 11.09)	182.53 ^{b}	90.0
MST-CT	Pond (Mixed sex)	52.52 ^{b} (± 36.93)	111.91 ^{ab} (± 13.93)	129.91 ^{c}	85.0
Mean		41.27	115.12	158.54	94.0
Probability		P<0.001	P=0.027	P<0.001	
GMT-MT	Cage (all-male)	39.03 ^{a} (± 15.19)	74.47 ^{a} (± 18.93)	139.61 ^{ac}	90.0
GMT-CT	Cage (all-male)	47.19 ^{bc} (± 9.25)	82.68 ^{a} (± 13.20)	132.47 ^{ab}	100.0
GFT-MT	Cage (all-male)	45.98 ^{c} (± 17.39)	104.83 ^{b} (± 13.92)	158.35 ^{c}	90.0
MST-MT	Cage (all-male)	53.57 ^{bd} (± 17.02)	111.19 ^{b} (± 19.88)	148.29 ^{ac}	100.0
MST-CT	Cage (all-male)	54.79 ^{d} (± 25.58)	76.17 ^{a} (± 24.96)	115.25 ^{b}	96.66
Mean		48.11	89.87	138.79	95.33
Probability		P<0.001	P<0.001	P<0.001	

Means in column within a culture environment with different letter subscripts are significantly different (P<0.05)



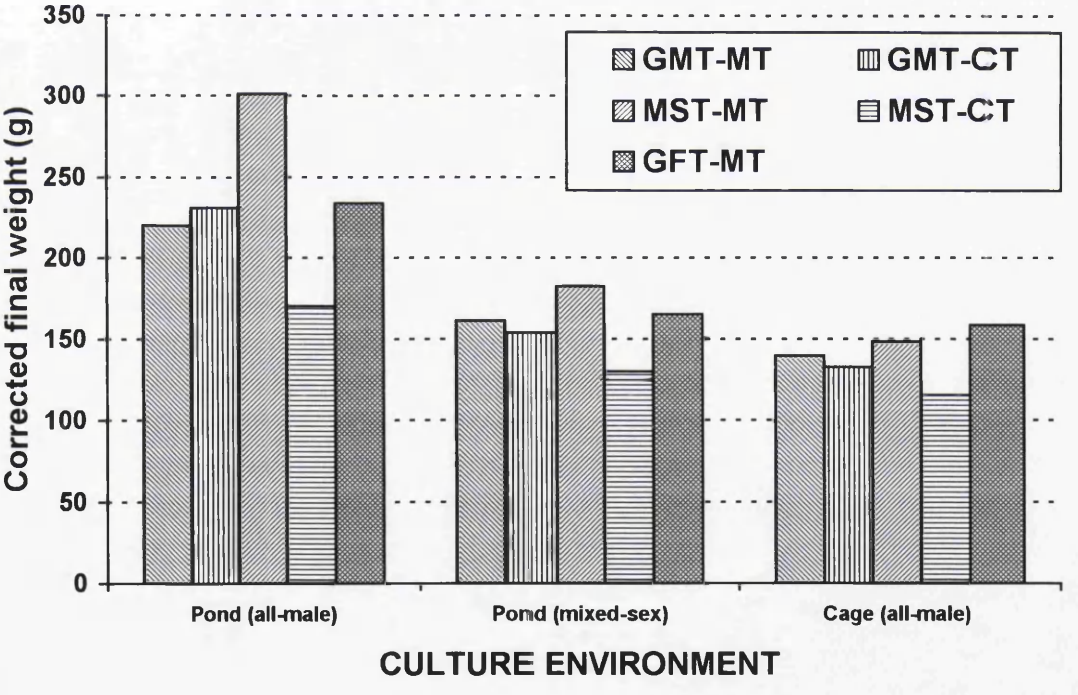


Figure 5.4. Histograms comparing the mean weight gains and corrected final mean weights of the methyltestosterone-treated and untreated control genotypes.

There is a significant genotype x culture environment interaction, both for the weight gain and corrected final weight ($P=0.038$ & $P<0.001$, respectively). The significant interaction for gain in weight could be associated with the change in ranking between the GMT-MT, GMT-CT and MST-CT (Figure 5.4). It appear that the presence and absence of females have affected the growth performance of the five male groups. Examining the mean gain in weights of the five groups in each environment, the mean values in all-male in pond (175.28 ±16.21) and in cage (89.87 ±16.99) are more variable than the mean values in mixed-sex in pond (115.12 ±6.40) as indicated by the higher standard deviation values. Similarly, the mean growth of males in an all-male culture environment in pond is higher by 52.26% (weight gain) and 45.95% (corrected final weight) compared to the males in mixed-sex culture environment.

The data for the gonadosomatic index (GSI) and percentages of dress-out and fillet weight of males are presented in Table 5.5. The mean GSI of the five groups in the all-male culture environment in pond are significantly variable ($P<0.001$) but the mean GSIs in mixed-sex in pond and all-male in cage culture environments are not statistically different. In the all-male in pond treatment, MST-MT has a significantly ($P<0.05$) lower GSI compared to the other groups. A significant genotype x culture environment interaction for GSI ($P=0.004$) is detected which indicates that the relative ranking of the five groups differs between culture environment (see Figure 5.5). The mean GSI of the three culture environments are significant different ($P<0.001$), with the mixed-sex in ponds having the highest mean value (0.53) followed by all-male in pond (0.40)

and all-male in cages (0.22). The relatively higher mean GSI of the fish in mixed-sex environment compared to those in all-male culture environment suggests that the presence of females might induce higher GSI in the males.

For the percentage dress-out weight, the trend is similar to that of the GSI. The mean values of the five groups in all-male in pond were significantly variable ($P=0.005$) but did not differ significantly in mixed-sex in pond and all-male in cage culture environments. A significant genotype x culture environment for dress-out weight ($P=0.011$) is also detected (see Figure 5.5). The mean values for the three culture environments are significantly different ($P<0.001$) with the all-male in pond having the highest (82.53%) followed by mixed-sex in pond (81.86%) and all-male in cage (79.59). The results of a correlation analysis shows the percentage dress-out weight is significantly negatively correlated with GSI ($n = 438$, $r=-0.190$, $P<0.001$).

The mean percentage fillet weights of the five groups in all-male environment and in pond and in cage are significantly variable ($P=0.002$ and $P<0.001$, respectively) but not significantly different in mixed-sex culture environment in pond. MST-MT had the highest fillet weight value in both all-male culture environments. A significant genotype x culture environment interaction ($P<0.001$) for fillet weight is also detected (see Figure 5.5). A correlation analysis shows that the percentage fillet weight is also significantly negatively correlated with GSI ($n=437$, $r=-0.341$, $P<0.001$). This indicates that those fish with a higher GSI tend to have a lower percentage dress-out and fillet weight (Figure 5.6).

Table 5.5. Mean gonadosomatic index (GSI) and percentages of dress-out and fillet weight of males in three hormone-treated genotypes and untreated controls grown in all-male and mixed-sex in ponds and all-male in cage culture environments. Means within a culture environment were compared by one-way ANOVA. The values in parenthesis represent standard deviation.

Treatment (genotype)	Culture environment	Mean GSI	Mean dress-out weight (%)	Mean fillet weight (%)
GMT-MT	Pond (all-male)	0.41 ^{a} (± 0.37)	82.57 ^{ab} (± 1.82)	34.25 ^{a} (± 2.45)
GMT-CT	Pond (all-male)	0.47 ^{a} (± 0.41)	82.86 ^{b} (± 1.36)	33.82 ^{a} (± 2.61)
GFT-MT	Pond (all-male)	0.57 ^{a} (± 0.42)	81.47 ^{a} (± 2.02)	33.94 ^{a} (± 3.16)
MST-MT	Pond (all-male)	0.16 ^{b} (± 0.13)	82.75 ^{b} (± 1.41)	36.19 ^{b} (± 1.81)
MST-CT	Pond (all-male)	0.41 ^{a} (± 0.33)	82.98 ^{b} (± 1.94)	34.77 ^{ab} (± 2.29)
Mean		0.40	82.53	34.59
Probability		P<0.001	P=0.005	P=0.002
GMT-MT	Pond (Mixed sex)	0.57 ^{a} (± 0.48)	82.02 ^{a} (± 1.67)	30.82 ^{a} (± 2.16)
GMT-CT	Pond (Mixed sex)	0.51 ^{a} (± 0.44)	82.00 ^{a} (± 2.11)	31.42 ^{a} (± 1.97)
GFT-MT	Pond (Mixed sex)	0.42 ^{a} (± 0.35)	81.96 ^{a} (± 1.46)	31.31 ^{a} (± 2.65)
MST-MT	Pond (Mixed sex)	0.53 ^{a} (± 0.41)	82.04 ^{a} (± 2.15)	31.16 ^{a} (± 1.79)
MST-CT	Pond (Mixed sex)	0.61 ^{a} (± 0.46)	81.27 ^{a} (± 2.00)	31.02 ^{a} (± 2.69)
Mean		0.53	81.86	31.15
Probability		P=0.510	P=0.478	P=0.865
GMT-MT	Cage (all-male)	0.19 ^{a} (± 0.15)	79.43 ^{a} (± 1.08)	30.79 ^{ac} (± 1.66)
GMT-CT	Cage (all-male)	0.20 ^{a} (± 0.13)	79.45 ^{a} (± 1.14)	31.29 ^{ab} (± 1.63)
GFT-MT	Cage (all-male)	0.25 ^{a} (± 0.15)	79.35 ^{a} (± 1.50)	30.76 ^{ac} (± 1.56)
MST-MT	Cage (all-male)	0.20 ^{a} (± 0.12)	79.43 ^{a} (± 1.41)	32.95 ^{b} (± 2.17)
MST-CT	Cage (all-male)	0.26 ^{a} (± 0.20)	80.28 ^{a} (± 1.29)	29.23 c (± 4.39)
Mean		0.22	79.59	30.60
Probability		P=0.301	P=0.054	P<0.001

Means in column within a culture environment with different letter subscripts are significantly different (P<0.05)

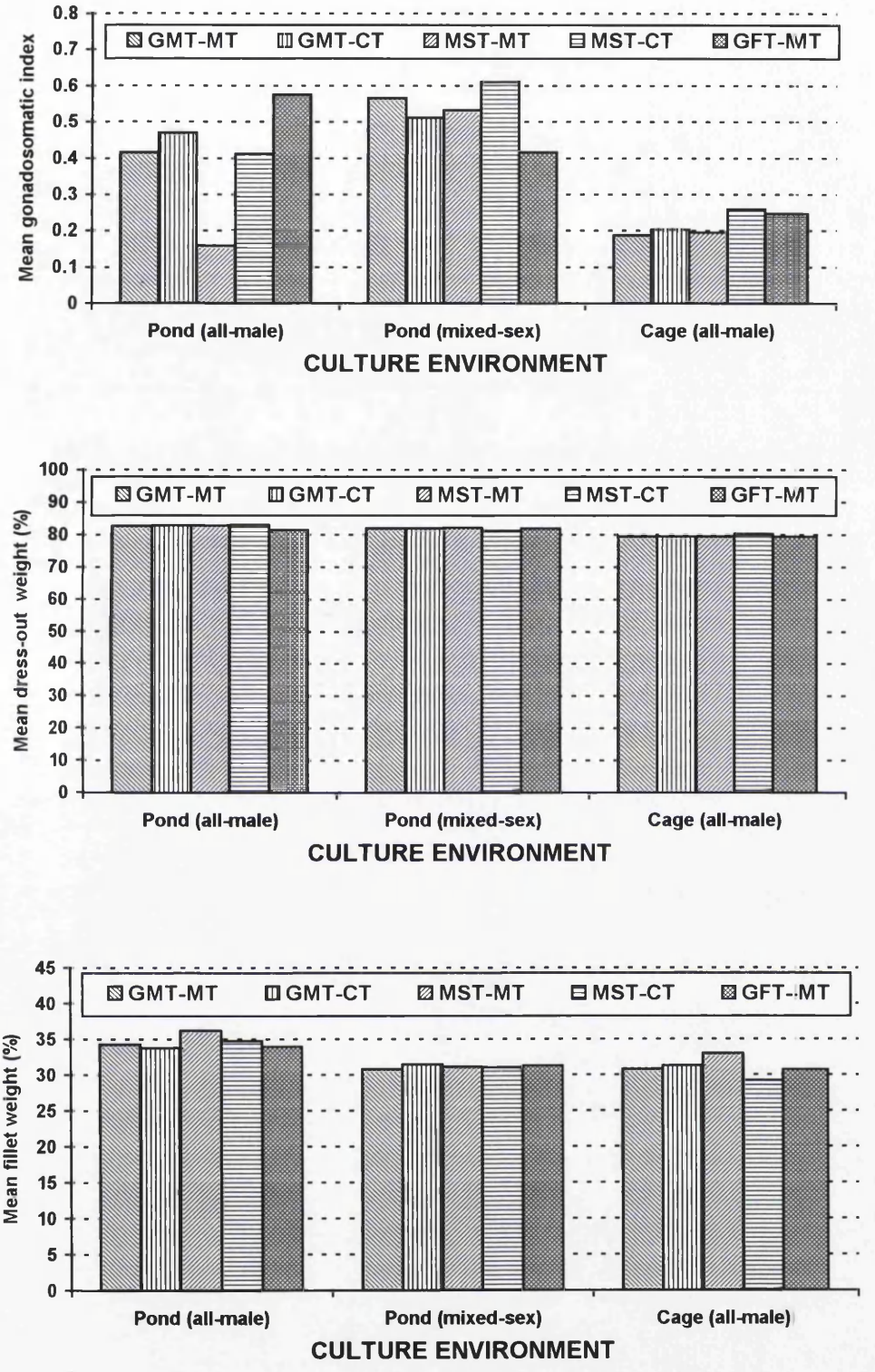


Figure 5.5. Histograms showing the mean gonadosomatic index, percentage of dress-out weight and percentage of fillet weight of the five male genotypes in all-male and mixed-sex culture environments in pond and all-male culture environment in cage.

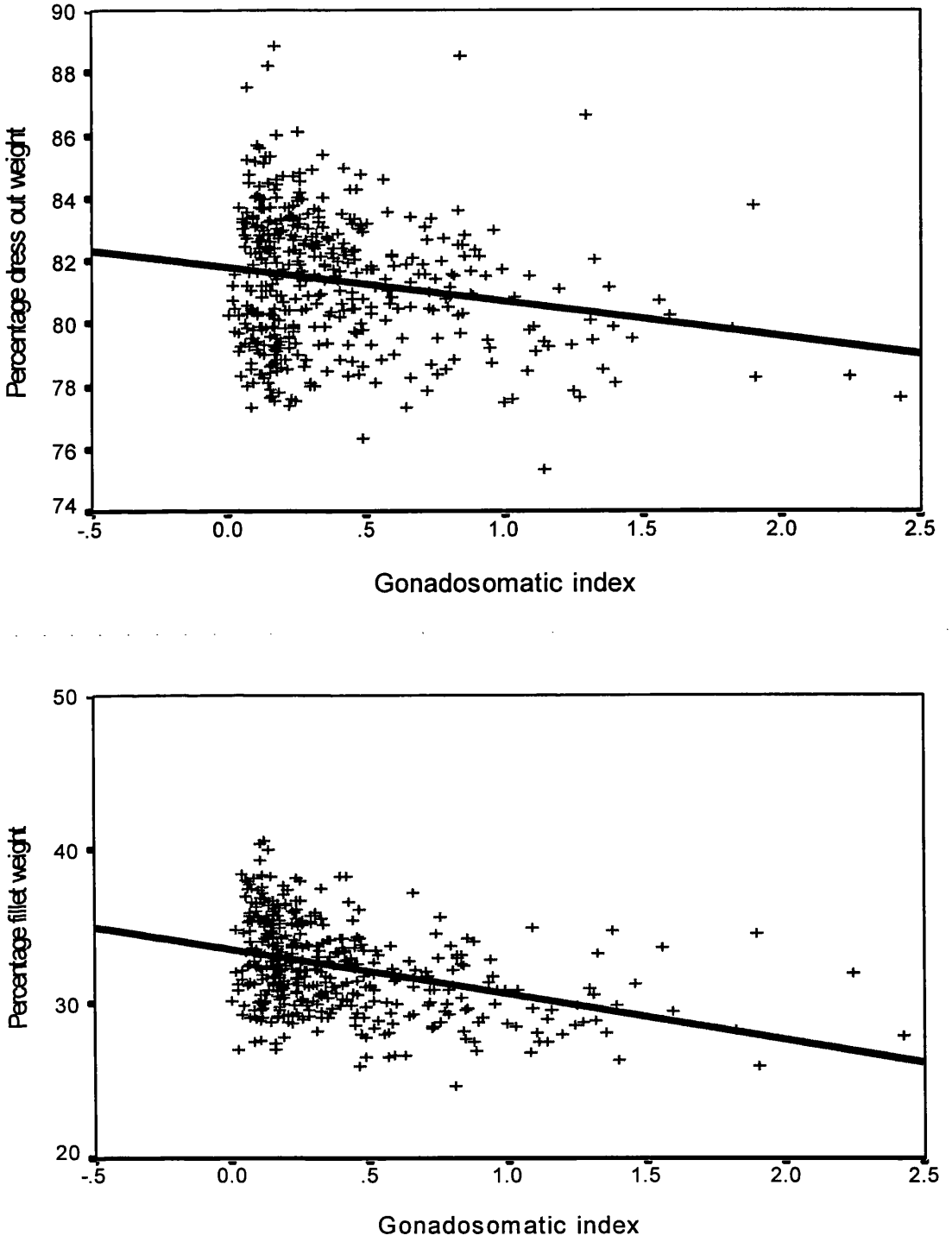


Figure 5.6. Scatter diagrams showing the plotted percentage of dress-out weight (upper figure), percentage of fillet weight (lower figure) and gonadosomatic index of males reared in all-male and mixed-sex culture environments.

5.3.4 Growth of females in all-female and mixed-sex culture environments

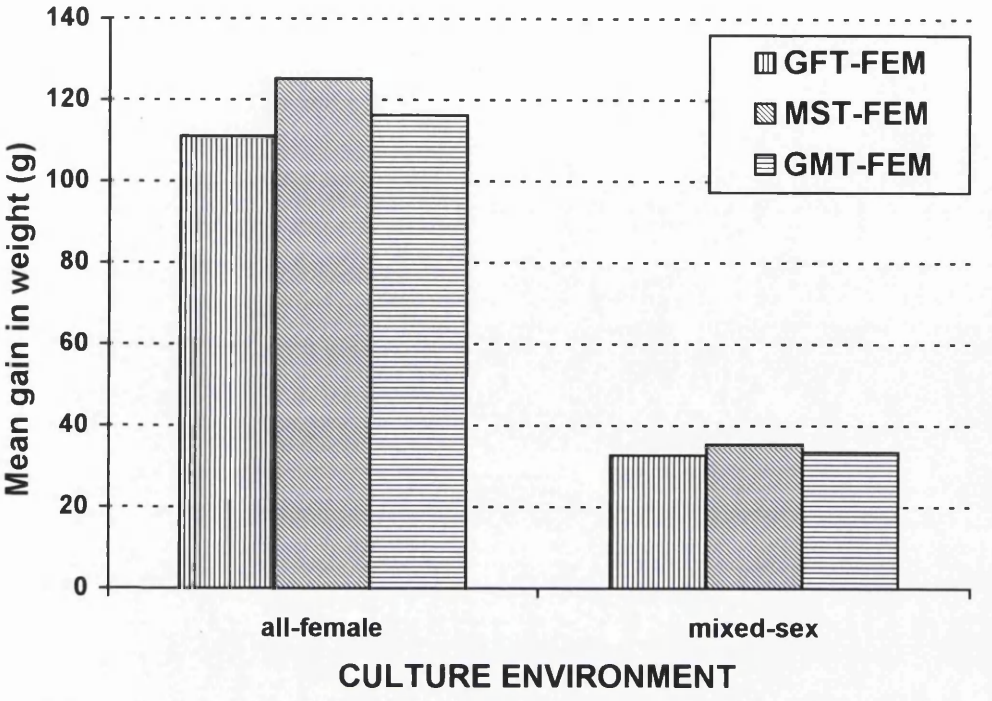
The results of the growth evaluation of three female genotypes in all-female and mixed-sex culture environments are presented in Table 5.6. As with the growth evaluation of males the initial weights in both culture environments are also significantly variable (P=0.008 & P<0.001, respectively). The weight gains and corrected final weights were calculated and presented following the method used in the growth evaluation of males.

The mean weight gains for the three female genotypes in all-female culture environment are significantly variable (P=0.003) but do not differ significantly in mixed-sex environment. The MST-FEM has the highest gain in weight in both culture environments. No significant genotype x culture environment interaction for gain in weight is detected (see Figure 5.7). The difference between the overall performance of the females in the two culture environments illustrates the potential of growing only females. Compared to the mean growth in mixed-sex environment, the females in an all-female culture environment grew bigger by an average of 246.75% (gain in weight) and 129.46% (corrected weight).

Table 5.6. Summary of the results of growth evaluation of three female genotypes cultured for 126 days in all-female and mixed-sex culture environments in ponds. The means within a culture environment were compared by one-way ANOVA. The values in parenthesis represent standard deviation of the actual weight of individual fish.

Treatment (genotype)	Culture environment	Initial mean weight (g)	Mean gain in weight (g)	Corrected mean final weight (g)	Mean survival (%)
GFT-FEM	All-female	29.57 ^{a} (± 31.10)	111.01 ^{a} (± 22.83)	151.83 ^{a}	97.39
MST-FEM	All-female	33.97 ^{b} (± 30.74)	125.17 ^{b} (± 24.33)	149.59 ^{a}	72.22
GMT-FEM	All-female	32.73 ^{ab} (± 32.19)	116.27 ^{ab} (± 26.10)	146.02 ^{a}	89.28
Mean		32.09	117.48	149.15	86.30
Probability		P=0.008	P=0.003	P=0.685	
GFT-FEM	Mixed-sex	27.06 ^{a} (± 30.36)	32.72 ^{a} (± 26.47)	68.74 ^{a}	89.53
MST-FEM	Mixed-sex	35.36 ^{b} (± 35.41)	35.46 ^{a} (± 30.80)	61.25 ^{a}	72.09
GMT-FEM	Mixed-sex	30.91 ^{ab} (± 25.82)	33.47 ^{a} (± 30.56)	65.03 ^{a}	85.71
Mean		31.11	33.88	65.01	82.44
Probability		P<0.001	P=0.687	P=0.070	

Means in column within a culture environment with different letter subscripts are significantly different (P<0.05)



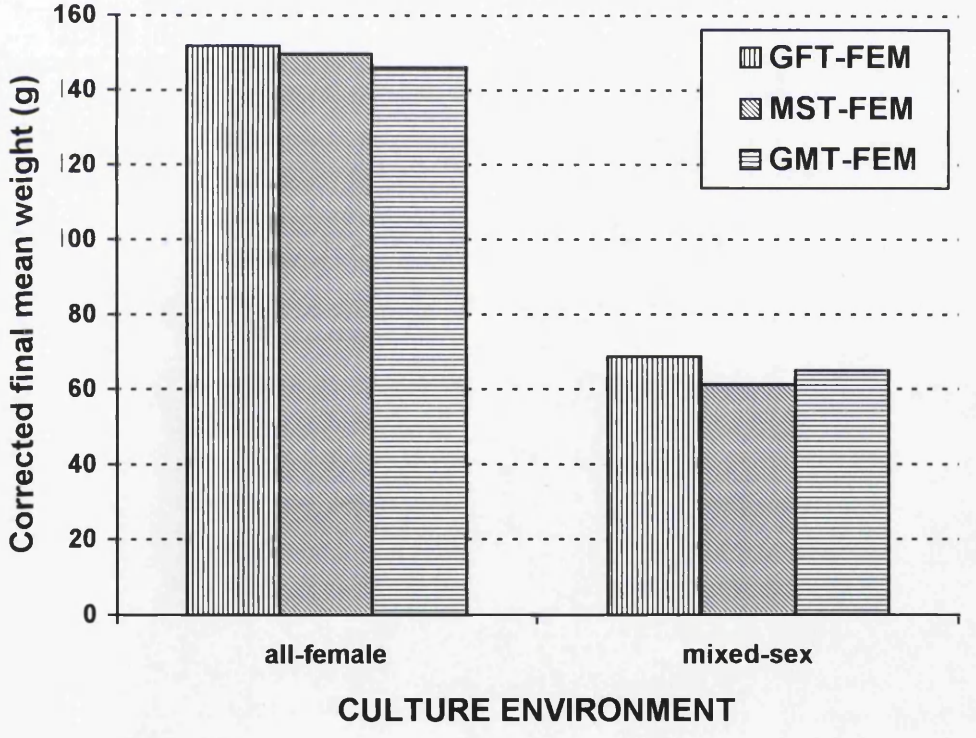


Figure 5.7. Histograms showing the mean gain in weights and corrected final weights of the three female genotypes in all-female and mixed-sex culture environments in ponds.

The data on the gonadosomatic index (GSI) and percentages of dress-out and fillet weight of three female genotypes in all-female and mixed-sex culture environments are presented in Table 5.7. The mean GSIs of the three groups within culture environment are not significantly variable. A significant genotype x culture environment interaction ($P<0.001$) is detected indicating that the relative ranking of the three female genotypes differed between the two culture environments (Figure 5.8). The mean GSI in all-female environment (3.09) is higher than that in the mixed-sex culture environment (2.37). Although the difference is not significant, the higher mean GSI in the all female environment could be partly associated with the absence of males. It was observed that the gonads of the females in the all-female environment were mostly composed of atretic eggs. Probably the absence of the males caused them to cease spawning resulting in retention of eggs in the gonad leading to higher GSI values.

The mean dress-out weights of the three female genotypes in the all-female environment are not significantly different but differed significantly in the mixed-sex culture environment ($P=0.026$). A significant genotype x culture environment for dress-out weight ($P=0.002$) is also detected (see Figure 5.8). The result of a correlation analysis show the dress-out weight is significantly negatively correlated with GSI ($n=169$, $r=-0.365$, $P<0.001$).

The mean fillet weights of the three female genotypes in the all-female environment are significantly variable ($P=0.001$) but do not differ significantly in the mixed-sex culture environment. A significant genotype x culture environment

interaction for fillet weight (P=0.027) is also detected (Figure 5.8). Overall, fillet weight is uncorrelated with GSI (n=169, r=0.004, P=0.957). When the analysis was performed only in an all-female environment there is a significant negative correlation with GSI (n=86, r=-0.271, P=0.012). As with the males, those females with a higher GSI also tend to have a lower percentage of dress out and fillet weight (Figure 5.9).

Table 5.7. Summary of gonadosomatic index and percentage of dress-out and fillet weights of three female genotypes in all-female and mixed-sex culture environments. The means within a culture environment were compared by one-way ANOVA. The values in parenthesis represent standard deviation (± sd).

Treatment (genotype)	Culture environment	Mean GSI	Mean dress-out weight (%)	Mean fillet weight (%)
GFT-FEM	All-female	3.09 ^{a} (± 1.54)	78.89 ^{a} (± 1.78)	30.02 ^{a} (± 1.91)
MST-FEM	All-female	2.64 ^{a} (± 1.64)	79.62 ^{a} (± 1.47)	31.79 ^{b} (± 2.04)
GMT-FEM	All-female	3.56 ^{a} (± 2.05)	79.99 ^{a} (± 2.15)	30.52 ^{a} (± 1.58)
Mean		3.09	79.50	30.78
Probability		P=0.152	P=0.070	P=0.001
GFT-FEM	Mixed-sex	1.95 ^{a} (± 1.79)	78.62 ^{a} (± 2.35)	27.32 ^{a} (± 1.95)
MST-FEM	Mixed-sex	2.49 ^{a} (± 2.14)	77.33 ^{ab} (± 1.99)	26.80 ^{a} (± 3.23)
GMT-FEM	Mixed-sex	2.68 ^{a} (± 1.83)	77.00 ^{b} (± 2.57)	26.28 ^{a} (± 2.89)
Mean		2.37	77.65	26.80
Probability		P=0.355	P=0.026	P=0.389

Means in column within a culture environment with different letter subscripts are significantly different (P<0.05)

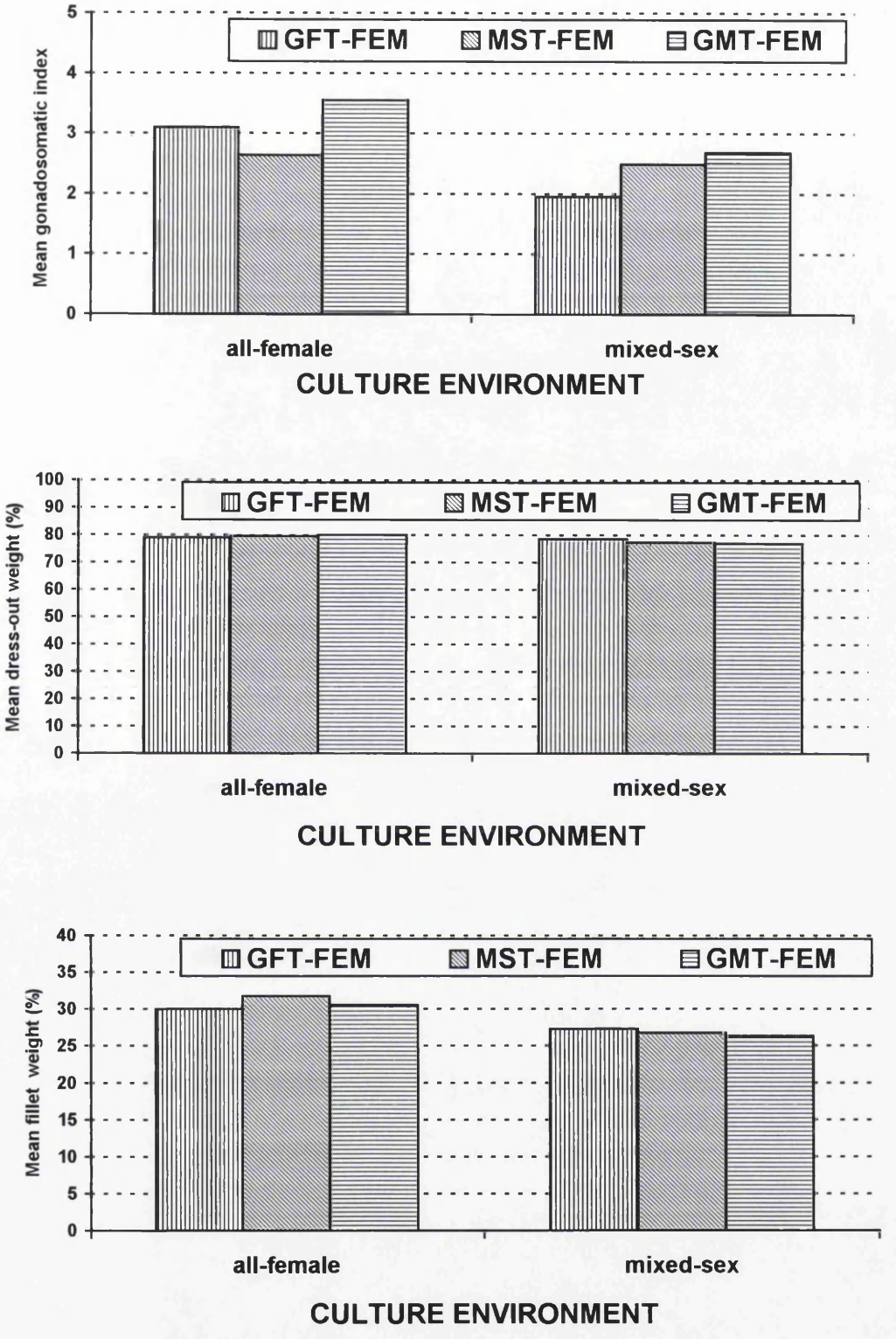


Figure 5.8. Histograms showing the mean gonadosomatic index and percentages of dress-out and fillet weight of three females genotypes grown in all-female and mixed-sex culture environments in ponds.

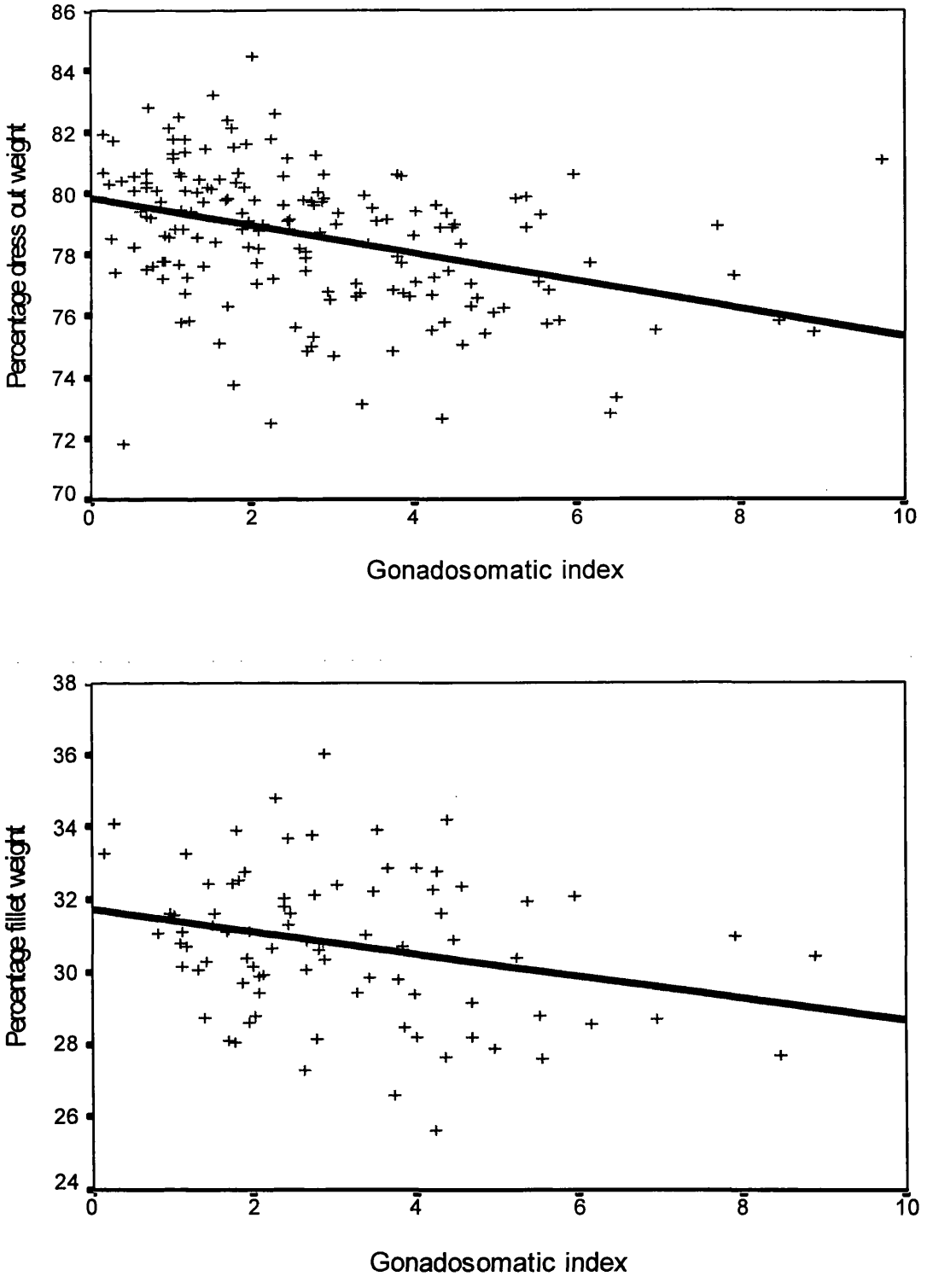


Figure 5.9. Scatter diagrams showing the plotted percentage dress out (upper figure), percentage fillet weight (all-female only - lower figure) and gonadosomatic index of females in all-female and mixed-sex culture environments.

5.4 Discussion

5.4.1 Growth during hormone treatment

The final results of the 28 days hormone treatment trial show that there was no significant difference in growth between the methyltestosterone-treated and untreated control fry. The lack of significant differences in the final growth indicates the absence of anabolic effect of the hormone used. This result supports the earlier observations on sex reversal of *O. spilurus* (Ridha and Lone, 1990) and *O. niloticus* (Phelps *et al.*, 1992; Green and Teichert-Coddington, 1994). These authors did not find a significant positive effect of 17α-methyltestosterone on growth during a period of hormone treatment to reverse the phenotypic sex.

The faster growth of GMT-MT and GMT-CT could be due to their being genetic males, although the physiological mechanism whereby genetic males would grow faster than the sex-reversed genetic females is not well understood. The result agree with the suggestion of Lester *et al.* (1989) that probably the higher the genetic tendency to become male the earlier the stimulation of male growth occurs. It may be possible that the faster growth could be due to the higher concentration of endogenous androgen in genetic males (Mol *et al.* (1994) even at an early age (Rothbard *et al.*, 1987). Johnstone *et al.* (1979) remarked that the acceleration of growth in male brook trout during their first year and in male rainbow trout during their second year, compared to females, is the result of

the anabolic activity of the increased concentration of circulating endogenous androgenic hormones in the males. Similarly the increase in androgen concentration as a result of feeding of hormone during grow-out have been shown to induce higher growth (Howerton *et al.*, 1992; Kuwaye *et al.*, 1993). Thus the presumably higher levels of testosterone in the GMT-MT and GMT-CT may have induced their faster growth.

The higher growth of the androgen-treated fish in previous studies (Guerrero, 1975; Mualungwe Muhaya, 1985; Macintosh *et al.*, 1988; Jo *et al.*, 1988) could be due to the difference in sex ratio compared to the mixed-sex control and also the duration of hormone treatment. As has been demonstrated earlier, the genetic males may have the tendency to grow faster than the genetic females even at an early age. If the treatment duration is extended beyond the assumed period of sex differentiation and the treated fish are still continuously fed with androgen hormone, this could result in a big advantage in growth over the untreated control fish. If the treated fish, being all- or nearly all-male, were still fed androgen-treated food after sex has already differentiated, the concentration of circulating androgen hormone in the body would probably increase due to the combined endogenous and exogenous androgen resulting in increase in growth. Guerrero (1975) treated *0. aureus* fry for a total of 25 days including three non-feeding days. The author mentioned that the age of the fry at the start of the treatment was already approximately 4-5 weeks. It may be possible that the fry were already part way through the estimated labile period of sex differentiation. After the fry were sex-reversed they were still continuously

fed with hormone until the termination of treatment. Similarly, Macintosh *et al.* (1988) treated *O. niloticus* fry for forty days. If the sex is expected to differentiate within 21 days after first feeding, then they had treated their fry for an additional 20 days.

It is also possible that the absence of an anabolic effect is caused by the feeding of hormone at a concentration greater than the optimal dose. Berger and Rothbard (1987) reported that the feeding of 120 PPM 17α-ethynyltestosterone significantly reduced the growth and increased mortality in red tilapia. Varadaraj and Pandian (1989b) also observed that doses of methyltestosterone higher than 30 $mg.kg^{-1}$ diet induced many abnormalities in *O. mossambicus*. During grow-out, the incorporation of high synthetic androgen hormone concentrations in the diet have also been observed to reduce growth (Basavaraja *et al.*, 1989; Gogoi and Keshavanath, 1988). Although, in the present study, the hormone dose used was the recommended to effect masculinizaton (Mair and Little, 1991), the results presented in chapter six of this thesis indicate the build up of hormone leachates and metabolites during hormone treatment, particularly when the water is infrequently changed. The combined effect of the ingested hormone and the hormone in the water could bring about a hormone concentration in the body higher than that normally required to effect sex reversal. During the present study, the tank used for hormone treatment was only cleaned once a week. Although fresh clean water was added continuously at a rate of 1 $l.min^{-1}$, it may have not been enough to prevent the accumulation of hormones in solution.

The possible exposure of the treated fish to a higher hormone concentration during treatment might have disrupted the normal physiological processes associated with the development of sex, particularly the genetic females, resulting in depressed growth. Komen *et al.* (1993) observed a similar result in hormone treatment of genetic females in common carp, *Cyprinus carpio*. The authors observed that the growth of the methyltestosterone-treated female carp was significantly depressed compared to the untreated controls.

5.4.2 Growth during post-treatment period

The evaluation of post-treatment growth showed that the mean gain in weight of the methyltestosterone-treated groups were not different from their respective controls. Although, overall, the mean gain in weight of the six groups were significantly variable, this was largely due to the difference in the proportion of males. When considering only males in the analysis no significant difference in the mean weights was observed. This finding conforms with the results of Meyer (1990) and Green and Teichert-Coddington (1994). These authors also observed that the growth of sex-reversed males did not differ from that of the untreated normal males during rearing following termination of hormone treatments. Androgen-treated fish have been reported to have faster growth than the untreated controls during hormone treatment due to an apparent anabolic effect of the hormone (Guerrero, 1975; Macintosh *et al.*, 1988; Jo *et al.*, 1988). However, the promoting effect of the hormone might only last for a short

period during the post-treatment rearing (Nagaraj and Satyanarayana Rao, 1988). This effect probably would lasts only until all the assimilated hormone has been excreted following termination of treatment. The similar growth of the methyltestosterone-treated and the untreated normal males in the present study indicates that the anticipated anabolic effect of the hormone may have ceased (assuming it occurred) after the withdrawal of hormone treatment.

Varadaraj and Pandian (1988) reported that in *O. mossambicus* the growth of mixed genetic males and sex-reversed genetic females (previously treated with methyltestosterone at 1.5 $\mu g.g^{-1}$ diet for 16 days) was significantly higher ($P>0.001$) compared to control genetic males (2.91g ±0.40) *vs.* 1.55g ±0.23) after a 70 day rearing period. Faster growth of the sex-reversed genetic females compared to genetic males was not observed in the present study. The growth of GFT-MT and MST-MT, which were composed mostly of sex-reversed genetic females, was lower and not significantly different from that of the GMT-MT and GMT-CT which were composed mostly of genetic males. It may be possible that the faster growth of sex-reversed genetic females is not expressed in a food limited environment or adverse conditions. In the present study, although the fish were provided with enough food (10% biomass) the culture condition may be inferior to the usual method of commercial cage culture.

The CV for final weight of the methyltestosterone-treated groups was observed to have increased while the CV for final weight of the controls decreased over this growth period, relative to the CV for initial weight. This result is somewhat contradictory to expectation that males should have a more uniform

size compared to mixed-sex. Vera Cruz and Mair (1994) observed similar results when they compared the growth of the methyltestosterone-treated untreated controls in earthen ponds over 112 days. They noted that the increase in weight heterogeneity of the treated fish may not have been apparent if the culture period has been extended. A possible explanation for this unexpected result could be the presence of two phenotypes in the methyltestosterone-treated groups; the genetic males and sex-reversed genetic females. It is possible that the two genotypes have grown differently resulting in more variable sizes.

5.4.3 Growth of males in all-male and mixed-sex culture environments

The MST-MT and GFT-MT which were composed mostly of sex-reversed genetic females had the fastest growth in all the culture environments. This is in contrast to the result of the evaluation of post-treatment growth where the MST-MT had the lowest mean gain in weight when only the males were compared. It appears that the effect of the hormone on sex-reversed genetic females is not expressed immediately following termination of treatment but is being expressed at a later stages of the life cycle. Macintosh *et al.* (1988) noted that in *O. niloticus* the hormone treatment had more than a temporary anabolic effect on growth and possibly included a subsequently reduced fertility and sexual activity. The authors reported that the histological analysis of testes of methyltestosterone-treated males revealed little evidence of viable sperm and the

fish rarely exhibit breeding coloration at harvest. Guerrero (1975) also observed marked proliferation of connective tissues and apparent degeneration of the germinal epithelium in the testes of methyltestosterone-treated males.

In other studies, sex-reversed genetic females (Baroiller, 1996; Pandian and Varadaraj, 1990) and sex-reversed genetic males (Lahav, 1993) were found to breed normally although the fecundity of the sex-reversed fish in these studies were not compared to that of normal males and females. In a comparative study of reproductive biology between sex-reversed genetic males (which the authors called "pseudofemales") and normal females in *0. aureus*, Desprez *et al.* (1995) reported a higher percentage of non-spawning sex-reversed genetic males (40%) compared to normal females (20%). They suggest that the sex-reversed genetic males could have a delayed maturity that would result in lower spawning frequency and percentage of spawning breeders. It is possible that any effect on the fecundity or fertility of the sex-reversed tilapia is related to the hormone dose used during treatment. Varadaraj and Pandian (1989b) noted that strong doses of steroids had deleterious effects such as gonadal suppression, paradoxical actions, high mortality and physical deformities. It is likely that fertility is also affected by the hormone dose although there are no available published studies on this subject. In other species such as common carp, *Cyprinus carpio* (Manzoor Ali and Satyanarayana Rao, 1989) and coho salmon, *Oncorhynchus kisutch* (Goetz *et al.*, 1979) the application of excess hormones commonly leads to permanent sterility.

The presumed reduction in fertility of the sex-reversed genetic females might be one reason for the significantly higher growth of the MST-MT and GFT-MT in the present study and the sex-reversed fish in the study of Hanson *et al.* (1983). In the latter study, however, the authors explained that the faster growth of the MT-treated groups in their study could be due to the more efficient food utilization. It is perhaps more likely that the apparent reduction in fertility and subsequently the sexual activity of the sex-reversed genetic females is a more logical reason for better growth. As the sex-reversed genetic females might have not been as reproductively active as the genetic males, most of their energies were utilized for somatic growth instead of reproduction. Manzoor Ali and Rao (1989) reported that hormone-treated sterile *Cyprinus carpio* had significantly faster growth than untreated control fish. The results of the present study contrast with those of Mair *et al.* (1995) where it was found that the sex-reversed tilapia (SRT) have a slower growth than the genetically male tilapia (GMT), although both were of the same strain and mostly males. Their data showed that the SRT had a higher GSI than the GMT which suggest that the former might have matured earlier and not have been affected by the previously used hormone.

The presumed difference in fertility and reproductive activity of the five male groups might have been the major contributing factor to the more variable mean weights in the all-male environment compared to that in the mixed-sex culture environment. The anticipated higher level of testosterone circulating in the more active males (Yaron *et al.*, 1983) might have made them more dominant

and aggressive (Johnsson and Bjornsson, 1994) than the other males. Yaron *et al.* (1983) monitored the level of testosterone in male *O. niloticus* held in a tank at 30°C for 40 days and observed that the testosterone levels fluctuated during that period. They suggest that the variations in the level of testosterone reflect the behavioural hierarchy among the males. Those with higher level of testosterone could have been the more dominant and aggressive males. Satyanarayana Rao *et al.* (1990) reported, in their earlier study in common carp, a reduction in the level of testosterone (176.8 $pg.g^{-1}$) in muscle of 17α-methyltestosterone treated sterile fish, compared to controls (756.8 $pg.g^{-1}$). Popma and Green (1990) noted the result of a study which demonstrated that the plasma testosterone level of sexually active, untreated males in the presence of females was actually higher than the levels found in the same age, sex-reversed fish in monosex culture environment.

Although in the absence of females, it is probable that males are less aggressive as they do not need to compete for females, the mature and more aggressive males may still exhibit their instinctive reproductive activity and try dominate other males (Peters, 1971; McGinty, 1985). In effect, a large proportion of their energies might still be utilized for nest building and guarding territories instead of for somatic growth. Similarly, the absence of females in the environment may also have slowed down the process of sexual maturation of some males, particularly those that are dominated. Vera Cruz and Mair (1994) remarked that the lower mean GSI in methyltestosterone-treated tilapia might be the direct effect of the hormone or, indirectly, a result of the absence or near

absence of females in the environment. The apparent social control of sexual maturation of males was also observed by Bushmann and Burns (1994) in the swordtail characin (*Corynopoma riisei*). The onset of maturation for the maturing males was being delayed by the presence of dominant adult males, although somatic growth still continued resulting in progressively larger fish. Those immature and less dominating males which were not engaging in any reproductive activity would tend to maximize their food intake and growth resulting in more variable sizes. Oliveira de-Fernandez and Volpato (1993) state that the increase in heterogeneous growth in *O. niloticus* is presumed to be partially promoted by the social stress imposed by the dominant fish on the subordinate fish.

In the mixed-sex environment, the presence of females may have accelerated the sexual maturation of the immature males. McGinty (1985) cited Kubaryk (1980) finding immature *O. niloticus* males that grew faster alone than in the presence of females. When grown with females, the immature males mature earlier spending a great proportion of their energies on reproductive activity partially inhibiting their somatic growth. In the present study the relatively similar gain in weight of GMT-MT, GMT-CT, GFT-MT and MST-CT is an indication that they may have participated equally in reproductive activity.

The mean GSI of the five male groups has been shown to have been affected by the presence and absence of females in the environment. The changes in ranking of the GSI between culture environments probably reflect the different reaction of each group to the change in the environment, i.e. the

presence or absence of the opposite sex. In Figure 5.5 it can be seen that in the mixed-sex culture environment, the MST-CT and GMT-MT had the highest GSI while the GFT-MT had the lowest. The higher mean GSI of the MST-CT and GMT-MT probably indicate their being reproductively more dominant than the other groups. It should be noted that the two groups were composed of genetic males and although the GMT-MT were hormonally treated their reproductive viability might not have been affected and might instead have been improved (Fagerland and Mcbride, 1975 as cited Jessy and Varghese, 1988; Lee *et al.*, 1991).

The results of the analysis of dress-out and fillet weight confirm further the explanation presented for the difference on GSI and conform to the result of Abucay (1996) which showed that the dress-out and fillet weight were negatively correlated with the GSI. As has been mentioned earlier, those fish with higher GSI might be more likely to be the more dominant and aggressive. Being dominant and aggressive would tend to result in expenditure of more energy to sustain the activities of the fish and consequently result in less growth of muscle and therefore lower fillet yield.

5.4.4 Growth of females in all-female and mixed-sex culture environments

The difference in the overall mean growth of females between the two environments was large. This should be expected considering the obvious difference between the two culture environments. Although, even with the absence of males it has been observed that matured females in an all-female environment still lay and incubate their unfertilised eggs (Capili, 1995). However, despite the exhibition of this instinctive habit it did not greatly affect the growth since the eggs were not fertilized and the females may only mouth incubate for two days. Similarly, unlike males which would still hold territories and dig nest even without females (McGinty, 1985), females do not hold territories and with the absence of males the energy spent for reproductive activities is greatly reduced. In guppies, *Poecilia reticulata*, Griffiths (1996) noted that males trade the costs of reduced foraging against the benefits of obtaining mating opportunities and potentially increasing reproductive success whilst the females attempt to maximise their feeding rate at all times, and that it is sexual harassment, not degree of satiation which constrains this rate. In tilapia, this could be a contributing factor as to why the females have higher growth than the males when grown separately, as observed by Capili (1995). Bolivar *et al.* (1993) noted that, based on the results of their study evaluating the growth and reproduction in different strains of *O. niloticus*, the growth of late spawning and virgin females was similar to that of males in some of the strains tested. The authors remarked that if reproduction can be delayed in the females, an average

growth rates comparable to those of an all-male population might be achieved. In the absence of the opposite sex and therefore the absence of reproductive activities and territorial boundaries, the females can expend more effort to search for food and utilise this for somatic growth. In contrast, males are busy guarding their territories, searching food only within the vicinity of their territory and exerting their energy to sustain these activities (Peters, 1971).

The results of the analysis of GSI, dress out and fillet weight for the females were similar to those for the males. The GSI was also found to be negatively correlated with the dress out and fillet weight. The lower dress out and fillet weight of those fish with high GSI show that a great proportion of their energies were sacrificed for the production of eggs instead of somatic growth.

5.5 Conclusion

The results of the study show that during the hormone treatment period itself, the MT-treated fry have similar growth patterns to their respective untreated controls, contrary to the results in some other studies. The better growth of genetic males is already apparent even at this early age, probably because of the likely higher level of androgen circulating in the body and also maybe due to genetic factors linked to the male sex determining locus or loci. The reported better growth of the hormone-treated fish over the control in other studies may be due to the extension of treatment duration beyond the sex

differentiation stage. The growth of males is likely to become faster when continuously fed with hormone resulting in the obvious better growth of the treated fish compared to the control, assuming a positive anabolic effect of the hormone on growth.

The similar growth of the methyltestosterone-treated genetic males and untreated genetic males during the evaluation of post-treatment growth indicate the absence of any residual effect of the hormone on growth of genetic males. The reported better post-treatment growth of the hormone-treated males compared to the control in other studies may not be due to any anabolic effect of the previously used hormone but more likely due to the difference in sex ratios. The hormone could have a longer or permanent effect on the sex-reversed genetic females, as indicated by the lower growth of MST-MT and GFT-MT during the post-treatment stage.

The growth of the sex-reversed genetic females (MST-MT) during the evaluation of growth in all-male and mixed-sex culture environments was significantly better than the genetic males. The application of hormones to genetic females successfully altered the phenotypic sex but may have caused delayed maturity or reduced fertility of the treated fish. The presumed lower fertility and sexual activity of the sex-reversed genetic females could have induced the fish to grow faster than the genetic males. The growth of monosex males alone is not always a guarantee for optimal growth, particularly when the fish are reproductively mature. Although the males do not reproduce in a monosex environment they may expend much of their energy on behavioural

interactions and production of gametes which will result in lower dress-out and fillet weight. The better growth of sex-reversed genetic females could be a major advantage when growing sex-reversed fish, although, this is only applicable to at least 50% of the males when using genetically mixed-sex progeny. A possible better alternative is to mass produce all-female progeny and then hormone treat them to produce sex-reversed genetic females. Similarly, the effectiveness of using sex reversal would be better if the application of hormone not only alters the sex but also make the fish sterile.

The culture of all-female fish appears also to have a great potential as seen in the excellent growth performance. The supposed lower interaction among individual females may be a major factor in the enhancement of growth.

CHAPTER SIX

FACTORS AFFECTING SEX REVERSAL

6.1 Introduction

The culture of monosex individuals in tilapia, particularly all-male, is preferred due to the faster growth of the males and the absence of recruits which usually constitute around 10 to 20% of the total harvest (Mair and Little,1991). The production of all-male progeny can be achieved in several ways (see Mair and Little, 1991), but up to now the technique of hormonal application for sex reversal has been the most widely used. Sex reversal is a process by which the phenotypic sex of an animal is altered to that of the opposite sex by application of exogenous hormones to the fish prior to the beginning and during the process of natural sex differentiation. In some species, particularly the tilapia, the phenotypic sex differentiates between 10 and 25 days posthatching when the fry are about 8 to 11mm (total length) in *Oreochromis mossambicus* (Nakamura and Takahashi, 1973) or between 30 and 33 days posthatching when the fry are about 9 to 12mm in *O. niloticus* (Alvendia-Casauay and Cariño, 1988). During the period of sex differentiation, the fish is said to be in a labile condition and the application of exogenous hormone can alter the phenotypic sex (Nakamura and Takahashi, 1973).

The administration of exogenous hormone to the fish may be carried out using one of three approaches; (i) through oral application by incorporating the hormone into the diet (Guerrero, 1975; Buddle, 1984b; Jo *et al.*, 1988; Ridha and Lone, 1990; Phelps and Cerezo, 1992; Hiott and Phelps, 1993; Vera Cruz and Mair, 1994), (ii) through a short or long term immersion in a dilute concentration

of the hormone in the rearing water (Eckstein and Spira, 1965; Varadaraj and Pandian, 1987; Baker *et al.*, 1988; Rosenstein and Hulata, 1992; Piferrer *et al.*, 1994a) or (iii) by combined immersion and feeding (Lone and Ridha, 1993; Gilling, 1994).

Hormone treatment can be applied in different treatment conditions such as; (i) tanks (Buddle, 1984b; Guerrero and Guerrero, 1988; Watanabe *et al.*, 1993b; Vera Cruz and Mair, 1994), (ii) fine mesh hapas suspended in earthen ponds (Buddle, 1984b; Berger and Rothbard, 1987; Popma and Green, 1990; Vera Cruz and Mair, 1994), (iii) fine mesh hapas suspended in tanks (Phelps *et al.*, 1992; Bocek *et al.*, 1992) or (iv) plastic pools (Guerrero, 1975; Basavaraja *et al.*, 1990).

The use of sex reversal offers the advantage that it is relatively easy to perform using simple facilities. Initially, it has been recommended that treatment should be done in clear water, free of natural food to achieve a high percentage of sex reversal (Yamamoto, 1969). To implement that recommendation would require specialized facilities where abundant clear and clean water is available with appropriate water supply and drainage system. However, Chambers (1984) demonstrated that the success of sex reversal was not necessarily affected by the presence of natural food when populations ranging from 98 to 100% males were produced in treatments irrespective of the presence or absence of natural food. Buddle (1984b) and Berger and Rothbard (1987) performed oral sex reversal in cages suspended in earthen ponds and achieved sex ratios close to 100% males. Phelps and Cerezo (1992) demonstrated that the success of sex

reversal was not affected when the hormone treatment was done directly in tanks at lower stocking density instead of treatment in fine hapas suspended in concrete tanks at a very high density. Vera Cruz and Mair (1994) reported that the efficiency of hormone treatment in fine mesh cages suspended in earthen ponds was similar to those treated in concrete tanks supplied with clear water.

It has been demonstrated that sex reversal can be done successfully on a commercial scale. Guerrero and Guerrero (1988) demonstrated the feasibility of commercial production of sex-reversed fingerlings in outdoor concrete tank facilities and reported that over a million fry were hormonally treated from May to November 1985. Popma and Green (1990) described in detail the procedures for commercial production of sex-reversed tilapia in earthen ponds.

However, in commercial conditions, sex reversal has often failed to achieve the degree of reliability which is required for widespread acceptance (Mair and Little, 1991). Despite the many studies conducted to evaluate and optimise the technique of sex reversal (see Hunter and Donaldson, 1983; Pandian and Sheela, 1995), there is still limited understanding of the processes taking place during the hormone treatment period. For example, there is still no definite standard for concentration of hormone for effective sex reversal. In most studies the recommended concentration for androgen, particularly methyltestosterone, is up to a maximum of 60 $mg.kg^{-1}$ diet (Guerrero, 1975; Nakamura, 1975; Popma and Green, 1990; Phelps and Cerezo, 1992; Mair and Little, 1991; Phelps *et al.*, 1992; Watanabe *et al.*, 1993b; Vera Cruz and Mair, 1994). Increasing the concentration beyond a certain optimal level has been

observed either to reduce the efficiency of sex reversal or increase mortality. Obi and Shelton (1983) observed that with methyltestosterone treatment in *O. hornorum* the percentage males at 60 $mg.kg^{-1}$ diet (97%) concentration was lower than that at 30mg (100% males). In sex reversal of red tilapia, increasing the concentration of ethynyltestosterone from 60 to 120 $mg.kg^{-1}$ diet resulted in significantly reduced growth and increased mortality compared to the control (Berger and Rothbard, 1987). Nakamura (1975) reported that in *O. mossambicus* when the concentration of methyltestosterone was increased from 50 to 1000 $mg.kg^{-1}$ diet, feminisation was induced rather than masculinisation. However, McGeachin *et al.* (1987) did not find any significant difference in the sex ratio and survival in *0. aureus*, when the concentrations of ethynyltestosterone and methyltestosterone were increased from 60 to 240mg and 60 to120 $mg.kg^{-1}$ diet, respectively.

In some feminisation experiments the inconsistencies of results has also been observed. Tayamen and Shelton (1978) treated *O. niloticus* with diethylstilbestrol at concentrations of 25 and 100 $mg.kg^{-1}$ diet and observed a results of 62 and 92% females, respectively. Varadaraj (1989) produced 100% female progeny in *O. mossambicus* treated with diethylstilbestrol at a concentration of 100 $mg.kg^{-1}$ diet for a minimum of 11 days. Basavaraja *et al.* (1990) reported that diethylstilbestrol at a concentration of 50 $mg.kg^{-1}$ diet was sufficient to induce 100% females in *O. mossambicus*. Rosenstein and Hulata (1993) achieved 100% females in *O. mossambicus* and in the F_1 of *O. mossambicus* x *O. urolepsis hornorum* treated with diethylstilbestrol and

ethynyltestradiol at concentrations >10 $mg.kg^{-1}$ and >50 $mg.kg^{-1}$ diet, respectively. However, similar or higher concentrations were found ineffective in other studies. Potts and Phelps (1995) treated *O. niloticus* with diethylstilbestrol at concentrations of 100 to 400 $mg.kg^{-1}$ and ethynylestradiol at 50 to 200 $mg.kg^{-1}$ diet and only observed 60 to 80% and 58 to 65% females, respectively. Jensen and Shelton (1979) treated *0. aureus* with the naturally occurring estrogens; estriol and 17β-estradiol, at concentrations ranging from 30 to 120 $mg.kg^{-1}$ diet beginning when the fry were about 8 to 11mm. The treatment failed to effect sex reversal which was claimed to be due to an insufficient dose of estrogen and treatment outside the labile period. Mair and Santiago (1994) treated *O. niloticus* fry with diethylstilbestrol at concentrations ranging from 50 to 1000 $mg.kg^{-1}$ diet and observed 57.0 to 82.7% females. In feminisation of YY males, fry were treated with diethylstilbestrol at concentration ranging from 250 to 1000 $mg.kg^{-1}$ diet at different feeding durations (10 to 20 days). It was observed that the rate of sex reversal was higher at higher concentrations and longer feeding durations (Vera Cruz *et al.*, 1996).

Administering hormone through rearing water has also been proven to be effective. Varadaraj and Pandian (1987) reported that in *O. mossambicus* 100% masculinisation was achieved by rearing fry in water containing 17α-methyl-5-androsten-3β-17β-diol at a concentration of 5 or 10 $\mu g.l^{-1}$ for a duration of 11 to 19 days. Gilling (1995) reared *O. niloticus* fry in water containing either β-estradiol, ethynyltestradiol or diethylstilbestrol at concentrations ranging from 4 to 500, 4 to 400, and 100 to 400 $\mu g.l^{-1}$, respectively, for 30 days treatment duration.

The results ranged from 52.3 to 91.4% females, with diethylstilbestrol at 100 $\mu g.l^{-1}$ giving the highest. However, the mortality increased with increase in hormone dose and almost all the fry reared at the highest concentration died. Gale *et al.* (1991) reported that immersion of *O. niloticus* fry in water containing 17α-methyltestosterone or mestanolone at a concentration of 100 and 500 $\mu g.l^{-1}$ for three hours at 10 and 13 days post fertilization produced greater than 93% males. However, Rosenstein and Hulata (1992) failed to achieve feminisation when eggs or embryos of *O. mossambicus* (L.) and *O. mossambicus* x *O. urolepsis hornorum* were immersed in water containing 100 to 5000 $\mu g.l^{-1}$ of oestrogenic hormones including 17β-estradiol, progesterone, flutamide and flutamide + progesterone for periods of 2 to 12 days. Eckstein and Spira (1965) also observed considerable mortality in *0. aureus* when immersed in water containing stilbestrol-diphosphate at concentration of $\geq$200 $\mu g.l^{-1}$, particularly during and after the third week of culture.

The effect of increasing the temperature during hormone treatment has been also investigated by several workers. Shelton *et al.* (1981) evaluated the sex ratio of *0. aureus* treated with ethynyltestosterone at a concentration of 60 $mg.kg^{-1}$ diet in water with a temperature of 21°C and 30°C. All-male populations were produced in 21 and 28 days at 21°C, but a few females persisted in the groups at 30°C. The reason was thought to be due to the higher metabolic rate and appetite of the fry thus requiring higher feeding rate to insure adequate supply of treated feed. In methyltestosterone treatment of common carp, *Cyprinus carpio*, Nagy *et al.* (1981) observed that a water temperature of 25°C

increased the effectiveness of the hormone treatment compared to that at 20°C, which frequently produced intersexes. In sex reversal of *O. mossambicus,* fry treated with methyltestosterone at a concentrations of 5 or 10 $mg.kg^{-1}$ diet for 11 days at 27°C produced all-male populations (Varadaraj *et al.*, 1994). At lower temperatures (22 and 25°C) females and a few intersexes were present, while a high percentage of intersexes was produced at 33°C and only females and intersexes were found after treatment at 38°C (Varadaraj *et al.*, 1994).

In a recent study designed to optimise feed quality and treatment duration in sex reversal treatments by oral applications of hormones, Abucay and Mair (in press a) observed the phenomenon of incidental sex reversal of control fish reared in separate but in the same water body as the treated fish. A similar observation was made in methyltestosterone treatment of common carp, *Cyprinus carpio* (Gomelsky *et al.*, 1994). Gomelsky and his fellow worker found that the sex ratio of the control fish, which received water from the same recirculating system as the treated fish, were also significantly modified from expectations. Chan and Yeung (1983) cited a work which demonstrated that in *Oryzias latipes*, close confinement results in the uptake of androgenic substances released by other fishes.

Elsewhere, it may also be possible that there are quite a few sex reversal experiments which have been conducted but, because of unsuccessful or inconsistent results, have never been published.

The general objective of the studies presented in this chapter was to determine factors which directly or indirectly affect the efficacy of sex reversal such as the source and effect of hormone residues in the water and the effect of water temperature during hormone treatment. The specific objectives were as follows;

1. To determine the effect of rearing fry in water previously used for oral sex reversal treatments (i.e. possible containing potent residues) on sex differentiation and survival,

2. To determine the effect of increasing the temperature above normal levels; during sex reversal hormone treatments and during rearing of fry in water previously used for hormone treatment, on sex ratio and survival,

3. To determine the possible leaching of hormone from hormone-treated food and the effect of these leachates on sex differentiation of untreated fry.

6.2 Materials and Methods

A series of experiments to determine factors affecting sex reversal were conducted. Sexually undifferentiated putative all-male and all-female progeny in the Egypt-Swansea strain were treated orally with diethylstilbestrol and methyltestosterone, respectively, in glass aquaria at a stocking density of 150 fish per replicate or approximately 8 fish.l^{-1} of water for a minimum period of 21 days after first feeding, to encompass the time of sex differentiation. Each treatment had consisted of either two or three replicates and each of the replicates was in separate cages in a single aquarium (see Photo 2.3 in Chapter two). After termination of treatment, the number of fish surviving was noted to determine the percentage survival after which the fish were stocked and grown in cages in earthen ponds for sexing by gonad squash to determine the sex ratio. The different experiments are described;

Experiment 1 - The effect of not changing the water during oral treatment was determined. Diethylstilbestrol at a concentrations of 100, 500 and 1000.kg^{-1} diet and methyltestosterone at 10, 20 and 40 mg.kg^{-1} diet (Treatments) were administered orally, for the feminisation and masculinisation experiments, respectively. Each treatment had consisted of three replicates. After termination of hormone treatment, new batches of the same genotype of sexually undifferentiated fish were reared in the same water previously used for hormone treatment (termed Immersion). The fish were fed with a hormone-free diet

throughout the rearing period. The feminisation and masculinisation experiments were performed twice (two trials) to further confirm the results.

Experiment 2 - Using the same rearing/treatment set up, hormones and hormone concentrations as in experiment 1, the effect of increasing the temperature to 33 and 36°C during hormone treatment was determined. However, unlike experiment 1, no immersion treatment was performed for experiment 2. No parallel treatment under ambient temperature conditions could be carried out due to limited availability of facilities. The specific temperatures were maintained throughout the rearing period. To determine the effect of temperature alone on sex ratio, a separate batch of untreated fish were maintained at ambient, at 33 and at 36°C temperatures, as controls (CT) for both feminisation and masculinisation treatments. Each treatment had consisted of three replicates.

Experiment 3 - The effect of high temperature during rearing of fry in the same water previously used for hormone treatment was determined. Oral treatment of DES at a concentration of 100 $mg.kg^{-1}$ and MT at 10 $mg.kg^{-1}$ diet, for the feminisation and masculinisation experiment, respectively, was first carried out at high (34°C) and at ambient temperatures. Each treatment was consisted of three replicates. Because of the observed effect of high temperature on sex ratio in experiment 2, a temperature lower than 36°C was maintained in the present experiment to minimise the possible effect of high temperature on sex ratio. After termination of oral treatments, new batches of the same genotype of sexually

undifferentiated fry were reared in the same water and at the same temperatures, and fed with a hormone-free diet throughout the rearing period (Immersion).

Experiment 4 - The possible leaching of hormone from hormone-treated food and the effect of these leachates on sex ratio was investigated. Sexually undifferentiated fry were stocked in two cages in an aquarium, the third cage was left empty. The fry were fed hormone-free food throughout the rearing period. During feeding, an amount of hormone-treated diet, equivalent to amount of untreated feed given to the fry, was dropped in the empty third cage and allowed to leach. To prevent the scattering of the hormone feed, the hormone was incorporated in a pelleted feed. For the control treatment in other aquaria, hormone-free food was dropped in the empty third cage. Each treatment had consisted of two replicates.

Statistical analysis - The difference between the percentage survival and sex ratio of three or more different treatments was determined by a one-way analysis of variance (ANOVA). For the comparison of two treatments only, the significance of the difference was determined using the Independent T-Test. The presence of temperature x hormone dose interaction was determined by a two-way ANOVA. Before analysis, all values in percentage form were first arcsine transformed. The comparison of among treatment means was performed using the Bonferroni multiple range test (three treatments) and Tukey multiple range test when comparing more than three treatment values.

6.3 Results

6.3.1 Effect of not changing the water during hormone treatment

The results of the feminisation and masculinisation experiments aimed at determining the effect of not changing the water during hormone treatment are presented in Tables 6.1 and 6.2, respectively. In all the trials, the sex ratios of the hormone-treated fish were significantly altered ($P<0.001$) compared to the control. However, no case of 100% sex reversal was achieved even with the highest and recommended doses (Mair and Santiago, 1994; Mair and Little, 1991). This may be partially attributed to the fact that it may be more difficult to effect 100% sex reversal in putatively monosex progeny (Melard, 1995). In mixed-sex progeny, only approximately 50% of the total number is needed to be sex-reversed so that 100% monosex is easier to achieve.

Generally, the proportion of sex reversal increased with increase in hormone dose (Figures 6.1 and 6.2). However, in most cases the percentage of sex reversal for the higher hormone dose was not significantly higher than those of the lower dose. In feminisation-2, the feminisation rate at 100 $mg.kg^{-1}$ diet concentration (87.67%) was higher than the rates at 500 mg and 1000 $mg.kg^{-1}$ (84.67 and 84.62%, respectively), whilst in masculinisation-2 the masculinisation rate at 20 $mg.kg^{-1}$ diet concentration (95.31%) was significantly higher than at 40 mg (84.66%).

The results indicate that by not changing the water during oral treatment it may be possible to reduce the hormone dose and still achieve relatively effective sex reversal.

The percentage survival values for feminisation 1 and 2 were both significantly variable ($P=0.003$ and $P<0.001$, respectively). The survival rates decreased with increase in hormone dose indicating the toxicity of diethylstilbestrol (Figure 6.1). The percentage survival values in masculinisation-1 were significantly variable ($P=0.032$) although no significant difference among treatments was detected. For masculinisation-2, the percentage of survival was not significantly variable. Generally, the survival rates for the treated fish were slightly higher than those of the control (Figure 6.2).

With the exception of the first masculinisation trial, the fish reared in water previously used for hormone treatment had sex ratios altered from that of the control, in the direction expected given the hormone used (see Figures 6.1 and 6.2). These differences were significant in both the feminisation-2 ($P=0.021$) and masculinisation-2 ($P=0.001$) experiments, and almost significantly different in feminisation-1 ($P=0.053$). The sex ratios in masculinisation-1 were somewhat contradictory to the expected result since the control (32.50%) had a higher proportion of males than those grown in water used for hormone treatments (12.65 to 21.62%). Nevertheless, there were clear increases in the proportion of females and proportion of males in the fry reared in water previously used for feminisation experiments 1 and 2 and masculinisation-2, respectively.

These results clearly indicate that some form of hormone and hormone residues have accumulated in the water which was able to effect sex reversal.

The survival rates of the fry reared in water previously used for feminisation-1 were not significantly variable but differed significantly in feminisation-2 ($P=0.001$). The amount of hormone residues accumulated in feminisation-1 might not be as high as in feminisation-2 so that the survival rate was not significantly affected. Similarly, the survival rates for masculinisation-1 were significantly variable ($P<0.001$) but the rates in masculinisation-2 did not differ significantly. The survival rates for feminisation-2 decreased with increase in hormone dose, a trend similar to those during oral treatment. The observed decrease in survival rate indicates that the increase in hormone concentration in the water corresponding to the previously used hormone dose. A similar trend was also observed for masculinisation-2, the rates decreased corresponding to the increase of the previously used hormone dose. However, the trend was contradictory to that during oral treatment. These results might indicate a significant effect of the accumulated hormone.

Table 6.1. Summary of the results of two feminisation experiments aimed at determining the effect of not changing the water during oral treatments on sex ratio. Initially diethylstilbestrol hormone was administered by incorporation into the feed at three doses. After termination of oral treatments, new batches of the same genotype of fish were reared in the same water but received hormone-free food (immersion). Numbers represent pooling of the fingerlings remaining from three replicates during sexing.

Experimental trials	Hormone dose ($mg.kg^{-1}$ diet)	Survival [1,2] (%)	Male	Female	Intersex	Females [2] (%)
Feminisation-1	Control	80.89 [a]	221	11	0	4.74 [c]
	100	74.89 [a]	89	85	17	44.50 [a]
	500	59.33 [ab]	122	72	4	36.36 [a]
	1000	45.11 [b]	29	107	10	73.29 [b]
Probability		P=0.003				P<0.001
Immersion	Control	49.33 [a]	130	13	0	9.09 [a]
	100	36.00 [a]	103	23	0	18.25 [a]
	500	65.78 [a]	126	52	0	29.21 [a]
	1000	51.11 [a]	124	38	0	23.46 [a]
Probability		P=0.128				P=0.053
Feminisation-2	Control	90.44 [c]	93	7	0	7.0 [b]
	100	68.22 [a]	7	64	2	87.67 [a]
	500	57.33 [ab]	16	116	5	84.67 [a]
	1000	41.33 [b]	9	110	11	84.62 [a]
Probability		P<0.001				P<0.001
Immersion	Control	56.67 [a]	122	11	0	8.27 [b]
	100	48.00 [a]	69	13	0	15.85 [ab]
	500	31.33 [b]	47	56	0	54.37 [ab]
	1000	31.33 [b]	14	28	0	66.67 [a]
Probability		P=0.001				P=0.021

[1] - Survival rates were based on counts of fry at the time of removal from aquaria at the end of the treatment
[2] - Mean values within a treatment group with different letter subscript are significantly different (P<0.05)

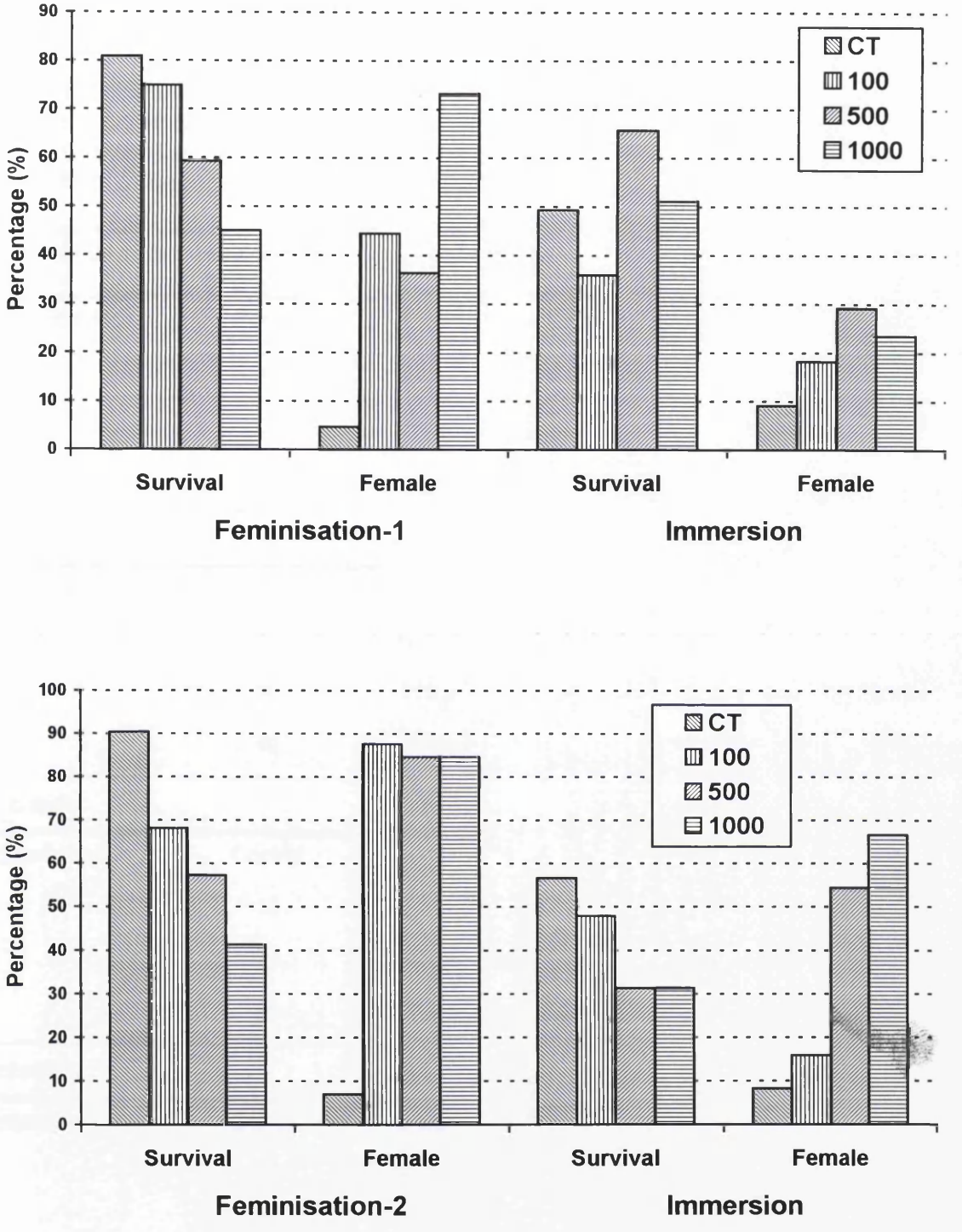


Figure 6.1. Histograms showing the mean percentage survival and percentage females in two feminisation trials aimed at determining the effect of not changing the water during oral treatments. After termination of oral treatments, new batches of the same genotype of fish were reared in the same water but received hormone-free food (immersion). The mean values were average of three replicates.

Table 6.2. Summary of the results of two masculinisation experiment trials aimed at determining the effect of not changing the water during oral treatments on sex ratio. Initially methyltestosterone hormone was administered by incorporation into the feed at three doses. After termination of oral treatments, new batches of the same genotype of fish were reared in the same water but received hormone-free food (immersion). Numbers represent pooling of the fingerlings remaining from three replicates during sexing.

Experimental trials	Hormone dose ($mg.kg^{-1}$ diet)	Survival [1, 2] (%)	Male	Female	Intersex	Males [2] (%)
Masculinisation-1	Control	74.89 [a]	32	314	0	9.25 [c]
	10	61.33 [a]	219	58	0	79.06 [a]
	20	76.89 [a]	291	29	0	90.94 [ab]
	40	76.89 [a]	295	8	0	97.36 [b]
Probability		P=0.032				P<0.001
Immersion	Control	77.11 [a]	78	162	0	32.50 [a]
	10	66.67 [a]	32	129	31	16.67 [a]
	20	76.44 [a]	48	90	84	21.62 [a]
	40	35.33 [b]	21	78	67	12.65 [a]
Probability		P<0.001				P=0.325
Masculinisation-2	Control	83.00 [a]	23	205	0	10.09 [c]
	10	93.11 [a]	177	58	0	75.32 [a]
	20	93.33 [a]	305	15	0	95.31 [b]
	40	92.00 [a]	149	27	0	84.66 [ab]
Probability		P=0.118				P<0.001
Immersion	Control	81.56 [a]	6	118	0	4.84 [c]
	10	78.44 [a]	38	82	6	30.16 [a]
	20	87.78 [a]	9	107	1	7.69 [bc]
	40	82.22 [a]	29	130	3	17.90 [ab]
Probability		P=0.214				P=0.001

[1] - Survival rates were based on counts of fry at the time of removal from aquaria at the end of the treatment
[2] - Mean values within a treatment group with different letter subscript are significantly different (P<0.05)

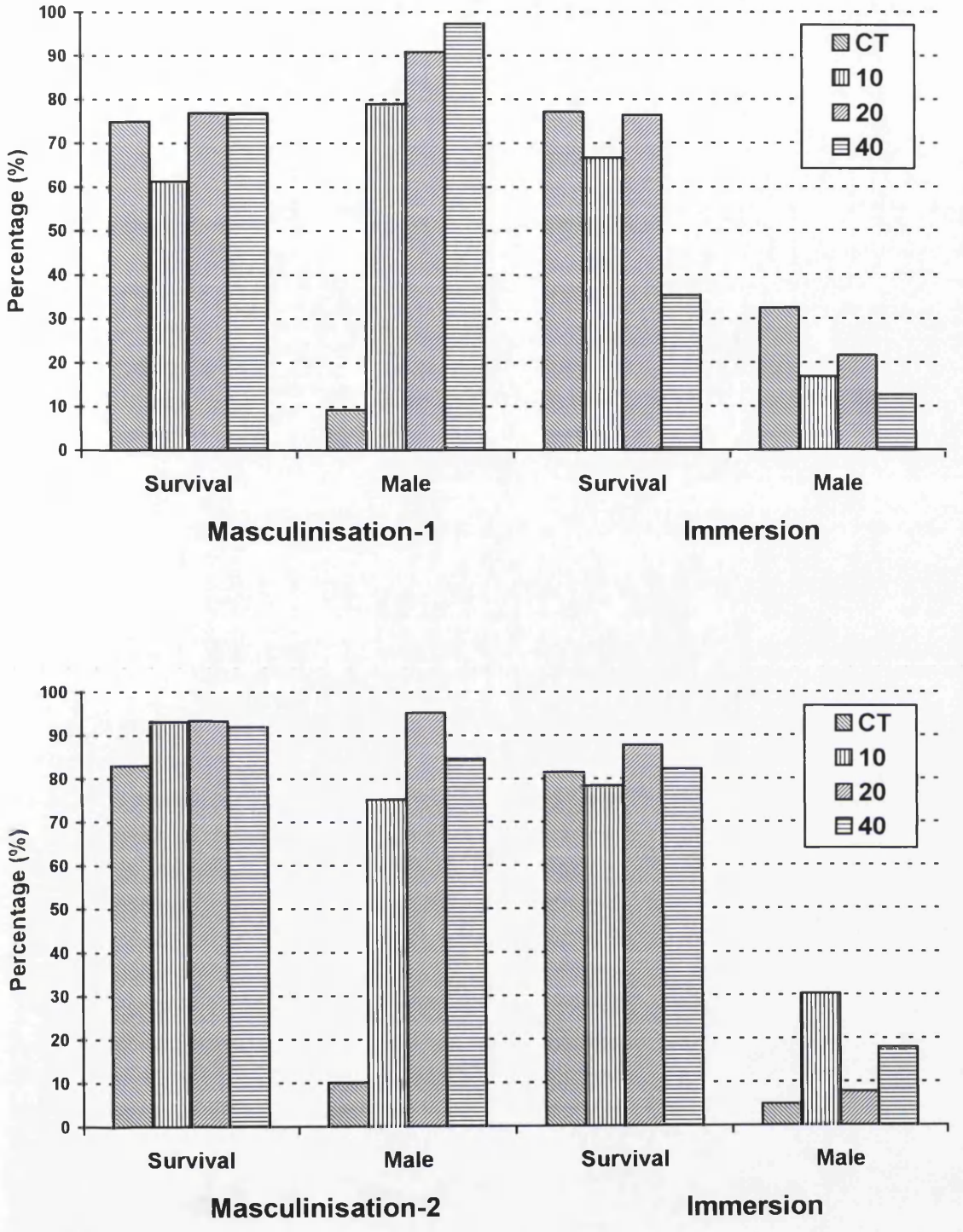


Figure 6.2. Histograms showing the mean percentage survival and percentage males in two masculinisation trials aimed at determining the effect of not changing the water during oral treatments. After termination of oral treatments, new batches of the same genotype of fish were reared in the same water but received hormone-free food (immersion). The mean values were average of three replicates.

6.3.2 Effect of increasing the temperature during hormone treatment

The results of the feminisation and masculinisation experiments at temperatures above normal level (33 and 36°C) during hormone treatment are presented in Tables 6.3 and 6.4. As in experiment 1, all the sex ratios of the hormone treated fish were significantly altered ($P<0.001$) in the direction expected given the hormone used. Compared to the results in the first experiment, which was performed at ambient temperature, the increase in temperature during hormone treatment appears to have improved the efficiency of the treatment. At 36°C, the feminisation rate at 1000 $mg.kg^{-1}$ diet concentration was close to 100% and the masculinisation rate at 40 $mg.kg^{-1}$ at 33 and 36°C were both 100% (see Figure 6.3). Similarly, comparing the results under the two temperature regimes (33 and 36°C), increasing the temperature to 36°C has improved sex reversal efficiency. A two-way ANOVA comparing the sex ratios of two temperature regimes and the hormone concentration show that, for the feminisation experiment, the mean percentage females at 36°C (61.95%) was significantly ($P=0.025$) higher than that at 33°C (52.98%). No significant temperature x hormone dose interaction for sex ratio was observed, indicating that the percentage of females at each hormone dose increases as the temperature increases. For the masculinisation experiment, a two-way ANOVA shows that the mean percentages of male in each temperature regime do not differ significantly. No significant temperature x hormone dose interaction for percentage males was observed. The apparent lack of response to the increase in temperature of the masculinisation treatment can be explained by the fact that

at 33°C the masculinisation rates, particularly at 20 mg (95.26%) and 40 $mg.kg^{-1}$ (100%) hormone doses, were already close to maximum efficiency. However, the masculinisation rate for 10 mg at 36°C (56.84%) did decrease relative to that at 33°C (85.98%). This result seems to suggest that the potency of methyltestosterone may be increase at 33°C but at a higher temperature (36°C) the hormone may start to degrade or be transformed into another compound resulting in lower masculinising effect.

The mean percentage survival for the feminisation experiment at 33 and at 36°C were both highly significantly variable ($P<0.001$). The trend was relatively similar to that of the same treatment in Experiment 1. The survival rates decreased with increase in hormone dose, with the exception of 500 mg at 36°C which had a higher rate than that at 1000 mg (Figure 6.3). The increase in temperature did not affect the percentage survival. The result of a two-way ANOVA shows that the mean percentage survival at 33°C (79.60%) was not significantly different from that at 36°C (76.22%). On the other hand, for the masculinisation treatments, the survival at 33 °C was not significantly variable but that at 36°C was significantly variable ($P=0.022$). A two-way ANOVA shows that the mean percentage survival for the masculinisation treatment at 36°C (82.80%) was significantly ($P=0.045$) higher than that at 33°C (76.04%).

Table 6.3. Summary of the results of a feminisation experiment aimed at determining the effect of increasing the temperature to 33 and 36°C on sex ratio. Diethylstilbestrol hormone was administered by incorporation into the feed at three doses. Two untreated controls (CT); at ambient and at high temperatures, were maintained to determined the effect of temperature on sex ratio alone. Numbers represent pooling of the fingerlings remaining from three replicates during sexing.

Experiment	Actual temp. (°C) (± sd)	Hormone dose ($mg.kg^{-1}$)	Survival [1, 2] (%)	Male	Female	Intersex	Females [2] (%)
Feminisation (≈33°C)	29.58 (± 0.53)	CT-1	95.56 [a]	91	13	0	12.50 [b]
	33.15 (± 0.32)	CT-2	90.00 [a]	153	46	3	22.77 [b]
	33.10 (± 0.33)	100	92.00 [a]	91	230	7	70.12 [a]
	33.99 (± 0.50)	500	61.56 [b]	26	96	3	76.80 [a]
	33.24 (± 0.29)	1000	58.89 [b]	27	134	1	82.72 [a]
Mean			79.60				52.98
Probability			P<0.001				P<0.001
Feminisation (≈36°C)	29.58 (± 0.53)	CT-1	95.56 [b]	91	13	0	12.50 [b]
	36.73 (± 0.13)	CT-2	91.78 [b]	96	26	0	21.31 [b]
	36.64 (± 0.36)	100	68.89 [a]	10	118	0	92.19 [a]
	36.43 (± 0.32)	500	86.00 [ab]	14	95	0	87.16 [a]
	36.73 (± 0.19)	1000	38.89 [c]	4	113	0	96.58 [a]
Mean			76.22				61.95
Probability			P<0.001				P<0.001

[1] - Survival rates were based on counts of fry at the time of removal from aquaria at the end of the treatment
[2] - Mean values within a treatment group with different letter subscript are significantly different (P<0.05)

Table 6.4. Summary of the results of a masculinisation experiment aimed at determining the effect of increasing the temperature to 33 and 36°C on sex ratio. Methyltestosterone hormone was administered by incorporation into the feed at three doses. Two untreated controls (CT); at ambient and at high temperatures, were maintained to determined the effect of temperature on sex ratio alone. Numbers represent pooling of the fingerlings remaining from three replicates during sexing.

Experiment	Actual temp. (°C) (± sd)	Hormone dose ($mg.kg^{-1}$)	Survival [1,2] (%)	Male	Female	Intersex	Males [2] (%)
Masculinisation (≈33°C)	29.22 (± 0.68)	CT-1	66.22 [a]	22	208	0	9.57 [c]
	33.19 (± 0.35)	CT-2	75.11 [a]	15	135	0	10.0 [c]
	33.04 (± 0.4)	10	82.89 [a]	92	15	0	85.98 [a]
	33.28 (± 0.35)	20	72.00 [a]	201	8	2	95.26 [ab]
	33.27 (± 0.33)	40	84.00 [a]	198	0	0	100.0 [b]
Mean			76.04				60.16
Probability			P=0.295				P<0.001
Masculinisation (≈36°C)	29.22 (± 0.68)	CT-1	66.22 [b]	22	208	0	9.57 [c]
	36.08 (± 0.8)	CT-2	84.00 [ab]	23	165	3	12.04 [c]
	36.88 (± 0.56)	10	84.89 [ab]	87	66	0	56.84 [a]
	36.75 (± 0.6)	20	89.78 [a]	136	1	0	99.27 [b]
	36.02 (± 0.45)	40	89.11 [a]	19	0	0	100.0 [b]
Mean			82.80				55.54
Probability			P=0.022				P<0.001

[1] - Survival rates were based on counts of fry at the time of removal from aquaria at the end of the treatment
[2] - Mean values within a treatment group with different letter subscript are significantly different (P<0.05)

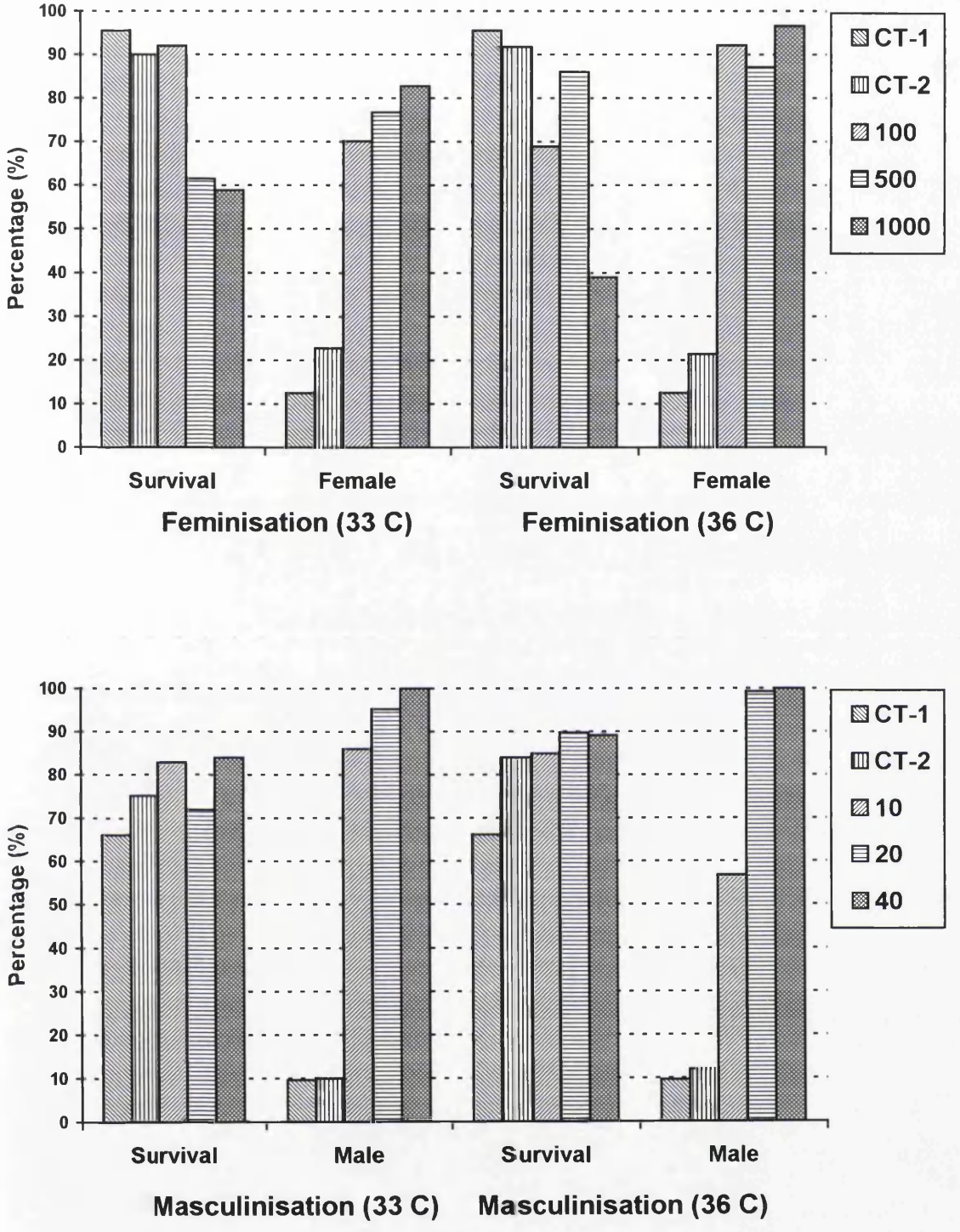


Figure 6.3. Histograms showing the mean percentages of survival and females in feminisation (upper graph), and percentages of survival and males in masculinisation (lower graph) experiments at 33 and 36°C. Two untreated controls (CT); at ambient and at high temperatures, were maintained to determined the effect of temperature on sex ratio alone. Mean values represent the mean of three replicates.

6.3.3 Effect of increasing the temperature during immersion treatment

The results of the experiment aimed at determining the effect of increasing the temperature during the "immersion phase" treatment (i.e. rearing fish in water previously used for oral treatment) are presented in Table 6.5. The mean sex ratios produced by the different hormone dose treatments during the initial treatment were all significantly variable ($P<0.001$). The sex ratios of the treated fish, both for feminisation and masculinisation experiments, are significantly ($P<0.05$) altered to the direction expected given the hormone used. The percentage sex reversal at high temperature is higher than that at ambient temperature, both for the feminisation and masculinisation experiment (Figure 6.4), which confirms further the earlier results that increasing the temperature could further improve the rate of sex reversal. The survival for the feminisation experiment is significantly variable for the different temperatures ($P<0.001$). The percentage survival at high temperature is significantly ($P<0.05$) lower than that at ambient temperature despite the fact that both treatments were treated with the same hormone dose. The result seems to indicate that the toxicity of diethylstilbestrol and its metabolites increases with increase in temperature. For the masculinisation experiment, there was no difference between the percentage survival values.

The percentage females of the fish reared in the same water previously used for feminisation treatment is significantly variable ($P=0.010$), where those at high and ambient temperatures have a higher percentage females than the control indicating that some were sex reversed (Figure 6.4). Although the

difference between the percentage females of that at high (19.05%) and at ambient temperature (28.1%) was not significant but assuming that the concentration of accumulated hormone residues in the two treatments were relatively similar (as both have received the same hormone dose), it appears that the potency of the accumulated hormone at high temperature was lower than those at ambient temperature. The percentage survival of the fish reared in the same water previously used for feminisation treatment is not significantly variable.

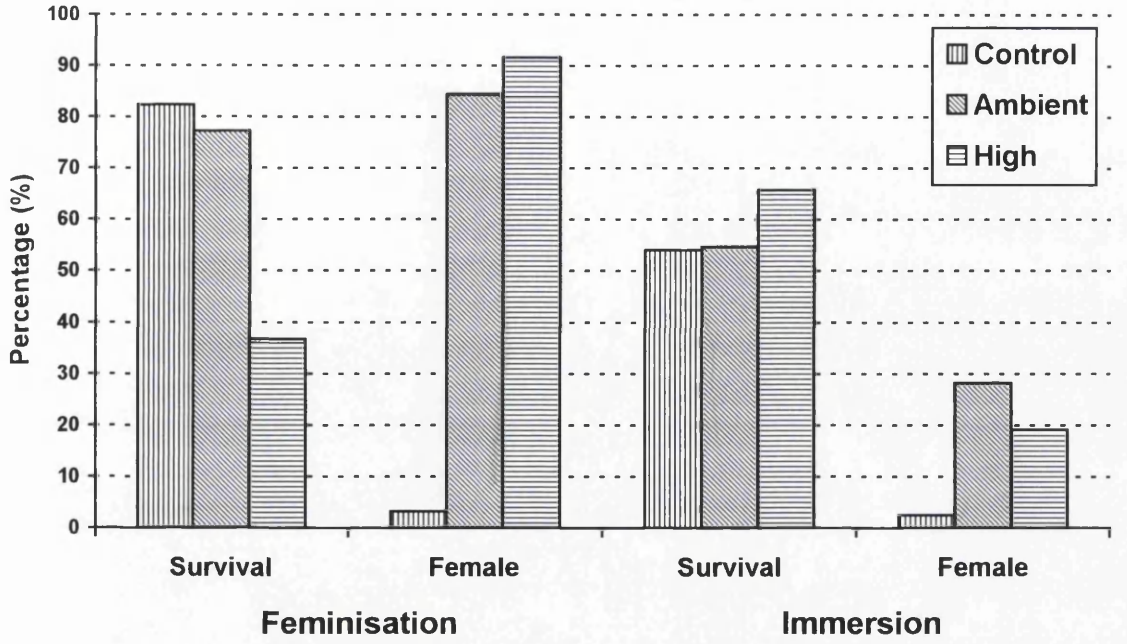
The sex ratio of the fish reared in the water previously used for masculinisation treatment produced the same phenomenon observed earlier in immersion treatment of masculinisation-1 in experiment 1. The mean percentage males of the fish reared at ambient temperature (24.68%) is slightly higher than the control (22.95%) but the percentage male at high temperature (15.52%) is lower (Figure 6.4).

Although the difference was not significant but considering that the survival rates were almost similar and the fact that this incident occurred twice, it appears that the accumulated hormone residues in water previously used for methyltestosterone treatment were effecting feminisation rather more than masculinisation.

Table 6.5. Summary of the results of the feminisation and masculinisation experiments aimed at determining the effect of increasing the temperature during oral and immersion treatment. Initially diethylstilbestrol and methyltestosterone hormones were administered by incorporating them into the feed at concentrations of 100 and 10 $mg.kg^{-1}$ diet, respectively. After termination of oral treatments, new batches of the same genotype of fish were reared in the same water and temperatures but received hormone-free food (immersion). Numbers represent pooling of the fingerlings remaining from three replicates during sexing.

Experiment	Temp. (°C) (± sd)	Survival [1,2] (%)	Male	Female	Intersex	Females [2] (%)
Fem. - control	28.90 (± 0.81)	82.44 [b]	148	5	0	3.27 [b]
Fem. - ambient	29.08 (± 0.50)	77.33 [b]	24	130	0	84.42 [a]
Fem. - high	34.79 (± 0.18)	36.67 [a]	5	110	5	91.67 [a]
Probability		P<0.001				P<0.001
Immerse - control	28.53 (± 0.51)	54.00 [a]	40	1	0	2.44 [a]
Immerse. - ambient	28.81 (± 0.5)	54.67 [a]	28	11	0	28.21 [b]
Immerse.- high	34.41 (± 0.09)	65.78 [a]	34	8	0	19.05 [ab]
Probability		P=0.134				P<0.001
						Males [2] (%)
Masc. - control	28.68 (± 0.55)	89.78 [a]	22	88	0	20.0 [b]
Masc. - ambient	28.69 (± 0.57)	83.33 [a]	142	8	0	94.67 [a]
Masc. - high	34.80 (± 0.22)	75.33 [a]	117	2	0	98.32 [a]
Probability		P=0.554				P<0.001
Immerse - control	28.42 (± 0.65)	93.54 [a]	14	47	0	22.95 [a]
Immerse. - ambient	27.96 (± 1.18)	89.66 [a]	19	58	0	24.68 [a]
Immerse. - high	34.43 (± 0.13)	91.20 [a]	9	49	0	15.52 [a]
Probability		P=0.685				P=0.148

[1] - Survival rates were based on counts of fry at the time of removal from aquaria at the end of the treatment
[2] - Mean values within a treatment group with different letter subscript are significantly different (P<0.05)



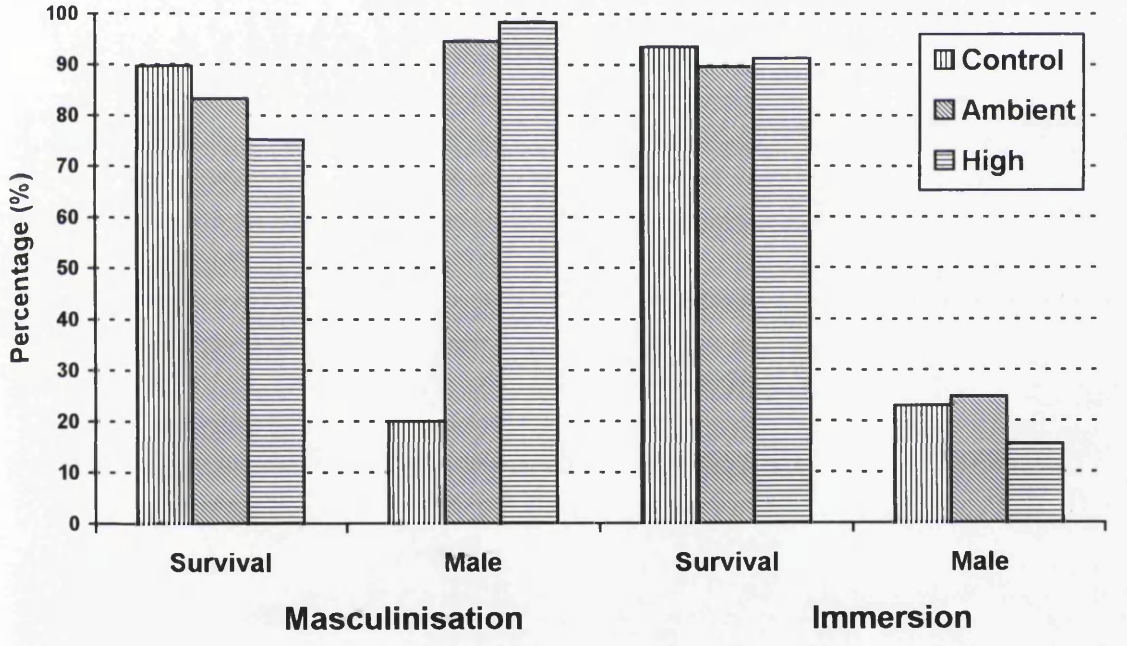


Figure 6.4. Histograms showing the mean percentages of survival and females in feminisation (upper chart), and percentages of survival and males in masculinisation (lower chart) experiments during oral and immersion treatments at high (34°C) and at ambient temperatures. After termination of oral treatments, new batches of the same genotype of fish were reared in the same water and temperatures but received hormone-free food (immersion). Mean values represent the means of three replicates.

6.3.4 Effect of leaching out from hormone-treated feed

With the presumed accumulation of hormone residues during oral administration of treated feeds, an experiment to determine the possible leaching of hormone from hormone treated feed and the effect of leachates on sex ratio was performed. The results of the experiment are presented in Table 6.6. The results demonstrate clearly that the sex ratio of the fry was altered by the addition of hormone treated feed to an empty cage within the same aquarium. This applies to both the diethylstilbestrol and methyltestosterone treated feed, with the effect being greatest for the methyltestosterone treatment (see Figure 6.5).

Although survival was lower in the fry reared in aquaria containing cages to which hormone treated was added, the magnitude of the effect on sex was so great to assume that the difference was due to the effect of differential mortality. As the fry in the cages were prevented from ingesting the hormone treated feed, these results clearly indicate that hormones have leached out of treated feeds and the leachates were still potent and capable of inducing sex reversal.

Table 6.6. Summary of the results of the feminisation and masculinisation experiments aimed at determining the possible leaching of hormone from hormone-treated food and the effect of leachates on sex ratio. Numbers represent pooling of the fingerlings remaining in two replicates at the time of sexing.

Experiment	Hormone dose ($mg.kg^{-1}$ diet)	Survival [1,2] (%)	Male	Female	Intersex	Females [2] (%)
Feminisation	Control	72.33 [b]	51	1	0	1.61 [b]
	1000	45.0 [a]	27	30	0	52.42 [a]
Probability		P=0.021				P=0.016
						Males [2] (%)
Masculinisation	Control	75.0 [a]	11	47	0	16.25 [b]
	40	64.67 [a]	70	2	0	96.99 [a]
Probability		P=0.296				P=0.003

[1] - Survival rates were based on counts of fry at the time of removal from aquaria at the end of the treatment
[2] - Mean values within a treatment group with different letter subscript are significantly different (P<0.05)

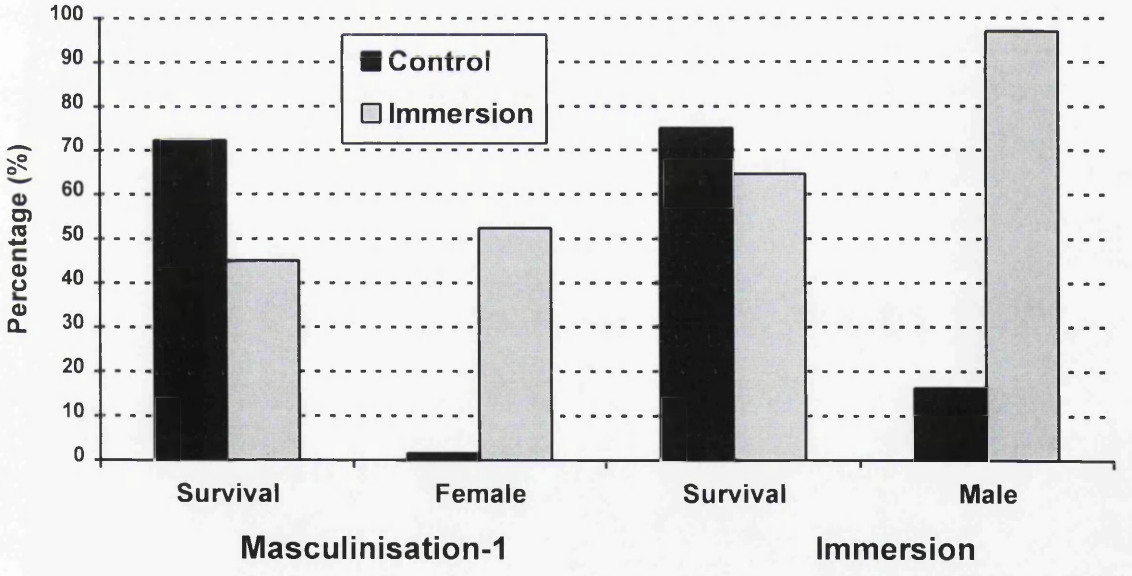


Figure 6.5. Histogram showing the mean percentages of survival and females in feminisation, and percentages of survival and males in masculinisation experiments aimed at determining the possible leaching of hormone from hormone-treated food and the effect of leachates on sex ratio. Mean values were average of two replicates.

6.4 Discussion

6.4.1 Effect of not changing the water during hormone treatment

In previous studies, it has been demonstrated that the hormone ingested by the treated fish does not accumulate in the tissue but is readily excreted, partly via the faeces and through the gall bladder, a few days after termination of oral treatments (Goudie, 1984; Johnstone *et al.*, 1983; Rothbard *et al.*, 1990; Satyanarayana Rao *et al.*, 1990; Curtis *et al.*, 1991). Although the authors only determined the concentration of an administered radiolabelled hormone in the fish body after termination of hormone feeding, it is likely that the treated fish would excrete steroidal metabolites soon after initiation of feeding of the treated feeds. Piferrer and Donaldson (1994) noted that in coho salmon (*Oncorhynchus kisutch*) the fry may be less sensitive to sex steroids compared to alevins because besides having a more developed gonad they are already in the process of sexual differentiation, with little or no remaining yolk to act as a steroid reservoir. This may be the case also in tilapia, during the stage of sexual differentiation the fry have already absorbed all the yolk which is why the ingested hormone are readily excreted. The level of metabolites excreted by the treated fish may be low at the beginning but gradually increase until the hormone concentration in the fish tissue has reached saturation, after which the amount of the excreted metabolites would be roughly equivalent to the ingested hormone.

With the ingested hormone readily excreted, it is likely that the metabolites would then be expected to accumulate in the water used during hormone treatment if this is not changed. Padilla (1997) measured the level of diethylstilbestrol (DES) residues present in the water, starting from initiation of hormone feeding, and observed the level increased gradually as the hormone treatment progressed. However, the hormone residue detected in water (Padilla, 1997) may be composed not only of the metabolites excreted by the treated fish but may also include leachates from uneaten food. The result from experiment 4 clearly demonstrates that hormone is leaching, in a potent form, from uneaten food. Although it is known that most of the commercially available synthetic hormones (i.e. diethylstilbestrol and methyltestosterone) are not readily soluble in water, it is possible that after being dissolved in alcohol, they become more soluble and available in water (Varadaraj and Pandian, 1987).

The sex reversal of the fish reared in water previously used for hormone treatment confirmed the theory that if the water used during hormone treatment is not changed the excreted metabolites would accumulate. Similarly, the result indicates that the accumulated hormone is still potent which corresponds to the observation of Padilla (1997) where the level of hormone residues measured in the water remained high after 15 days of feed withdrawal. Thus it is apparent that the hormone takes a substantial time to degrade in the water.

In earlier studies the phenomenon of iincidental sex reversal of control fish sharing the same water body as treated fish was reported for carp (Gomelsky *et al.*, 1995) and tilapia (Abucay and Mair, in press a). The incidental sex reversal

was thought to be due to hormone metabolites excreted by the treated fish which have affected the control fish found in the same water. However, the sex reversal of the control fish happened during the treatment period. Thus there is the possibility that hormone-treated feed from an adjacent cage or container might have diffused and been ingested by control fish. The present results suggest that the observed incidental sex reversal was due to the accumulated hormone residues in water. The fish were reared in the same water used following hormone treatment and given hormone-free food so it is very likely that the incidental sex reversal was due to the hormone residues present in the water.

The presence of hormone residues in the water appears to have improved the efficiency of sex reversal in those fish that have received lower hormone dose. The sex ratios of the fish which have received higher hormone dose during the initial treatments in experiment 1 were, in general, not significantly different from those that have received lower dose. Gomelsky *et al.* (1995) and Abucay and Mair (in press a) observed in their study that the frequency of sex reversal in control fish was actually higher than in the treated fish. Gomelsky *et al.* (1995) remarked that the higher percentage of inverted males in the control could be due to the fact that the treated fish were subjected to double treatment; the hormone in food and the hormone in the surrounding water could have caused paradoxical sex reversal. In the present study, since the water was not changed, the hormone metabolites excreted by the treated fish and the leachates from uneaten treated food combined with the ingested food may have reached

an effective concentration so that even at lower hormone dose a high percentage of sex reversal has been achieved.

Basavaraja *et al.* (1990) achieved 100% feminisation in *O. mossambicus* treated with diethylstilbestrol at a concentration of 50 $mg.kg^{-1}$ of food in outdoor plastic pools with stagnant water. In that study the plastic pools were cleaned only once per week and fresh water was only added to replenished evaporation losses. It may be possible that hormone residues accumulated in the water and, in combination with ingested hormone, resulted in 100% feminisation. On the other hand, the 25 $mg.kg^{-1}$ concentration failed to induce complete feminisation probably because the hormone concentration was too low and the amount of treated food given was very small due to low stocking density (6.6 $fish.l^{-1}$). It might be possible that the hormone residues in the water in combination with the ingested hormone could not reach a concentration which can affect sex reversal. In a similar study using the same hormone and the same species, Varadaraj (1989) only achieved 100% feminisation at 100 $mg.kg^{-1}$ concentration, with lower doses being ineffective. The treatment was done in aquaria which were cleaned once in two days. The frequency of cleaning might have prevented the accumulation of hormone residues in the water.

Watanabe *et al.* (1993b) treated red tilapia with ethynyltestosterone at 60 $mg.kg^{-1}$ at different feeding durations (7, 14, 21, or 28 days) in tanks supplied with recirculated brackish water. In this study, the fish in terminated treatments remained in the tanks until all treatments were completed. The percentage of males in the different treatments were all very high (94.3 to 98.1%) and not

significantly different and also the percentage of males in the control was high (61.7%). Based on these results, the authors concluded that it is possible to achieve high rates of sex reversal even at a short feeding duration of seven days. However, it might also be possible that the high rate of sex reversal of the fish treated for seven days could have been due to fact that they remained in the tank until the 28th day, receiving water containing hormone residues. Phelps *et al.* (1992) treated *O. niloticus* fry with fluoxymesterone at concentrations ranging from 0.2 to 25 $mg.kg^{-1}$ and methyltestosterone at 60 $mg.kg^{-1}$ diet in small hapas suspended in a 20.4 m^2 outdoor concrete tank. The percentage males of the different treatments were all high (87.3 to 100%) and were not significantly different. The fish being located in the same tank might have caused the production of a high proportion of males in all treatments.

When feeding high hormone dose, there could be the tendency to overdose the fish due to the combined effect of the ingested hormone and the hormone residues in the water. The actual hormone dose received by the fish may be more than the expected level based on the hormone concentration in the treated feed. There are reports which show that high hormone dose could result in lower sex ratio and survival (Obi and Shelton, 1983; Berger and Rothbard, 1987; Varadaraj and Pandian, 1989b). In the present study, most of the sex ratios generally increased with increase in hormone dose. However, the percentage survival, particularly in those fish treated with diethylstilbestrol, have decreased with increase in hormone dose indicating that the toxicity increased as the hormone concentration increased. For the masculinisation experiment, no

effect of the hormone dose on percentage survival was observed. Although it could be likely that hormone residues have accumulated, particularly at 40 $mg.kg^{-1}$ which was the highest dose used, this may not be high enough to reach a toxic level.

McGeachin *et al.* (1987) treated *0. aureus* with high concentrations of ethynyltestosterone (60 to 240 $mg.kg^{-1}$ diet) and methyltestosterone (60 to 120 $mg.kg^{-1}$ diet) and did not observe any significant effect on sex ratio and survival. Moreover, the percentage of males and survival of the methyltestosterone and the percentage survival of ethynyltestosterone treated fish were slightly decreased as the hormone dose increased. However, it should be noted that the hormone treatment in this particular study was done in replicated 38 L capacity aquaria supplied with a continuous flow of well water at 0.51 $l.min^{-1}$, so that the water was totally replenished approximately every 76 minutes, and thus hormone residues could not accumulate.

It seems probable that the excretion of hormone metabolites and leaching of hormones can be exploited to optimize further sex reversal procedures. The presence of hormone residues in the water would be likely to enhance the effect of hormone administered orally. It had been noted that the potency of metabolites, supposed to be soluble from surrounding water is stronger than that of methyltestosterone administered orally (Gomelsky *et al.*, 1995). Gomelsky *et al.* (1995) suggest that the methyltestosterone consumed by fish may be transformed in the liver into soluble, active metabolites and excreted with the bile. Pelissero and Sumpter (1992) mentioned that when steroid or steroids-like

substances are conjugated, their solubility in aqueous solutions is increased. In a study which monitored the elimination of radiolabelled-hormone (^{3}H-MT) after feeding to Nile tilapia fry, Curtis *et al.* (1991) reported that gallbladder bile appeared to be a major site of ^{3}H-MT disposition and most residues were polar metabolites. Gomelsky *et al.* (1995) cited an earlier work which added bile from common carp that were fed a diet containing methyltestosterone at a high dose to water in an aquarium containing guppy (*Poecilia reticulata*) fry and obtained development of secondary male sex characteristics in all fish, including genotypic females. Although it is not known yet whether the excreted metabolites are still chemically similar as the parent compound but the capacity to alter sex seems to contradict Johnstone's *et al.* hypothesis (1983) that the metabolites of 17α-methyltestosterone would show little or no physiological activity.

The seemingly higher potency of metabolites, supposed to be soluble in the water, could be due to the fact these can be absorbed easily by the fish through gills to the blood stream. The principle in sex reversal is to raise the level of steroid in the bloodstream of sexually undifferentiated fry before they become physically male or female. Piferrer and Donaldson (1994) mentioned that the gills are viewed as the major route for the uptake and excretion of the drugs from the main circulation system when juvenile or adult fish received immersion treatments. Hurk and Lambert (1982) reported that in rainbow trout, *Oncorhynchus mykiss*, progesterone (P4) does not influence sex ratio when added to food contrary to its feminising effect when suspended in water. The authors hypothesized that P4 taken in by the alimentary tract is possibly more

easily subjected to degradation by the liver than when taken up by the gills. In *O. mossambicus*, Varadaraj and Pandian (1987) were able to achieve 100% masculinisation by rearing fry in water containing 17α-methyl-5-androsten-3β-17β-diol at a concentration of 5 $\mu g.l^{-1}$. However, the failure to achieve successful sex reversal in short and long term immersion treatment in other studies (Eckstein and Spira, 1965; Varadaraj and Pandian, 1987; Baker *et al.*, 1988; Rosenstein and Hulata, 1992; Piferrer *et al.*, 1994a; Lone and Ridha, 1993; Gilling, 1994) could be due to the treatment duration and the hormone dose used. As mentioned earlier, tilapia fry do not have the capacity to accumulate hormone in the body thus immersion outside the labile period is likely to have no effect. Similarly, since the hormone is absorbed by the gills directly to the blood stream, the hormone, especially when applied at a high dose, is likely to be more toxic to the fish than when administered orally. The ingested hormones during oral administration still undergo the usual processes of digestion and assimilation where a great proportion is excreted as metabolites, reducing the actual concentration reaching the blood stream as compared to the hormone absorbed by the gills.

6.4.2 Effect of increasing the temperature during hormone treatment

Increasing the temperature above the normal level during hormone treatment has also been observed to improve the efficiency of sex reversal. The result conforms to earlier observations of Nagy *et al.* (1981) on oral administration of methyltestosterone in common carp. The increase in efficiency of sex reversal could be due to the higher metabolic rate and feeding rates of the treated fish at higher temperature (Watanabe *et al.*, 1993a) and consequent increase in the intake of hormone (Shelton *et al.*, 1981). Similarly, when comparing the sex ratios of the controls at ambient and at high temperatures, there was a small proportion of sex reversal affected by the increase in temperature. The effect of high temperature on sex ratio has been demonstrated in the work described in Chapter three of this thesis. The increased rate of sex reversal in both feminisation and masculinisation treatment could have been partly due to the small effect of high temperature on sex ratio.

The present results differ from the results of Varadaraj *et al.* (1994) on sex reversal of *O. mossambicus*. The difference could be due to two possible factors. First, it could be that the actual hormone concentration in the treated feed used was much lower than the expected. In our laboratory in the Philippines, hormone is usually stored in the fridge at 4°C. However, the treated feeds were stored at room temperature and exposed to light and air. Varadaraj *et al.* (1994) have demonstrated that storing either hormones or the treated feeds at room temperature and exposed to light and air could result in degradation or

decreased in the potency of the hormone. The possible higher potency of the treated feeds used by Varadaraj *et al.* (1994) could have resulted in paradoxical feminisation at higher temperature (discussed further later in this chapter). Second, it could be possible that *O. mossambicus* are more sensitive to paradoxical feminisation compared to *O. niloticus,* a phenomenon for which there is little evidence in this species.

The increase in temperature, however, appears to promote faster degradation of the hormone compared to that at ambient temperature. In Experiment 3, the percentage sex reversal during the rearing of fry in water previously used for hormone treatment in feminisation experiment at high temperature, was higher than that at ambient temperature. Considering that both treatments have received the same hormone dose, it could be possible that the hormone (diethylstilbestrol) degraded faster at higher temperature. Whilst for the masculinisation treatment, the percentage of males of the fish reared in water previously used for hormone treatment at high temperature was lower than that of the control. It appears that the accumulated hormone in the water at high temperature did not degrade but were transformed into estrogen. This phenomenon was first observed in immersion treatment of Masculinisation-1 in Experiment 1, although, unlike Experiment 3, the treatment was performed at ambient temperature. The observed phenomenon preceded an oral treatment where a high percentage of masculinisation was achieved. It is possible that the treated fish have excreted a large amount of metabolites, particularly under higher temperature, as in the case of experiment 3. It should be noted that the

fish were fed *ad libitum*. The excreted metabolites might have been transformed into estrogen through the action of aromatase (Nakamura, 1975; Goudie *et al.*, 1983; Davis *et al.*, 1990; Piferrer and Donaldson, 1991) resulting in paradoxical feminisation instead of masculinisation of the fish. However, it is not known whether the transformation process took place before or after the metabolites were excreted.

Among many androgen hormones, methyltestosterone is known to be easily aromatised into estrogen (Piferrer and Donaldson, 1991). The process of aromatisation is enhanced when the hormone is administered at high concentration and for prolonged feeding durations (Nakamura, 1975; also see Hunter and Donaldson, 1983). In the present study, an unexpected sex ratios were observed following an initial hormone treatment where a high percentage of sex reversal was achieved. It is possible that a high level of hormone residues has accumulated in the system inducing aromatisation and consequently paradoxical feminisation. At higher temperature, the metabolic rate and appetite of the treated fish is increased which consequently also increases the amount of feed being ingested and excreted. Similarly, the conversion of androgen to estrogen is catalyzed by an enzyme aromatase (Piferrer *et al.*, 1994). It is generally known that the activity of this enzyme is increased with the increase in temperature.

6.5 Conclusion

This study has demonstrated that hormone residues accumulate in the water if it is not changed. The residues may be due to the excreted metabolites of the treated fish and/or the leachates from uneaten food. The last experiment demonstrated that leachates from uneaten food can effect sex reversal of fish held in the same water body. The hormone residues are still potent and capable of effecting sex reversal. The presence and the difference in concentration of hormone residues in rearing water apparently may have caused the inconsistency of observed sex ratio in other sex reversal studies using the same hormone and hormone dose.

By not changing the water during treatment, the hormone residues could be exploited to improve the efficiency of sex reversal. The efficiency can be further improved by increasing the temperature, particularly in feminisation treatment. However, excessive hormone residues in water, particularly at high temperature, tend to enhance paradoxical feminisation in masculinisation treatments.

CHAPTER SEVEN

GENERAL DISCUSSION

7.1 Introduction

In this thesis the results of a number of studies in major aspects including sex determination, growth improvement and sex reversal in Nile tilapia were presented. The studies focused on various relevant issues pertaining to the further improvement of the YY male technology. In this chapter, the results of the different studies will be highlighted and their implications are discussed.

7.2 Highlights of the Results and their Implications

7.2.1 Genetic and environmental factors affecting sex ratio

A broad understanding on how sex is determined is of considerable interest in tilapia. This is particularly important because of the obvious sexual dimorphism in growth where males grow faster and larger than females. Furthermore the culture of mixed-sex populations, particularly in earthen ponds, usually results in precocious reproduction and overcrowding which eventually has a negative impact on the growth of the stocked fish. While growing all-male populations is not necessarily a requirement, it assures higher chances of a better yield and return on investment.

In recent years, several approaches have been developed to control excessive reproduction and/or to produce monosex populations. However, each

of the approaches has its limitations (Mair and Little, 1991). Recently, a model for the mass production of monosex populations in *Oreochromis niloticus*, known as YY male technology, has been proposed (Mair *et al.* 1988, Scott *et al.*, 1989, Mair and Little, 1991). A project was initiated to develop and verify this technology and results have shown the technology to be feasible (Mair *et al.*, 1997a).

Males with YY genotypes, which when cross to a normal XX females are expected to produce all-male (XY) progeny, have been developed. In a series of progeny tests in within strain (Egypt-Swansea) crosses of XX♀ x YY♂, male proportions ranging from 71.6 to 100% with a mean of 97% male have been observed (Mair *et al.*, 1992). The results show that YY males are capable siring all-male progeny, although some YY males produce small proportions of females. While the average proportion of males is high, this could change (either increase or decrease) if one happens to use those YY males that produces high or lower proportion of males in their progeny. It has been demonstrated that even a small percentage of mature females (≥5%) is capable of generating excessive recruitment and can significantly reduce the growth of both males and females in a populations (Lovshin *et al.*, 1990; Mair and van Dam, 1996). In order to fully harness the potential of the YY male technology very high proportions of male progeny should be produced, preferably 100% male.

The small number of unexpected males or females in the progeny of putatively genetically monosex populations has been thought to be due to autosomal and/or environmental sex modifying factors (Penman *et al.*, 1987;

Scott *et al.*, 1989; Mair *et al.*, 1991a). The results of the studies presented in Chapter three tend to confirm these views. High temperature (36°C) was observed to affect sex ratio in either direction. The sensitivity to high temperature of the progeny used for the experiment is thought to be due to autosomal sex-modifying genes and the difference in sensitivity is believe to be due to differences in the proportions of male and female determining genes among individual fish.

Assuming that the hypothesis that autosomal genes exist and play a major role in causing the observed unexpected sex ratio is correct, the question now is what influences or cause the disproportionate representation of these genes in different individuals? In Chapter three, it was noted that the all-female and all YY male progeny used in the experiments described were produced from crosses of XX♀ x XX♂ and YY♀ x YY♂. During the generation of these XX males and YY females, there might have been an unconscious selection for either male or female autosomal sex-modifying genes (see Discussion in Chapter three). For example, a genetic female (XX genotype) which carries a high proportion of "male" autosomal genes might, in an extreme condition such as high temperature, have the autosomal effects intensified and override the sex chromosomes factors resulting in the development of male rather than a female phenotypic sex. If all-female and all YY male progeny are continuously produced in this way following the same process in each generation, the tendency will be towards an increase or decrease in the proportion of male and/or female genes. This may eventually produce a situation where sex ratio is behaving as a

continuous rather than discrete trait, which would be characteristic of a polygenic sex determining mechanism model, as Lester *et al.* (1989) have suggested exists in *Oreochromis* species.

While the results of the experiments presented in Chapter three were observed in controlled conditions (constant temperature), the effect of high temperature on sex ratio might also be occurring in other strains reared in less controlled conditions. For example, in the Egypt-AIT strain the sex ratios were observed to be very variable and it has been hypothesised based on results seen in this strain that sex determination may be evolving from monofactorial to a polygenic sex determining mechanism (Tuan, 1997). In Table 4.9 in Chapter four, it can be seen that the sex ratios of YY males in crosses with females of the Egypt-AIT strain were more variable than those with Egypt-BFAR females. The source of females is the likely reason for the difference in variation of sex ratio as the YY males were of the same strain. Differences in sample size could also be a factor. These two strains are known to be of similar genetic background, both being derived from the same introduction of *O. niloticus* to Thailand. The seemingly more stable sex ratio in the Egypt-BFAR strain suggests that at one time Egypt-AIT may have also exhibited the same stability.

Tuan (1997) noted that the Egypt-AIT strain is already considered as an isolated stock of the Jitralada strain having been reproduced intensively in a hapa-in-pond breeding system for at least ten generations. Suspended hapas are usually submerged to a depth of just 50 cm. In this rearing system, the fish have no option but being subjected to what ever condition exist in that limited

space. Based on personal observations (under Philippine conditions), at noon time, particularly during the summer time, the temperature near the water surface commonly reaches 37°C. During the period covering the ten generations it may be possible that temperature has partly influence the development of sex in that strain. The temperature effect might have intensify the disproportion of the loading of autosomal sex modifying genes among individual fish.

This hypothesis may be supported by the result of an evaluation of sex ratio of the progeny of YY males in Egypt-Swansea strain crossed with females of the Egypt-AIT strain reared in hapas suspended in earthen ponds. Tuan (1997) observed a negative correlation between the proportion of males and temperature. In spite of the fry being reared in normal conditions, with temperature only occasionally rising above normal levels during noon time, a significant effect of the increase in temperature on sex ratio was observed.

While other potential factors affecting sex ratio are not being discounted here, the results of the present study provide evidence of the effect of temperature on sex ratio and a basis for a possible influence on the evolution of sex determining mechanisms. With the observation that the intensity of temperature effect appears to be higher in a more inbred population (see section 3.3.3 in Chapter three), the evolution of sex determining mechanism in an inbred population is likely to be faster or may be lower.

When investigating sex determining mechanisms based on the observed sex ratio of test fish, the nature of the culture environment during period of sexual

differentiation and the degree of inbreeding in the population being investigated should be given prior consideration. Likewise, the origin and history of the strain or species, how the population has been maintained and the method of generation of future broodstock should also be considered. In the event of the results of the present study, it may well be that environment and genetic quality have played a vital role in the populations investigated in other studies but this has not been given much attention.

7.2.2 Genetics and environmental factors affecting growth

The result of the growth evaluation experiment presented in Chapter four showed that the hybrids did not grow better than the larger parent, although they did grow better than the smaller parental cross (Egypt-Swansea strain). As has already been mentioned the Egypt-Swansea strain is considered to be somewhat inbred due to having been through a number of genetic bottlenecks (Mair *et al.*, 1995). Moreover, the decrease in genetic variability or inbreeding depression of the YY males developed in that strain is likely to be higher because mating of closely related individuals cannot be avoided during the development process of the technology.

The negative effect of inbreeding depression has been mentioned in previous chapters. The higher growth of hybrid GMT compared to the Egypt-Swansea parental strain shows that the negative effect of inbreeding on growth

may be regained through crossbreeding. However, the lower growth of the hybrids compared to the larger parent (Egypt-ICLARM strain) suggest that crossbreeding does not necessarily improve growth but simply regains what has been lost. Although the Egypt-ICLARM YY males used may also be considered inbred for having undergone similar process as the Egypt-Swansea strain, this did not influence a higher growth for the hybrids over the former strain.

Wohlfarth (1993) noted that the advantage of crossbreds over purebreds is apparent when no unusually good pure line is available. For the Egypt-Swansea YY males, although the on-station (Mair *et al.*, 1995) and on-farm (Mair *et al.*, 1997b) growth evaluation trials of pure Egypt-Swansea strain GMT showed satisfactory results, crossbreeding could further improve the growth. For the Egypt-ICLARM YY males, growing purebred GMT may be a better approach, although, it may be possible to produce a larger hybrids if cross to a genetically better and faster growing strain. However, the only limitation in using crossbreeding is that the potential improvement in growth of the hybrids is reached during the first generation and if no further testing and selection is practiced there will be no more progress (Gjedrem, 1985). To further improve the growth of hybrids, a combined crossbreeding and selection approach may be desired (see Figure 7.1). In this approach, selective breeding is performed to improve the growth performance of the parents and these qualities are combine to produce a superior hybrids (see Gjedrem, 1985).

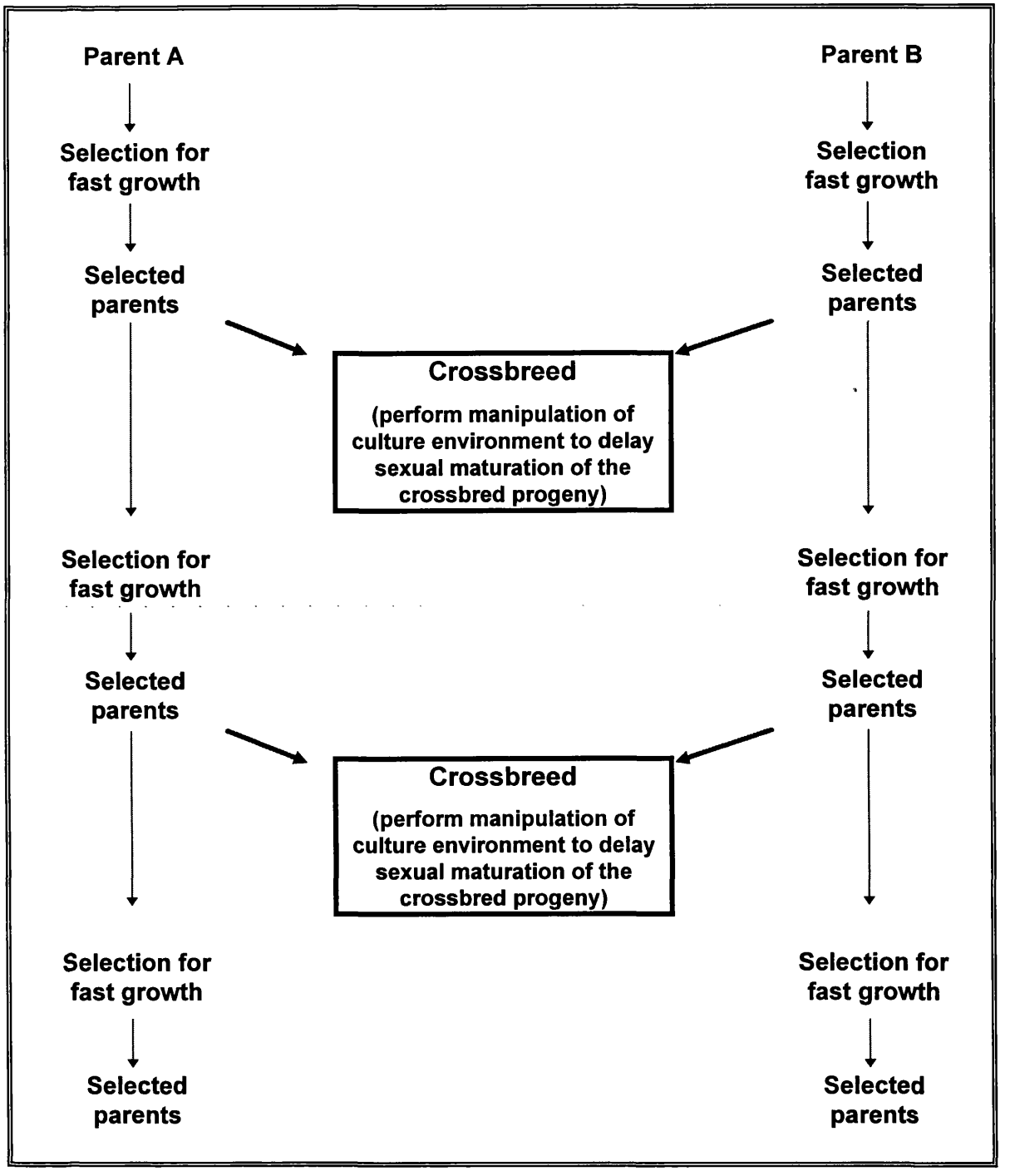


Figure 7.1. Schematic diagram of a reciprocal recurrent selection (RRS) for growth rate. Two strains previously identified to produce heterotic hybrids are crossbreed. To further improve the quality of the hybrids, selection for fast growth is performed separately on both parental strains (modified from Tave, 1986).

To further exploit the potential of growing all-male progeny, the nature of the culture environment and the behaviour of the stocked fish taking place during culture should also be considered and their implications to growth understood. The results of the growth evaluation experiments presented in Chapter five demonstrate that during the later stage of the life cycle, later maturing individuals appear to have a faster growth. Sexually mature males tend to be more aggressive for the purpose of reproduction and in their attempts to dominate other fish within the culture system. Feeding is usually sacrificed in favour of aggression and reproduction and a great of deal energy is being expended to sustain these activities, resulting in reduced growth. Early sexual maturation may be influenced by the presence of the opposite sex and also if the culture environment is conducive for reproduction.

In Chapter five, it is shown that the growth of females in an all-female environment is lower than that of males in an all-male environment but is higher than that of males in mixed-sex environment. A supposed lower social aggression among females may have been a factor in their better growth. However, there are severe limitations in the culture of all-female populations compared to all-males. The accidental mixing of very small numbers of males would be enough to excite a great majority of the females to spawn with resulting suppression of growth. On the other hand, in all male populations, contamination by a small proportion of females would not result in sufficient reproduction to cause overpopulation. However, the presence of a few females may be enough to influence the males to become sexually mature earlier and subsequently

induce further aggression. Hence, culturing of fast growing, 100% late maturing males, should be the objective for maximizing growth.

When evaluating growth of different strains or groups of fish, the difference in the degree of sexual maturation at a certain life stage should be given prior considerations. The results presented in Chapter five showed that MST-MT had a higher growth compared to respective control MST-CT and all the other groups. The better growth MST-MT is unlikely due to better genetic quality but rather due to a delayed sexual maturity. Wohlfarth (1993) noted that in common carp, heterosis is more emphasized among younger and smaller fish but this slowly disappeared as the fish grow bigger (>1 kg in weight). It is possible that by this later stage, the fish were already sexually matured and start exhibiting reproduction and aggression. McGinty (1985) remarked that such social interactions are primarily environmental in origin and their effects could confound growth evaluation experiments. The results of a one year generation of selection for growth in *O. niloticus* (Lester *et al.*, 1989) showed that the offspring of fish selected as adults have growth rates exceeding the unselected population by 15% for females and 10% males. The higher response to selection in females could be due to fact that they do not exhibit intense social interaction as males do so that the difference in growth among females is due more to genetic than environmental effects. Moreover, Hershberger (1993) noted that the somewhat lower heritabilities observed in fish compared to terrestrial animals could reflect their nature of being poikilothermic and a consequent responsiveness to external environmental factors. The author further noted that

habitat and husbandry changes may have more effects on fish than has been observed in terrestrial animals.

7.2.3 Factors affecting sex reversal and the potential effect of hormone residues

In recent years, a great deal of experimental works has been focused on the use of sex reversal to produce monosex progeny for culture (see reviews by Hunter and Donaldson, 1983 and Pandian and Sheela, 1995). The growing interest in the use of sex reversal may be attributed to the simplicity and the apparent relative ease of application of the technique. However, the perceived advantage of sex reversal has not been fully realised because of the failure to produce consistently high percentages of sex reversal (Mair and Little, 1991). The failure to achieve the desired result may be due to a limited understanding on the processes that are taking place during the hormone treatment period.

Due to failure to produce high proportions of sex reversal, there has been the tendency to increase hormone dose use (see Introduction in Chapter six). Yet, in some cases the results are not significantly better or even worse than those of the lower dose. The experiments described in Chapter six were designed to determined factors affecting sex reversal. Previous studies showed that ingested hormones are readily excreted (Johnstone *et al.*, 1983; Goudie, 1984; Rothbard *et al.*, 1990; Satyanarayana Rao *et al.*, 1990; Curtis *et al.*, 1991). In addition, in the research presented in Chapter six was been demonstrated that

hormone does leach from hormone-treated feed. The hormone residues excreted by the treated fish and the leachates from uneaten hormone-treated feed would accumulate if the water it is seldom or never changed. These hormone residues are still capable of effecting sex reversal. In addition, it has also been observed that temperatures higher than ambient (34 to 36°C) appear to increase the efficiency of sex reversal. The observed increase in efficiency may be partly due to the effect of high temperature on sex ratio (see Chapter three). High temperatures also increase the metabolic rate of the fish and consequently their appetite resulting in a higher intake of hormone (Shelton *et al.*, 1981). High temperature may also increase the potency of the hormone (Lester *et al.*, 1989).

During periods of hormone treatment, the frequency of water change may be a major factor in the inconsistency of results common among sex reversal studies. Similarly the difference in water temperature of the treatment facilities may also be factor. A difference in the intake of hormone and consequently the amount of excreted metabolites may result in difference in the amount of accumulated hormone in the surrounding water. In addition high temperature appears to enhance the degradation of hormone, particularly diethylstilbestrol. For methyltestosterone, high temperature appeared to enhance the aromatization of the hormone to estrogen which may result to a phenomenon of paradoxical feminisation.

The current difficulty to achieve successful sex reversal using the immersion approach make oral application still the most effective method for sex

reversal. The excreted metabolites from the treated fish and the leachates from hormone-treated feed could be exploited to further improve the efficiency of sex reversal. By way of systematic management such as by not changing or recirculating the water being used during treatment, the hormone metabolites and leachates can accumulate to further improve the success of sex reversal. Similarly, through this approach, it should also be possible to reduce the hormone dose used. Furthermore, the problem of unequal access to the feed due to high stocking density and hierarchical interactions, which may result in lower success of sex reversal (Varadaraj and Pandian, 1987), could be reduced as the hormone is present in solution. Bocek *et al.* (1992) did not find any difference in the percentage of males between small (96.6%) and large fish (97.6%) in a population of methyltestosterone-treated fish (*O. niloticus*) in hapas held in concrete tank with almost stagnant water.

In commercial hatcheries, the apparent excretion and leaching of hormones may pose a threat to the environment (Johnstone *et al.*, 1983), particularly those in the developing countries which use methyltestosterone extensively to produce all-male progeny for stocking. Discharging water used for hormone treatment, which may contain large amounts of hormone residues into rivers or canals may effect sex reversal or render toxicity to the fish found in the surrounding water. Howell *et al.* (1980) reported that the effluent discharge of a paper mill caused phenotypic masculinisation in the normally gonochoristic mosquito fish *Gambusia affinis,* presumably due to the presence of androgenic type compounds in the effluent. Macintosh *et al.* (1988) observed that, at AIT in

Thailand, the young *O. niloticus* brood fish raised in a water recirculating system receiving waste water containing low level of hormone from MT-treatment tanks exhibited a duct blockage syndrome which may have been due to an incomplete sex reversal. Moreover, discharging water containing large amounts of diethylstilbestrol residues in bodies of water, especially where this might be used for domestic purposes, may also pose a threat to human health (Singh and Lal, 1989). Diethylstilbestrol is known to be carcinogenic, particularly to women.

7.3 Recommendations and Suggestions for Future Studies

7.3.1 Sex determination

The results of the experiments described in Chapter three provide evidence for the effect of temperature on sex ratio. However, the experimental work was all performed in controlled conditions with only minimal temperature fluctuation. It may be interesting to know if the same genotype of fish tested would show response to higher but fluctuating temperatures that might prevail in certain outdoor conditions.

Similarly, the data presented in Table 3.3 in Chapter three show that the putatively all-female progeny (GFT) reared at low temperature appeared to have responded to a decrease in temperature during sex differentiation with higher proportions of males present. However, a differential in mortality could not be

discounted as a possible reason for the observed decrease in percentage of males at low temperature. The putatively all-male and all YY male progeny, which have a relatively higher percentage of survival at normal temperature, did not show response to decreased temperature. Further studies should be performed to investigate the effect of low temperature on sex ratio. If it was found that sex ratio only responds to increases in temperature, then it could be suggested that all fish, particularly those intended to be use as broodstock, should be reared at low temperature in controlled conditions at an early age encompassing the stage of sex differentiation.

The observed sensitivity of the different fish genotypes tested to high temperature may be due to differential loading of autosomal sex modifying genes which, in turn, may have been caused by an unconscious selection for male or female genes. It would be more meaningful if an empirical data is generated to prove the relevance of this hypothesis. In addition, experiments should also be conducted to determine if different genotypes of fish differ in sensitivity to hormone treatment for sex reversal as mentioned earlier based on the results of the studies of other workers.

The level of inbreeding has also been thought to have an effect on the sensitivity of sex differentiation in the tested fish to high temperature. The purebred YY males (Egypt-Swansea) were observed to be more sensitive to high temperature than the crossbred YY males (Egypt-Swansea YY♀ x Egypt-ICLARM YY♂). However, a strain effect could not be discounted as a possible reason, the crossbreds being generated from mating of two different strains. It

may be possible that the Egypt-ICLARM strain is more tolerant to high temperature than the Egypt-Swansea strain. Generating an inbred line by sib-mating in both the Egypt-Swansea and Egypt-ICLARM strains and comparing the sensitivity to high temperature of the inbred progeny with those within strain randombred or crossbred YY male progeny will certainly elucidate the validity of this hypothesis.

Mbahinzireki and Dabrowski (1997) observed that, in *O. niloticus*, fry reared at high temperature (35 ±1°C) during sex differentiation stage, fed more vigorously and showed faster growth compared to those fry reared at control temperature (22 ±1°C), when they were grown as juvenile fish (12 ±2.7g) at different diets in recirculation systems. The faster growth of the fish reared in high temperature has also been noticed in some of the experiments presented in Chapter three although no systematic data was collected. Although, it may be possible that the apparent faster growth is due to higher percentage of males of fish reared at high temperature. However, it would be interesting to know whether being reared at higher temperature during early stages, when the important systems of the body are developing, causes the fish to develop a faster and more efficient assimilation process even when subsequently grown in normal temperature conditions. In the light of an attempt to improve further the growth performance of GMT this may be a very interesting topic for further research.

7.3.2 Growth improvement

Presently, the improvement of growth performance in tilapia, particularly *O. niloticus* has placed too much emphasis on the genetic manipulation approach. While the significant contribution cannot discounted but it should be borne in mind that the quality of the culture environment also plays an important role. Based from the results of the experiments described in Chapter five it appears that a delay in sexual maturation would improve the growth of the fish further and also produce a higher dressing and fillet yield. The development of a systematic culture approach that would prevent or delay sexual maturation will increase further the potential yield of culturing genetically improved fish.

7.3.3 Sex reversal

A delay in sexual maturity or permanent sterility which result in no reproduction appears to improve the growth of the fish further. It would be more advantageous if techniques in sex reversal should not only be focused on the production of all-male progeny but also on the possibility of rendering the treated fish permanently sterile.

The apparent accumulation of hormone residues excreted by the hormone-treated fish and/or leachates from hormone-treated feed, and the capacity to effect sex reversal even at termination of hormone treatment, has only been evaluated using methyltestosterone and diethylstilbestrol hormones. It

would be interesting to know if similar finding can be observed using other available synthetic and natural hormones.

Finally, it would be very interest to know if the exploitation of the factors described to improve the efficiency of sex reversal is proven to be also applicable in condition in commercial hatcheries. It is only this way wherein the significant contribution of the research on this aspect in this thesis could be measured.

REFERENCES

Abban, E.K., 1988. Taxonomy and biochemical genetics of some African freshwater fish species. University of Wales, Wales. *Ph.D. Thesis.*

Abella, T.A., 1990. Within family selection for growth rate with rotation mating in *Oreochromis niloticus. In* R. Hirano and I. Hanyu (eds.) The Second Asian Fisheries Forum, 991 p. Asian Fisheries Society, Manila, Philippines.

Abella, T.A., 1994. Genetical studies on the inheritance of selected traits in *Oreochromis niloticus* and red tilapia. University of Wales Swansea, Wales. *Ph.D. Thesis.*

Abucay, J.S., 1996. Maternal strain effects on the sex ratio, growth and survival of the progenies of YY males in the *Oreochromis niloticus* L. Central Luzon State University, Nueva Ecija, Philippines. *M.Sc. Thesis.*

Abucay, J.S., 1997. The YY male technology and its products, pp. 14-26. *In* G.C. Mair and T.A. Abella (eds.) Technoguide on the Production of Genetically Male Tilapia (GMT). Freshwater Aquaculture Center, Central Luzon State University, Philippines.

Abucay, J.S. and Mair, G.C., in press a. Sex reversal in tilapia: effect of hormone treatment in closed water system. *Aquaculture Research.*

Abucay, J.S. and Mair, G.C., in press b. Methods for the identification of males with a YY genotype in *Oreochromis niloticus* L. Proceedings of the Second ASEAN-EEC Aquaculture Development Coordination Programme (AADCP) International Workshops on Genetics in Aquaculture and Fisheries Management, 7-11 November 1994, Phuket, Thailand.

Alvendia-Casauay, A. and and Cariño, V.S., 1988. Gonadal sex differentiation in *Oreochromis niloticus*, p. 121-124. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings **15**, 623 p. Department of Fisheries, Bangkok, Thailand and International Center for Living Aquatic Resources Management, Manila, Philippines.

Anonymous, 1993. Tilapia beats its critics in the US, pp. 18-19. *Fish Farmer*, September/October 1993.

Ayles, G.B. and Baker, R.F., 1983. Genetic differences in growth and survival between strains and hybrids of rainbow trout (*Salmo gairdneri*) stocked in aquaculture lakes in the Canadian prairies. *Aquaculture*, **33**: 269-280.

Avault, J.W. and Shell, E.W., 1968. Preliminary studies with the hybrid tilapia, *Tilapia nilotica* and *Tilapia mossambica*. *FAO Fish Rep.*, **44**(4): 237-242.

Avtalion, R.R. and Hammerman, I.S., 1978. Sex determination in *Sarotherodon* (Tilapia) 1. Introduction to a theory of autosomal influence. *The Israeli Journal of Aquaculture - Bamidgeh*, **30**: 110-115.

Babiker, M.M. and Ibrahim, H., 1979. Studies on the biology of reproduction in the cichlid *Tilapia nilotica* (L.): gonadal maturation and fecundity. *J. Fish Biol.*, **14**: 437-448.

Baker, I.J., Solar, I.I. and Donaldson, E.M., 1988. Masculinization of chinook salmon (*Oncorhynchus tshawytscha*) by immersion treatments using 17α-methyltestosterone around the time of hatching. *Aquaculture*, **72**: 359-367.

Bakos, J. and Gorda, S., 1995. Genetic improvement of common carp strains using intrastrain hybridization. *Aquaculture*, **129**: 183-186.

Balarin, J.D. and Hatton, J. P., 1979. Tilapia: a guide to their biology and culture in Africa. University of Stirling, Scotland.

Balarin, J.D. and Haller, R.D., 1983. Commercial tank culture of tilapia, pp. 473-483. *In* L. Fishelson and Z. Yaron (eds.) Proceedings of the International Symposium on Tilapia in Aquaculture, May 1983, Tel Aviv University, Nazareth, Israel.

Bardach, J.E., Ryther, J.H. and McLarney, W.O., 1972. Aquaculture: the farming and husbandry of freshwater and marine organisms. Wiley-Interscience, a division of John Wiley and Sons, Inc.

Baroiller, J.-F. and Jalabert, B., 1989. Contribution of research in reproductive physiology to the culture tilapias. *Aquat. Living Resour.*, **2**: 105-116.

Baroiller, J.-F., Chourrout, D., Fostier, A. and Jalabert, B., 1995. Temperature and sex chromosomes govern sex-ratios of the mouthbrooding cichlids fish *Oreochromis niloticus. The Journal of Experimental Zoology*, **272**.

Baroiller, J.-F., 1996. Significant proportions of unexpected males in progenies from single pair matings with sibling sex-reversed males in *Oreochromis niloticus*. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D.Pauly (eds.) Third International Symposium in Aquaculture. ICLARM Conf. Proc. **41**, 575 p. International Center for Living Aquatic Resources Management, Manila, Philippines.

Baroiller, J.-F., Fostier, A., Cauty, C., Rognon, X. and Jalabert, B., 1996. Effects of high rearing temperature on the sex ratio of progeny from sex-reversed males of *Orechromis niloticus. In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D.Pauly (eds.) Third International Symposium in Aquaculture. ICLARM Conf. Proc. **41**, 575 p. International Center for Living Aquatic Resources Management, Manila, Philippines.

Bartolome, Z.P., 1997. Extensive and semi-intensive pond based tilapia hatchery production systems, pp. 52-57. *In* G.C. Mair and T.A. Abella (eds.) Technoguide on the Production of Genetically Male Tilapia (GMT). Freshwater Aquaculture Center, Central Luzon State University, Philippines.

Basavaraja, N., Srikanth, G.K., Nanadeesha, M.C. and Varghese, T.J., 1989. Impact of testosterone-incorporated diets on growth, body composition and organoleptic quality of the common carp, *Cyprinus carpio* (Linnaeus). *J. Aqua. Trop.*, **4**:21-28.

Basavaraja, N., Nandeesha, M.C., Varghese, T.J., Keshavanath, P. and Srikanth, G.K., 1990. Induction of sex reversal in *Oreochromis mossambicus* by diethylstilbestrol. *J. Appl. Ichthyol.*, **6**: 46-50.

Basavaraju, Y., Keshavappa, G.Y., Seenappa, D., Devaraj, K.V. and Mohire, K.V., 1990. Growth of *Labeo rohita* x *Labeo fimbriatus* hybrid in comparison with parental species. *In* Hirano, R. and I. Hanyu (eds.) The second Asian Fisheries Forum, 991 p. Asian Fisheries Society, Manila, Philippines.

Bautista, A.M., Carlos, M.H., Acosta, B.O. and Valera, P.B., 1988. Cage culture of *Oreochromis aureus* and *O. niloticus* hybrid in Laguna Lake, Philippines. *Journal of Aquaculture in the Tropics*, **3**(2).

Beamish, F.W.H., 1993. Environmental sex determination in Southern Brook Lamprey, *Ichthyomyzon gagei. Can. J. Fish. Aquat. Sci.*, **50**:1299-1307.

Behrends, L.L. and Smitherman, R.O., 1984. Development of a cold-tolerant population of red tilapia through introgressive hybridization. *J. World Maric. Soc.*, **15**: 172-178.

Behrends, L.L., Kingsley, J.B. and Price, A.H., 1986. Performance of two red tilapia hybrids in polyculture with channel catfish and freshwater prawn. *Aquaculture*, **57**: 360-361.

Berger, A. and Rothbard, S., 1987. Androgen induced sex reversal of red tilapia fry stocked in cages within pond. *The Israeli Journal of Aquaculture - Bamidgeh*, **39**: 49-57.

Bimbao, M.P. and Ahmed, M., 1990. Market potential of tilapia in the Philippines: an assessment, pp. 000-000. *In* A.E. Eknath, B.O. Acosta and R.S.V. Pullin (eds.) Proceedings on Present Progress, Future Directions and Needs in Fish Genetics Research in Asia, Pacific and Africa. ICLARM Conference Proceedings No. **38**. International Center for Living Aquatic Resources Management, Manila, Philippines.

Bocek, A., Phelps, R.P. and Popma, T.J., 1992. Effect of feeding frequency on sex reversal and on growth of Nile tilapia, *Oreochromis niloticus*. *Journal of Applied Aquaculture*, **1**(3): 97-103.

Bolivar, R.B., Eknath, E.A., Bolivar, H.L. and Abella, T.A., 1993. Growth and reproduction of individually tagged Nile tilapia (*Oreochromis niloticus*) of different strains. *Aquaculture*, **111**: 159-169.

Bolivar, R.B., 1993. National fish breeding programs in the Philippines. *In* K.L. Main and B. Reynolds (eds.) Selective Breeding of Fishes in Asia and the United States. Published by the Oceanic Institute, Makapuu Point, Hanolulu, Hawaii.

Bolivar, H.L., de Vera, M.P., Reyes, R.A., Bolivar, R.B., Bentsen, H.B. and Eknath, A.E., 1994. Early growth and survival of eight strains of Nile tilapia (*Oreochromis niloticus*) and their crosses, pp. 552-555. *In* L.M. Chou, A.D. Munro, T.J. Lam, T.W. Chen, L.K.K. Cheong, J.K. Ding, K.K. Hooi, H.W. Khoo, V.P.E. Phang, K.F. Shim and C.H. Tan (eds.) The Third Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Bondari, K., Threadgill, E.D. and Bender, J.A., 1983. Tilapia culture in conjunction with irrigation and urban farming, pp. 484-493. *In* L. Fishelson and Z. Yaron (eds.) Proceedings of the International Symposium on Tilapia in Aquaculture, May 1983, Tel Aviv University, Nazareth, Israel.

Bongers, A.B.J., Holland, M.C.H., Leenen, R.H.M., Komen, J. and Richter, C.J.J., 1991. Effect of 17β-estradiol on the sex differentiation in inbred (**XX**; mas-1/mas-1) males of common carp, *Cyprinus carpio* L. *In* A.P. Scott, J.P. Sumpter, D.E. Kime and M.S. Rolfe (eds.) Proceedings of the Fourth International Symposium on the Reproductive Physiology of Fish, 7-12 July 1991, University of Anglia, Norwich, U.K.

Brzeski, V.J., Doyle, R.W., Newkirk, G.F., Shackell, N.L. and Topp-Nowen, B.A., 1989. Manual for aquaculture genetics network in Asia. *In* B. Field (ed.) International Development Research Centre. Nova Scotia, Canada.

Buddle, C.R., 1984a. Monosex tilapia fry production. ICLARM Newsletter, January 1984.

Buddle, C.R., 1984b. Androgen-induced sex-inversion of *Oreochromis* (Trewavas) hybrid fry stocked into cages standing in an earthen pond. *Aquaculture*, **40**: 233-239.

Bull, J.J., 1980. Sex determination in reptiles. *The Quarterly Reviews of Biology*, **55**(1).

Bull, J.J., Vogt, R.C. and Bulmer, M.G., 1982. Heritability of sex ratio in turtles with environmental sex determination. *Evolution*, **36**(2): 333-341.

Bushmann, P.J. and Burns, J.R., 1994. Social control of male sexual maturation in the swordtail characin, *Corynopoma riisei. Journal of Fish Biology*, **44**: 263-272.

Calhoun, W.E. and Shelton, W.L., 1983. Sex ratios of progeny from mass spawnings of sex-reversed broodstock of *Tilapia nilotica. Aquaculture*, **33**: 365-371.

Capili, J.B., 1995. Growth and sex determination in the Nile tilapia, *Oreochromis niloticus* (L.). University of Wales Swansea, Wales. *Ph.D. Thesis.*

Chambers, S.A.C., 1984. Sex reversal of Nile tilapia in the presence of natural food. Auburn University, Alabama, USA. *M.Sc. Thesis*, 37 p.

Chan, S.T.H. and Yeung, W.S.B., 1983. Sex control and sex reversal in fish under natural conditions. *In* W.S. Hoar, D.J. Randall and E.M. Donaldson (eds.) Fish Physiology. Academic Press, New York, IXB: 171-213.

Charnov, E.L. and Bull, J.J., 1977. When is sex environmentally determined? *Nature*, **266**: 828-830.

Chen, F.Y., 1969. Preliminary studies on the sex determining mechanism of *Tilapia mossambica* and *T. hornorum* Trewavas. *Verh. Internat. Verein. Limnol.*, **17**: 719-724.

Chevassus, B., 1979. Hybridization in salmonids: results and perspective. *Aquaculture*, **17**: 113-128.

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

Clayton, G.M. and Price, D.J., 1994. Heterosis in resistance to *Ichthyophthirius multifliis* infections in poeciliid fish. *Journal of Fish Biology*, **44**: 59-66.

Coche, A.G., 1982. Cage culture of tilapias, pp. 205-246. *In* R.S.V. Pullin and R.H. Lowe-McConnell (eds.) The Biology and Culture of Tilapias. ICLARM Conference Proceedings **7**, 432 p. International Center for Living Aquatic Resources Management, Manila, Philippines.

Conover, D.O. and Kynard, B.E., 1981. Environmental sex determination: interaction of temperature and genotype in a fish. *Science*, **213**, 31 July 1981.

Cruz, C.R. dela, 1980. Integrated agriculture-aquaculture farming systems in the Philippines, with two case studies on simultaneous and rotational rice-fish culture. *In* R.S.V Pullin and Z.H. Shehadeh (eds.) Integrated Agriculture-Aquaculture Farming Systems. ICLARM Conference Proceedings **4**, 258 p. International Center for Living Aquatic Resources Management, Manila and the Southeast Asian Center for Graduate Study and Research in Agriculture, College, Los Baños, Laguna, Philippines.

Cruz, E.M. and Shehadeh, Z.H., 1980. Preliminary results of integrated pig-fish and duck-fish production tests. *In* R.S.V Pullin and Z.H. Shehadeh (eds.) Integrated Agriculture-Aquaculture Farming Systems. ICLARM Conference Proceedings **4**, 258 p. International Center for Living Aquatic Resources Management, Manila and the Southeast Asian Center for Graduate Study and Research in Agriculture, College, Los Baños, Laguna, Philippines.

Cuisset, B., Pelissero, C., Nuñez-Rodrigues, J. and Le Menn, F., 1991. Use of plasma 11-ketotestosterone for sex determination in Siberian sturgeon, *Acipenser baeri* B. *In* A.P. Scott, J.P. Sumpter, D.E. Kime and M.S. Rolfe (eds.) Proceedings of the Fourth International Symposium on the Reproductive Physiology of Fish, 7-12 July 1991, University of Anglia, Norwich, U.K.

Cunningham, E.P. and Connolly, J., 1989. Efficient design of crossbreeding experiment. *Theor. Appl. Genet.*, **78**: 381-386.

Curtis, L.R., Diren, F.T., Hurley, M.D., Seim, W.K. and Tubb, R.A., 1991. Disposition and elimination of 17α-methyltestosterone in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, **99**: 193-201.

Davis, K.B., Simco, B.A., Goudie, C.A., Parker, N.C., Cauldwell, W. and Snellgrove, R., 1990. Hormonal sex manipulation and evidence for female homogamety in Channel catfish. *General and Comparative Endocrinology*, **78**: 218-223.

Deb, D. and Varghese, T.J., 1988. Effect of 17α-methyltestosterone on the growth and food conversion of major carps, pp. 69-71. *In* M. Mohan Joseph (ed.) Proceedings, The First Indian Fisheries Forum. Asian Fisheries Society, Indian Branch, Mangalore.

Deeming, C. and Ferguson, M., 1989. In the heat of the nest. *New Scientist*, 25 March 1989.

Desprez, D., Melard, C. and Philippart, J.C., 1995. Production of high percentage of male offspring with 17α-ethynylestradiol sex-reversed *Oreochromis aureus*. II. Comparative reproductive biology of females and F_2 pseudofemales and large-scale production of male progeny. *Aquaculture*, **130**: 31-41.

Don, J. and Avtalion, R.R., 1990. Production of viable tetraploid tilapias using the cold shock technique. *The Israeli Journal of Aquaculture-Bamidgeh*, **40**(1): 17-21.

Donaldson, E.M., Fagerlund, U.H.M., Higgs, D.A. and McBride, J.R., 1979. Hormonal enhancement of growth, pp. 455-497. *In* W.S. Hoar, D.J. Randall and J.R. Brett (eds.) Fish Physiology. Academic Press, New York.

Dunham, R.A., Smitherman, R.O. and Webber, C., 1983a. Relative tolerance of Channel x Blue hybrid and Channel catfish to low oxygen concentrations. *Prog. Fish-Cult.*, **45**(1).

Dunham, R.A., Smitherman, R.O., Horn, J.L. and Bice, T.O., 1983b. Reproductive performances of crossbred and pure-strain channel catfish brood stocks. *Transactions of the American Fisheries Society*, **112**: 436-440.

Dunham, R.A. and Smitherman, R.O., 1983. Crossbreeding channel catfish for improvement of body weight in earthen ponds. *Growth*, **47**: 97-103.

Dunham, R.A., 1986. Selection and crossbreeding response for cultured fish. Proceedings of the Third World Congress on Genetic Applications to Livestock Production.

Dunham, R.A., Smitherman, R.O., Goodman, R.K. and Kemp, P., 1986. Comparison of strains, crossbreeds and hybrids of channel catfish for vulnerability to angling. *Aquaculture*, **57**: 193-201.

Dunham, R.A., 1990. Production and use of monosex or sterile fishes in aquaculture. *Reviews In Aquatic Sciences*, **2**(1).

Eckstein, B. and Spira, M., 1965. Effect of sex hormones on gonadal differentiation in a cichlid, *Tilapia aurea*. *Biological Bulletin*, **129**: 482-487.

Eknath, A.E., Bentsen, H.B., Gjerde, B., Tayamen, M.M., Abella, T.A., Gjedrem, T. and Pullin, R.S.V., 1991. Approaches to national fish breeding programs: pointers from a tilapia pilot study. *NAGA, The ICLARM Quarterly*, **14**(2): 10-12.

Eknath, A.E., Tayamen, M.M., Palada-de Vera, M.S., Danting, J.C., Reyes, R.A., Dionisio, E.E., Capili, J.B., Bolivar, H.L., Abella, T.A., Circa, A.V., Bentsen, H.B., Gjerde, B., Gjedrem, T. and Pullin, R.S.V., 1993. Genetic improvement of farmed tilapias: the growth performance of eight strains of *Oreochromis niloticus* tested in different farm environments. *Aquaculture*, **111**: 171-188.

Falconer, D.S. and Mackay, T.F.C., 1996. Introduction to quantitative genetics. Fourth edition. Longman Group Ltd.

FAO, 1995. Aquaculture production statistics. FAO Fisheries Circular 815, Rev. 7, 186 p.

Final Report, 1994. Renewable natural resources research strategy: 1995-2005. Natural Resources Research Department, British Overseas Development Administration, U.K.

Gale, W.L., Fitzpatrick and Schreck, C.B., 1991. Immersion of Nile tilapia (*Oreochromis niloticus*) in 17α-methyltestosterone and mestanolone for the production of all-male populations. *In* A.P. Scott, J.P. Sumpter, D.E. Kime and M.S. Rolfe (eds.) Proceedings of the Fourth International Symposium on the Reproductive Physiology of Fish, 7-12 July 1991, University of Anglia, Norwich, U.K.

Garcia-Abiado, M.A.G., 1995. Reproductive biology and chromosome manipulation in the Nile tilapia, *Oreochromis niloticus* Linnaeus. University of Wales Swansea, Wales. *Ph.D. Thesis.*

Gilling, C.J., 1994. Application of gynogenesis and sex reversal to improvement of tilapia. University of Wales Swansea, Wales. *Ph.D. Thesis.*

Gjedrem, T., 1985. Improvement of productivity through breeding schemes. *Geo Journal*, **10**(3): 233-241.

Gjedrem, T., 1992. Breeding plans for rainbow trout. *Aquaculture*, **100**: 73-83.

Gjerde, B., 1988. Complete diallele cross between six inbred groups of rainbow trout, *Salmo gairdneri*. *Aquaculture*, **75**: 71-87.

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**: 267-78.

Gogoi, D. and Keshavanath, P., 1988. Digestability and growth promoting potential of 17α-methyltestosterone incorporated diets in the mahseer, Tor khudree, pp. 99-102. *In* M. Mohan Joseph (ed.) Proceedings, The First Indian Fisheries Forum. Asian Fisheries Society, Indian Branch, Mangalore.

Gomelsky, B., Cherfas, N.B., Peretz, Y., Ben-Dom, N. and Hulata, G., 1994. Hormonal sex inversion in the common carp (*Cyprinus carpio* (L.). *Aquaculture*, **126**: 265-270.

Gordon, M., 1947. Genetics of *Platypoecilus maculatus*. IV. The sex determining mechanism in two wild populations of the Mexican platyfish. *Genetics*, **32**: 8-17.

Goudie, C.A., Redner, B.D., Simco, B.A. and Davis, K.B., 1983. Feminization of channel catfish by oral administration of steroids sex hormones. *Transactions of the American Fisheries Society*, **112**: 670-672.

Goudie, C.A., 1984. Tissue distribution and elimination of radiolabelled-methyltestosterone administered in the diet to sexually undifferentiated and adult tilapia, *Tilapia* (*Oreochromis*) *aurea*. Auburn University, Alabama, USA. *Ph.D. Thesis*, 151 p.

Green, B.W., 1992. Substitution of organic manure for pelleted feed in tilapia production. *Aquaculture*, **101**: 213-222.

Green, B.W. and Teichert-Coddington, D.R., 1994. Growth of control and androgen-treated Nile tilapia, *Oreochromis niloticus* (L.), during treatment, nursery and grow-out phases in tropical fish ponds. *Aquaculture and Fisheries Management*, **25**: 613-621.

Griffiths, W.S., 1996. Sex differences in the trade-off between feeding and mating in the guppy. *Journal of Fish Biology*, **48**: 891-898.

Guerrero III, R.D. and Shelton. W.L., 1974. An aceto-carmine squash method of sexing juvenile fishes. *Prog. Fish. Cult.*, **36**: 56.

Guerrero III, R.D., 1975. Use of androgens for the production of all-male *Tilapia aurea* (Steindachner). *Trans. Amer. Fish. Soc.*, **2**: 342-347.

Guerrero III, R.D., 1976. *Tilapia mossambica* and *Tilapia zilli* treated with ethynyltestosterone for sex reversal. *Kalikasan, Philipp. J. Biol.* **5**: 187-192.

Guerrero III, R.D., 1985. Tilapia farming in the Philippines: practices, problems and prospects, pp. 3-14. *In* I.R. Smith, E.B. Torres and E.O. Tan (eds.) Philippine Tilapia Economics. ICLARM Conference Proceedings **12**, 261 p. Philippine Council for Agriculture and Resources Research and Development, Los Baños, Laguna and International Center for Living Aquatic Resources Management Center, Manila, Philippines.

Guerrero III, R.D., 1987. Tilapia Farming in the Philippines. Technology and Livelihood Resource Center. Manila, Philippines.

Guerrero III, R.D. and Guerrero, L.A., 1988. Feasibility of commercial production of sex-reversed Nile tilapia fingerlings in the Philippines, p. 183-186. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings **15**, 623 p. Department of Fisheries, Bangkok, Thailand and International Center for Living Aquatic Resources Management, Manila, Philippines.

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. *Thoer. Appl. Genet.*, **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 (eds.) Proceedings of the International Symposium on Tilapia in Aquaculture, May 1983, Tel Aviv University, Nazareth, Israel.

Hepher, B. and Pruginin, Y., 1982. Tilapia culture in ponds under controlled conditions, pp. 185-203. *In* R.S.V. Pullin and R.H. Lowe-McConnell (eds.) The Biology and Culture of Tilapias. ICLARM Conference Proceedings **7**, 432 p. International Center for Living Aquatic Resources Management, Manila, Philippines.

Hershberger, W.K., 1993. Genetic resources for future finfish aquaculture, pp. 98-109. *In* K.L. Main and B. Reynolds (eds.) Selective Breeding of Fishes in Asia and the United States. Published by the Oceanic Institute, Makapuu Point, Hanolulu, Hawaii.

Hickling, C.F., 1960. The Malacca tilapia hybrids. *J. Genet.*, **57**: 1-10.

Hiott, A.E. and Phelps R.P., 1993. Effects of initial age and size on sex reversal of *Oroechromis niloticus* fry using methyltestosterone. *Aquaculture*, **112**: 301-308.

Holsinger, K.E., 1988. Inbreeding depression doesn't matter: the genetic basis of mating-system evolution. *Evolution*, **42**(6): 1235-1244.

Horstgen-Schwark, G., Fricke, H. and Langholz, H.-J., 1986. The effect of crossing on the production performance in rainbow trout. *Aquaculture*, **57**: 141-152.

Howell, W.M., Black, D.A. and Bortone, S.A., 1980. Abnormal expression of secondary sex characters in a population of mosquito fish, *Gambusia affinis Holbrooki.* Evidence for environmentally induced masculinization. *Copeia*, pp. 676-440.

Howerton, R.D., Okimoto, D.K. and Grau, E.G., 1992. The effect of orally administered 17α-methyltestosterone and 3.3'-triiodo-L-thyronine on growth of seawater-adapted tilapia, *Oreochromis mossambicus* (Peters). *Aquaculture and Fisheries Management*, **23**: 123-128.

Huang, C.-M. and Liao, I.-C., 1990. Response to mass selection for growth rate in *Oreochromis niloticus. Aquaculture*, **85**: 199-205.

Hulata, G., Wohlfarth, G.W. and Rothbard, S., 1983. Progeny-testing selection of tilapia broodstocks producing all-male hybrid progenies - preliminary. *Aquaculture*, **33**: 263-268.

Hulata, G., Rothbard, J., Itzkovich, G., Wohlfarth, G. and Halevy, A., 1985. Differences in hybrid fry production between two strains of the Nile tilapia. *Prog. Fish-Cult.*, **47**(1).

Hulata, G., Wohlfarth, G.W. and Halevy, A., 1986. Mass selection for growth rate in the Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, **57**: 177-184.

Hulata, G., Wohlfarth, G.W. and Halevy, A., 1988. Comparative growth tests of *Oreochromis niloticus* x *0. aureus* hybrids derived from different farms in Israel, in polyculture, pp. 191-195. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings **15**, 623 p. Department of Fisheries, Bangkok, Thailand and International Center for Living Aquatic Resources Management, Manila, Philippines.

Hulata, G., Wohlfarth, G.W., Karplus, I., Schoeder, G.L., Harpaz, S., Halevy, A., Rothbard, S., Cohen, S., Israel, I. and Kavessa, M., 1993. Evaluation of *Oreochromis niloticus* x *0. aureus* hybrid progeny of different geographical isolates, reared under varying management regimes. *Aquaculture*, **115**: 253-271.

Hulata, G., 1995a. A review of genetic improvement of common carp (*Cyprinus carpio* L.) and other cyprinids by crossbreeding, hybridization and selection. *Aquaculture*, **129**: 143-155.

Hulata, G., 1995b. The history and current status of aquaculture genetics in Israel. *The Israeli Journal of Aquaculture-Bamidgeh*, **47**(3-4): 142-154.

Hunter, G.A. and Donaldson, E.M., 1983. Hormonal sex control and its application to fish culture, pp. 223-304. *In* W.S. Hoar and D.S. Randall (eds.) Fish Physiology. Academic Press, New York.

Hurk, R. van den and Lambert, J.G.D., 1982. Temperature and steroid effects on gonadal sex differentiation in Rainbow trout. Proceedings of the International Symposium on Reproductive Physiology of Fish, 2-6 August 1982, Wageningen, the Netherlands.

Hussain, M.G., 1992. Genetic manipulation studies in *Oreochromis niloticus* L. University of Stirling, Scotland. *Ph.D. Thesis.*

ICLARM, 1994. ICLARM Report for year 1993. International Center for Living Aquatic Resources Management, Manila, Philippines. 101 p.

Jayaprakas, V., Tave, D. and Smitherman, R.O., 1988. Growth of two strains of *Oreochromis niloticus* and their F_1, F_2 and backcross hybrids, pp. 197-201. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings **15**, 623 p. Department of Fisheries, Bangkok, Thailand and International Center for Living Aquatic Resources Management, Manila, Philippines.

Jensen, G.L. and Shelton W.L., 1979. Effects of estrogens on *Tilapia aurea*: implication for production of monosex genetic male tilapia. *Aquaculture*, **16**: 233-242.

Jessy, D. and Varghese, T.J., 1988. Hormonal sex control in *Betta splendens* Regan and *Xiphophorus helleri* Heckel, pp. 123-124. *In* M. Mohan Joseph (ed.) Proceedings, The First Indian Fisheries Forum. Asian Fisheries Society, Indian Branch, Mangalore.

Jhingran, A.G. and Gupta, R.A., 1989. The relevance of selective breeding for genetic improvement of farmed fish, pp. 135-140. *In* Das and Jhingran (eds.) Fish Genetics in India.

Jo, J.-Y., Smitherman, R.O. and Behrends, L.L., 1988. Effects of dietary 17α-methyltestosterone on sex reversal and growth of *0reochromis aureus,* pp. 203-207. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings **15**, 623 p. Department of Fisheries, Bangkok, Thailand and International Center for Living Aquatic Resources Management, Manila, Philippines.

Johnsson, J.I. and Bjornsson, B.T., 1994. Growth hormone increases growth rate, appetite and dominance in juvenile rainbow trout, *Oncorhynchus mykiss. Anim. Behav.*, **48**: 177-186.

Johnsson, J.I., Clarke, W.C. and Blackburn, J., 1994. Hybridization with domesticated rainbow trout reduces seasonal variation in sea water adaptability of steelhead trout (*Oncorhynchus mykiss*). *Aquaculture*, **121**: 73-77.

Johnstone, R., Simpson, T.H. and Walker, A.F., 1979. Sex reversal in salmonid culture. Part III. The production and performance of all-female populations of brook trout. *Aquaculture*, **18**: 241-252.

Johnstone, R., Macintosh, D.J. and Wright, R.S., 1983. Elimination of orally administered 17α-methyltestosterone by *Oreochromis mossambicus* (tilapia) and *Salmo gairdneri* (rainbow trout) juveniles. *Aquaculture*, **35**: 249-257.

Kallman, K.D., 1984. A new look at sex determination in *Poeciliid* fishes, pp. 95-171. *In* B.J. Turner (ed.) Evolutionary Genetics of Fishes. Plenun Press, New York, NY.

Kavumpurath, S. and Pandian, T.J., 1992. Hybridization and gynogenesis in two species of the genus *Brachydanio*. *Aquaculture*, **105**: 107-116.

Khater, A.A.E., 1985. Identification and comparison of three *Tilapia nilotica* strains for selected aquacultural traits. Auburn University, Alabama, USA. *Ph.D. Thesis*, 67 p.

Kincaid, H.L., 1977. Rotational line crossing: an approach to the reduction of inbreeding accumulation in trout brood stocks. *Prog. Fish Cult.*, **39**(4).

Kincaid, H.L., 1983. Inbreeding in fish populations used for aquaculture. *Aquaculture*, **33**: 215-227.

Komen, J., Eding, E.H., Bongers, A.B.J. and Ritcher, C.J.J., 1993. Gynogenesis in common carp (*Cyprinus carpio*) IV. Growth, phenotypic variation and gonad differentiation in normal and methyltestosterone-treated homozygous clones and F_1 hybrids. *Aquaculture*, **111**: 271-280.

Kulikovsky, Z., Wohlfarth, G.W. and Avtalion, R.R., 1994. The association between initial weight and growth in tilapias during communal testing. II. Cage testing. *The Israeli Journal of Aquaculture - Bamidgeh*, **46**(2): 89-94.

Kuwaye, T.T., Okimoto, D.K., Shimoda, S.K., Howerton, R.D., Lin, H.R., Pang, P.K.T. and Grau, E.G., 1993. Effect of 17α-methyltestosterone on the growth of the euryhaline tilapia, *Oreochromis mossambicus*, in fresh water and in sea water. *Aquaculture*, **113**: 137-152.

Lagomarsino, I.V. and Conover, D.O., 1993. Variation in environmental and genotypic sex-determining mechanisms across a latitudinal gradient in the fish, *Menidia menidia*. *Evolution*, **47**(2): 487-494.

Lahav, E., 1993. Use of sex-reversed females to produce all-male tilapia (*Oreochromis aureus*) fry. *The Israeli Journal of Aquaculture - Bamidgeh*, **45**(3): 131-136.

Lee, C.-S., Kelley, C.D., Tamaru, C.S., Moriwake, A., Miyamoto, G. and Aida, K., 1991. The effects of testosterone and 17α-methyltestosterone capsules on male maturation in the milkfish, *Chanos chanos*. *In* A.P. Scott, J.P. Sumpter, D.E. Kime and M.S. Rolfe (eds.) Proceedings of the Fourth International Symposium on the Reproductive Physiology of Fish, 7-12 July 1991, University of Anglia, Norwich, U.K.

Lester, L.J., Lawson, K.S., Abella, T.A. and Palada, M.S., 1989. Estimated heritability of sex ratio and sexual dimorphism in tilapia. *Aquaculture and Fisheries Management*, **20**: 369-380.

Little, D. and Muir, J., 1987. A guide to integrated warm water aquaculture. Institute of Aquaculture Publications, University of Stirling, Scotland.

Lone, K.P. and Ridha, M.T., 1993. Sex reversal and growth of *Oreochromis spilurus* (Gunther) in brackish and sea water by feeding 17α-methyltestosterone. *Aquaculture and Fisheries Management*, **24**: 593-602.

Lovshin, L.L. and Pretto, R.M., 1983. A strategy for the use of tilapias in rural Latin America: the Panamian integrated approach, pp. 494-505. *In* L. Fishelson and Z. Yaron (eds.) Proceedings of the International Symposium on Tilapia in Aquaculture, May 1983, Tel Aviv University, Nazareth, Israel.

Lovshin, L.L., Da Silva, A.B., Carneiro-Sobrinho, A. and Melo, F.R., 1990. Effects of *Oreochromis niloticus* females on the growth and yield of male hybrids (*O. niloticus* ♀ x *O. hornorum* ♂) cultured in earthen ponds. *Aquaculture*, **88**: 55-60.

Macaranas, J.M., Taniguchi, N., Pante, M.J.R., Capili, J.B. and Pullin, R.S.V., 1986. Electrophoretic evidence for extensive hybrid gene introgression into commercial *Oreochromis niloticus* (L.) stocks in the Philippines. *Aquaculture and Fisheries management*, **17**: 249-258.

Macintosh, D.J., Singh, T.B., Little, D.C. and Edwards, P., 1988. Growth and sexual development of 17α-methyltestosterone- and progesterone-treated Nile tilapia (*Oreochromis niloticus*) reared in earthen ponds. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings **15**, 623 p. Department of Fisheries, Bangkok, Thailand and International Center for Living Aquatic Resources Management, Manila, Philippines.

Macintosh, D.J. and Little, D.C., 1995. Nile tilapia (*Oreochromis niloticus*), pp. 277-320. *In* N.R. Bromage and R.J. Roberts (eds.) Broodstock Management and Egg and Larval Quality. Blackwell Science, University Press, Cambridge.

Mair, G.C., Penman, D.J., Scott, A., Skibinski, D.O.F. and Beardmore, J.A., 1987. Hormonal sex-reversal and the mechanisms of sex determination in *Oreochromis*. *In* Proc. World Symp. on Selection, Hybridization, and Genetic Engineering in Aquaculture Vol. II, 27-30 May 1986, Bordeaux, Berlin.

Mair, G.C., 1988. Studies on sex determining mechanisms in *Oreochromis species*. University of Wales, Wales. *Ph.D. Thesis.*

Mair, G.C., Beardmore, J.A. and Skibinski, D.O.F., 1990. Experimental evidence for environmental sex determination in *Oreochromis spp. In* R. Hirano and I. Hanyu (eds.) The Second Asian Fisheries Forum. 991 p. Asian Fisheries Society, Manila, Philippines.

Mair, G.C., Scott, A.G., Penman, D.J., Beardmore, J.A. and Skibinski, D.O.F., 1991a. Sex determination in the genus *Oreochromis.* 1. sex reversal, gynogeneis and triploidy in *O. niloticus* (L.). *Theor Appl Genet*, **82**: 144-152.

Mair, G.C., Scott, A.G., Penman, D.J., Skibinski, D.O.F. and Beardmore, J.A. 1991b. Sex determination in the genus *Oreochromis.* 2. Sex reversal, hybridisation, gynogenesis and triploidy in *O. aureus* Steindachner. *Theor Appl Genet*, **82**: 153-160.

Mair, G.C. and Little, D.C., 1991. Population control in farmed tilapia. *NAGA, The ICLARM Quarterly.*

Mair, G.C., Capili, J.B., Beardmore, J.A. and Skibinski, D.O.F., 1992. The YY-male technology for the mass production of monosex male tilapia, *Oreochromis niloticus* (L.), pp. 93-95. *In* D.J. Penman, N. Roongratri and B.J. McAndrew (eds.) Proceedings of the First ASEAN-EEC aquaculture Development Coordination Programme (AADCP) International Workshops on Genetics in Aquaculture and Fisheries Management, 31 August - 4 September 1992, University of Stirling, Scotland.

Mair, G.C., 1993. Chromosome-set manipulation in tilapia - techniques, problems and prospects. *Aquaculture*, **111**: 227-244.

Mair, G.C. and Santiago. L.P., 1994. Feminization of Nile tilapia, *Oreochromis niloticus* L., by oral application of diethylstilbestrol (DES), pp. 94-97. *In* L.M. Chou, A.D. Munro, T.J. Lam, T.W. Chen, L.K.K. Cheong, J.K. Ding, K.K. Hooi, H.W. Khoo, V.P.E. Phang, K.F. Shim and C.H. Tan (eds.) The Third Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Mair, G.C., Abucay, J.S., Beardmore, J.A. and Skibinski, D.O.F., 1995. Growth performance trials of genetically male tilapia (GMT) derived from YY males in *Oreochromis niloticus* L.: on station comparisons with mixed-sex and sex-reversed populations. *Aquaculture*, **137**: 313-322.

Mair, G.C. and van Dam, A.A., 1996. The effect of sex ratio at stocking on growth and recruitment in Nile tilapia (*Oreochromis niloticus* L.) ponds, pp. 100-107. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D.Pauly (eds.) Third International Symposium in Aquaculture. ICLARM Conf. Proc. **41**, 575 p. International Center for Living Aquatic Resources Management, Manila, Philippines.

Mair, G.C., Abucay, J.S., Skibinski, D.O.F., Abella, T.A. and Beardmore, J.A., 1997a. Genetic manipulation of sex ratio for the large scale production of all-male tilapia, *Oreochromis niloticus* L. *Canadian Journal of Fisheries and Aquatic Sciences,* **54**(2): 396-404.

Mair, G.C., Dahilig, L.R., Morales, E.R., Beardmore, J.A. and Skibinski, D.O.F., 1997b. Application of genetic techniques for the production of monosex male tilapia in aquaculture: early experiences from the Philippines, pp. 225-227. Proceedings of the Fourth Central America Symposium on Aquaculture, 22-24 April 1997, Tegucigalpa, Honduras.

Mair, G.C. and Skibinski, D.O.F., 1997. Selective improvement of genetically male tilapia. Final report, British Overseas Development Research Project R6058.

Majumdar, K.C. and McAndrew, B.J., 1983. Sex ratios from interspecific crosses within the tilapias, pp. 261-269. *In* L. Fishelson and Z. Yaron (eds.) Proceedings of the International Symposium on Tilapia in Aquaculture, May 1983, Tel Aviv University, Nazareth, Israel.

Manzano, V.B., 1989. Polyculture of grouper (*Epinephelus tauvina*) and tilapia (*Oreochromis niloticus*) in brackishwater ponds, pp. 171-181. *In* E.A. Huisman, N. Zonneveld and A.H.M. Boumans (eds.) Aquaculture Research in Asia: Management Techniques and Nutrition. International Fisheries Society, Malang, Indonesia.

Manzoor Ali, P.K.M. and Satyanarayana Rao, G.P., 1989. Growth improvement in carp, *Cyprinus carpio* (Linnaeus), sterilized with 17α-methyltestosterone. *Aquaculture*, **76**: 157-167.

Mather, K. and Jinks, J.L., 1977. Introduction to biometric genetics. Chapman and Hall Ltd., London.

Matty, A.J. and Cheema, I.R., 1978. The effect of some steroids hormones on the growth and protein metabolism of rainbow trout. *Aquaculture*, **14**: 163-178.

Mbahinzireki, G. and Dabrowski, K., 1997. Production of male tilapia fry by heat-treatment of embryos and growth on different diets in rericulation systems. A paper presented during the World Aquaculture Society Conference, 19-3 February 1997, Washington State Convention Center, Seattle, USA.

McAndrew, B.J. and Majumdar, K.C., 1989. Growth studies on juvenile tilapia using pure species, hormone-treated and nine interspecific hybrids. *Aquaculture and Fisheries Management*, **20**: 35-47.

McGeachin, R.B., Robinson, E.H. and Neil, W.H., 1987. Effect of feeding high levels of androgens on the sex ratio of *0reochromis Aureus*. *Aquaculture*, **61**: 317-321.

McGinty, A.S., 1985. Effect of size at stocking on competition and growth of all-male tilapia hybrids. *J. World Maricul. Soc.*, **16**: 52-56.

McGinty, A.S., 1991. Tilapia production in cages: effect of cage size and number of non-caged fish. *The Progressive Fish-Culturist*, **53**: 46-249.

Melard, C., 1995. Production of high percentage of male offspring with 17α-ethynylestradiol sex-reversed *Oreochromis aureus*. I. Estrogen sex reversal and production of F_2 pseudofemales. *Aquaculture*, **130**: 25-34.

Meyer, D.E., 1990. Growth, survival and sex ratios of *Orechromis hornorum*, *O. niloticus* and their hybrid (*O. niloticus* ♀ x *O. hornorum* ♂) treated with 17α-methyltestosterone. Auburn University, Alabama, USA. *Ph.D. Thesis*, 75 p.

Mitton, J.B. and Grant, M.C., 1984. Association among protein heterozygosity, growth rate, and developmental homeostasis. *Ann. Rev. Ecol. Syst.*, **15**: 479-499.

Moav, R., Brody, T. and Hulata, G., 1978. Genetic improvement of wild fish populations. *Science*, Vol. **201**.

Mol, K., Byamungu, N., Cuisset, B., Yaron, Z., Ofir, M., Melard, C., Castelli, M. and Kuehn, E.R., 1994. Hormonal profile of growing male and female diploids and triploids of the blue tilapia, *0reochromis aureus*, reared in intensive culture. *Fish. Physiol. Bio. Chem.*, **13**(3): 209-218.

Morales, E.J., 1997. Intensive tilapia hatchery production systems, pp. 39-51. *In* G.C. Mair and T.A. Abella (eds.) Technoguide on the Production of Genetically Male Tilapia (GMT). Freshwater Aquaculture Center, Central Luzon State University, Philippines.

Morrison, J.R., Deavours, W.L., Jones, J.C. and Tabb, M.A., 1995. Maximizing first-year growth of mixed-sex tilapia (*Oreochromis aureus*) in polyculture with catfish (*Ictalurus spp.*). *J. World Aquacult. Soc.*, **26**(4): 447-452.

Mualungwe Muhaya, B.B., 1985. Growth comparison of *Tilapia nilotica* males produced through oral administration of methyltestosterone at varying levels and duration. Auburn University, Alabama, USA. *M.Sc. Thesis*, 28 p.

Muller-Belecke, A. and Hortsgen-Schwark, G., 1995. Sex determination in tilapia (*Oreochromis niloticus*) sex ratios in homozygous gynogenetic progeny and their offspring. *Aquaculture*, **137**: 57-65.

Murugesan, V.K. and Selvaraj, C., 1991. Observations on fin-clipping of carps cultivated in reservoir, pp. 153-155. Proceedings, The Second Indian Fisheries Forum, 27-31 May 1990, Mangalore, India.

Nagaraj, C.G. and Rao, G.P.S., 1988. Effect of testosterone Acetate and estradiol benzoate on sexuality and growth of *Cyprinus carpio* (Linn.), pp. 115-117. *In* M. Mohan Joseph (ed.) Proceedings, The First Indian Fisheries Forum. Asian Fisheries Society, Indian Branch, Mangalore.

Nagy, A., Bercsenyi, M. and Csanyi, V., 1981. Sex reversal in carp (*Cyprinus carpio*) by oral administration of methyltestosterone. *Can. J. Aquat. Sci.*, **38**: 725-728.

Nakamura, M. and Takahashi, H., 1973. Gonadal sex differentiation in *Tilapia mossambica* with special regard to the time of estrogen treatment effective in inducing complete feminization of genetic males. *Bull. Fac. Fish.*, **42**(1): 1-13.

Nakamura, M., 1975. Dosage-dependent changes in the effect of oral administration of methyltestosterone on gonadal differentiation in *Tilapia mossambica*. *Bull. Fac. Fish. Hokkaido Univ.*, **26**(2): 99-108.

Obi, A. and Shelton, W.L., 1983. Androgen sex reversal in *Tilapia hornorum*, pp. 165-173. *In* L. Fishelson and Z. Yaron (eds.) Proceedings of the International Symposium on Tilapia in Aquaculture, May 1983, Tel Aviv University, Nazareth, Israel.

Oliveira de-Fernandez, -M. and Volpato, -G. L., 1993. Heterogeneous growth in the Nile tilapia: social stress and carbohydrate metabolism. *Physiol. - Behav.*, **54**(2): 319-323.

Padilla, M.G., 1997. Diethylstilbestrol residue in sex-reversed Nile tilapia. Central Luzon State University, Nueva Ecija, Philippines. *B.Sc. Thesis.*

Palada-de Vera, M.S. and Eknath, E.A., 1993. Predictability of individual growth rates in tilapia. *Aquaculture*, **111**: 147-158.

Pandian, T.J. and Varadaraj, K., 1990. Development of monosex female *Oreochromis mossambicus* broodstock by integrating gynogenetic technique with endocrine sex reversal. *The Journal of Experimental Zoology*, **255**: 88-96.

Pandian, T.J. and Sheela, S.G., 1995. Hormonal induction of sex reversal in fish. *Aquaculture*, **138**: 1-22.

Patil, J.G. and Vargese, T.J., 1994. Production of monosex progeny of tilapia *Oreochromis mossambicus* (Peters) by integrating hormonal sex reversal and selective breeding techniques, pp. 90-93. *In* L.M. Chou, A.D. Munro, T.J. Lam, T.W. Chen, L.K.K. Cheong, J.K. Ding, K.K. Hooi, H.W. Khoo, V.P.E. Phang, K.F. Shim and C.H. Tan (eds.) The Third Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Pelissero, C. and Sumpter, J.P., 1992. Steroids and "steroid-like" substances in fish diets. *Aquaculture*, **107**: 283-301.

Penman, D.J., Shah, M.S., Beardmore, J.A. and Skibinski, D.O.F., 1987. Sex ratios of gynogenetic and triploid tilapia. Proc. World Symp. on Selection, Hybridization, and Genetic Engineering in Aquaculture Vol. II, 27-30 May 1986, Bordeaux, Berlin.

Peters, H.M., 1971. Testis weights in *Tilapia* (Cichlidae). *Copeia*, (1): 13-17.

Phelps, R.P. and Cerezo, G., 1992. The effect of confinement in hapas on sex reversal and growth of *Oreochromis niloticus*. *Journal of Applied Aquaculture*, **1**(4): 73-81.

Phelps, R.P., Cole, W. and Katz, T., 1992. Effect of fluoxymesterone on sex ratio and growth of Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture and Fisheries Management*, **23**: 405-410.

Philippart, J-CL. and Ruwet, J.CL., 1982. Ecology and distribution of tilapias, pp. 15-59. *In* R.S.V. Pullin and R.H. Lowe-McConnell (eds.) The Biology and Culture of Tilapias. ICLARM Conference Proceedings **7**, 432 p. International Center for Living Aquatic Resources Management, Manila, Philippines.

Piferrer, F. and Donaldson, E.M., 1991. Dosage-dependent differences in the effect of aromatizable and non-aromatizable androgens on the resulting phenotype of coho salmon (*Oncorhynchus kisutch*). *Fish Physiology and Chemistry*, **9**(2): 145-150.

Piferrer, F., Carillo, M., Hofstede, P.T. and Zanuy, S., 1991. Effects of dietary methyltestosterone on one year old sea bass (Dicentrarchus labrax, L.). *In* A.P. Scott, J.P. Sumpter, D.E. Kime and M.S. Rolfe (eds.) Proceedings of the Fourth International Symposium on the Reproductive Physiology of Fish, 7-12 July 1991, University of Anglia, Norwich, U.K.

Piferrer, F., Carillo, M., Zanuy, S., Solar, I.I. and Donaldson, E.M., 1994a. Induction of sterility in coho salmon (*Oncorhynchus kisutch*) by androgen immersion before first feeding. *Aquaculture*, **119**: 409-423.

Piferrer, F., Zanuy, S., Carillo, M., Solar, I.I., Devlin, R.H. and Donaldson, E.M., 1994b. Brief treatment with an aromatase inhibitor during sex differentiation causes chromosomally female salmon to develop as normal, functional males. *The Journal of Experimental Zoology*, **270**: 255-262.

Piferrer, F. and Donaldson, E., 1994. Uptake and clearance of exogenous estradiol-17β and testosterone during the early development of coho salmon (*Oncorhynchus kisutch*), including eggs, alevins and fry. *Fish Physiology and Biochemistry*, **13**(2): 219-232.

Pillay, T.V.R., 1990. Recent advances in aquaculture. Fishing News Book Ltd., Publisher.

Pongsri, C., 1994. Genetic approaches to improvement of tilapia culture in Thailand. University of Wales Swansea, Wales. *Ph.D. Thesis.*

Popma, T.J. and Green, B.W., 1990. Sex reversal of tilapia in earthen ponds. Aquaculture Production Manual, Research and Development Series No. **35**. International Center for Aquaculture, Alabama Agricultural Experiment Station, Auburn University, Alabama, USA.

Potts, A.C. and Phelps, R.P., 1995. Use of diethylestilbestrol and ethynyltestradiol to feminize Nile tilapia, *Oreochromis niloticus* (L) in an outdoor environment. *Journal of Applied Ichthyology*, **11**: 111-117.

Price, D.J., 1984. Genetics of sex determination in fishes - a brief review, pp. 77-89. *In* G.W. Potts and R.J. Wootton (eds.) Fish Reproduction: Strategies and Tactics. Academic Press, New York, NY.

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., 1985. Tilapias: "Everyman's fish". *Biologist*, **32**(2): 84-48.

Pullin, R.S.V. and Capili, J.B., 1988. Genetic improvement of tilapias: problems and prospects, pp. 259-266. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings **15**, 623 p. Department of Fisheries, Bangkok, Thailand and International Center for Living Aquatic Resources Management, Manila, Philippines.

Purdom, C.E., 1986. Genetic techniques for control of sexuality in fish farming. *Fish Physiology and Biochemistry*, **2**(1-4): 3-8.

Purdom, C.E., 1993. Genetics and fish breeding. Fish and Fisheries Series **8**. Chapman and Hall, Publisher.

Quartararo, N. and Bell, J.D., 1992. Effect of intraperitoneal passive implantable transponder (PIT) tags on the growth and survival of juvenile snapper, *Pagrus auratus* (Bloch and Schneider). *Aquaculture and Fisheries Management*, **23**: 543-548.

Reyes, R.A. and Eknath, A.E., 1991. Environment effects on expression of genetic potential for growth in seven strains of Nile tilapia (*Oreochromis niloticus*) and its implication for applied breeding programs. Paper presented at the Fourth International Symposium on Genetics in Aquaculture, 29 April - 3 May 1991, Wuhan, China.

Ridha, M.T. and Lone, K.P., 1990. Effect of oral administration of different levels of 17α-methyltestosterone on the sex reversal, growth, and food conversion efficiency of the tilapia *Oreochromis spilurus* (Gunther) in brackish water. *Aquaculture and Fisheries Management*, **21**: 391-397.

Romer, U. and Beisenherz, W., 1996. Environmental determination of sex in *Apistogramma* (Cichlidae) and two other freshwater fishes (Teleostei). *Journal of Fish Biology*, **48**: 714-725.

Ross, B. and Ross, L.G., 1983. The oxygen requirements of *Oreochromis niloticus* under adverse condition, pp. 134-143. *In* L. Fishelson and Z. Yaron (eds.) Proceedings of the International Symposium on Tilapia in Aquaculture, May 1983, Tel Aviv University, Nazareth, Israel.

Rosentein, S., 1990. Hormonal sex inversion of male tilapia (*Oreochromis spp.*) to female, as a step in the development of brood-stock yielding all male progeny. Hebrew University, Jerusalem. *M.Sc. Thesis,* 66 p.

Rosenstein, S. and Hulata, G., 1992. Sex reversal in the genus *Oreochromis*. 1. Immersion of eggs and embryos in oestrogen solutions is ineffective. *Aquaculture and Fisheries Management*, **23**: 669-678.

Rothbard, S., Moav, B. and Yaron, Z., 1987. Changes in steroid concentrations during sexual ontogenesis in tilapia. *Aquaculture*, **61**: 59-74.

Rosentein, S. and Hulata, G., 1993. Sex reversal in genus *Oreochromis*: optimization of feminization protocol. *Aquaculture and Fisheries Management*, **4**: 329-339.

Rothbard, S., Zohar, Y., Zmora, N., Levavi-Sivan, B. Moav, B. and Yaron, Z., 1990. Clearance of 17α-methyltestosterone from muscle of sex-inversed tilapia hybrids treated for growth enhancement with two doses of the androgen. *Aquaculture*, **89**: 365-376.

Roy, A.K., Datta, A.K., Sen, P.R. and Saha, B.K., 1991. Preliminary studies on the effect of pectoral fin clipping in carps on growth, survival and regeneration rate. *J. Aqua. Trop.*, **6**: 89-98.

Rubin, D.A., 1985. Effect of pH on sex ratio in *Cichlids* and a *Poecilliid* (Teleostei). *Copeia*, (1): 233-235.

Samonte, G.P.B., Agbayani, R.F. and Tumaliuan, R.E., 1991. Economics feasibility of polyculture of tiger shrimp (*Penaeus monodon*) with Nile tilapia (*Oreochromis niloticus*) in brackishwater ponds. *Asian Fisheries Science*, **4**(3): 335-343.

Sarig, S., 1983. A review of tilapia culture in Israel, pp. 116-122. *In* L. Fishelson and Z. Yaron (eds.) Proceedings of the International Symposium on Tilapia in Aquaculture, May 1983, Tel Aviv University, Nazareth, Israel.

Sarig, S. and Arieli, Y., 1980. Growth capacity of tilapia in intensive culture. *The Israeli Journal of Aquaculture-Bamidgeh*, **32**(3): 57-65.

Satyanarayana Rao, G.P., Sharma, P.V., Keshava and Keshavappa, G.Y., 1990. Elimination of testosterone in the fry of common carp, *Cyprinus carpio* L, pp. 87-89. Proceedings, The second Indian Fisheries Forum, May 21-31, 1990, Mangalore, India.

Sbordoni, V., Matthaeis, E., Cobolli Sbordoni, M., La Rosa, G. and Mattoccia, M., 1986. Bottleneck effects and the depression of genetic variability in hatchery stocks of *Penaeus japonicus* (Crustacea, Decapoda). *Aquaculture*, **57**: 239-251.

Schoenen, P., 1982. A bibliography of important tilapia (Pisces: Cichlidae) for aquaculture. ICLARM Bibliographies. **3**. International Center for Living Aquatic and Resources Management, Manila, Philippines.

Scott, A.G., Penman, D.J., Beardmore, J.A. and Skibinski, D.O.F., 1989. The 'YY' supermale in *Oreochromis niloticus* (L.) and its potential in aquaculture. *Aquaculture*, **78**: 237-251.

Shackell, N., 1991. Growth and developmental stability of inbred, outbred and inter-strain hybrid tilapia (*Oreochromis niloticus*) in two diet regimes. Dalhousie University, Halifax, Nova Scotia. *Ph.D. Thesis.*

Shah, M.S., 1988. Female homogamety in Tilapia (*Oreochromis niloticus*) revealed by gynogenesis. *Asian Fisheries Science*, **1**: 215-219.

Shelton, W.L., Rodriguez-Guerrero, D. and Lopez-Macias, J., 1981. 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*, pp. 270-280. *In* L. Fishelson and Z. Yaron (eds.) Proceedings of the International Symposium on Tilapia in Aquaculture, May 1983, Tel Aviv University, Nazareth, Israel.

Singh, T.P. and Lal, B., 1989. Sex control and sex reversal in teleost: a review. *In* Das and Jhingran (eds.) Fish Genetics in India. Today and Tomorrow's Printers and Publishers, New Delhi, India.

Smith, I.R. and Pullin, R.S.V., 1984. The tilapia booms in the Philippines. *ICLARM Newsletter*, **7**(1): 7-9.

Smith, M.H. and Chesser, R.K., 1981. Rationale for conserving genetic variation of fish gene pools. *In* N. Ryman (ed.) Fish Gene Pools. *Ecol. Bull.* (Stockholm) **34**: 13-20.

Smitherman, R.O. and Dunham, R.A., 1985. Genetics and breeding. *In* C.S. Tucker (ed.) Channel Catfish Culture. Elsevier Science Publication, Amsterdam, the Netherlands.

Smitherman, R.O. and Tave, D., 1987. Maintenance of genetic quality in cultured tilapia. *Asian Fisheries Science*, **1**: 75-82.

Subba Rao, B. and Chandrasekaran, G., 1978. Preliminary report on hybridization experiments in trout - growth and survival of F_1 hybrids. *Aquaculture*, **115**: 297-300.

Taniguchi, N., Macaranas, J.M. and Pullin, R.S.V., 1985. Introgressive hybridization in cultured tilapia stocks in the Philippines. *Bulletin of the Japanese Society of Scientific Fisheries*, **51**(8): 1219-1224.

Tave, D. and Smitherman, R.O., 1980. Predicted response to selection for early growth in *Tilapia nilotica*. *Trans. Am. Fish. Soc.*, **109**: 439-455.

Tave, D., 1984. Effective breeding efficiency: an index to quantify the effects that different breeding programs and sex ratios have on inbreeding and genetic drift. *Prog. Fish-Cult.*, **46**: 262-268.

Tave, D., 1986. Genetics for hatchery managers. The AVI Publishing Company, Inc., Westport, Connecticut, USA.

Tave, D., 1988. Genetics and breeding of tilapia: a review, pp. 285-293. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings **15**, 623 p. Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Tave, D., Jayaprakas V. and Smitherman, R.O., 1990. Effects of intraspecific hybridization in *Tilapia nilotica* on survival under ambient winter temperature in Alabama. *Journal of the World Aquaculture Society*, **21**(3): 201-204.

Tayamen, M.M. and Shelton, W.L., 1978. Inducement of sex reversal in *Sarotherodon niloticus* (Linnaeus). *Aquaculture*, **14**: 349-354.

Tayamen, M.M., 1992. Progress in fish genetics research in the Philippines. Proceedings of the First ASEAN-EEC aquaculture Development Coordination Programme (AADCP) International Workshops on Genetics in Aquaculture and Fisheries Management, 31 August - 4 September 1992, University of Stirling, Scotland.

Trewavas, E., 1966. A preliminary review of fishes of the genus *Tilapia* in the eastward flowing rivers of Africa, with proposals for two new specific names. *Revue de Zoologie et Botanique Africaine*, **74**: 394-424.

Trewavas, E. 1982. Tilapias: taxonomy and speciation, pp. 3-13. *In* R.S.V. Pullin and R.H. Lowe-McConnell (eds.) The Biology and Culture of Tilapias. ICLARM Conference Proceedings **7**, 432 p. International Center for Living Aquatic Resources Management, Manila, Philippines.

Trombka, D. and Avtalion, R., 1993. Sex determination in tilapia- a review. *The Israeli Journal of Aquaculture - Bamidgeh*, **45**(1): 26-37.

Tuan, P.A., 1997. Options for genetic control of sex ratio in Nile tilapia, *Oreochromis niloticus* (L.). University of Wales Swansea, Wales. *Ph.D. Thesis*, 279 p.

Tuan, P.A., Mair, G.C., Little, D.C. and Beardmore, J.A., 1997. Sex determination and the feasibility of genetically male tilapia (GMT) production in the Thai-Chitralada strain of *Oreochromis niloticus* (L.). Paper presented the Sixth International Symposium on Genetics in Aquaculture, 24-28 June 1997, Institute of Aquaculture, University of Stirling, Scotland.

Ufodike, E.B.C. and Madu, C.T., 1986. Effects of methyltestosterone on food untilization and growth of *Sarotherodon niloticus* fry. *Bulletin of the Japanese Society of Scientific Fisheries*, **5**(11): 1919-1922.

Varadaraj, K. and Pandian, T.J., 1987. Masculinization of *Oreochromis mossambicus* by administration of 17α-methyl-5-androsten-3β-18β-dil through rearing water. *Current Science*, **56**(9): 412-413.

Varadaraj, K. and Pandian, T.J., 1988. Food consumption and growth efficiency of normal and phenotypic male *Oreochromis mossambicus*, pp. 429-432. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings **15**, 623 p. Department of Fisheries, Bangkok, Thailand and International Center for Living Aquatic Resources Management, Manila, Philippines.

Varadaraj, K., 1989. Feminization of *Oreochromis mossambicus* by the administration of diethylstilbestrol. *Aquaculture*, **80**: 337-341.

Varadaraj, K. and Pandian, T.J., 1989a. First report on the production of supermale tilapia by integrating endocrine sex reversal with gynogenetic technique. *Current Science*, **58**(8): 434-441.

Varadaraj, K. and Pandian, T.J., 1989b. Monosex male broods of *Oreochromis mossambicus* produced through artificial sex reversal with 17α-methyl-4-androsten-17β-01-3-one. *In* Das and Jhingran (eds.) Fish Genetics in India. Today and Tomorrow's Printers and Publishers, New Delhi.

Varadaraj, K., Sindhu Kumari, S. and Pandian T.J., 1994. Comparison of conditions for hormonal sex reversal of Mozambique tilapias. *The Progressive Fish-Culturist*, **56**: 81-90.

Vera Cruz, E.M. and Mair, G.C., 1994. Conditions for effective androgen sex reversal in *Oreochromis niloticus* (L.). *Aquaculture*, **122**: 237-248.

Vera Cruz, E.M., Mair, G.C. and Marino, R.P., 1996. Feminization of genotypically YY Nile tilapia *Oreochromis niloticus* L. *Asian Fisheries Science*, **9**: 161-167.

Villegas, C.T., 1990. Growth and survival of *Oreochromis niloticus*, *O. mossambicus* and their F_1 hybrids at various salinities. *In* Hirano, R. and I. Hanyu (eds.) The second Asian Fisheries Forum, 991 p. Asian Fisheries Society, Manila, Philippines.

Vuorinen, J., 1984. Reduction of genetic variability in a hatchery stock of brown trout, *Salmo trutta* L. *Fish Biol.*, **24**: 339-348.

Watanabe, W.O, Ernst, D.H., Chasar, M.P., Wicklund, R.I. and Olla, B.L., 1993a. The effects of temperature and salinity on growth and feed utilization of juvenile, sex-reversed male Florida red tilapia cultured in a recirculating system. *Aquaculture*, **112**: 309-320.

Watanabe, W.O., Mueller, K.W., Head, W.D. and Ellis, S.C., 1993b. Sex reversal of Florida red tilapia in brackish water tanks under different treatment durations of 17α-ehtynyltestosterone administered in feed. *Journal of Applied Aquaculture*, **2**(1).

Welcomme, R.L., 1981. Register of international transfer of inland fish species. *FAO Fish. Tech. Pap.* **213**, 120 p.

Welcomme, R.L., 1988. International introductions of inland aquatic species. *FAO Fish. Tech. Pap.*, **294**, 318 p.

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).

Wohlfarth, G.W., 1983. Genetics of fish: applications to warm water fishes. *Aqauculture*, **33**: 373-381.

Wohlfarth, G.W. and Hulata, G., 1983. Applied genetics of tilapias. *ICLARM Studies and Reviews*, **6**: 1-26.

Wohlfarth, G.W. and Moav, R., 1985. Communal testing, a method of testing the growth of different genetic groups of common carp in earthen ponds. *Aquaculture*, **48**: 143-157.

Wohlfarth, G.W. and Wedekind, H., 1991. The heredity of sex determination in tilapias. *Aquaculture*, **92**: 143-156.

Wohlfarth, G.W., 1992. Association between initial weight and weight gain in brook trout, *Salvelinus fontinalis* Mitchill, and brown trout, *Salmo trutta* L. *Aquaculture and Fisheries Management*, **23**: 549-556.

Wohlfarth, G.W., 1993. Heterosis for growth rate in common carp. *Aquaculture*, **113**: 31-46.

Wohlfarth, G.W. and Moav, R., 1993. Genetic testing of common carp in cages. 2. Influence of variation in initial weight on weight gain. *Aquaculture*, **109**: 245-256.

Wohlfarth, G.W., 1994. The unexploited potential of tilapia hybrids in aquaculture. *Aquaculture and Fisheries Management*, **25**: 781-788.

Wolters, W.R. and DeMay, R., 1996. Production characteristics of Striped bass x White bass and Striped bass x Yellow bass hybrids. *Journal of the World Aquaculture Society*, **27**(2).

Xia, D. and Wu, T., 1993. A review of modern fish breeding research and practices in China. *In* K.L. Main and B. Reynolds (eds.) Selective Breeding of Fishes in Asia and the United States. Published by the Oceanic Institute, Makapuu Point, Hanolulu, Hawaii.

Yamamoto, T.O., 1969. Sex differentiation. *In* W.S. Hoar and D.J. Randall (eds.) *Fish Physiology*, Academic Press, New York and London, Vol. **3**: 117-175.

Yang, Y., Zhang, Z., Lin, K., Wei, Y., Huang, E., Gao, A., Xu, Z., Ke, S. and Wei, J., 1980. Use of three line combination for production of genetic all-male *Tilapia mossambica*. *Acta Scientica Sinica*, **7**: 241-246.

Yant, D.R., Smitherman, R.O. and Green, O.L., 1975. Production of hybrid (Blue x Channel) catfish in ponds. Proc. Conf. Southeast Assoc. Game and Fish Comm., **29**: 86-91.

Yaron, Z., Ilan, Z., Bogomolnaya, A., Blauer, Z. and Vermaak, J.F., 1983. Steroid hormone in two tilapia species: *0reochromis aureus* and *O. niloticus,* pp. 153-164. *In* L. Fishelson and Z. Yaron (eds.) Proceedings of the International Symposium on Tilapia in Aquaculture, May 1983, Tel Aviv University, Nazareth, Israel.

Yater, L.R. and Smith, I.R., 1985. Economics of private tilapia hatcheries in Laguna and Rizal Provinces, Philippines, pp. 15-32. *In* I.R. Smith, E.B. Torres and E.O. Tan (eds.) Philippine Tilapia Economics. ICLARM Conference Proceedings **12**, 261 p. Philippine Council for Agriculture and Resources Research and Development, Los Baños, Laguna and International Center for Living Aquatic Resources Management Center, Manila, Philippines.

APPENDICES

Appendix 1. Sex ratios of progenies from a 2 x 2 diallele cross of Egypt-Swansea (ES) and Egypt-ICLARM (EI) strains using YY males as broodstock.

Family	Strain cross (XX♀ x YY♂)	Number of fry sexed	Sex ratio (♂ : ♀)	% male	χ^2 (1:1)
1	**ES x ES**	50	50 : 0	100.00	50.00 ***
2		27	27 : 0	100.00	27.00 ***
3		24	24 : 0	100.00	4.00 *
4		100	98 : 2	98.00	92.16 ***
5		10	10 : 0	100.00	10.00 **
6		25	23 : 2	92.00	17.64 ***
7		28	28 : 0	100.00	28.00 ***
8		62	62 : 0	100.00	62.00 ***
9		15	15 : 0	100.00	15.00 ***
10		32	32 : 0	100.00	32.00 ***
11		45	45 : 0	100.00	45.00 ***
12		245	242 : 3	98.78	233.15 ***
13		41	41 : 0	100.00	41.00 ***
14		100	100 : 0	100.00	100.00 ***
15		154	153 : 1	99.35	150.03 ***
16		44	44 : 0	100.00	44.00 ***
17		36	36 : 0	100.00	36.00 ***
18		50	50 : 0	100.00	50.00 ***
19		100	100 : 0	100.00	100.00 ***
20		105	105 : 0	100.00	105.00 ***
21		100	100 : 0	100.00	100.00 ***
22		27	27 : 0	100.00	27.00 ***
23		27	27 : 0	100.00	27.00 ***
24		38	38 : 0	100.00	38.00 ***
25		56	54 : 2	96.43	48.29 ***
26		18	18 : 0	100.00	18.00 ***
27		89	85 : 4	95.51	73.72 ***
28		78	76 : 2	97.44	70.21 ***
29		100	98 : 2	98.00	92.16 ***
30		50	50 : 0	100.00	50.00 ***
31		26	26 : 0	100.00	6.00 *
32		46	46 : 0	100.00	46.00 ***
33		61	61 : 0	100.00	61.00 ***
34		75	75 : 0	100.00	75.00 ***
35		36	36 : 0	100.00	36.00 ***
36		47	47 : 0	100.00	47.00 ***
37		7	7 : 0	100.00	7.00 **
38		15	15 : 0	100.00	15.00 ***
39		50	50 : 0	100.00	50.00 ***
40		35	35 : 0	100.00	35.00 ***
41		60	52 : 8	86.67	32.27 ***
1	**EI x ES**	50	50 : 0	100.00	50.00 ***
2		32	32 : 0	100.00	32.00 ***
3		16	16 : 0	100.00	16.00 ***
4		33	33 : 0	100.00	33.00 ***
5		28	28 : 0	100.00	28.00 ***
6		57	57 : 0	100.00	57.00 ***
7		49	49 : 0	100.00	49.00 ***
8		48	48 : 0	100.00	48.00 ***
9		54	52 : 2	96.30	46.30 ***
10		49	49 : 0	100.00	49.00 ***
11		59	59 : 0	100.00	59.00 ***
12		26	14 : 12	53.85	0.15 ns
13		70	69 : 1	98.57	66.06 ***

Appendix 1. Continuation

Family	Strain cross (XX♀ x YY♂)	Number of fry sexed	Sex ratio (♂ : ♀)	% male	χ^2 (1:1)
14		34	34 : 0	100.00	34.00 ***
15		63	63 : 0	100.00	63.00 ***
16		61	61 : 0	100.00	61.00 ***
17		56	56 : 0	100.00	56.00 ***
18		40	40 : 0	100.00	40.00 ***
19		59	59 : 0	100.00	59.00 ***
20		48	48 : 0	100.00	48.00 ***
21		65	61 : 4	93.85	49.98 ***
22		74	74 : 0	100.00	74.00 ***
23		49	48 : 1	97.96	45.08 ***
24		148	148 : 0	100.00	148.00 ***
1	EI x EI	46	46 : 0	100.00	46.00 ***
2		37	37 : 0	100.00	37.00 ***
3		35	35 : 0	100.00	35.00 ***
4		51	51 : 0	100.00	51.00 ***
5		34	34 : 0	100.00	34.00 ***
6		47	47 : 0	100.00	47.00 ***
7		64	64 : 0	100.00	64.00 ***
8		37	37: 0	100.00	37.00 ***
9		36	36 : 0	100.00	36.00 ***
10		41	41 : 0	100.00	41.00 ***
11		48	48 : 0	100.00	48.00 ***
12		49	49 : 0	100.00	49.00 ***
13		10	9 : 1	90.00	6.40 *
14		17	17 : 0	100.00	17.00***
1	ES x EI	50	50 : 0	100.00	50.00 ***
2		55	55 : 0	100.00	55.00 ***
3		50	50 : 0	100.00	50.00 ***
4		49	49 : 0	100.00	49.00 ***
5		72	72 : 0	100.00	72.00 ***
6		50	50 : 0	100.00	50.00 ***
7		44	44 : 0	100.00	44.00 ***
8		50	49 : 1	98.00	46.08 ***
9		72	70 : 2	97.22	64.22 ***
10		10	10 : 0	100.00	10.00 **
11		29	29 : 0	100.00	29.00 ***
12		27	27 : 0	100.00	27.00 ***
13		46	46 : 0	100.00	46.00 ***
14		58	58 : 0	100.00	58.00 ***
15		29	29 : 0	100.00	29.00 ***
16		25	25 : 0	100.00	25.00 ***
17		46	46 : 0	100.00	46.00 ***
18		43	43 : 0	100.00	43.00 ***
19		9	9 : 0	100.00	9.00 **
20		46	46 : 0	100.00	46.00 ***
21		20	20 : 0	100.00	20.00 ***
22		37	36 : 1	97.30	33.11 ***
23		39	38 : 1	97.44	35.10 ***
24		29	28 : 1	96.55	25.14 ***
25		19	19 : 0	100.00	19.00 ***
26		22	21 : 1	95.45	18.18 ***
27		15	13 : 2	86.67	8.07 **
28		27	23 : 4	85.19	13.37 ***
29		15	15 : 0	100.00	15.00 ***
30		29	29 : 0	100.00	29.00 ***
31		22	22 : 0	100.00	22.00 ***
32		44	40 : 4	90.91	29.45 ***

Appendix 1. Continuation

Family	Strain cross (XX♀ x YY♂)	Number of fry sexed	Sex ratio (♂ : ♀)	% male	χ^2 (1:1)
33		56	56 : 0	100.00	56.00 ***
34		52	52 : 0	100.00	52.00 ***
35		64	64 : 0	100.00	64.00 ***
36		63	63 : 0	100.00	63.00 ***
37		64	64 : 0	100.00	64.00 ***
38		61	61 : 0	100.00	61.00 ***
39		50	50 : 0	100.00	50.00 ***
40		32	32 : 0	100.00	32.00 ***
41		56	54 : 2	96.43	48.29 ***
42		44	44 : 0	100.00	44.00 ***
43		53	53 : 0	100.00	53.00 ***
44		64	64 : 0	100.00	64.00 ***
45		50	50 : 0	100.00	50.00 ***
46		36	35 : 1	97.22	32.11 ***
47		79	78 : 1	98.73	75.05 ***
48		81	81 : 0	100.00	81.00 ***
49		60	60 : 0	100.00	60.00 ***
50		35	35 : 0	100.00	35.00 ***
51		26	26 : 0	100.00	26.00 ***
52		39	39 : 0	100.00	39.00 ***
53		37	37 : 0	100.00	37.00 ***
54		51	51 : 0	100.00	51.00 ***
55		67	67 : 0	100.00	67.00 ***
56		11	7 : 4	63.64	0.82 ns
57		35	35 : 0	100.00	35.00 ***

Appendix 2. Sex ratios of the progenies of YY males in Egypt-Swansea strain in crosses with females from different strain in *O. niloticus*.

Family	Female strain	Number of fry sexed	Sex ratio (♂ : ♀)	% male	χ^2 (1:1)
1	E.-Stirling	32	32 : 0	100.00	32.00 ***
2		58	58 : 0	100.00	58.00 ***
3		50	49 : 1	98.00	46.08 ***
4		129	127 : 2	98.45	121.12 ***
5		51	51 : 0	100.00	51.00 ***
6		31	31 : 0	100.00	31.00 ***
7		25	25 : 0	100.00	25.00 ***
8		101	100 : 1	99.01	97.04 ***
9		105	105 : 0	100.00	105.00 ***
10		49	49 : 0	100.00	49.00 ***
11		29	29 : 0	100.00	29.00 ***
12		60	60 : 0	100.00	60.00 ***
13		85	85 : 0	100.00	85.00 ***
14		27	26 :1	96.30	23.15 ***
15		33	33 : 0	100.00	33.00 ***
16		56	51 : 5	91.07	37.79 ***
17		31	27 : 4	87.10	17.06 ***
18		10	10 : 0	100.00	10.00 **
19		25	25 : 0	100.00	25.00 ***
20		17	16 : 1	94.12	13.24 ***
21		36	26 : 10	72.22	7.11 **
22		37	24 : 13	64.86	3.27 ns
23		36	25 : 11	69.44	5.44 *
24		33	32 : 1	96.97	29.12***
25		50	50 : 0	100.00	50.00 ***
26		26	24 : 2	92.31	18.62 ***
27		80	74 : 6	92.50	57.80 ***
28		47	47 : 0	100.00	47.00 ***
29		32	32 : 0	100.00	32.00 ***
30		44	44 : 0	100.00	44.00 ***
31		33	28 : 5	84.85	16.03 ***
32		73	65 : 8	89.04	44.51 ***
33		41	41 : 0	100.00	41.00 ***
34		51	51 : 0	100.00	51.00 ***
1	E.-AIT	113	110 : 3	97.35	101.32 ***
2		62	62 : 0	100.00	62.00 ***
3		37	35 : 2	94.59	29.43 ***
4		66	66 : 0	100.00	66.00 ***
5		25	22 : 3	88.00	14.14 ***
6		62	34 : 28	54.84	0.58 ns
7		182	156 : 26	85.71	92.86 ***
8		40	39 : 1	97.50	36.10 ***
9		90	89 : 1	98.89	86.04 ***
10		105	100 : 5	95.24	85.95 ***
11		48	48 : 0	100.00	48.00 ***
12		108	93 : 15	86.11	56.33 ***
13		69	67 : 2	97.10	61.23 ***
14		70	54 : 16	77.40	20.63 ***
15		90	90 : 0	100.00	90.00 ***
16		78	78 : 0	100.00	78.00 ***
17		106	100 : 6	94.34	83.36 ***
18		29	29 : 0	100.00	29.00 ***
19		20	18 : 2	90.00	12.80 ***
20		13	10 : 3	76.92	3.77 ns
21		20	20 : 0	100.00	20.00 ***
22		31	31 : 0	100.00	31.00 ***

Appendix 2. Continuation

Family	Female strain	Number of fry sexed	Sex ratio (♂ : ♀)	% male	χ^2 (1:1)
23		41	41 : 0	100.00	41.00 ***
24		31	29 : 2	93.55	23.52 ***
25		30	28 : 2	93.33	22.53 ***
26		32	19 : 13	59.38	1.13 ns
27		35	31 : 4	88.57	20.83 ***
28		43	43 : 0	100.00	43.00 ***
29		37	34 : 3	91.89	25.97 ***
30		19	19 : 0	100.00	19.00 ***
31		10	10 : 0	100.00	10.00 **
32		30	30 : 0	100.00	30.00 ***
33		55	55 : 0	100.00	55.00 ***
34		40	39 : 1	97.50	36.10 ***
35		21	19 : 2	90.48	13.76 ***
36		52	52 : 0	100.00	52.00 ***
37		66	66 : 0	100.00	66.00 ***
38		68	51 : 17	75.00	17.00 ***
39		27	25 : 2	92.59	19.59 ***
40		178	178 : 0	100.00	178.00 ***
41		32	32 : 0	100.00	32.00 ***
1	E.-BFAR	22	22 : 0	100.00	22.00 ***
2		29	28 : 1	96.55	25.14 ***
3		28	28 : 0	100.00	28.00 ***
4		31	31 : 0	100.00	31.00 ***
5		58	58 : 0	100.00	58.00 ***
6		29	26 : 3	89.66	18.24 ***
7		32	32 : 0	100.00	32.00 ***
8		23	23 : 0	100.00	23.00 ***
9		54	54 : 0	100.00	54.00 ***
1	K.-Turkana	86	86 : 0	100.00	86.00 ***
2		91	91 : 0	100.00	91.00 ***
3		50	50 : 0	100.00	50.00 ***
4		69	66 : 3	95.65	57.52 ***
5		50	50 : 0	100.00	50.00 ***
6		100	100 : 0	100.00	100.00 ***
7		42	42 : 0	100.00	42.00 ***
8		56	56 : 0	100.00	56.00 ***
9		59	59 : 0	100.00	59.00 ***
10		17	17 : 0	100.00	17.00 ***
11		18	18 : 0	100.00	18.00 ***
12		31	30 : 1	96.77	27.13 ***
13		20	20 : 0	100.00	20.00 ***
14		24	24 : 0	100.00	24.00 ***
15		50	50 : 0	100.00	50.00 ***
16		42	40 : 2	95.24	34.38 ***
17		27	27 : 0	100.00	27.00 ***
18		25	25 : 0	100.00	25.00 ***
19		24	24 : 0	100.00	24.00 ***
20		43	43 : 0	100.00	43.00 ***
21		67	67 : 0	100.00	67.00 ***
22		11	11 : 0	100.00	11.00 ***
23		43	36 : 7	83.72	19.56 ***
24		34	34 : 0	100.00	34.00 ***
25		64	64 : 0	100.00	64.00 ***
26		22	22 : 0	100.00	22.00 ***
27		51	51 : 0	100.00	51.00 ***

Appendix 2. Continuation

Family	Female strain	Number of fry sexed	Sex ratio (♂ : ♀)	% male	χ^2 (1:1)
1	K.-Baringo	61	61 : 0	100.00	61.00 ***
2		11	11 : 0	100.00	11.00 ***
3		55	55 : 0	100.00	55.00 ***
4		8	8 : 0	100.00	8.00 **
5		67	67 : 0	100.00	67.00 ***
1	K.-Baobab	31	29 : 2	93.55	23.52 ***
2		49	39 : 10	79.59	17.16 ***
3		250	237 : 13	94.80	200.70 ***
4		35	35 : 0	100.00	35.00 ***
5		31	31 : 0	100.00	31.00 ***
6		53	52 : 1	98.11	49.08 ***
7		23	13 : 10	56.52	0.39 ns
8		41	41 : 0	100.00	41.00 ***
9		27	27 : 0	100.00	27.00 ***
1	GIFT fish	97	97 : 0	100.00	97.00 ***
2		53	53 : 0	100.00	53.00 ***
3		25	21 : 4	84.00	11.56 ***
4		13	12 : 1	92.31	9.31 **
5		133	133 : 0	100.00	133.00***
6		85	84 : 1	98.82	81.05 ***
7		43	35 : 8	81.40	16.95 ***
8		35	35 : 0	100.00	35.00 ***
9		55	55 : 0	100.00	55.00 ***
10		29	28 : 1	96.55	25.14 ***
11		25	21 : 4	84.00	11.56 ***
12		46	46 : 0	100.00	46.00 ***
13		43	42 : 1	97.67	39.09 ***
14		15	15 : 0	100.00	15.00 ***
1	IDRC fish	107	106 : 1	99.07	103.04 ***
2		22	19 : 3	86.36	11.64 ***
3		81	80 : 1	98.77	77.05 ***
4		211	208 : 3	98.58	199.17 ***
5		19	19 : 0	100.00	19.00 ***
6		45	45 : 0	100.00	45.00 ***
7		52	52 : 0	100.00	52.00 ***
8		56	55 : 1	98.21	52.07 ***
9		46	43 : 3	93.48	34.78 ***
10		93	93 : 0	100.00	93.00 ***
11		39	39 : 0	100.00	39.00 ***
12		52	52 : 0	100.00	52.00 ***
13		7	7 : 0	100.00	7.00 **
14		41	41 : 0	100.00	41.00 ***
15		35	23 : 12	65.71	3.46 ns
16		34	34 : 0	100.00	34.00 ***

Appendix 3. Average weight (g), standard deviation (in parenthesis) and number of fish sampled (n) during growth evaluation of Egypt-Swansea (ES) and Egypt-ICLARM (EI) strains and their reciprocal hybrids in earthen ponds. Sampling was performed every after 21 days from stocking.

Treatment	Replic.	0	n	21	n	42	n	63	n
ES x ES	1	9.60 (2.39)	60	28.61 (4.54)	25	65.35 (11.00)	25	87.77 (13.90)	29
	2	9.00 (2.15)	60	27.87 (5.07)	25	48.63 (9.41)	22	71.38 (12.88)	24
	3	9.27 (2.30)	60	32.21 (4.08)	25	66.17 (11.77)	28	95.81 (16.53)	31
Mean		**9.29**		**29.56**		**60.05**		**84.99**	
EI x ES	1	10.66 (2.25)	60	33.60 (5.57)	24	79.55 (9.92)	22	112.50 (10.78)	22
	2	9.31 (1.63)	60	31.30 (4.29)	25	60.78 (5.09)	12	82.04 (7.23)	29
	3	10.18 (1.79)	60	37.04 (4.06)	21	78.50 (9.64)	31	116.42 (11.63)	36
Mean		**10.05**		**33.98**		**72.94**		**103.65**	
EI x EI	1	11.80 (3.05)	60	33.24 (7.89)	9	78.78 (9.76)	11	113.71 (15.82)	30
	2	9.55 (3.09)	60	31.09 (5.28)	12	55.11 (6.50)	18	81.49 (12.24)	34
	3	11.65 (2.90)	60	39.88 (5.98)	9	78.64 (15.53)	38	108.69 (19.34)	29
Mean		**11.0**		**34.74**		**70.85**		**101.30**	
ES x EI	1	13.62 (4.79)	60	37.26 (6.42)	21	80.44 (9.88)	11	119.47 (16.19)	26
	2	9.79 (2.40)	60	33.86 (6.26)	23	60.92 (7.13)	9	82.83 (10.92)	21
	3	12.91 (3.78)	60	40.56 (5.34)	24	78.37 (9.49)	22	103.41 (17.45)	24
Mean		**12.11**		**37.22**		**73.24**		**101.91**	

Appendix 3. Continuation

Treatment	Replic.	84	n	105	n	126	n	147	n
ES x ES	1	116.60 (19.78)	26	148.21 (25.98)	30	168.14 (31.72)	35	219.88 (46.62)	58
	2	101.43 (17.39)	31	123.05 (23.61)	28	151.41 (29.23)	26	182.84 (40.04)	49
	3	134.07 (21.70)	20	167.66 (26.66)	24	188.69 (47.29)	34	225.97 (48.84)	57
Mean		**117.37**		**146.31**		**169.41**		**209.56**	
EI x ES	1	146.36 (20.98)	16	182.52 (23.09)	13	209.56 (25.38)	31	260.64 (38.61)	55
	2	111.95 (16.12)	17	142.51 (16.70)	19	174.47 (18.40)	24	212.18 (22.72)	55
	3	165.51 (22.81)	21	188.04 (26.42)	27	211.69 (33.39)	29	259.75 (44.25)	59
Mean		**141.28**		**171.02**		**198.57**		**244.19**	
EI x EI	1	149.98 (20.32)	33	172.03 (21.67)	21	220.71 (31.41)	22	270.75 (39.85)	55
	2	113.24 (14.81)	15	137.39 (17.86)	19	173.05 (28.10)	21	219.34 (32.57)	49
	3	150.20 (25.03)	24	176.20 (21.02)	12	205.66 (22.47)	22	246.15 (35.92)	52
Mean		**137.80**		**161.87**		**199.81**		**245.41**	
ES x EI	1	148.39 (19.42)	17	187.02 (23.81)	17	207.69 (25.90)	24	273.78 (39.63)	59
	2	118.55 (19.90)	27	142.25 (24.97)	15	179.01 (25.41)	21	223.94 (34.18)	59
	3	144.06 (21.22)	12	183.46 (25.62)	26	209.14 (29.82)	23	239.28 (49.07)	54
Mean		**137.00**		**170.91**		**198.61**		**245.67**	

Appendix 4. Mean weight (g), standard deviation (in parenthesis) and number of fish sampled (n) during growth evaluation of Egypt-Swansea (ES) and Egypt-ICLARM (EI) strains and their reciprocal hybrids in cages-in-pond (Cage-1). Sampling was performed every after 21 days from stocking.

Treatment	Replic.	0	n	21	n	42	n	63	n
ES x ES	1	13.78 (1.95)	45	15.74 (1.98)	45	21.78 (2.65)	44	28.91 (3.06)	44
	2	12.90 (2.70)	45	15.97 (2.66)	44	20.73 (3.13)	36	29.48 (3.55)	36
	3	13.00 (2.80)	45	15.60 (2.89)	45	22.77 (3.49)	44	30.43 (3.29)	44
Mean		**13.23**		**15.77**		**21.76**		**29.61**	
EI x ES	1	11.43 (1.87)	45	12.72 (1.87)	45	19.94 (2.23)	45	26.88 (2.93)	45
	2	12.30 (1.98)	45	14.80 (1.92)	45	19.34 (1.82)	32	28.02 (3.00)	32
	3	11.99 (1.67)	45	14.76 (1.95)	45	22.50 (2.36)	44	30.51 (2.96)	43
Mean		**11.91**		**14.09**		**20.59**		**28.47**	
EI x EI	1	13.92 (2.76)	45	15.34 (2.59)	45	22.03 (3.15)	44	28.70 (3.86)	45
	2	13.99 (1.80)	45	15.64 (2.23)	43	21.67 (2.48)	28	30.57 (3.10)	28
	3	16.34 (2.64)	45	17.12 (2.36)	45	24.67 (3.09)	44	31.10 (3.45)	44
Mean		**14.75**		**16.03**		**22.79**		**30.12**	
ES x EI	1	11.60 (1.74)	45	14.54 (1.77)	44	21.59 (2.37)	44	28.93 (2.80)	44
	2	11.64 (1.88)	45	14.74 (2.48)	43	20.86 (3.58)	32	30.43 (3.76)	33
	3	11.37 (1.92)	45	13.19 (1.79)	44	20.53 (3.09)	44	28.22 (4.14)	44
Mean		**11.54**		**14.16**		**20.99**		**29.19**	

Appendix 4. Continuation

Treatment	Replic.	84	n	105	n	126	n	147	n
ES x ES	1	33.03 (3.16)	42	36.69 (4.21)	43	40.18 (5.57)	40	51.19 (6.51)	43
	2	34.61 (4.06)	36	40.61 (4.90)	37	44.43 (5.62)	35	57.54 (6.40)	34
	3	36.68 (5.13)	44	43.30 (5.35)	44	46.86 (6.51)	41	58.94 (8.10)	42
Mean		**34.77**		**40.20**		**43.82**		**55.89**	
EI x ES	1	31.49 (3.54)	45	36.98 (4.32)	45	41.16 (5.37)	45	53.50 (6.84)	45
	2	34.24 (3.55)	30	41.27 (4.43)	31	46.29 (5.45)	33	59.62 (9.12)	32
	3	38.00 (4.08)	41	45.17 (5.15)	39	50.74 (5.99)	41	64.20 (7.92)	41
Mean		**34.58**		**41.14**		**46.06**		**59.10**	
EI x EI	1	34.78 (4.44)	44	39.02 (6.38)	45	44.56 (6.02)	45	61.76 (8.78)	45
	2	37.00 (3.86)	27	44.82 (4.42)	27	50.35 (5.35)	27	66.69 (6.75)	27
	3	37.10 (4.10)	44	42.94 (5.35)	43	46.99 (6.84)	45	60.84 (9.30)	43
Mean		**36.30**		**42.26**		**47.30**		**63.09**	
ES x EI	1	32.33 (3.04)	45	37.22 (4.06)	44	41.80 (4.97)	44	56.58 (5.76)	44
	2	36.19 (4.65)	34	43.07 (4.59)	32	47.84 (5.32)	30	63.61 (6.91)	32
	3	34.03 (5.36)	43	41.02 (6.53)	42	45.02 (8.81)	42	58.35 (11.38)	42
Mean		**34.19**		**40.44**		**44.89**		**59.52**	

Appendix 5. Mean weight (g), standard deviation (in parenthesis) and number of fish sampled (n) during growth evaluation of Egypt-Swansea (ES) and Egypt-ICLARM (EI) strains and their reciprocal hybrids in cages-in-pond (cage-2). Sampling was performed every after 21 days from stocking.

Treatment	Replicate	0	n	21	n	42	n
ES x ES	1	52.77 (5.22)	30	57.37 (6.64)	23	67.19 (7.77)	22
	2	52.87 (6.63)	30	63.76 (7.92)	30	74.06 (9.80)	30
	3	58.37 (8.32)	30	67.88 (9.17)	30	82.49 (10.54)	30
Mean		**5634**		**63.00**		**74.58**	
EI x ES	1	55.19 (7.04)	30	58.51 (7.74)	19	70.96 (9.32)	17
	2	58.79 (7.51)	30	66.25 (9.01)	30	80.65 (8.09)	25
	3	63.92 (7.50)	30	75.19 (8.65)	29	91.17 (10.33)	30
Mean		**59.30**		**66.65**		**80.93**	
EI x EI	1	60.98 (9.26)	30	64.92 (11.26)	22	80.33 (13.59)	22
	2	66.69 (6.75)	27	74.98 (7.93)	27	89.15 (10.44)	26
	3	61.01 (9.75)	30	72.33 (11.19)	30	87.59 (13.69)	30
Mean		**62.89**		**70.74**		**85.69**	
ES x EI	1	56.66 (6.46)	30	64.40 (7.22)	27	75.51 (8.67)	30
	2	63.72 (7.03)	30	72.52 (8.37)	30	84.30 (9.42)	29
	3	56.22 (11.06)	30	66.98 (13.62)	29	79.34 (16.56)	29
Mean		**58.87**		**67.96**		**79.72**	

Appendix 5. Continuation

Treatment	Replicate	63	n	84	n	105	n
ES x ES	1	72.44 (8.79)	21	82.89 (10.64)	22	102.57 (11.33)	22
	2	78.92 (11.00)	30	86.55 (12.16)	30	111.61 (15.80)	30
	3	88.26 (12.15)	29	95.00 (12.39)	27	118.34 (15.70)	28
Mean		**79.87**		**88.15**		**110.84**	
EI x ES	1	76.97 (10.91)	16	89.48 (12.61)	16	116.03 (15.96)	16
	2	85.53 (9.86)	28	95.28 (11.71)	30	122.53 (15.45)	28
	3	98.59 (12.10)	29	108.83 (14.15)	28	136.01 (16.09)	28
Mean		**87.03**		**97.86**		**124.86**	
EI x EI	1	82.73 (13.89)	22	93.49 (17.20)	23	121.42 (21.99)	23
	2	96.23 (11.22)	28	105.07 (14.30)	27	136.54 (19.01)	27
	3	94.08 (14.95)	30	100.01 (15.95)	30	130.02 (18.39)	28
Mean		**91.01**		**99.52**		**129.33**	
ES x EI	1	81.82 (11.07)	30	9.65 (12.55)	29	120.30 (16.78)	29
	2	92.35 (9.84)	25	101.06 (12.88)	28	130.32 (15.42)	30
	3	86.04 (18.73)	29	92.76 (22.06)	28	117.52 (28.38)	28
Mean		**86.74**		**95.49**		**122.71**	

Appendix 6. Average weight (mg) and total number fish in each replicate (n) during methyltestosterone (MT) treatment of putative genetic male tilapia (GMT), genetic female tilapia (GFT) and mixed-sex tilapia (MST) in concrete tanks. Sampling was performed every after seven days from stocking.

Treatment	Repli.	0	n	7	n	14	n	21	n	28	n
GMT-MT	1	10.0	150	23.7	139	39.2	120	61.9	105	89.0	100
	2	10.0	150	24.6	134	36.9	122	57.1	105	82.0	100
	3	9.3	150	26.1	134	40.2	117	62.8	94	93.2	88
Mean		**9.8**		**24.8**		**38.7**		**60.6**		**88.1**	
GMT-CT	1	8.7	150	15.9	132	27.6	105	54.3	94	85.4	89
	2	9.3	150	21.1	128	39.3	117	59.8	112	84.8	105
	3	9.3	150	19.8	126	34.9	126	63.2	106	81.6	103
Mean		**9.1**		**18.9**		**34.0**		**59.1**		**83.9**	
GFT-MT	1	8.3	150	19.6	143	28.8	139	38.4	99	59.1	88
	2	8.3	150	17.7	147	25.5	145	34.1	132	47.7	128
	3	8.7	150	16.6	145	19.8	121	29.4	68	59.4	64
Mean		**8.4**		**17.9**		**24.7**		**34.0**		**55.4**	
GFT-CT	1	8.7	150	19.2	146	30.4	135	46.2	132	58.5	130
	2	8.7	150	15.2	145	23.7	131	40.3	129	59.5	126
	3	8.0	150	14.4	139	23.4	128	38.7	124	50.4	121
Mean		**8.4**		**16.2**		**25.8**		**41.7**		**56.1**	
MST-MT	1	8.7	150	22.2	144	26.8	142	35.3	116	58.7	98
	2	10.0	150	20.7	150	26.9	145	37.8	119	59.6	109
	3	10.0	150	20.1	149	27.4	146	37.3	126	54.3	116
Mean		**9.6**		**21.0**		**27.0**		**36.8**		**57.5**	
MST-CT	1	8.7	150	16.8	149	28.9	142	39.0	141	52.0	123
	2	10.0	150	18.9	148	28.5	137	42.6	136	54.8	135
	3	9.3	150	16.9	148	25.7	140	43.0	128	57.1	126
Mean		**9.3**		**17.5**		**27.7**		**41.5**		**54.7**	

Appendix 7. Average total length (mm) and standard deviation (sd) of 10 random fish samples from each replicate during methyltestosterone (MT) treatment of putative genetic male tilapia (GMT), genetic female tilapia (GFT) and mixed-sex tilapia (MST) in concrete tanks. Sampling was performed every after seven days from stocking.

Treatment	Repli.	0	sd	7	sd	14	sd	21	sd	28	sd
GMT-MT	1	8.98	0.96	11.70	1.73	12.10	2.21	14.24	3.61	17.09	4.95
	2	9.28	0.91	12.10	2.53	12.9	2.38	15.52	3.97	16.49	4.37
	3	9.27	1.21	11.87	2.64	12.88	2.05	14.80	3.48	17.42	4.45
Mean		**9.18**		**11.89**		**12.42**		**14.85**		**17.00**	
GMT-CT	1	8.80	1.09	11.50	1.42	11.71	1.82	14.15	2.21	16.90	4.32
	2	8.82	0.95	11.51	2.11	12.34	1.95	14.78	3.08	17.74	4.15
	3	9.26	1.23	11.16	1.73	12.11	1.62	14.07	3.20	17.84	4.25
Mean		**8.96**		**11.39**		**12.05**		**14.33**		**17.49**	
GFT-MT	1	8.60	0.31	11.00	0.87	12.28	1.18	13.80	2.02	15.53	3.05
	2	8.38	0.47	10.74	0.46	11.39	1.27	12.60	1.19	14.52	2.75
	3	8.06	0.32	10.64	0.49	10.94	0.68	12.75	1.55	15.05	2.44
Mean		**8.35**		**10.83**		**11.54**		**13.05**		**15.03**	
GFT-CT	1	8.67	0.16	10.99	0.65	12.05	1.37	13.76	1.66	15.88	2.26
	2	8.57	0.54	10.31	0.47	11.17	1.11	12.93	1.95	15.77	2.23
	3	8.28	0.25	10.48	0.34	11.17	1.08	13.72	1.41	14.51	2.37
Mean		**8.51**		**10.59**		**11.46**		**13.47**		**15.51**	
MST-MT	1	8.92	0.15	11.59	0.69	12.09	1.03	13.25	2.57	16.49	3.16
	2	8.97	0.24	11.27	0.48	12.11	0.75	12.87	0.87	14.57	2.40
	3	8.65	0.68	11.12	0.53	11.74	0.74	13.56	1.53	14.25	2.47
Mean		**8.85**		**11.33**		**11.98**		**13.23**		**15.10**	
MST-CT	1	9.16	0.15	10.73	0.52	11.81	0.82	13.27	1.27	15.01	2.05
	2	8.96	0.16	11.00	0.58	11.47	0.80	13.01	1.23	14.57	2.43
	3	8.98	0.40	11.04	0.73	11.85	0.93	12.97	1.56	15.06	2.09
Mean		**9.03**		**10.92**		**11.71**		**13.08**		**14.88**	

Appendix 8. Average weight (g), standard deviation (in parenthesis) and number of fish sampled (n) during evaluation of post-treatment growth of methyltestosterone-treated putative genetic male tilapia (GMT), genetic female tilapia (GFT) and mixed-sex tilapia (MST) in cages-in-pond. Sampling was performed every after 14 days from stocking.

Treatment	Repli.	0	n	14	n	28	n	42	n	56	n
GMT-MT	1	1.44 (0.60)	50	2.91 (1.47)	50	5.39 (2.71)	50	9.16 (4.41)	50	12.03 (6.95)	50
	2	1.55 (0.62)	50	3.44 (1.58)	50	5.58 (3.22)	50	10.56 (5.02)	50	13.81 (7.44)	50
	3	1.97 (0.79)	50	2.97 (1.47)	50	5.00 (2.62)	50	9.31 (4.17)	50	12.77 (5.93)	50
Mean		**1.65**		**3.11**		**5.33**		**9.68**		**12.87**	
GMT-CT	1	1.36 (0.72)	50	2.85 (1.46)	50	4.80 (2.29)	50	9.10 (3.60)	50	11.45 (5.64)	50
	2	1.48 (0.60)	50	2.86 (1.34)	50	5.58 (2.72)	50	10.21 (4.56)	50	13.67 (5.96)	50
	3	1.28 (0.54)	50	2.39 (0.98)	50	4.57 (2.00)	50	8.19 (3.43)	50	12.68 (4.89)	50
Mean		**1.37**		**2.70**		**4.98**		**9.16**		**12.60**	
GFT-MT	1	1.29 (0.52)	50	2.30 (1.14)	50	4.58 (2.15)	50	8.35 (3.41)	50	10.70 (4.85)	50
	2	1.16 (0.51)	50	2.76 (1.13)	50	4.59 (2.27)	50	8.42 (3.64)	50	13.04 (5.17)	50
	3	1.33 (0.50)	50	2.63 (0.91)	50	4.13 (2.35)	50	8.84 (3.05)	50	11.22 (5.91)	50
Mean		**1.26**		**2.56**		**4.43**		**8.54**		**11.65**	
GFT-CT	1	0.88 (0.23)	59	1.94 (0.51)	59	3.66 (1.09)	59	5.86 (1.69)	59	7.98 (2.51)	59
	2	0.87 (0.29)	50	2.34 (0.65)	50	4.14 (1.31)	50	7.14 (2.23)	50	10.12 (3.27)	50
	3	0.94 (0.32)	50	1.88 (0.54)	50	3.24 (1.30)	50	6.14 (1.91)	50	9.34 (3.10)	50
Mean		**0.90**		**2.05**		**3.68**		**6.39**		**9.15**	
MST-MT	1	1.22 (0.46)	50	2.57 (1.04)	50	3.90 (1.59)	50	7.81 (3.13)	50	10.36 (4.56)	50
	2	1.12 (0.41)	50	2.04 (0.85)	50	4.53 (1.85)	50	7.71 (3.21)	50	9.54 (5.15)	50
	3	1.09 (0.42)	50	2.02 (0.76)	50	4.33 (1.28)	50	6.31 (2.27)	50	9.29 (3.68)	50
Mean		**1.14**		**2.21**		**4.25**		**7.28**		**9.73**	
MST-CT	1	0.82 (0.23)	50	2.05 (0.55)	50	4.05 (1.11)	50	6.48 (2.03)	50	8.32 (3.18)	50
	2	0.91 (0.25)	50	1.89 (0.63)	50	3.06 (1.11)	50	5.89 (1.66)	50	8.17 (2.80)	50
	3	0.83 (0.41)	50	1.64 (0.63)	50	3.08 (1.40)	50	5.80 (2.07)	50	8.28 (3.26)	50
Mean		**0.85**		**1.86**		**3.39**		**6.05**		**8.26**	

Appendix 8. Continuation

Treatment	Repli.	70	n	84	n	98	n	112	n	126	n
GMT-MT	1	17.69 (7.41)	50	22.36 (9.66)	50	21.55 (10.80)	78	24.93 (12.10)	78	31.45 (14.86)	78
	2	18.29 (10.59)	50	25.44 (10.50)	50	23.65 (12.06)	73	27.41 (12.77)	73	32.83 (13.98)	73
	3	16.52 (7.59)	50	21.65 (9.10)	50	21.60 (9.76)	75	26.27 (10.96)	75	32.31 (12.12)	74
Mean		**17.50**		**23.15**		**22.27**		**26.20**		**32.20**	
GMT-CT	1	14.74 (6.87)	50	20.26 (8.07)	50	18.72 (9.36)	84	22.33 (9.69)	84	26.67 (10.49)	83
	2	15.19 (7.76)	50	21.57 (10.30)	50	23.23 (11.04)	76	29.78 (12.37)	76	35.53 (12.67)	76
	3	13.44 (6.55)	50	18.06 (7.38)	50	19.05 (8.77)	79	23.73 (9.78)	79	29.38 (10.95)	79
Mean		**14.46**		**19.96**		**20.33**		**25.28**		**30.53**	
GFT-MT	1	12.32 (6.94)	50	19.11 (7.20)	50	19.89 (8.81)	75	23.98 (10.30)	75	27.51 (11.65)	74
	2	15.99 (6.99)	50	21.19 (8.06)	50	22.19 (10.43)	74	27.92 (12.06)	75	34.70 (13.49)	74
	3	17.86 (5.85)	50	20.55 (7.44)	50	19.71 (9.36)	73	24.60 (10.67)	71	29.54 (12.64)	70
Mean		**15.39**		**20.28**		**20.59**		**25.50**		**30.58**	
GFT-CT	1	12.06 (3.56)	59	16.34 (3.92)	59	16.25 (5.23)	80	19.67 (5.62)	80	23.53 (6.40)	80
	2	13.32 (3.26)	50	16.79 (4.15)	50	17.99 (5.52)	74	22.60 (5.95)	74	26.29 (6.70)	73
	3	12.45 (4.35)	50	15.50 (4.23)	50	15.14 (5.29)	77	18.34 (5.47)	78	22.04 (5.95)	74
Mean		**12.58**		**16.21**		**16.46**		**20.20**		**23.95**	
MST-MT	1	13.65 (5.31)	50	17.29 (6.98)	50	17.80 (8.16)	79	24.22 (10.62)	77	27.51 (11.90)	78
	2	14.08 (6.52)	50	17.68 (8.38)	50	18.35 (8.79)	77	23.22 (10.29)	79	27.92 (12.05)	79
	3	12.35 (4.63)	50	17.49 (6.03)	50	16.45 (7.87)	75	19.65 (9.23)	75	25.57 (11.80)	75
Mean		**13.36**		**17.48**		**17.53**		**22.37**		**27.00**	
MST-CT	1	12.27 (3.58)	50	16.57 (3.96)	50	17.76 (4.76)	73	21.86 (5.54)	75	27.26 (5.72)	73
	2	11.77 (3.94)	50	15.59 (5.05)	50	17.61 (6.17)	74	22.96 (7.05)	71	27.11 (7.68)	70
	3	10.92 (4.19)	50	15.10 (5.00)	50	15.28 (6.62)	70	19.58 (7.96)	72	24.51 (8.41)	70
Mean		**11.66**		**15.75**		**16.88**		**21.47**		**25.29**	

Appendix 9. Average weight (g), standard deviation (in parenthesis) and number of fish sampled (n) during growth evaluation of methyltestosterone-treated (MT) and untreated control males (CT) genetically males (GMT), genetically females (GFT) and mixed-sex tilapia (MST) in an all-male (A) and mixed-sex (B) culture environments in earthen ponds. Sampling was performed every after 21 days from stocking.

Treatment		0	n	21	n	42	n	63	n	84	n	105	n	126	n
GMT-MT	A	44.93 (14.02)	50	85.29 (23.19)	39	124.15 (29.43)	22	162.44 (35.75)	27	189.26 (33.20)	16	192.27 (38.07)	34	205.75 (48.94)	80
GMT-CT	A	42.91 (10.85)	50	83.86 (16.95)	40	127.21 (21.47)	27	155.79 (27.53)	23	183.51 (30.23)	19	186.53 (34.23)	33	204.54 (34.50)	73
GFT-MT	A	46.26 (8.27)	50	92.82 (12.57)	49	142.31 (21.72)	21	169.41 (25.43)	24	186.69 (23.06)	23	200.22 (42.42)	44	224.96 (38.14)	80
MST-MT	A	38.47 (12.14)	50	79.01 (20.36)	35	117.04 (25.72)	16	151.93 (35.99)	16	184.87 (34.77)	11	205.29 (47.53)	26	239.12 (53.80)	72
MST-CT	A	68.89 (19.27)	50	111.70 (19.22)	36	146.93 (26.49)	12	184.98 (19.21)	13	197.96 (28.89)	16	220.18 (42.51)	20	243.48 (37.93)	72
GMT-MT	B	39.30 (16.53)	40	76.62 (16.99)	22	117.86 (16.62)	10	128.28 (20.07)	17	142.38 (20.55)	19	150.57 (19.98)	21	153.75 (26.04)	40
GMT-CT	B	40.35 (11.89)	40	70.37 (14.29)	33	98.44 (20.87)	24	122.88 (18.85)	20	124.40 (20.53)	20	139.04 (24.25)	18	150.80 (22.76)	38
GFT-MT	B	37.49 (14.46)	40	65.69 (10.44)	28	100.57 (16.36)	26	125.67 (17.53)	17	124.19 (16.88)	23	146.33 (21.42)	23	150.01 (22.71)	40
MST-MT	B	36.71 (12.45)	40	76.44 (14.01)	23	113.58 (19.06)	18	129.02 (19.68)	12	132.59 (15.82)	9	159.08 (15.05)	19	162.98 (18.08)	36
MST-CT	B	52.52 (19.39)	40	84.46 (16.25)	21	114.54 (18.32)	16	133.82 (14.62)	12	138.70 (12.89)	11	157.61 (18.77)	15	164.44 (22.90)	34

Appendix 10. Average weight (g), standard deviation (in parenthesis) and number of fish sampled (n) during growth evaluation of methyltestosterone-treated (MT) and untreated control males (CT) genetically males (GMT), genetically females (GFT) and mixed-sex tilapia (MST) in a cage-in-pond culture environment. Sampling was performed every after 21 days from stocking.

Treatment	0	n	21	n	42	n	63	n	84	n	105	n	126	n	147	n	168	n	189	n
GMT-MT	39.03 (5.93)	30	53.29 (5.70)	30	51.06 (7.92)	30	59.65 (7.95)	28	67.09 (9.54)	28	74.02 (11.21)	28	85.02 (14.02)	26	88.61 (15.33)	27	100.06 (18.92)	27	113.50 (21.49)	27
GMT-CT	47.19 (4.37)	30	45.73 (6.51)	30	57.41 (5.77)	30	66.66 (6.88)	30	76.76 (10.13)	29	84.68 (9.08)	28	98.06 (11.60)	28	101.41 (12.81)	30	114.73 (14.65)	29	129.87 (17.14)	30
GFT-MT	45.98 (8.00)	30	54.88 (8.09)	30	62.21 (9.02)	30	71.01 (10.26)	29	80.86 (12.41)	29	91.88 (14.45)	28	110.54 (18.16)	29	113.72 (15.88)	27	129.33 (17.27)	27	150.81 (20.99)	27
MST-MT	53.57 (9.12)	30	61.39 (9.81)	30	65.63 (11.92)	30	77.48 (12.45)	30	89.53 (15.39)	30	102.35 (17.53)	30	119.05 (21.81)	30	128.14 (25.91)	30	140.30 (29.42)	30	162.92 (32.39)	30
MST-CT	54.79 (14.01)	30	60.32 (14.47)	30	65.12 (15.09)	30	71.79 (16.25)	30	78.59 (22.62)	30	93.82 (28.48)	30	105.70 (21.91)	30	108.32 (24.92)	29	115.74 (27.98)	30	130.96 (32.69)	29

Appendix 11. Average weight (g), standard deviation (in parenthesis) and number of fish sampled (n) during growth evaluation three genotypes of females (FEM) from different sources; putative genetic all-female (GFT), mixed-sex (MST) and putative genetic all-male (GMT) in an all-female (A) and mixed-sex (B) culture environments in earthen ponds. Sampling was performed every after 21 days from stocking.

Treatment		0	n	21	n	42	n	63	n	84	n	105	n	126	n
GFT-FEM	A	29.57 (9.20)	100	58.92 (11.75)	43	93.47 (20.08)	50	114.34 (24.87)	50	125.38 (25.43)	51	130.92 (28.36)	88	140.58 (32.09)	224
MST-FEM	A	33.97 (10.44)	100	72.09 (14.57)	40	105.68 (24.69)	31	125.90 (24.00)	24	152.02 (39.51)	18	143.02 (28.43)	30	159.15 (38.72)	104
GMT-FEM	A	32.73 (10.53)	27	64.05 (14.09)	13	106.37 (23.98)	10	125.96 (16.80)	8	142.70 (25.80)	8	143.78 (26.43)	12	149.00 (38.89)	25
GFT-FEM	B	27.06 (8.21)	50	43.86 (10.00)	50	50.30 (12.90)	40	53.65 (8.93)	22	60.92 (13.67)	29	62.95 (16.00)	30	59.78 (15.82)	77
MST-FEM	B	35.36 (12.52)	50	47.59 (14.03)	28	57.10 (15.80)	18	66.69 (15.49)	13	67.11 (24.78)	18	73.66 (19.87)	15	69.60 (21.44)	62
GMT-FEM	B	30.91 (7.98)	28	52.91 (11.01)	14	65.17 (21.35)	14	66.78 (21.55)	8	60.75 (13.53)	6	65.56 (20.54)	14	64.39 (19.68)	24