GENETIC STUDIES IN TILAPIAS

by

KSHITISH C. MAJUMDAR

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TO MY PARENTS

DECLARATION

I declare that this thesis has been composed by myself and that it embodies the results of my own research. It has neither been accepted nor is being submitted for any other degree. Where appropriate I have acknowledged the nature and extent of work carried out in collaboration with others and included in the thesis.

Kshitish Chandra Majumdar

KSHITISH C. MAJUMDAR

ABSTRACT

Tilapia as a group have suffered from a lack of detailed genetic research in a number of important applied and basic areas. Sex determination in tilapia is of great importance because of the need to produce monosex populations for aquaculture. In this study sex ratio data produced from a total of 41 intra- and interspecific crosses utilizing pure species have been analysed. The results so far obtained do not support the existing hypothesis of chromosomal sex determination. On the basis of the existing data it seems that sex in these fishes is determined by a polygenic system.

Cytogenetic studies on chromosome number, chromosome morphology and DNA content show many similarities between the 7 species belonging to three genera. The DNA value varies between 0.84pg for O. macrochir and S. galilaeus and up to 1.21pg for O. aureus. The chromosome number (2n = 44, n = 22) is the same in all the species. No heteromorphic sex specific chromosome pair has been found in any species. Arm number (NF) difference in the species indicates the involvement of pericentric inversions in the karyotypic evolution of these species. C-banding of the metaphase chromosomes shows that the heterochromatin is localised around the centromere in all the species of Oreochromis and Sarotherodon, but T. zillii has more heterochromatin with six chromosomes having their short arm completely C-positive. O. mossambicus and O. spilurus, two closely related species, can also be distinguished by their C-banding pattern.

Comparative growth trials on different species, their hybrids and the effect of hormone treatment on growth performance have all been analysed.

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Comparative growth trials on different species, their hybrids and the effect of hormone treatment on growth performance have all been analysed.

Different species showed large differences in growth performance based on SGR and FCR values, when compared under standardized conditions. Comparison between hybrids and their parental species showed no significant heterosis for SGR and FCR except in the hybrid between O. spilurus female and O. niloticus male. Hormone treatment of fry improved growth performance but did not alter the relative growth found in the untreated group.

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CONTENTS

CHAPTER I

GENERAL INTRODUCTION

Since human beings are land dwellers, it is logical that they applied scientific and engineering skills to the production of terrestrial organisms and hence the domestication of animals and plants has been known for the last 10,000 years (Wilkins, 1981). Fish, however, have remained wild, man relying on hunting and gathering fish from the sea and inland waters rather than growing them. This process of collection is therefore an age-old practice, but has received considerable technological inputs in recent years so that the amount of sea fish harvested per man per year has increased considerably (Bardach et al., 1972). Natural resources are limited and continuous harvesting has led to the depletion of many species. To meet the shortfall between demand and production of fish "aquaculture" has developed rapidly in the last twenty years and now makes a significant contribution to overall fish production (FAO, 1978).

The term "aquaculture" is used in general to denote the production of aquatic animals and plants under artificial and semi-artificial conditions (Wilkins, 1981). Aquaculture can be divided into three main categories on the basis of the food supply in the water bodies. According to Coche (1982) these are as follows:

1) Intensive systems where organisms are grown exclusively on an artificial supply of food.

2) Semi-intensive systems where natural food supply and to a certain extent artificial food supplementation are used to grow the organisms.

3) Extensive systems where organisms are grown without any supplementation of artificial feed and rely exclusively on the primary production of the water bodies.

The culture of fish is known to have existed before 2,000 BC in ancient China and Egypt (Fryer and Iles, 1972; Bardach et al., 1972; Huet, 1972; Calaprice, 1976) but was probably limited to some form of fattening the fish in ponds, although spawning of captive common carp (Cyprinus carpio) in China was described and was advocated as a profitable business by Fan Li as early as 475 BC (mentioned in Bardach et al., 1972). Throughout Indo-China and South East Asia fish culture is expanding under the pressure of the increasing protein demands from the growing populations. This is leading to the modernization of fish culture techniques to improve yield by improving husbandry techniques utilizing the water body more efficiently by using the system of polyculture, artificial fertilization and artificial feeding etc. But unfortunately little emphasis has so far been given to the genetic improvement of existing fish stocks. Although fish culture is an age-old practice, domestication of fish has hardly begun. The only exception is the common carp which is thought to have been domesticated for a considerable time period in China and Europe (Wohlfarth et al., 1975).

The domestication of livestock and plants has been known for a long time and the improvement that has been achieved in their growth, yield and disease resistance and other commercial traits is quite astonishing. The knowledge of genetic principles at the beginning of the twentieth century gave a boost to the improvement of such stocks by the manipulation of desired characteristics using genetic techniques. The genetic principles used so far are mainly restricted to the following: breeding from selected parents; culling inferior offspring; cross breeding with other species and producing and crossing identifiable strains within a species. The role of genetics in livestock management is still today largely seen

to emcompass these procedures (Gjedrem, 1975, 1976) and it would be difficult to envisage modern agriculture and animal production prospering in the absence of such genetic management (Wilkins, 1981).

As aquaculture has expanded and production levels in established cultured species have stabilized, so the interest in the genetic improvement of commercially used species to improve profitability has increased. Much discussion has centred around how these genetic improvements should be carried out and how feasible it is to utilize the techniques developed for other organisms. Skjervold (1976) and Wilkins (1981) mentioned that fish exhibit a number of traits which should make the process of genetic manipulation and improvement easier and more effective than it has been with other organisms. These are as follows:

1) Genetic variability in the form of heterozygosity and individual loci is higher in fishes than most other vertebrates.

2) Compared to present day livestock fishes are wild, so the genomes of fishes are basically unaltered as artificial selection has not yet been used.

3) High fecundity and external fertilization generally in fishes make it possible to raise many more siblings and selection studies could be done much more intensively raising full sib and half sib families.

4) Sex determination is much more plastic in fishes which allows production of monosex, gynogenetic and androgenetic populations leading to the production of inbred lines in fewer generations.

5) Intergeneric and interspecific hybrids are very often viable and fertile in fishes which allows the possibility of obtaining "tailor made" stocks through the combination of several commercially important characteristics from different species.

Besides the above mentioned advantages, some disadvantages are also apparent. These include:

1) Longer generation time, especially in temperate species such as salmon and trout.

2) Low level of knowledge concerning the technology of fish farming.

3) Fish in a population generally develop a hierarchy which interferes with the experimental design.

4) Lack of visible markers associated with sex or other commercially important characters.

An example of how genetic improvement can help increase productivity is in the rainbow trout (Salmo gairdneri) which has been selected for a number of traits by the California Department of Fish and Game (see, Bardach et al., 1972). It has been shown here that selection does not take long to achieve some worthwhile results:

1) Increase in the number of trout spawning at 2 years of age from 53% to 98% in three generations.

2) More than double the average weight of yearlings in five generations.

3) Increased egg production by 2 year old females, fourfold in six generations.

All these achievements can be ascertained as being due to the understanding and utilization of the genetic principles as well as control of reproduction. Unfortunately the knowledge regarding genetic characteristics of many cultured aquatic organisms is very limited, especially the species used in the tropical countries. In most cases the species used in any aquaculture programme are not domesticated as exemplified by the collection

of fry from riverine sources and their ongrowing in ponds (intensive, semi-intensive and extensive) for consumption. Thus no control over their reproduction has yet been achieved, a vital point for designing any genetic improvement programme. Another problem associated in such aquacultural situations is the indiscriminate introduction of species without understanding their biology and the success of the species in the given ecosystem. All these factors make the work on genetic improvement of a species much more difficult.

A typical example of the problems associated with genetic improvement has been in the tilapias. "Tilapia" is a common name given to a group of fishes (Tilapiines) belonging to the family Cichlidae and which includes about 70 species in three genera, viz. Oreochromis, Sarotherodon and Tilapia (Trewavas, 1982a). All of these species are endemic to Africa and some areas of the Middle East (Wohlfarth and Hulata, 1983). Tilapias have a great potential in warm water aquaculture (Schwartz, 1983) and their many beneficial characteristics have been extensively reviewed by Balarin and Hatton (1979) and Pullin and Lowe-McConnell (1982). These include high yield potential, short generation time, tolerance to a wide range of ecological conditions, adaptable food habits, tolerance to crowding, disease resistance, survival at low oxygen tension and their tolerance to poor water quality. Because of their many beneficial characters, tilapias, especially O. mossambicus, were thought to be a "wonder fish" and have been widely transplanted throughout the world (Chimits, 1957). The initial enthusiasm for tilapia as a candidate for aquaculture can be found in the two bibliographic accounts by Chimits (1955, 1957). Unfortunately later studies on O. mossambicus showed that it was unsuitable for many of the environmental conditions and culture

practices to which it had been introduced. This resulted in poor yields and to the failure of many aquaculture programmes. This early bias towards a single species also hindered the comparative work needed to find other species more suited to different culture and environmental conditions (Chimits, 1957). The other problem with tilapias is that they breed in the warm water at a high rate (Fryer and Iles, 1972) which causes overpopulation in a pond leading to the stunting of the growth and the production of unmarketable small sized fishes under poorly managed conditions (Hickling, 1960, 1968). Because of these problems the initial enthusiasm for utilizing tilapias in aquaculture has been disillusioned, but recently the interest has been renewed which has led to a spate of books and symposia on this subject (Smitherman et al., 1978; Pullin and Lowe-McConnell, 1982; Wilkins and Gosling, 1983; Wohlfarth and Hulata, 1983; Fishelson and Yaron, 1983). From all these reviews it could be ascertained that the work on the control of reproduction of these species and the culture potential of individual species have received most attention.

The methods of reproductive control so far attempted in tilapias have been reviewed by Balarin and Hatton (1979) and Guerrero (1982). These include monosex culture by separating the males and females manually, predator association in the culture system, cage culture, high stocking density, sterilization by ionizing radiation and chemicals, monosex interspecific hybridization, and sex reversal using sex steroids. Of these methods interspecific hybridization and hormonal sex reversal have been given special importance as can be judged by the extensive work on these aspects. The experimental interspecific hybridization in tilapias was discovered accidentally by Hickling (1960) who found all male F1 progeny

in a cross between female O. mossambicus and O. hornorum male. He proposed that this procedure could be used as an effective method of population control as the absence of the opposite sex in the progeny will inhibit further reproduction. So far 114 different hybrids have been reported from as many as 30 different species (Schwartz, 1983). The results obtained show that none of the crosses consistently gave one hundred percent success. The failure to produce 100% males in these experiments has often been blamed on the impurity of the species used, this being caused by poor management and husbandry. The justification of such statements, however, needs careful experimental verification. The results of hybridization in the form of sex ratio data in the F1 progeny have been used by several authors (see, Wohlfarth and Hulata, 1983) to explain the sex determining mechanism in these fishes and several hypotheses have been put forward. Earlier hypotheses assume the presence of several sex chromosomes expressed in the form of male and female heterogamety in different species. In the most recent hypothesis a number of sex chromosomes along with a pair of autosomes are thought to be responsible for the primary sex of any individual (Avtalion and Hammerman, 1978; Hammerman and Avtalion, 1979). Unfortunately the experimental results do not fully support any of the above mentioned chromosomal hypotheses of sex determination. Similarly no evidence for the presence of a sex chromosome pair has been found on the basis of chromosome morphology.

The hormone sex reversal experiments in tilapias are designed to produce monosex populations and have been based on the chromosomal sex determination hypothesis. Yamamoto (1969) mentioned that the homogametic sex reversed individuals when crossed with the opposite sex which is normal

(untreated group) and also homogametic in its chromosome constitution will result in progeny with only one sex and the reciprocal cross will lead to 1:1 sex ratio. In the case of tilapia several authors used sex steroids along with the inhibition of endogenous sex hormone production to obtain sex reversed individuals (Guerrero, 1982; Yamazaki, 1983). Shelton et al. (1978) proposed a scheme which combines sex reversal and a specific breeding programme which should lead to the production of monosex progeny if sex is chromosomally determined. Unfortunately so far the results do not support the hypothesis. This definitely indicates that the assumption of sex chromosomes and the sex determination mechanism in these fishes is questionable and needs thorough investigation, a fact which has been highlighted in all the previously mentioned symposia.

Although hybridization and the use of sex hormones are well known to tilapia biologists, little is known about the advantages to be achieved by utilizing these techniques over normal mixed sex culture with pure species.

The comparative growth trials between hybrids and their parent species are equivocal in their results. Some authors showed that "heterosis" has often been found (Avault and Shell, 1968; Hickling, 1968; Kuo, 1969), whereas others are of the opinion that tilapia hybrids do not show superiority of growth over their parents (Dunseth, 1977; Lovshin et al., 1977). Many of the above reports have been based on experiments carried out in ponds and the "heterosis" or lack of it has often been an additional observation rather than the main aim of the experiment. The strict experimental regime and environmental controls necessary to detect differences

in growth or performance are not generally possible under pond conditions and this makes many of these results unreliable. Similarly in the hormone studies the main emphasis has been on the achievement of sex reversed individuals (Guerrero, 1982). The effect of these hormones which are known to have an anabolic effect (Donaldson et al., 1979), on growth have not been studied critically. Evidence for the growth promoting effect of steroid hormones on tilapias is contradictory (Guerrero, 1975; Anderson and Smitherman, 1978; Tayamen and Shelton, 1978). Once again these studies have also been done in ponds and control over the food intake has not been taken into serious consideration which will undoubtedly affect the outcome of the experiments.

Although work on the culture of tilapia was seriously started during the early 1950's in Africa it has now spread gradually all over the world. Despite the widespread use of these fish the biological requirements and therefore the optimum conditions for the culture of many of these species is still unknown. Wohlfarth and Hulata (1983) in the review on the applied genetics of tilapia wrote

> "a first step towards improving the characteristics of cultured tilapias is the proper choice of species."

They also mentioned that tilapia as a group consists of several species and that selection work will be rather difficult if it is started indiscriminately without the proper understanding of the requirements of a particular situation and the species suitability. At the recent International Symposium on Tilapia in Aquaculture held in Israel (1983) it was agreed and highlighted by all the scientists that species choice should be given priority because this was hindering further work on genetic improvement.

The cytogenetics of tilapia is still in its infancy and much more work is needed even in the basic area of chromosome number. It has been mentioned by several authors that the tilapias are phylogenetically closely related. The evidence has been gathered from morphological, biochemical and developmental studies (Peters and Berns, 1978, 1982; Kornfield et al., 1979; Trewavas, 1980, 1982b, 1983; McAndrew and Majumdar, 1983, 1984). It is also known that the use of cytogenetical information, viz. chromosome cytology, C-value determination, and DNA reassociation kinetics can yield fruitful results in recognising the genetic interrelationships in the closely related species (White, 1973; Dobzhansky et al., 1977). On the other hand the karyotype of only 12 species of tilapias are known out of 70 different species. The karyotypic data in the majority of cases are confusing, even those pertaining to their diploid chromosome numbers.

Despite the wide environmental tolerances of the tilapia, their overall morphology is very similar. This has led to many problems in taxonomy and species identification. The classical example of misidentification is that of O. hornorum as O. mossambicus (Zanzibar strain) by Hickling (1960) and this is only one of many. With the advancement of biochemical genetics and the utilization of enzyme markers specific for a species, such problems have been partially solved (Cruz et al., 1982; McAndrew and Majumdar, 1983). These biochemical markers as well being used for species identification are a useful tool for tagging different strains and therefore aiding in the design of a selection programme (Moav et al., 1978). Similarly the studies on the chromosomes should also be given stress as the karyotype is one of the many characters (which do not change with environmental influence) which could be used

in taxonomic interpretation and also as an additional marker which might help in identifying a species (pure or hybridized) and in designing a selection programme. The results of such studies could then be used in practical aquaculture, e.g. gynogenesis, androgenesis, triploidy, tetraploidy, etc. (Gold, 1979; Sola et al., 1981).

From the above discussion it is obvious that there is still much genetic research to be done on tilapia, and that there are a number of areas in which there should be a concentrated effort:

1) Choice of species;
2) Determination of variation of commercially important characters in a species;
3) Understanding the sex determination mechanism;
4) Interspecific hybridization and any heterotic effects for commercially important characters;
5) Use of hormones as a husbandry tool other than sex reversal;
6) Utilization of cytogenetical markers along with biochemical genetics for designing selection work.

Only a few of the above mentioned aspects have been considered in the present dissertation and are presented separately (Chapters II to V). The second chapter deals specifically with interspecific hybridization. The hybridization work has been done on pure species checked through electrophoresis in controlled experimental conditions which eliminate the poor management conditions blamed for the failure of monosex production. On the basis of all the results of hybridization found in the literature as well as the present studies the validity of the chromosomal

sex determination hypothesis has been questioned. The next chapter (III) is devoted to the chromosomal studies (mitotic and meiotic) of several species of tilapias along with the determination of DNA values in the diploid nuclei. The study includes more species and improved chromosome analysis techniques. Chapter IV is the compilation of the results of the comparative growth studies of different tilapias and the effects of two anabolic steroids on the growth of three commercially important species. The comparative growth trials were completed in much more controlled conditions which allowed precise measurements of growth criteria in the form of specific growth rate and food conversion ratio. The last chapter (V) deals with the comparative growth trials of different hybrids as well as their parents. Because of the similarity in the experimental conditions it was possible to calculate the "heterosis" in the hybrids precisely for the most commercially important character, i.e. growth (Gjedrem, 1983).

CHAPTER II

INTRASPECIFIC AND INTERSPECIFIC HYBRIDIZATION IN *OREOCHROMIS* WITH REFERENCE TO SEX DETERMINATION

INTRODUCTION

It is over twenty years since Hickling (1960) made the chance discovery of all-male F1 hybrid fish in a cross between female *Oreochromis mossambicus* and male *O. hornorum*. This appeared to be a promising method for population control of tilapia species which are prolific breeders in mixed sex pond culture, an undesirable characteristic for any cultured species. Since then many crosses have been performed between different species/genera (see Balarin and Hatton, 1979; Wohlfarth and Hulata, 1983, and Table 1) throughout the world. However, in practice it has been very difficult to obtain consistent results, which could be applied regularly as a population control method in tilapia (Hulata *et al.*, 1981).

Although interspecific hybridization has been used to obtain skewed sex ratios in the F1 progeny, only a few authors have tried to use their results to propose a possible sex determination mechanism in these species.

A chromosomal mechanism for sex determination in tilapia was proposed by Hickling (1960), analogous to the chromosomal system of *Xiphophorus* sp., as mentioned by Gordon (1947). According to this system both XX/XY male heterogamety and WY/YY female heterogamety are to be found in different populations. This model predicts that a cross between a homogametic male and female from two different strains will lead to 100% male, and 1:1 sex ratio in the reciprocal cross. Hickling (1960) assumed that in *O. mossambicus* the sex chromosomes were XX female:XY male whereas in *O. hornorum* it was WY female:YY male. When the two homogametic sexes were crossed he obtained 100% males. He also proposed that

TABLE 1. Intraspecific and interspecific hybridization reported by previous workers.

Species and Strain	% Male	Reference
Oreochromis mossambicus		
Malayan, pair mating	49-70	Hickling (1960)
Malayan, mass breeding	55	"
Trinidad, mass breeding	41-51	"
Malayan, pair mating	3-12	Hickling (1968)
Unknown (Oklahoma Univ. Station)	33-77	Clemens and Inslee (1968)
Unknown (Lukang Fish Station)	51	Kuo (1969)
Unknown	53	Hsiao (1980)
Oreochromis hornorum		
Zanzibar, pair mating	53-61	Hickling (1960)
Zanzibar, mass breeding	53	"
Unknown (Puerto Rico)	54-56	Pinto (1982)

Species and Strain	% Male	Reference
Oreochromis niloticus		
Kenya, mass breeding	50.3	Chervinski (1967)
Unknown (Lukang Fish Station)	31	Kuo (1969)
Nile, pair mating	42-69	Jalabert *et al.* (1974)
Volta, pair mating	45-65	"
Uganda female) Israeli male) pair mating	45.4	Pruginin *et al.* (1975)
Uganda, pair mating	46-57	"
Israeli, pair mating	50-57	Tayamen and Shelton (1978)
Unknown	47-87	Hsiao (1980)
Ghana, pair mating	78	Owusu-Frimpong and Nijjhar (1981)
Unknown (Puerto Rico)	55-57	Pinto (1982)
Israeli, pair mating	31-77	Shelton *et al.* (1983)
Oreochromis macrochir		
Cameroon, pair mating	47-54	Jalabert *et al.* (1974)
Oreochromis vulcani		
Kenya, pair mating	42	Pruginin *et al.* (1975)

Species and Strain	% Male	Reference
Oreochromis aureus		
Israeli, mass breeding	50	Chervinski (1967)
Israeli, pair mating	56	Guerrero (1975)
Israeli, pair mating	46	Pruginin *et al.* (1975)
Israeli, mass breeding	47-53	"
Israeli, mass breeding	47-56	Shelton *et al.* (1978)
Unknown	51-56	Hsiao (1980)
Unknown (Puerto Rico)	42-59	Pinto (1982)
Israeli, mass breeding (37♀:37♂)	54	Shelton *et al.* (1983)
Israeli, pair mating (13♀ with 4 different ♂)	28-100	"
O. mossambicus x *O. aureus*		
O. mossambicus aquarist stock *O. aureus* Israeli	70.6	Avault and Shell (1968)
Unknown (Plainfield, Vermont, USA)	89	Pierce (1980)
Unknown	91	Hsiao (1980)

Species and Strain	% Male	Reference
O. aureus x *O. mossambicus*		
O. mossambicus aquarist stock *O. aureus* Israeli	71.6	Avault and Shell (1968)
Unknown (Plainfield, Vermont, USA)	77	Hsiao (1980)
O. aureus Israeli *O. mossambicus* South Africa	80	Hulata *et al.* (1981)
O. niloticus x *O. mossambicus*		
Unknown (Lukang Fish Station)	56	Kuo (1969)
Unknown	72	Hsiao (1980)
O. mossambicus x *O. niloticus*		
Same as above	15	Kuo (1969)
Same as above	64	Hsiao (1980)
O. niloticus x *O. macrochir*		
O. niloticus Nile-Volta Basin *O. macrochir* Cameroon	100	Lessent (1968)
" pair mating	100	Jalabert *et al.* (1971)

Species and Strain		% Male	Reference
O. macrochir x *O. niloticus*			
O. niloticus Nile-Volta Basin *O. macrochir* Cameroon	pair mating	68-77	Lessent (1968)
"	pair mating	75	Jalabert *et al.* (1971)
O. mossambicus x *O. hornorum*			
O. mossambicus Malayan *O. hornorum* Zanzibar	pair mating	49.6-100	Hickling (1960)
"	mass breeding	96-100	"
"	mass breeding	100	Chen (1969)
O. mossambicus South Africa *O. hornorum* Ivory Coast		100	Hulata *et al.* (1981)
O. hornorum x *O. mossambicus*			
Same as above	pair mating	48-80	Hickling (1960)
"	mass breeding	70-86	"
"	mass breeding	76	Chen (1969)
"	pair mating	66-90	Hulata *et al.* (1981)

Species and Strain		% Male	Reference
O. niloticus x *O. hornorum*			
O. niloticus Uganda *O. hornorum* Uganda	mass breeding	100	Pruginin (1967)
O. niloticus Brazil *O. hornorum* Brazil	mass breeding	100 (one cross gave some female)	Lovshin and Da Silva (1975)
O. niloticus Brazil *O. hornorum* Ivory Coast	pair mating	100	Hulata *et al.* (1981)
O. niloticus Ghana *O. hornorum* Ivory Coast		100	"
Unknown (Puerto Rico)		100	Pinto (1982)
O. hornorum x *O. niloticus*			
O. niloticus Brazil *O. hornorum* Ivory Coast	pair mating	66-100	Hulata *et al.* (1981)
O. niloticus Ghana *O. hornorum* Ivory Coast	pair mating	50-80	"
O. hornorum x *O. aureus*			
O. hornorum Ivory Coast *O. aureus* Israeli		97	Hulata *et al.* (1981)
Unknown (Puerto Rico)		77	Pinto (1982)

Species and Strain		% Male	Reference
O. aureus x *O. hornorum*			
Unknown (Puerto Rico)		100	Pinto (1982)
O. vulcani x *O. aureus*			
O. vulcani Lake Rudolph *O. aureus* Israeli		95-98	Pruginin *et al.* (1975)
Same stock mass breeding		52-60	"
O. vulcani x *O. hornorum*			
O. vulcani Lake Rudolph *O. hornorum* Ivory Coast	pair mating	90	Pruginin *et al.* (1975)
O. niloticus x *O. aureus*			
O. niloticus Tel Aviv *O. aureus* Israeli Farm Stock		100	Fishelson (1962)

Species and Strain		% Male	Reference
O. niloticus Kenya O. aureus Israeli		82.6	Chervinski (1967)
Israeli mass breeding		61-62	Mires (1969)
O. niloticus Uganda O. aureus Sdeh Eliyahu	pair mating	98-100	Pruginin et al. (1975)
O. niloticus Uganda O. aureus Tel Aviv	pair mating	96-99.5	"
O. niloticus Uganda O. aureus Ein Feshkha	pair mating	96-100	"
O. niloticus Israeli O. aureus Sdeh Eliyahu	pair mating	61-99	"
O. niloticus Israeli O. aureus Tel Aviv	pair mating	52-81	"
O. niloticus Israeli O. aureus Ein Feshkha	pair mating	80-100	"
O. niloticus Uganda O. aureus Israeli	mass breeding	100	"
Unknown		73.5-100	Hsiao (1980)
O. niloticus Ghana O. aureus Israeli	pair mating	66-100	Hulata et al. (1981)
Unknown (Puerto Rico)		65	Pinto (1982)

Species and Strain		% Male	Reference
O. aureus x *O. niloticus*			
O. aureus Israeli *O. niloticus* Kenya		63.5	Chervinski (1967)
O. aureus Israeli *O. niloticus* Uganda		77.2	Pruginin *et al.* (1975)
Unknown		83.3	Hsiao (1980)
O. aureus Israeli *O. niloticus* Brazil	pair mating	33-50	Hulata *et al.* (1981)
Unknown (Puerto Rico)		50	Pinto (1982)

the Y chromosome had the male determining factors. However, the reciprocal cross gave a 3 male:1 female ratio, which could not be explained on the basis of such a model.

Chen (1969) extended the crosses initiated by Hickling (1960) and on the basis of his results he postulated that O. mossambicus had an XX:XY chromosome system, but that O. hornorum had a WZ female:ZZ male chromosomal system. Chen (1969) also proposed that when the homogametic female O. mossambicus (XX) was crossed with the homogametic male O. hornorum (ZZ) all the F1 progeny were XZ and male, so that the male determining genes on the Z chromosome were dominant over the female determining genes on the X chromosome. The reciprocal cross gave a sex ratio of 3 male:1 female and was explained by the fact that the male determining genes on the Y chromosome were stronger than the female determining genes on the W chromosome. To verify the proposed model Chen (1969) performed further crosses leading up to F4 generation, and a series of backcrosses. However, some crosses gave unexpected results. When a WX female was crossed to a ZX male he obtained two different sets of female offspring percentages, 53-66% and 69-73% which came from large and small broods respectively. From his theoretical proposition the sex ratio in the above cross should be 3 female:1 male; instead he had both 1:1 and 3:1 ratios. To explain the different sex ratios in different broods he postulated that differential mortality of females in the large brood was responsible for such deviations. This seems unrealistic as the progeny number obtained in different broods was more or less the same.

In another cross between WW female and O. hornorum (ZZ) male according to the proposed model the sex ratio should be all female. In practice Chen (1969) obtained a 1:1 sex ratio in 3 out of 4 crosses. The remaining cross

yielded almost all males(2 female:441 male). Clearly the involvement of three sex chromosomes is not sufficient to explain all the experimental results.

Jalabert et al. (1971) reported the results of different crosses between O. niloticus and O. macrochir. The cross between O. niloticus female and O. macrochir male resulted in 100% males, and the reciprocal cross sex ratio was 3 male:1 female. To explain their results they designated the chromosomes as xx female:xY male in O. niloticus and Xy female:yy male in O. macrochir. Further, when the F1 all-male hybrid of the constitution xy was crossed separately to females of O. niloticus and O. macrochir equal numbers of male and female progeny resulted as predicted by the model. As mentioned above the cross between O. macrochir female and O. niloticus male yielded 1 female (xX) and 3 different types of males (xy, XY, Yy). The backcross of this female (xX) with O. niloticus male resulted in the following sex ratios (♂:♀); 2:1, 3:1, 1.47:1, 1.63:1 and 1.78:1 in seven different crosses. Similarly, backcrosses with O. macrochir males and the same xX females resulted in the following progeny sex ratios (♂:♀); 1:1, 2:1, 1.18:1, and 0.63:1 in six different crosses. According to the chromosomal assignment the sex ratio should be 1:1. The situation becomes more complicated when the F1 males of the above mentioned cross were backcrossed to females of O. niloticus. This resulted in the following sex ratios (♂:♀); 3:1, 2:1, 1.54:1, and 1.70:1 in eight different crosses. Theoretically two types of sex ratios were expected from the three types of males, i.e. 1:1 and 100% male. Similarly two types of sex ratios were expected when F1 hybrid males are crossed to female O. macrochir, i.e. 1:1 and 3:1 (male:female). In practice the sex ratios obtained from seven such crossings were (male:female); 3:1, 1:1 and 2:1 (Jalabert et al., 1971). The deviations from the predicted ratios can not be

explained on the basis of differential mortality of one sex alone as the progeny numbers in all the crosses were more or less equal. To explain their results Jalabert et al. (1974) stressed that there may be autosomes which along with the sex chromosomes were responsible for the final determination of the sex of the individuals.

Recently a model has been proposed by Avtalion and Hammerman (1978) and Hammerman and Avtalion (1979) to explain the results of crosses between O. mossambicus and O. hornorum reported by Chen (1969). They also claimed that using the same model the results of the hybridization of O. macrochir and O. niloticus (Jalabert et al., 1971) could similarly be explained. They strongly believe that sex determination in tilapia is mainly governed by the autosome along with the sex chromosomes (gonosomes). The hypothesis is based on the following assumption: a) there are three gonosomes (W, X, Y) and two autosomes (A, a) involved; b) within one species the autosome pair is identical (AA or aa); c) the chromosome complement thus for these two species may be AAXX(♀)/AAXY(♂) in the O. mossambicus and aaWY (♀)/aaYY (♂) or aaWX(♀)/aaWW (♂) in the O. hornorum; d) each chromosome has a fixed strength which is not influenced by the other chromosomes; e) the additive effect of individual sex influencing chromosomes determine the primary sex; f) if the sum is greater than a certain threshold, the individual develops into a male: if less into a female. They also postulate that

> "in crosses between related species of tilapia all male generating parents could be identified as homogametic, while those pairs giving a 3 male:1 female ratio could be identified as being heterogametic. In this respect O. hornorum, O. macrochir and O. aureus could be considered as homogametic in the male and heterogametic in the female,

with the opposite being the case for O. mossambicus, O. niloticus and O. nigra."

The autosome/gonosome hypothesis not only explains the results of Chen (1969) but also predicts some unique sex ratios, i.e. (female:male); 3:5, 5:3, and 9:7 which can be obtained by appropriate crossings, these not being expected in a sex chromosome hypothesis alone. This hypothesis also predicts that a) in a pure species the sex ratio will be 1:1; b) in interspecific crosses where chromosome complements are the same the sex ratio will be 1:1; c) in interspecific crosses where the chromosome complements are different the results may be skewed but should agree to Mendelian ratios. So accordingly crosses between O. niloticus and O. mossambicus should result in a 1:1 ratio. The same should be for the crosses between O. hornorum, O. macrochir and O. aureus. On the other hand crosses between the females of the former group with the males of the latter group should result in all male progeny whereas the reciprocal cross between these groups should give a 3 male:1 female ratio.

The reported results of such crosses do not match the theoretical predictions (Table 1). The cross O. niloticus x O. aureus has been reported by a number of researchers. Pruginin et al. (1975) reported that the male percentage varied between 52-100% in different pair matings. Hulata et al. (1981) have obtained very similar male percentages and have found that an all-male F1 progeny was an exception rather than a rule. These are difficult to explain on the basis of the autosome/gonosome hypothesis.

The autosome/gonosome hypothesis also does not explain the variability of the sex ratios observed in pure species crosses. Hickling (1968)

reported that a few pure O. mossambicus males when crossed with females from the same stock resulted in fry batches with only 3-12% males.

Table 1 summarises the results on the sex ratio of different intra- and interspecific crosses reported in the literature. It is clear that there is a great deal of variation in the sex ratios observed, much of which cannot be explained by the existing hypothesis on the sex determination of tilapias.

It is obvious from the literature that no one hypothesis can explain all the results. The incredible variability in sex ratios observed, even in the same cross may be explained as underlying genetic variation or as being the product of accidentally hybridized brood stocks. The many cases of misidentification which are observed in the literature means that many of the crosses may not even be between the species they are said to be. Many of the earlier hybridization experiments were performed in ponds and the risk of contamination from wild fish fry and multiple paternity of fry batches was an ever present problem.

It was therefore necessary to repeat many of the previous crosses, by utilizing pure broodstock from a number of different species under stringent laboratory conditions. Crosses under these conditions would show whether it was caused by hybridized broodstock or poor experimental conditions.

With the development of electrophoretic methods of tilapia species identification (McAndrew and Majumdar, 1983) using molecular variation, individual fish could be unequivocally identified as either pure species or a hybrid individual which overcomes many of the problems associated with earlier studies.

In this chapter results of a series of intra- and interspecific crosses using electrophoretically tested individuals are presented. All hybridization was performed in glass aquaria and only single pair mating was used. It was hoped that by repeating many of the hybrid crosses under these stringent conditions much of the criticism of previous trials would be removed, and the results reflect the inherent genetic make up of the individual species.

MATERIALS AND METHODS

The species used and their origin are given in Table 2. The species were maintained in warm water recirculating systems at the Institute of Aquaculture Tropical Aquarium facility. All the intraspecific and interspecific crosses were made in 200 litre glass aquaria equipped with an internal undergravel filter bed and water temperature was maintained at $28^{o} \pm 1^{o}C$ by thermostatically controlled heaters. Additional air supply was maintained through forced air bubbling from a central air pump system. Each tank was stocked with one male and two or three females. Once the male had fertilized the females he was removed, so the females would have the minimum of disturbance. Some crosses were performed by induced spawning and by the artificial fertilization method of Rothbard and Pruginin (1975). The fertilized eggs of these crosses were incubated in inverted bottles fitted with a warm water ($28^{o} \pm 1^{o}C$) recirculating system. The flow through the bottles being regulated by a clamp, so the eggs were gently swirled.

When the fry had absorbed their yolk sacs they were gently collected from the incubator and were stocked in 20 litre tanks. The stocking density was 10 fry/litre and fry were initially fed with micronized trout pellets (250 micron). The feed size was gradually increased as the fish grew. When the fish attained about 3gms they were transferred to 60 litre tanks at a stocking density of one fish per two litres of water. They remained in these tanks until they could be sexed (20-30gms). The fish were sexed by inspecting the genitalia and by direct observation of the gonads. Any doubtful individuals were also checked by gross histological examination of gonad tissue for the presence of developing eggs or sperms.

TABLE 2. Source and location of species hybridized.

Species	Abbreviation	Source
Oreochromis aureus	(O.a.)	Egypt (Lake Manzala)
Oreochromis macrochir	(O.m.)	Botswana
Oreochromis mossambicus	(O.mo.)	Mozambique (Aquarist stock)
Oreochromis niloticus	(O.n.)	Egypt (Lake Manzala)
Oreochromis spilurus	(O.s.)	Kenya (River Tana)

RESULTS

The results of the crosses are given in Table 3 along with the number of progeny, percentage of males, sex ratios and Chi-squared values for goodness-of-fit to different sex ratios. All crosses follow the genetic practice of giving the female component first.

The intraspecific crosses for four of the species used are interesting in that within a species the sex ratio seems to be biased in one direction or another. These differences although not statistically significant seem to be consistent particularly in O. spilurus and O. aureus, the former in favour of females and the latter in favour of males. In the interspecific crosses, O. spilurus has not been previously recorded as having been used in any hybridization experiments. The crosses between O. mossambicus x O. spilurus (Nos. 15-17) consistently gave only 30-36% males. All these crosses significantly differ from a 1:1 ratio and are not significantly different from a 3 ^{o}:1 o ratio. The reciprocal cross is more variable but once again it has an excess of females (Nos. 18-19). In the case of O. spilurus x O. niloticus the ratio is 1:1 (Nos. 20-21). The cross between O. spilurus x O. macrochir (Nos. 22-24) gives very near to all-male. Of the three crosses only one has a single female progeny. O. mossambicus x O. niloticus (Nos. 26-27) gives a 1:1 sex ratio. The reciprocal cross (Nos. 28-29) gives nearly all female progeny. In two crosses only 7 females have been found out of 111 progeny. The crosses between O. niloticus x O. aureus show a male percentage which varied from 65 (No. 34) to 86 (No. 32). The mean for all crosses is 79.16%. The reciprocal crosses between these species gave variable results, i.e. 52% (No. 30) and 88% (No. 31). The crosses O. mossambicus x O. aureus (No. 25) and O. niloticus x

O. macrochir (Nos. 39-40) gave a 1:1 sex ratio. The other cross gave 100% males in O. mossambicus x O. macrochir (No. 41).

TABLE 3. Table of the Intraspecific and Interspecific crosses, number of progeny, percentage of males, sex ratios, and goodness-of-fit to expected ratios. Any crosses which were produced by stripping are denoted by (s) next to the cross number.

Cross No.	Female	Male	F1 progeny Female (F)	F1 progeny Male (M)	% Male	(F:M)	x^2 expected on 1:1 (F:M)	3:1 (F:M)
1	O.mo.	O.mo.	102	115	52.99	0.89:1	0.66	
2	"	"	79	83	51.23	0.95:1	0.04	
3	"	"	30	32	51.61	0.94:1	0.01	
4	"	"	54	42	43.75	1.28:1	1.26	
		TOTAL	265	272	50.65	0.97:1	0.09	
5	O.s.	O.s.	26	24	48.00	1.08:1	0.02	
6	"	"	29	21	42.00	1.38:1	0.98	
7	"	"	27	20	42.55	1.35:1	0.76	
8	"	"	26	18	40.91	1.44:1	1.10	
		TOTAL	108	83	43.45	1.30:1	3.00	
9	O.n.	O.n.	71	76	51.70	0.93:1	0.10	
10	"	"	28	30	51.72	0.93:1	0.01	
11	"	"	35	29	45.31	1.20:1	0.38	
		TOTAL	134	135	50.18	0.99:1		
12	O.a.	O.a.	70	75	51.72	0.93:1	0.10	
13	"	"	43	46	51.68	0.93:1	0.04	
14	"	"	51	60	54.05	0.85:1	0.56	
		TOTAL	164	181	52.46	0.91:1	0.74	
15	O.mo.	O.s.	34	15	30.61	2.26:1	6.61**	0.54
16	"	"	33	16	32.65	2.06:1	5.22*	1.14
17	"	"	44	25	36.23	1.76:1	4.68*	4.05
		TOTAL	111	56	33.53	1.98:1	17.46***	6.02*

Cross No.	Female	Male	F1 progeny Female (F)	F1 progeny Male (M)	% Male	(F:M)	x^2 expected on 1:1 (F:M)	3:1 (F:M)
18(s)	O.s.	O.mo.	15	12	44.44	1.25:1	0.14	4.45*
19(s)	"	"	19	7	26.92	2.71:1	4.65*	0.02
		TOTAL	34	19	35.84	1.78:1	3.68	2.77
20	O.s.	O.n.	25	25	50.00	1.00:1	-	
21	"	"	25	23	47.92	1.08:1	0.02	
		TOTAL	50	48	48.97	1.04:1	0.01	
22	O.s.	O.m.	1	16	94.11	6.25:1		
23	"	"	0	16	100.00	-		
24	"	"	0	14	100.00	-		
		TOTAL	1	46	97.87	2.17:1		
25	O.mo.	O.a.	91	88	49.16	1.03:1	0.02	
26	O.mo.	O.n.	58	46	44.23	1.26:1	1.16	
27	"	"	77	74	49.00	1.04:1	0.02	
		TOTAL	135	120	47.05	1.12:1	0.76	
28	O.n.	O.mo.	54	2	3.57	27:1		
29	"	"	50	5	9.09	10:1		
		TOTAL	104	7	6.30	14.85:1		
								M:F
30	O.a.	O.n.	104	113	52.07	0.92:1	0.29	
31	"	"	11	83	88.29	0.13:1	53.62***	8.16**
		TOTAL	115	196	63.02	0.58:1	20.36***	23.16 ***

Cross No.	Female	Male	F1 progeny Female (F)	F1 progeny Male (M)	% Male	(F:M)	x^2 expected on 1:1 (F:M)	3:1 (M:F)
32	O.n.	O.a.	7	42	85.71	0.17:1	23.58***	2.45
33	"	"	13	32	71.11	0.41:1	7.20**	0.17
34	"	"	16	30	65.22	0.53:1	3.67	1.85
35	"	"	9	40	81.63	0.22:1	18.36***	0.81
36	"	"	15	74	83.15	0.20:1	37.78***	2.72
37	"	"	13	58	81.69	0.22:1	27.26***	1.34
38	"	"	17	66	79.52	0.26:1	27.74***	0.66
		TOTAL	90	342	79.16	0.26:1	145.82***	3.77
39(s)	O.n.	O.m.	10	13	56.52	0.77:1	0.17	
40(s)	"	"	16	22	57.89	0.73:1	0.61	
		TOTAL	26	35	57.37	0.74:1	1.04	
41(s)	O.mo.	O.m.	0	10	100.00			

*p <.05 **p <.01 ***p <.001

DISCUSSION

The main reason cited in the literature for the failure to obtain all-male hybrid offspring from interspecific hybridization is broodstock impurity due to gene introgression or species misidentification (Lovshin and Da Silva, 1975; Wohlfarth and Hulata, 1983). Many of the criticisms levelled at previous studies cannot account for the wide range of variation found in this series of crosses. The greatest care was taken in the selection of broodstock and the risk of contamination was kept to a minimum because of the rigid experimental controls. The results thus obtained in the present series of experiments might be attributed to the real genetic variation inherent within the sex determining mechanisms of these fishes and are not due to artifact.

A summary of all the comparative interspecific crosses between this and other reported studies is given in Table 4.

It can be seen that the results from this study in general exhibit the same level of variability shown in previous works. It is clear that species purity is not the main reason for the differences between observed sex ratios and those predicted by the various chromosomal sex determining mechanisms.

Much attention has been paid to the sex ratios of interspecific crosses and little to the variation in the sex ratio of single pair pure species crosses.

In O. mossambicus several authors have reported from different locations using different strains, that the sex ratio is 1:1 (Whitehead, 1962;

TABLE 4. Comparative data on the sex ratio in interspecific hybridization.

Cross and Strain	% Male	Reference
O. niloticus x *O. aureus*		
Israeli	100	Fishelson (1962)
O. niloticus Kenya *O. aureus* Israeli	82.6	Chervinski (1967)
Israeli	61-62	Mires (1969)
O. niloticus Uganda *O. aureus* Israeli	96-100	Pruginin *et al.* (1975)
Israeli	52-100	"
Unknown	74-100	Hsiao (1980)
O. niloticus Ghana *O. aureus* Israeli	66-100	Hulata *et al.* (1981)
Unknown (Puerto Rico)	65	Pinto (1982)
Egypt	65-86	Present Study

Cross and Strain	% Male	Reference
O. aureus x *O. niloticus*		
O. aureus Israeli *O. niloticus* Kenya	63.5	Chervinski (1967)
O. aureus Israeli *O. niloticus* Uganda	77.2	Pruginin *et al.* (1975)
Unknown	83.3	Hsiao (1980)
O. aureus Israeli *O. niloticus* Brazil	33-50	Hulata *et al.* (1981)
Unknown (Puerto Rico)	50	Pinto (1982)
Egypt	52-88	Present Study
O. mossambicus x *O. aureus*		
O. mossambicus Aquarist stock *O. aureus* Israeli	70.6	Avault and Shell (1968)
Unknown (Plainfield, Vermont, USA)	89	Pierce (1980)
Unknown	90.9	Hsiao (1980)
O. mossambicus Mozambique *O. aureus* Egypt	49.1	Present Study

Cross and Strain	% Male	Reference
O. niloticus x *O. mossambicus*		
Unknown (Lukang Fish Station)	56	Kuo (1969)
Unknown	72	Hsiao (1980)
O. niloticus Egypt *O. mossambicus* Mozambique	4-10	Present Study
O. mossambicus x *O. niloticus*		
Unknown (Lukang Fish Station)	15	Kuo (1969)
Unknown	64	Hsiao (1980)
O. mossambicus Mozambique *O. niloticus* Egypt	44-49	Present Study
O. niloticus x *O. macrochir*		
O. niloticus Nile-Volta Basin *O. macrochir* Cameroon	100	Lessent (1968)
"	100	Jalabert *et al.* (1971)
O. niloticus Egypt *O. macrochir* Botswana	56-58	Present Study

Kuo, 1969; Hsiao, 1980 and the present work). Hickling (1960) in his classical report mentioned that 41-51% and 55% males were obtained in two strains in Malaya and Trinidad respectively, when bred in ponds. He also reported that the Malayan strain when pair mated gave a wide range of variation (49-70% males). Clemens and Inslee (1968) have found 33-77% males in their crosses. A few unusual males have been reported by Hickling (1968), which when crossed with different females of the same strain consistently gave 3-12% males in the broods.

In O. niloticus variability in the male frequency is high. Tayamen and Shelton (1978), and Pinto (1982) reported equal numbers of males and females in their crosses. Similar results have been found in the present crosses. Kuo (1969) reported only 31% males. Jalabert et al. (1974) in a series of single pair crosses in two different strains, Nile and Volta, obtained 42-69% and 45-65% males respectively. A similar range of variations has been reported by Pruginin et al. (1975) from two other strains (Israeli and Uganda). Hsiao (1980) mentioned that the percentage of males in his crosses varied between 47-86%. Owusu-Frimpong and Nijjhar (1981) consistently obtained 78% males from a Ghana stock. The widest range of variation was reported by Shelton et al. (1983), where they found between 31-77% males in their crosses.

Most of the sex ratio data on O. aureus fits with a 1:1 ratio. Exceptional results have been reported by Shelton et al. (1983). They have obtained offsprings from spawning of 13 females with 4 different males with a result of 28-100% males, whereas the results of 37 ♀ when mated with 37 ♂ of the same strain gave a 1:1 sex ratio (overall total average of 54% males).

Evidence that this variability in sex ratios has some genetic basis, and is not a result of random error comes from the work of Jalabert et al. (1974). They have obtained repeated spawnings from the same parental pairs of O. niloticus. Two of the 8 pairs they used show similar deviations from a 1:1 sex ratio consistently in four successive spawnings. Two other pairs show different sex ratios in the subsequent spawnings. The remaining 4 pairs show consistently a 1:1 sex ratio in all the spawnings. Similar results have also been reported by Shelton et al. (1983). In the case of O. aureus again Shelton et al. (1983) have shown that from an unspecified number of consecutive spawnings in 4 pairs the sex ratio remained the same in each pair.

The homogametic/heterogametic chromosome hypothesis does gain some support in sex reversal studies using different sex hormones, the results of which are summarised by Jensen and Shelton (1979). According to their model the sex reversed male from female (normal homogametic) when crossed to a normal female will lead to all female populations. The same cross using a species with heterogametic females should lead to a 1:1 sex ratio. The cited studies show that crosses involving sex reversed males of O. mossambicus, O. niloticus and O. aureus (Clemens and Inslee, 1968; Jalabert et al., 1974; Guerrero, 1975) or females of O. aureus (Liu, 1977) result in broods with aberrent sex ratios. There are several odd results in these data sets which have not been discussed. Clemens and Inslee (1968) reported that seven crosses of presumed sex reversed males with normal females of O. mossambicus gave all female broods leading to the assumption of homogametic females and heterogametic males. However, the sex ratio of the seven control crosses varied between 33-79% males. The other crosses involving hormone treated individuals with normal females (untreated) gave the sex ratio range of 10-72% males. If we

assume that these fishes although hormone treated are not sex reversed, the sex ratio data from this group as well as the control group does not fit with the simple homogametic/heterogametic sex chromosome hypothesis. Jalabert et al. (1974) reported the results of sex reversed O. niloticus males when test crossed with O. niloticus females which gave three types of sex ratio : 4 out of 8 crosses gave an all-female progeny, one gave a 1:1 ratio, one gave 2 female:1 male ratio, one gave 3 female:1 male and the other one gave 7 female:1 male ratio. Among crosses of testosterone treated individuals with normal females of O. aureus reported by Guerrero (1975) there were 15 crosses which gave sex ratios not significantly different from 1:1 and 9 crosses that were significantly different with male percentages that varied between 24-62. On the basis of the results mentioned, O. mossambicus and O. niloticus were said to have homogametic females and heterogametic males, and O. aureus had the opposite designation. But the aberrent sex ratio data obtained both in the control crosses as well as the test crosses suggest that a simple sex chromosomal mechanism is not sufficient for sex determination.

From the earlier discussion it is clear that variation in the sex ratio data in both intraspecific and interspecific crosses is a generalised phenomenon, which has not been explained by any of the hypotheses proposed for sex determination in tilapia.

The sex determination mechanism in other aquarium fishes has been resolved by using different mutant markers which are found to be sex linked, i.e. X or Y (review by Yamamoto, 1969, 1975; Kallman, 1975). Unfortunately in tilapia no mutant has so far been discovered which shows sex linkage. The only mutant (polymorphism?) known is the "red tilapia". A recent study on the "philippine strain" of red tilapia by Galman and Avtalion (1983)

shows that this fish is a product of the hybridization between O. mossambicus, O. niloticus and O. hornorum along with some degree of deficiency in pigment formation on the skin. Recently in our laboratory we have encountered a male O. niloticus which is red (pink) in colour. This male when crossed to female O. niloticus (dark coloured), yields both male and female F1 progeny at a frequency of 9 male:1 female. But colour segregation is also not suggestive to the sex linked inheritance pattern. Another report by Avtalion et al. (1976) claimed that they have found an extra band in the male of O. aureus, O. vulcani and Sarotherodon galilaeus which is absent in the females after gel electrophoresis of serum protein in 7.5% polyacrylamide. Their study did not conclude whether such an extra protein band is sex linked or a sex limited expression (Wohlfarth and Hulata, 1983).

In higher organisms like mammals, birds and some of the ophidians there are differential sex chromosome pairs, which can be identified because of their conspicuous size differences as well as composition (Ohno, 1967; Singh et al., 1976, 1981; Jones and Singh, 1981). But in tilapia no sex chromosome pair has yet been discovered (Wohlfarth and Hulata, 1983; Chapter III of this thesis). Although in fishes there are reports on both male and female heteromorphic sex chromosomes (Ohno, 1967; Gold, 1979) nobody has shown how much they are directly involved in primary sex determination. Ohno (1974) argued that

> "although in most species of fish, the sex cannot be recognised by morphological means yet great majority of fish species are bisexual and for this very reason, they must possess sex determining genes."

But it is still to be ascertained how many sex determining genes there are in the genome.

The most well studied animal in genetics is Drosophila and even here nobody knows how many genes are responsible for primary sex determination, although it is known that the balance between autosome pairs and sex chromosomes (X) is responsible for sex determination. The problem becomes more complicated as so many modifier genes have been reported which can affect the sex of this animal (Lindsley and Grell, 1968).

According to Haldane (1922)

> "When in the F1 offspring of two different animal races one sex is absent, rare or sterile, that sex is the heterozygous sex."

He cited examples from interspecific crosses in different avian and mammalian species. This might hold true for those groups of vertebrates where the sex determination mechanism has been more or less fixed, because the heteromorphism generally prevents crossing over and so keeps the sex linked genes together (Ohno, 1967). In fishes although genetically and chromosomally determined heterogamety is reported, extensive crossing over in the heterochromosome pair has also been reported (Ohno, 1967, 1974; Yamamoto, 1969, 1975; Kallman, 1975) indicating the primitive state of sex chromosomal evolution.

One plausible explanation of the wide range of variation in the sex ratios in the tilapia is that the sex in these fishes is determined by a number of genes rather than a few sex chromosomes. The polygenic sex determination system assumes that there are a series of alleles dispersed throughout the genome. The ultimate sex is determined by the additive action of all these alleles. Kosswig (1964) assuming only three genes responsible for maleness out of 4 loci (A/a, B/b, C/c, D/d) has predicted 16 different combinations as follows:

1. Aa Bb Cc dd
2. Aa Bb cc Dd
3. Aa bb Cc Dd
4. aa Bb Cc Dd
5. AA Bb cc dd
6. AA bb Cc dd
7. AA bb cc Dd
8. aa BB Cc dd
9. aa BB cc Dd
10. aa bb CC Dd
11. aa bb Cc DD
12. Aa BB cc dd
13. Aa bb CC dd
14. Aa bb cc DD
15. aa Bb CC dd
16. aa Bb cc DD

Taking only five different males and four females the wide range of sex ratio variation can be calculated (Table 5). Table 5 also represents the possibility of occurrence of different sex ratios when the same male is crossed with different females. Recently we have found such a male in O. mossambicus which when crossed to two different females yielded different sex ratios as shown in Table 6.

TABLE 6. Sex ratio data from single male crossed with two different females of O. mossambicus.

Total	Female	Male	% Male	Ratio (♀:♂)
91	32	59	64.83	0.54:1
84	80	4	4.76	20.0:1

Kosswig (1964) from his theoretical predictions also concluded that

> "there is an enormous degree of relativity in the sex determination process on the basis of polymeric genes, because the sexual strength of an individual is dependent (1) on the number of sex genes it bears, and (2) on the degree of heterozygoty for the participating genes."

As has already been mentioned, there is no clear answer for how many

TABLE 5. Theoretical prediction of sex ratio in crosses between different genotypic females and males

	Female			
	aabbccdd	aabbccDd	aabbccDD	aabbCcDd
Male	♂ : ♀	♂ : ♀	♂ : ♀	♂ : ♀
AABBCCDD	1 : 0	1 : 0	1 : 0	1 : 0
AaBbCcdd	1 : 7	1 : 3	1 : 1	13 : 19
AABbccdd	0 : 1	1 : 3	1 : 1	1 : 1
aaBBccDd	0 : 1	1 : 3	1 : 1	1 : 1
AabbCCdd	0 : 1	1 : 3	1 : 1	1 : 1

genes are involved in sex determination in tilapia and at present no means of measuring their heterozygosity. Measurement of gene heterozygosity by electrophoretic studies has shown that there is an enormous amount of molecular variation in most organisms (Lewontin, 1974; Selander, 1976). If this type of variation is representative of the genome as a whole then it would not be surprising to find variation at any loci involved in sex determination.

In a polygenic system, the strength of individual alleles determines the sex. A multiallelic system with different strengths of alleles and loci would lead to much greater variation (Kosswig, 1964). Kosswig (1964) also mentioned that in an interspecific cross the sex ratio will be "doubtful" with wider variation in the case of polygenic system. The results of interspecific crosses in tilapia show greater variability than the intraspecific crosses which supports the above assumption.

The other prediction in the polygenic system is the presence of "strong" and "weak" individuals of the same sex in the population. The "strong" individuals when crossed to the opposite sex will give a progeny of all (or nearly all) of its own kind whereas the "weak" individuals will give a progeny of the opposite sex. Examples of such males are found in two species of Oreochromis. Hickling (1968) reported that a few males of Malayan strain of O. mossambicus when crossed to the females of the same strain gave 88-97% females. In O. aureus a few males were found which gave 100% males when crossed to different females (Shelton et al., 1983).

One way of proving the polygenic hypothesis is by producing lines rich in males and others rich in females by continuous selection over several generations. The selection programme will be difficult if there are a

large number of alleles and loci responsible for sex determination in the species concerned and if the strengths of the individual alleles are different. Hulata et al. (1981) proposed a model for such selection. They used the O. niloticus x O. aureus cross to select out parents which give all-male progeny. In the reciprocal cross they selected the parents which gave a 1 female:3 male ratio. Using such criteria they selected seven female O. niloticus and four male O. aureus which gave all-male progeny but in the reciprocal cross they have yet to find any individual pairs which fit with their expectations (Hulata et al., 1983). The failure of such studies might be because they used selection criteria on two species simultaneously. This procedure will give pairs of two species which will produce higher males in those combinations only, and not a strain of one species which can be used as a base stock. Selection within a species for higher or lower percentages of males, to produce separate lines is likely to be more practical. These selected strains when inbred should consistently produce higher or lower male percentages in their offspring, which can be used for culture. This method of selection will give an additional advantage over hybridization where problems of introgression is higher in poorly managed systems.

CHAPTER III

RELATIVE DNA CONTENTS OF SOMATIC NUCLEI AND CHROMOSOMAL STUDIES IN SOME COMMERCIALLY IMPORTANT TILAPIINES

INTRODUCTION

Fish comprise a polyphyletic group which includes over 23,000 species (Ohno, 1974). Of all those, the chromosome complement of only 850 - 900 species are known (Manna and Khuda-Bukhsh, 1977; Sola et al., 1981). Unfortunately a state of confusion still exists in many species, even about their diploid chromosome numbers (Chiarelli and Capanna, 1973; Ohno, 1974; Park, 1974; Ojima et al., 1976; Vasilyev, 1981; Sola et al., 1981). In comparison with the placental mammals where 30% or more of the species have been studied chromosomally, only about 3% are known in fishes. This is probably because the fishes generally have a large number of tiny chromosomes which makes a detailed analysis very difficult (Nogusa, 1960). Regarding fish chromosome cytology White (1973) remarked that

> "detailed studies of the karyotypes have hardly begun and will obviously be too far easy."

Several methods of chromosome preparations have so far been developed for fish. These include tissue culture and chromosome banding techniques (Ojima et al., 1970; Denton, 1973; Blaxhall, 1975, 1983a, 1983b, 1983c; Hartley and Horne, 1983). Despite this information the field of fish cytogenetics is still in its infancy (Gold, 1979).

Regarding the understanding of the cytogenetics of the fishes Ohno (1974) questioned whether the study of chromosomes of the whole group would provide much information. He also mentioned that

> "a comparative study of chromosome complement in fish is meaningless unless accompanied by information on their respective genome size."

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> "a comparative study of chromosome complement in fish is meaningless unless accompanied by information on their respective genome size."

It is known that the genome size or C-value (Swift, 1950) in fishes varies between 0.5 x 10^{-9}mg and 125 x 10^{-9}mg (Ohno and Atkin, 1966; Hinegardner, 1968; Hinegardner and Rosen, 1972) and that the advanced fishes are found to possess the lower DNA amounts (Bachmann et al., 1974; Ohno, 1974). It has also been shown that more than a two-fold difference in genome size can be found among members of the same family or even the same genus (Ohno et al., 1967; Wolf et al., 1969). So comparative analysis of chromosomes between species without the knowledge of genome size may well lead to mistakes if these data are used for evolutionary studies.

Tilapia, a group of fish belonging to the family Cichlidae are exclusively endemic to the African continent (see Balarin and Hatton, 1979). They have adapted to a wide range of environmental conditions and food habits. The ecological diversity and distribution have been reviewed by Trewavas (1982b, 1983), Lowe-McConnel (1982) and Philippart and Ruwet (1982). Despite the highly diverse ecological niches occupied by this group, their morphological diversity is such that ordinary morphological characteristics are very unreliable in resolving their taxonomical groupings. Trewavas (1982a) recently reclassified this group into three genera, viz. Oreochromis, Sarotherodon and Tilapia which includes about 70 species. The classification is mainly based on the breeding and brooding behaviour along with morphology and body colouration. The Oreochromis is restricted to the maternal mouthbrooders, the Sarotherodon includes both the biparental (female and male) and paternal (male) mouthbrooders, and the Tilapia includes all the substrate spawning species. Enzyme electrophoretic studies (Kornfield et al., 1979; McAndrew and Majumdar, 1983, 1984) generally confirm the taxonomic groupings by

Trewavas (1982a).

Only 12 species of tilapias have been studied cytogenetically and of these, in two species only the chromosome number is known (Table 1). Where the same species has been studied by different authors there are often conflicting reports on the chromosome number, e.g. T. zillii and O. niloticus. Some confusion is also present over the chromosome morphology. In O. mossambicus the karyotype is reported to consist of only metacentric chromosomes by Natarajan and Subrahmanyam (1968), whereas several other authors reported that it consists of mostly subterminal and terminal chromosomes (Prasad and Manna, 1976; Krishnaja and Rege, 1980; Thompson, 1981). Such contradictory results (Table 1) on the chromosome data may be due to the presence of natural polymorphism in the chromosome system or due to technical difficulties. As it has been mentioned, fish chromosomes are tiny and the methodology is not advanced enough to give unequivocal identification of each individual chromosome pair in a species, which might lead to the chromosomal formulation being rather erroneous. To overcome these problems chromosomal techniques need to be standardized to allow direct comparisons of results obtained by various authors.

The DNA value of only a few tilapia species (5) is known but once again the results are highly variable. Hinegardner (1968) and Hinegardner and Rosen (1972) reported that T. zillii, O. niloticus and O. leucosticta all have the same amount of DNA in their nuclei. On the other hand, Kornfield et al. (1979) reported a higher amount of DNA in O. aureus but similar levels to those reported by the other authors of T. zillii. It seems that although tilapias are closely related, the C-value is not the same in every species. A similar phenomenon has been reported in

TABLE 1. Chromosomal Data on Tilapias

Species	2n	n	Karyotype	Arm No. (NF)	Reference
O. mossambicus	44	22	All metacentrics	88?	Natarajan and Subrahmanyam (1968)
	44				Denton (1973)
	44			62	Fukoka and Muramoto (1975) in Vasilyev (1981)
	44	22	3st + 2t + 17T	44	Prasad and Manna (1976)
	44	22	3st + 2t + 17T	44	Krishnaja and Rege (1980)
	44		3(sm?) + 19st	44-50	Thompson (1981)
O. aureus	44		5sm + 17st	54	Kornfield et al. (1979)
	44		3(sm?) + 19st	44-50	Thompson (1981)
O. niloticus	44				Jalabert et al. (1971)
	40		1sm + 19st	42	Badr and El-Dib (1977)
	44	22	ZW(♀); ZZ(♂)		Nijjhar et al. (1983)
O. grahami	48	24			Post (1965)
	48				Denton (1973)
	48				Park (1974)
O. multifasciatus	44	22	ZW(♀); ZZ(♂)		Nijjhar et al. (1983)
S. galilaeus	44				Badr and El-Dib (1977)
	44		5sm + 17st	54	Kornfield et al. (1979)
S. melanotheron		16			Jakowska (1950)
T. zillii	38		1M + 1sm + 17st	42	Badr and El-Dib (1977)
	44		5sm + 17st	54	Kornfield et al. (1979)
T. rendalli	44		4sm + 18st	52	Michele and Takahashi (1977)
	44	22			Foresti et al. (1983)

Species	2n	n	Karyotype	Arm No. (NF)	Reference
T. sparrmanii	42		1(m?) + 3(sm?) + 17st	46-50	Thompson (1981)
T. mariae	40		1(m?) + 3(sm?) + 16st	44-48	Thompson (1981)
T. busumana	44	22	ZW(♀); ZZ(♂)		Nijjhar et al. (1983)

several species of animals leading to the C-value paradox (review by Bachmann et al., 1974; Hinegardner, 1976). To understand whether the C-value paradox is a generalised phenomenon in tilapias, a greater number of species will need to be studied.

C-banding analysis in these species has been done by Kornfield et al., (1979). They concluded that except in T. zillii all the other two species (O. aureus and S. galilaeus) do not vary much in their constitutive heterochromatin content. In T. zillii a few chromosomes have much more C-heterochromatin. It has been reported by several authors that closely related species sometimes show a difference in the C-heterochromatin distribution which may indicate karyotype evolution (Pathak et al., 1973; Mizuno and Macgregor, 1974), so C-banding in tilapia could also be utilized to understand the chromosomal interrelationships (if any).

Another important aspect in the study of chromosomes is to see whether sex determination is governed by a chromosomal mechanism. Several authors proposed models based on sex reversal and hybridization studies in tilapias suggesting that some species are XX:XY (male heterogametic) and other species are ZW:ZZ (female heterogametic) (Wohlfarth and Hulata, 1983; Chapter II). But none of these workers could show any sex specific chromosome pair in these fishes. Only one report by Nijjhar et al., (1983) claims that heteromorphic sex chromosomes can be seen in three species: O. niloticus, O. multifasciatus and T. busmana, all have a ZZ male and ZW female sex chromosome pair. Unfortunately the detailed karyotype has not been provided by the authors.

It seems that the enormous discrepancies on the chromosome cytology of these species might be due to technical shortcomings or some other reasons, e.g. species purity, species identification, etc. So to minimise such variation much more attention should be given to methodology of chromosome preparation (Sola et al., 1981) and also many more pure species to be included before generalization on the chromosome data are made.

In the present Chapter data on somatic and meiotic chromosomes of seven tilapia species belonging to three genera are presented. The C-value of six species out of the seven is calculated from Feulgen stained nuclei of blood cells by microdensitometry. C-banding studies on the somatic chromosomes of all these species are also presented. The results of these studies are discussed in the light of chromosomal interrelationships in this group.

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MATERIALS AND METHODS

The following are the species used for the present series of experiments: Oreochromis mossambicus, O. spilurus, O. aureus, O. niloticus, O. macrochir, Sarotherodon galilaeus and Tilapia zillii. The origin of these species and their maintenance procedure is given in Chapter II and Chapter IV, except S. galilaeus which has been procured from Lake Turkana (Kenya).

DNA Value Determination using Feulgen Staining

Blood was collected by puncturing the caudal vein of the fish which was anaesthetised by benzocaine (1:10,000), using #18 gauge needle fitted with a heparinised syringe. A blood film was made on clean slides. After air drying the preparation was fixed in acetic acid:absolute ethanol (1:3) at 0 - 4°C for 30 minutes. After fixation the slides were passed through ascending grades of alcohol (90%, 100%), two changes each for 5 minutes, and were air dried. The slides were then stored in light-proof airtight boxes at 0 - 4°C until used. Usually within two weeks of the preparation, the slides were stained.

Feulgen staining was done according to the procedure given by Sumner and Buckland (1976), which was as follows: The slides were post-fixed in formalin for one hour at room temperature, washed thoroughly in distilled water, incubated for 30 minutes in freshly prepared 5 N HCl at room temperature. (The hydrolysis time of 30 minutes gives maximum staining of the nuclei). After hydrolysis the slides were washed in distilled water, stained in Schiff's reagent for one and a half hours in the dark at room temperature. The slides thus stained were washed in running

tap water for about 10-15 minutes (Demalsy and Callebaut, 1967) and were dehydrated through ascending grades of alcohol and finally were mounted in DPX (G.T.Gurr).

Nuclei were measured using a Vickers M 86 integrating microdensitometer. Only morphologically normal, undamaged nuclei were measured. All measurements were made at a wavelength of 560nm, using 100 X objective. To reduce glare the field stop was reduced to its minimum size and residual glare compensated for electronically (Goldstein, 1970). To reduce distributional error as far as practicable, the smallest size of the scanning spot (equivalent to 0.2 μm in the objective plane) was used (Goldstein, 1971). All the measurements were made with the background set to a density of about 0.07. Erythrocyte nuclei from carp (Cyprinus carpio) were prepared simultaneously and were used as a standard on all slides. The measurements of absorbance and nuclear area were taken from 25 nuclei from each specimen. For each species five different individuals were used for DNA measurements. The absorbance and nuclear area are expressed as arbitrary units.

A statistical test using two-way nested anovar (Sokal and Rohlf, 1969) was performed on the absorbance. To test the significance of ranking between species, multiple range analysis (Duncan, 1955) was done on mean absorbance of individuals of the species together.

Chromosome preparation: somatic: Mitosis was induced in vivo in kidney and spleen tissues of the fishes by a phytohaemagglutinin (PHA) injection as described by Majumdar (1979) and Kornfield et al.(1979). Live fishes weighing 25-30gms received 0.05-0.1ml of PHA (phytohaemagglutinin P/M, Difco) intraperitoneally. Altogether two injections of PHA were given at

an interval of 24 hours. The animals received a colchicine injection (0.05ml of 0.025% colchicine solution) four hours before being killed. At about 48 hours from the start of the first injection of PHA, the fishes were killed. The kidney and spleen were taken out in 0.075M KCl solution in a cavity block. The tissues were gently washed twice in KCl solution to remove any excess blood. The tissues were then finely chopped or minced using a fine pair of scissors and forceps. The cell suspension thus obtained was gently decanted in a centrifuge tube, helping to avoid the contamination of blocks of tissues. The tube was then filled with more KCl solution and was kept at room temperature for 10-15 minutes. After the hypotonic treatment a few drops of fixative (1 acetic acid:3 methanol) were added and mixed thoroughly. The cells were then pelleted by centrifugation at about 1,000rpm for 5-8 minutes. The cell pellet was then fixed with fresh fixative. The centrifugation and replacement of fixative procedures were repeated several times which allows better fixation and removal of debris from the cell suspension.

For slide preparation two procedures were followed:

1) Flame Drying: A few drops of cell suspension were poured onto a slide and then brought near a naked flame which instantly burned the alcohol off the suspension leaving the cells firmly attached to the slide surface.

2) Air Drying: The clean slides were placed horizontally on a glass plate. Two drops of cell suspension were allowed to fall onto the middle of each slide from a distance of about 12-18 inches, and were left to dry.

Both these procedures of slide preparation gave good results as far as the chromosome spreading, but for C-banding only air dried slides were used (Hartley, S., personal communication, 1982).

C-banding

C-banding was done by the procedure mentioned in Sumner (1972) with a few minor modifications. The air dried slides were treated with 0.2 N HCl for one hour at room temperature, and then washed thoroughly in deionized water. The slides were then treated in 5% aqueous $Ba(OH)_2 \cdot 8H_2O$ solution for 5 minutes at 55°C in a water bath and washed thoroughly in deionized water. The slides were then incubated in 2 X SSC (SSC = 0.15 M NaCl, 0.015 M Na-citrate, pH 7.0) for one to one and a half hours at 60-65°C in a water bath. The slides were stained for 15-30 minutes in 5% Giemsa in a phosphate buffer (0.1 M) solution (pH 6.8-7.0) after washing.

Preparation of Meiotic Chromosomes

A single intraperitoneal colchicine injection was administered (0.05ml of 0.025% colchicine solution) 4 hours before killing the fish. The testes were removed with the least amount of fat bodies and blood. The small pieces of testes were squeezed a little in KCl solution to remove excess sperm, then teased thoroughly which yielded a homogeneous cell suspension. The cells were then treated in hypotonic solution (0.075 M KCl) for 15-20 minutes at room temperature. After hypotonic treatment the cells were pelleted and fixed in acetic acid: methanol (1:3) solution as mentioned for the somatic chromosomes. Slides were prepared by the flame drying method.

Determination of Chromosome Number

Chromosomes of more than 100 metaphase spreads prepared from 5 male and 5 female individuals of each species were counted. The frequency in percentage was calculated for each chromosome number obtained in different metaphase plates. The chromosome number whose frequency occurrence was highest was considered as the diploid number for that species. Variations in chromosome number (both hypo- and hyper-diploid) were probably the result of errors inherent in the preparation technique.

Morphometric Measurements of the Chromosome

Karyotypes were made from the photographs of individual metaphase plates from males and females. Comparison of the male and female karyotypes was made to find out the presence or absence of sex specific chromosome pairs. Individual chromosomes of each karyotype (total of five) were measured by a slide caliper. The relative length (L^R) of each chromosome as percentage of the total haploid length was calculated. The centromeric position of each chromosome was expressed as the centromeric index (I^C) calculated on the basis of the percentage of the short arm to that of the total length of that chromosome.

RESULTS

DNA Value

DNA value data are shown in Table 2 which includes absorbance, nuclear area, DNA value relative to carp (Cyprinus carpio) and the C-value for all the six species of tilapia. The absorbance and nuclear area are expressed as arbitrary units. For every species 5 individuals were used and measurements from 25 nuclei were made from each individual for absorbance and nuclear area. To check the significance of the differences in the absorbance a two-way nested anovar analysis (Sokal and Rohlf, 1969) was used for the absorbance (Table 3). Significance was tested using the F-test. Data on absorbance show that there are no significant differences among individuals of the same species, whereas there is a significant difference between species. Using Duncan's (1955) multiple range test for ranking, the species could be placed into the following three groups: a) with least absorbance, e.g. O. macrochir (33.62), S. galilaeus (33.87); b) with the intermediate absorbance, e.g. O. niloticus (38.16), O. spilurus (38.02), O. mossambicus (40.70); c) with the highest absorbance, e.g. O. aureus (48.43). Using the same statistics on the nuclear area data it was possible to conclude that the nuclear area does not differ significantly in different species. Nuclear DNA relative to carp in different species varied between 0.494 (O. macrochir and S. galilaeus) and 0.711 (O. aureus). Using Cyprinus carpio haploid DNA content as 1.7pg (Hinegardner, 1968) the haploid DNA content for all these species was calculated which shows the range between 0.84pg DNA (O. macrochir and S. galilaeus) and 1.21pg DNA in O. aureus. In relation to mammalian genome (3.5×10^{-12}g, Ohno, 1974) the percentage

TABLE 2. Estimated Nuclear Area, C-Values, C-Values Relative to Mammals in Six Different Tilapias

Species	No. of cells per specimen (No. of individuals)	Absorbance Arbitrary Unit Mean ± S.E.	Nuclear Area Arbitrary Unit Mean ± S.E.	DNA value Relative to Carp Mean ± S.E.	C-value (Haploid nuclei) approximately 10^{-12}g	% to mammals (3.5×10^{-12}g = 100%)
O. mossambicus	25 (5)	40.70^{b} ± 0.26	10.00^{a} ± 0.13	0.588 ± 0.003	1.00	28.57
O. spilurus	25 (5)	38.02^{b} ± 0.15	13.42^{a} ± 0.13	0.558 ± 0.002	0.95	27.14
O. niloticus	25 (5)	38.16^{b} ± 0.08	11.30^{a} ± 0.20	0.560 ± 0.001	0.95	27.14
O. aureus	25 (5)	48.43^{c} ± 0.09	13.25^{a} ± 0.20	0.711 ± 0.001	1.21	34.57
O. macrochir	25 (5)	33.62^{a} ± 0.21	11.59^{a} ± 0.12	0.494 ± 0.003	0.84	24.00
S. galilaeus	25 (5)	33.87^{a} ± 0.14	11.15^{a} ± 0.13	0.497 ± 0.002	0.84	24.00

Common superscripts in the same column are not statistically significant ($P > 0.01$)

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TABLE 3. Results of Two Way Nested Anovar on the Absorbance of the Nuclei After Feulgen Staining in Tilapia

Sources of Variation	df	SS	MS	Fs
Among species	5	18544.3	3708.8	806.2*
Among individuals within species	24	111.1	4.6	1.7 N.S.
Between measurements within each individual	720	2001.3	2.7	
TOTAL	749	20656.7		

*$P < 0.01$

genome size in these fishes varied between 24 (O. macrochir and S. galilaeus) and 34.57 (O. aureus).

Chromosome Number

Diploid chromosome number data obtained from spleen and kidney cells are given in Table 4. This includes the number of cells with different chromosome numbers and their frequency in percentages. From the Table it is evident that most of the cells (over 94%) found possess 44 chromosomes in every species. The widest variation of chromosome number was recorded for O. niloticus and S. galilaeus (from 40-45) whereas the least variation was observed in O. macrochir and T. zillii (from 42-44). The distribution of the chromosome frequency seems quite skewed and peaks at 44. So it seems that the commonest diploid chromosome number for all these species is 44. The random karyotyping from both hypodiploid and hyperdiploid metaphase plates shows that loss or addition is the cause of such different chromosome numbers. This also suggests that the technique for chromosome preparation gives less chance of addition than loss of a chromosome. Only 0.35% of the cells show hyperdiploidy as compared to 4.01% of the cells which have hypodiploid chromosome number.

Chromosome Morphology

The karyotypes of different species have been described according to the classification proposed by Levan et al. (1964) on the basis of centromeric index (I^C). The karyotypes were presented according to the length of the chromosomes in descending order denoted by the increasing number. So the 1st chromosome is the longest and the 22nd is the smallest in any karyotype. In every species the 1st and 2nd chromosome pair can be distinguished from the rest of the chromosome pairs because of their

TABLE 4. Distribution of Diploid Chromosome Number in Tilapias

Species		Chromosome Number						
		40	41	42	43	44	45	Total
O. mossambicus	Cell No.		2	3	5	247	1	258
	%		0.78	1.16	1.93	95.74	0.39	
O. spilurus	Cell No.		2	5	11	379	3	400
	%		0.50	1.25	2.75	94.75	0.75	
O. niloticus	Cell No.	1	3	2	13	587	4	610
	%	0.16	0.48	0.32	2.13	96.27	0.64	
O. aureus	Cell No.		1	5	8	264		278
	%		0.36	1.80	2.88	94.96		
O. macrochir	Cell No.			2	5	270		277
	%			0.72	1.80	97.48		
T. zillii	Cell No.			2	5	272		279
	%			0.72	1.79	97.49		
S. galilaeus	Cell No.	1	3	9	14	408	1	436
	%	0.23	0.69	2.07	3.22	93.56	0.23	
TOTAL	Cell No.	2	11	28	61	2427	9	2538
	%	0.08	0.43	1.10	2.40	95.64	0.35	100

conspicuous size difference. The chromosomes nos. 3-22 are so close in their length that any groupings within them was not possible. Comparison of karyotypes from male and female somatic cells did not reveal any sex specific heteromorphic pairs.

The borderline chromosomes in the class submedian (sm) and subterminal (st) are considered as single armed because of the overlapping of their 95% confidence limit.

O. mossambicus (Fig. 1, Table 5): The karyotype consists of 3 pairs of submedian (Nos. 5, 6 and 13), 15 pairs of subterminal (Nos. 1-4, 7-12, 14, 16 and 2o-22) and 4 pairs of submedian-subterminal (sm-st) borderline chromosomes (Nos. 15 and 17-19). The chromosome length varies between 3.72% and 11.73%. The diploid arm number (N.F., Matthey, 1945) in this species is 50.

O. spilurus (Fig. 2, Table 6): The karyotype consists of 3 pairs of sm (Nos. 3, 5 and 6), 10 pairs of st (Nos. 1, 2, 7, 8, 11, 12, 15, 16, 21 and 22) and 9 pairs of sm-st (Nos. 4, 9, 10, 13, 14 and 17-20) chromosomes. The chromosome length varies between 3.62% and 11.24%. The NF is 50. The karyotype is very similar to O. mossambicus.

O. niloticus (Fig. 3, Table 7): The karyotype consists of 1 pair of median (m) (No. 6), 9 pairs of sm (Nos. 3-5, 7, 8, 11, 12, 14 and 15), 7 pairs of st (Nos. 1, 2, 9, 17 and 20-22) and the remaining 5 pairs of sm-st (Nos. 10, 13, 16, 18 and 19) chromosomes. The chromosome length varies between 3.76% and 12.01%. The NF is 64 in this species.

TABLE 5. Metrical Data on the Chromosomes of *Oreochromis mossambicus*

No.	$L^R \pm$ S.E.	95% C.L.	$I^C \pm$ S.E.	95% C.L.	Classification after Levan *et al.* (1964)
1	11.73 ± 0.25	11.24 - 12.22	13.71 ± 0.21	13.29 - 14.12	st
2	6.23 ± 0.07	6.09 - 6.37	22.54 ± 0.53	21.50 - 23.58	st
3	4.53 ± 0.12	4.29 - 4.76	20.19 ± 0.25	19.70 - 20.68	st
4	4.46 ± 0.09	4.28 - 4.64	21.69 ± 0.27	21.16 - 22.22	st
5	4.31 ± 0.04	4.23 - 4.39	34.85 ± 0.06	34.73 - 34.97	sm
6	4.25 ± 0.07	4.11 - 4.39	28.40 ± 0.34	27.73 - 29.06	sm
7	4.24 ± 0.07	4.10 - 4.38	19.13 ± 0.24	18.66 - 19.60	st
8	4.23 ± 0.09	4.05 - 4.41	24.05 ± 0.21	23.64 - 24.46	st
9	4.21 ± 0.03	4.15 - 4.27	21.91 ± 0.26	21.40 - 22.42	st
10	4.17 ± 0.04	4.09 - 4.25	21.91 ± 0.22	21.48 - 22.34	st
11	4.11 ± 0.05	4.01 - 4.21	23.26 ± 0.36	22.55 - 23.96	st
12	4.08 ± 0.17	3.75 - 4.41	22.03 ± 0.32	21.40 - 22.66	st
13	4.02 ± 0.08	3.86 - 4.18	31.98 ± 0.69	30.63 - 33.33	sm
14	4.02 ± 0.06	3.90 - 4.14	23.25 ± 0.15	22.96 - 23.54	st
15	3.98 ± 0.05	3.88 - 4.08	25.17 ± 0.38	24.42 - 25.91	sm-st
16	3.98 ± 0.09	3.80 - 4.15	24.10 ± 0.25	23.61 - 24.59	st
17	3.95 ± 0.11	3.73 - 4.16	25.26 ± 0.79	23.71 - 26.81	sm-st
18	3.94 ± 0.11	3.72 - 4.15	25.51 ± 0.31	24.90 - 26.12	sm-st

No.	L^R ± S.E.	95% C.L.	I^C ± S.E.	95% C.L.	Classification after Levan et al. (1964)
19	3.86 ± 0.05	3.76 - 3.96	25.06 ± 0.39	24.29 - 25.82	sm-st
20	3.80 ± 0.09	3.62 - 3.97	23.98 ± 0.36	23.27 - 24.68	st
21	3.75 ± 0.08	3.59 - 3.91	23.13 ± 0.28	22.58 - 23.68	st
22	3.72 ± 0.11	3.50 - 3.93	23.85 ± 0.39	23.08 - 24.61	st

No.	L^R ± S.E.	95% C.L.	I^C ± S.E.	95% C.L.	Classification after Levan et al. (1964)
19	3.86 ± 0.05	3.76 - 3.96	25.06 ± 0.39	24.29 - 25.82	sm-st
20	3.80 ± 0.09	3.62 - 3.97	23.98 ± 0.36	23.27 - 24.68	st
21	3.75 ± 0.08	3.59 - 3.91	23.13 ± 0.28	22.58 - 23.68	st
22	3.72 ± 0.11	3.50 - 3.93	23.85 ± 0.39	23.08 - 24.61	st

TABLE 6. Metrical Data on the Chromosomes of Oreochromis spilurus

No.	L^R ± S.E.	95% C.L.	I^C ± S.E.	95% C.L.	Classification after Levan et al. (1964)
1	11.24 ± 0.37	10.51 - 11.96	9.88 ± 0.67	8.57 11.19	st
2	6.30 ± 0.06	6.18 - 6.42	21.58 ± 0.64	20.32 - 22.83	st
3	4.52 ± 0.14	4.24 - 4.79	26.67 ± 0.66	25.37 - 27.96	sm
4	4.43 ± 0.10	4.23 - 4.63	25.90 ± 0.33	25.25 - 26.54	sm-st
5	4.39 ± 0.09	4.21 - 4.57	34.05 ± 0.95	32.19 - 35.91	sm
6	4.37 ± 0.10	4.17 - 4.57	30.91 ± 0.42	30.08 - 31.73	sm
7	4.34 ± 0.02	4.30 - 4.38	22.55 ± 0.82	20.94 - 24.16	st
8	4.34 ± 0.07	4.20 - 4.48	21.74 ± 0.75	20.27 - 23.21	st
9	4.30 ± 0.13	4.04 - 4.55	25.08 ± 0.21	24.67 - 25.49	sm-st
10	4.19 ± 0.07	4.05 - 4.33	25.85 ± 0.98	23.93 - 27.77	sm-st
11	4.12 ± 0.05	4.02 - 4.22	22.81 ± 0.83	21.18 - 24.44	st
12	4.12 ± 0.17	3.79 - 4.45	21.75 ± 0.93	19.93 - 23.57	st
13	4.11 ± 0.03	4.05 - 4.17	25.12 ± 0.67	23.73 - 26.51	sm-st
14	4.09 ± 0.05	3.99 - 4.19	26.56 ± 0.71	25.17 - 27.95	sm-st
15	4.04 ± 0.08	3.88 - 4.20	24.06 ± 0.61	22.86 - 25.25	st
16	4.00 ± 0.09	3.82 - 4.18	19.02 ± 0.62	17.80 - 20.23	st
17	3.89 ± 0.06	3.77 - 4.01	25.79 ± 0.20	25.39 - 26.18	sm-st
18	3.80 ± 0.01	3.78 - 3.82	25.16 ± 0.48	24.22 - 26.10	sm-st

No.	L^R ± S.E.	95% C.L.	I^C ± S.E.	95% C.L.	Classification after Levan et al. (1964)
19	3.79 ± 0.08	3.63 - 3.94	25.72 ± 0.54	24.66 - 26.78	sm-st
20	3.77 ± 0.10	3.57 - 3.97	25.57 ± 0.53	24.53 - 26.61	sm-st
21	3.75 ± 0.10	3.55 - 3.95	22.43 ± 0.78	20.90 - 23.96	st
22	3.62 ± 0.13	3.36 - 3.87	22.77 ± 0.65	21.49 - 24.04	st

FIG. 1 Representative karyotype of O. mossambicus consisting of 3 sm(Nos. 5,6 and 13), 4 sm-st(Nos. 15 and 17-19) and 15 st chromosome pairs.

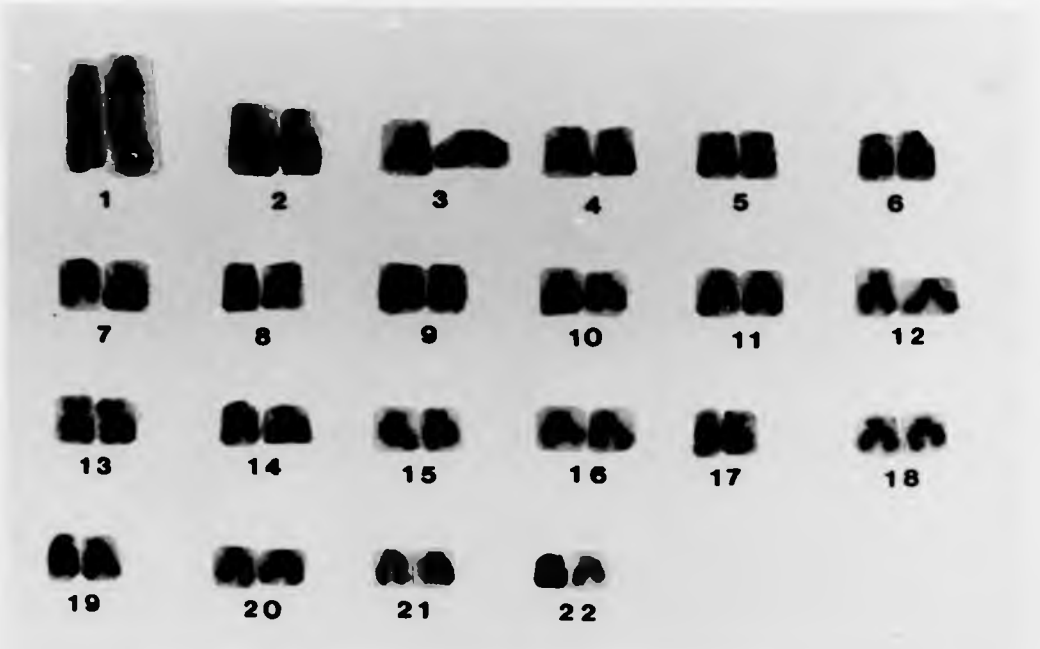

FIG. 2 Representative karyotype of O. spilurus consisting of 3 sm(Nos. 3,5 and 6), 9 sm-st(Nos. 4,9,10,13,14 and 17-20) and 10 st chromosome pairs.

FIG. 1 Representative karyotype of O. mossambicus consisting of 3 sm(Nos. 5,6 and 13), 4 sm-st(Nos. 15 and 17-19) and 15 st chromosome pairs.

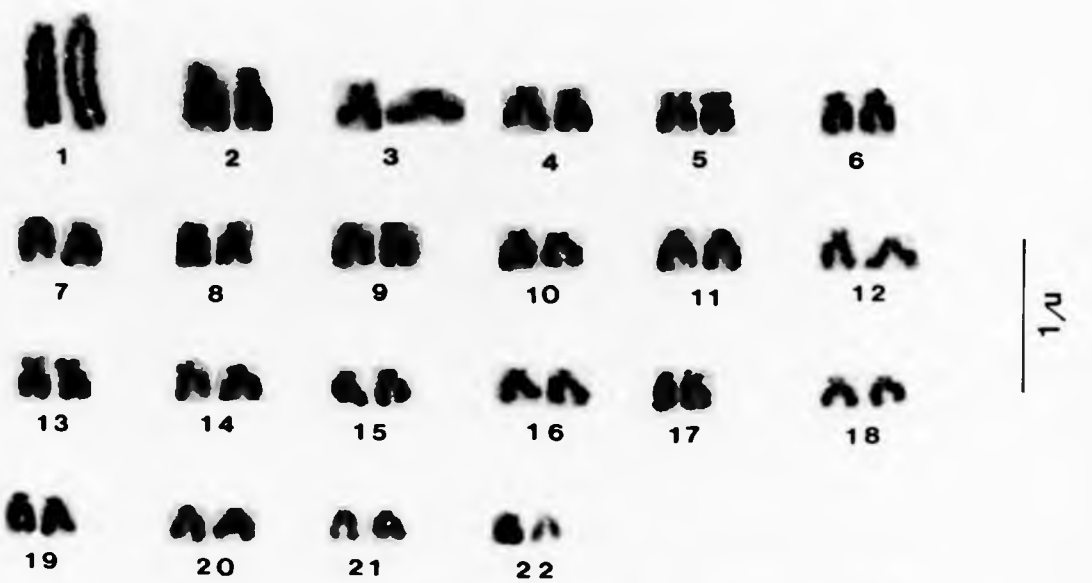

FIG. 2 Representative karyotype of O. spilurus consisting of 3 sm(Nos. 3,5 and 6), 9 sm-st(Nos. 4,9,10,13,14 and 17-20) and 10 st chromosome pairs.

TABLE 7. Metrical Data on the Chromosomes of Oreochromis niloticus

No.	L^R ± S.E.	95% C.L.	I^C ± S.E.	95% C.L.	Classification after Levan et al. (1964)
1	12.01 ± 0.21	11.60 - 12.42	12.11 ± 0.61	10.91 -13.30	st
2	6.07 ± 0.14	5.79 - 6.34	27.04 ± 0.62	25.82 - 28.25	st
3	4.60 ± 0.24	4.13 - 5.07	29.83 ± 0.59	28.67 - 30.98	sm
4	4.41 ± 0.15	4.12 - 4.70	29.46 ± 0.28	28.91 - 30.01	sm
5	4.37 ± 0.10	4.17 - 4.57	34.75 ± 0.76	33.26 - 36.24	sm
6	4.36 ± 0.09	4.18 - 4.54	39.34 ± 0.37	38.61 - 40.06	m
7	4.36 ± 0.09	4.18 - 4.54	30.34 ± 0.71	28.95 - 31.73	sm
8	4.29 ± 0.06	4.17 - 4.41	28.49 ± 0.30	27.90 - 29.08	sm
9	4.29 ± 0.05	4.19 - 4.39	21.80 ± 0.64	20.54 - 23.05	st
10	4.18 ± 0.09	4.01 - 4.36	25.17 ± 0.19	24.80 - 25.54	sm-st
11	4.15 ± 0.05	4.05 - 4.25	30.44 ± 0.26	29.93 - 30.95	sm
12	4.13 ± 0.06	4.01 - 4.25	27.20 ± 0.30	26.61 - 27.79	sm
13	4.11 ± 0.07	3.97 - 4.25	25.98 ± 0.18	25.63 - 26.33	sm-st
14	4.07 ± 0.04	3.99 - 4.15	30.96 ± 0.40	30.18 - 31.74	sm
15	4.07 ± 0.12	3.83 - 4.30	30.47 ± 0.53	29.43 - 31.51	sm
16	4.02 ± 0.07	3.88 - 4.16	25.99 ± 0.56	24.89 - 27.09	sm-st
17	4.00 ± 0.04	3.92 - 4.08	24.99 ± 0.82	23.38 - 26.60	st
18	3.98 ± 0.05	3.88 - 4.08	25.24 ± 0.27	24.71 - 25.77	sm-st

No.	L^R ± S.E.	95% C.L.	I^C ± S.E.	95% C.L.	Classification after Levan et al. (1964)
19	3.96 ± 0.07	3.82 - 4.09	25.78 ± 0.36	25.07 - 26.48	sm-st
20	3.96 ± 0.08	3.80 - 4.12	23.54 ± 0.46	22.64 - 24.44	st
21	3.80 ± 0.11	3.58 - 4.01	23.72 ± 0.56	22.62 - 24.82	st
22	3.76 ± 0.06	3.64 - 3.88	20.06 ± 0.60	18.88 - 21.24	st

O. aureus (Fig. 4, Table 8): The karyotype consists of 7 pairs of sm (Nos. 3, 6, 7, 9, 12, 14 and 18), 7 pairs of st (Nos. 1, 2, 15, 16 and 20-22) and the remaining 8 pairs of sm-st (Nos. 4, 5, 8, 10, 11, 13, 17 and 19) chromosomes. The L^R of chromosomes varies between 3.61% and 10.89%. The NF is 58 in this species.

O. macrochir (Fig. 5, Table 9): The karyotype consists of 5 pairs of sm (Nos. 2, 4, 11, 15 and 19), 8 pairs of st (Nos. 1, 3, 10, 12, 17, 18, 21 and 22) and 9 pairs of sm-st (Nos. 5-9, 13, 14, 16 and 20) chromosomes. The L^R varies between 3.66% and 12.65%. The NF is 54 for this species.

T. zillii (Fig. 6, Table 10): The karyotype consists of 2 pairs of m (Nos. 5 and 10), 9 pairs of sm(Nos. 2, 3, 7, 9, 11, 13-15 and 18), 7 pairs of st (Nos. 1, 8, 12, 16 and 20-22) and 4 pairs of sm-st (Nos. 4, 6, 17 and 19) chromosomes. The L^R varies between 3.71% and 10.84%. The NF is 66 in this species.

S. galilaeus (Fig. 7, Table 11): The karyotype consists of 2 pairs of sm (Nos. 9 and 17), 4 pairs of sm-st (Nos. 3, 8, 10 and 13) and the rest, st chromosomes. The L^R varies between 3.37% and 12.32% and the NF is 48.

The summary of the chromosome formulae and NF of each species is given in Table 12.

TABLE 8. Metrical Data on the Chromosomes of Oreochromis aureus

No.	L^R ± S.E.	95% C.L.	I^C ± S.E.	95% C.L.	Classification after Levan et al. (1964)
1	10.89 ± 0.25	10.40 - 11.38	11.72 ± 0.54	10.66 - 12.78	st
2	5.93 ± 0.15	5.64 - 6.22	25.00 ± 0.78	23.47 - 26.53	st
3	4.58 ± 0.04	4.50 - 4.66	28.84 ± 0.73	27.41 - 30.27	sm
4	4.52 ± 0.12	4.28 - 4.75	25.72 ± 0.70	24.35 - 27.09	sm-st
5	4.52 ± 0.09	4.34 - 4.69	25.85 ± 0.58	24.71 - 26.98	sm-st
6	4.41 ± 0.12	4.17 - 4.64	34.40 ± 0.71	33.01 - 35.79	sm
7	4.40 ± 0.14	4.12 - 4.67	28.80 ± 0.61	27.60 - 29.99	sm
8	4.39 ± 0.11	4.17 - 4.60	25.73 ± 0.64	24.47 - 26.98	sm-st
9	4.26 ± 0.08	4.10 - 4.42	31.61 ± 0.83	29.98 - 33.24	sm
10	4.22 ± 0.09	4.04 - 4.39	25.79 ± 0.99	23.85 - 27.73	sm-st
11	4.22 ± 0.05	4.12 - 4.32	25.27 ± 0.99	23.33 - 27.21	sm-st
12	4.21 ± 0.13	3.95 - 4.46	31.23 ± 0.76	29.74 - 32.72	sm
13	4.21 ± 0.10	4.01 - 4.41	25.78 ± 0.70	24.41 - 27.15	sm-st
14	4.13 ± 0.11	3.91 - 4.34	26.21 ± 0.45	25.33 - 27.09	sm
15	4.06 ± 0.04	3.98 - 4.14	23.36 ± 0.73	21.93 - 24.79	st
16	4.05 ± 0.08	3.89 - 4.21	20.54 ± 0.84	18.89 - 22.19	st
17	4.02 ± 0.08	3.86 - 4.18	25.15 ± 0.90	23.38 - 26.91	sm-st
18	3.98 ± 0.10	3.78 - 4.18	27.99 ± 0.99	26.04 - 29.93	sm

No.	L^R ± S.E.	95% C.L.	I^C ± S.E.	95% C.L.	Classification after Levan et al. (1964)
19	3.95 ± 0.07	3.81 - 4.09	25.34 ± 0.49	24.38 - 26.30	sm-st
20	3.91 ± 0.12	3.67 - 4.14	22.38 ± 0.79	20.83 - 23.93	st
21	3.77 ± 0.09	3.59 - 3.95	22.54 ± 0.62	21.32 - 23.75	st
22	3.61 ± 0.13	3.35 - 3.86	20.84 ± 0.93	19.02 - 22.66	st

TABLE 9. Metrical Data on the Chromosomes of Oreochromis macrochir

No.	L^R ± S.E.	95% C.L.	I^C ± S.E.	95% C.L.	Classification after Levan et al. (1964)
1	12.65 ± 0.25	12.16 - 13.14	13.90 ± 0.37	13.17 - 14.62	st
2	6.12 ± 0.03	6.06 - 6.18	26.02 ± 0.61	24.82 - 27.21	sm
3	4.54 ± 0.08	4.38 - 4.69	24.87 ± 0.44	24.01 - 25.73	st
4	4.53 ± 0.08	4.37 - 4.69	30.60 ± 0.33	29.95 - 31.25	sm
5	4.37 ± 0.11	4.15 - 4.58	25.70 ± 0.66	24.41 - 26.99	sm-st
6	4.23 ± 0.14	3.95 - 4.50	25.35 ± 0.47	24.43 - 26.27	sm-st
7	4.20 ± 0.07	4.06 - 4.34	25.08 ± 0.45	24.20 - 25.96	sm-st
8	4.15 ± 0.07	4.01 - 4.29	25.04 ± 0.62	23.82 - 26.25	sm-st
9	4.14 ± 0.06	4.02 - 4.26	25.45 ± 0.55	24.37 - 26.53	sm-st
10	4.14 ± 0.09	3.96 - 4.32	24.19 ± 0.16	23.87 - 24.50	st
11	4.13 ± 0.07	3.99 - 4.27	30.86 ± 0.59	29.70 - 32.01	sm
12	4.07 ± 0.06	3.95 - 4.19	20.39 ± 0.98	18.47 - 22.31	st
13	4.03 ± 0.12	3.79 - 4.26	25.33 ± 0.58	24.19 - 26.47	sm-st
14	3.96 ± 0.07	3.82 - 4.10	25.99 ± 0.16	25.67 - 26.30	sm-st
15	3.92 ± 0.08	3.76 - 4.08	33.47 ± 0.28	32.92 - 34.02	sm
16	3.89 ± 0.07	3.75 - 4.03	25.18 ± 0.52	24.16 - 26.20	sm-st
17	3.89 ± 0.05	3.79 - 3.99	20.70 ± 0.45	19.82 - 21.58	st
18	3.85 ± 0.07	3.71 - 3.99	22.59 ± 0.60	21.41 - 23.76	st

No.	L^R ± S.E.	95% C.L.	I^C ± S.E.	95% C.L.	Classification after Levan et al. (1964)
19	3.81 ± 0.08	3.65 - 3.97	32.19 ± 0.50	31.21 - 33.17	sm
20	3.79 ± 0.05	3.69 - 3.89	25.37 ± 0.47	24.45 - 26.29	sm-st
21	3.77 ± 0.09	3.59 - 3.95	22.42 ± 0.56	21.32 - 23.52	st
22	3.66 ± 0.11	3.44 - 3.87	20.13 ± 0.65	18.86 - 21.40	st

TABLE 10. Metrical Data on the Chromosomes of Tilapia zillii

No.	L^R ± S.E.	95% C.L.	I^C ± S.E.	95% C.L.	Classification after Levan et al. (1964)
1	10.84 ± 0.13	10.58 - 11.09	19.55 ± 0.29	18.98 - 20.12	st
2	6.23 ± 0.11	6.01 - 6.44	28.54 ± 0.49	27.58 - 29.50	sm
3	4.62 ± 0.04	4.54 - 4.70	36.49 ± 0.30	35.90 - 37.08	sm
4	4.45 ± 0.10	4.25 - 4.65	25.18 ± 0.42	24.36 - 26.00	sm-st
5	4.39 ± 0.02	4.35 - 4.43	44.26 ± 0.50	43.28 - 45.24	m
6	4.34 ± 0.09	4.16 - 4.52	25.05 ± 0.53	24.01 - 26.09	sm-st
7	4.33 ± 0.07	4.19 - 4.47	32.30 ± 0.51	31.30 - 33.30	sm
8	4.26 ± 0.07	4.12 - 4.40	22.72 ± 0.41	21.91 - 23.52	st
9	4.22 ± 0.09	4.04 - 4.40	35.60 ± 0.96	33.72 - 37.48	sm
10	4.19 ± 0.11	3.97 - 4.40	39.14 ± 0.27	38.61 - 39.67	m
11	4.17 ± 0.10	3.97 - 4.37	32.54 ± 0.60	31.36 - 33.72	sm
12	4.16 ± 0.06	4.04 - 4.28	22.44 ± 0.40	21.66 - 23.22	st
13	4.14 ± 0.08	3.98 - 4.30	36.38 ± 0.27	35.85 - 36.91	sm
14	4.13 ± 0.05	4.03 - 4.23	29.48 ± 0.35	28.79 - 30.17	sm
15	4.12 ± 0.09	3.94 - 4.30	26.04 ± 0.36	25.33 - 26.74	sm
16	4.04 ± 0.10	3.84 - 4.24	24.28 ± 0.42	23.46 - 25.10	st
17	4.03 ± 0.04	3.95 - 4.11	25.72 ± 0.71	24.33 - 27.11	sm-st
18	3.99 ± 0.05	3.89 - 4.09	27.29 ± 0.42	26.47 - 28.11	sm

No.	L^R ± S.E.	95% C.L.	I^C ± S.E.	95% C.L.	Classification after Levan et al. (1964)
19	3.90 ± 0.14	3.62 - 4.17	25.18 ± 0.19	24.81 - 25.55	sm-st
20	3.90 ± 0.07	3.76 - 4.04	23.92 ± 0.59	22.76 - 25.07	st
21	3.84 ± 0.10	3.64 - 4.04	24.37 ± 0.49	23.41 - 25.33	st
22	3.71 ± 0.09	3.53 - 3.89	22.71 ± 0.36	22.00 - 23.41	st

TABLE 11. Metrical Data on the Chromosomes of Sarotherodon galilaeus

No.	L^R ± S.E.	95% C.L.	I^C ± S.E.	95% C.L.	Classification after Levan et al. (1964)
1	12.32 ± 0.25	11.83 - 12.81	12.93 ± 0.35	12.24 - 13.61	st
2	6.38 ± 0.07	6.24 - 6.52	23.82 ± 0.60	22.64 - 24.99	st
3	4.72 ± 0.04	4.64 - 4.80	25.50 ± 0.43	24.66 - 26.34	sm-st
4	4.57 ± 0.03	4.51 - 4.63	23.31 ± 0.42	22.48 - 24.13	st
5	4.47 ± 0.02	4.43 - 4.51	24.54 ± 0.62	23.32 - 25.75	st
6	4.44 ± 0.02	4.40 - 4.48	23.24 ± 0.52	22.22 - 24.26	st
7	4.39 ± 0.03	4.33 - 4.45	22.58 ± 0.64	21.32 - 23.83	st
8	4.33 ± 0.03	4.27 - 4.39	25.45 ± 0.58	24.31 - 26.58	sm-st
9	4.26 ± 0.02	4.22 - 4.30	29.89 ± 0.58	28.75 - 31.03	sm
10	4.18 ± 0.04	4.10 - 4.26	25.69 ± 0.51	24.69 - 26.69	sm-st
11	4.15 ± 0.03	4.09 - 4.21	23.65 ± 0.08	23.49 - 23.81	st
12	4.11 ± 0.03	4.05 - 4.17	23.09 ± 0.46	22.19 - 23.99	st
13	4.08 ± 0.03	4.02 - 4.14	25.28 ± 0.09	25.10 - 25.45	sm-st
14	4.05 ± 0.04	3.97 - 4.13	20.99 ± 0.15	20.69 - 21.28	st
15	3.99 ± 0.03	3.93 - 4.05	22.86 ± 0.49	21.90 - 23.82	st
16	3.92 ± 0.04	3.84 - 4.00	23.62 ± 0.32	22.99 - 24.25	st
17	3.87 ± 0.03	3.81 - 3.93	32.69 ± 0.34	32.00 - 33.35	sm
18	3.78 ± 0.04	3.70 - 3.86	24.29 ± 0.58	23.15 - 25.42	st

No.	L^R ± S.E.	95% C.L.	I^C ± S.E.	95% C.L.	Classification after Levan et al. (1964)
19	3.74 ± 0.04	3.66 - 3.82	24.92 ± 0.06	24.80 - 25.40	st
20	3.63 ± 0.04	3.55 - 3.71	23.95 ± 0.29	23.38 - 24.52	st
21	3.55 ± 0.05	3.45 - 3.65	22.58 ± 0.24	22.11 - 23.05	st
22	3.37 ± 0.08	3.21 - 3.53	20.16 ± 0.28	19.61 - 20.71	st

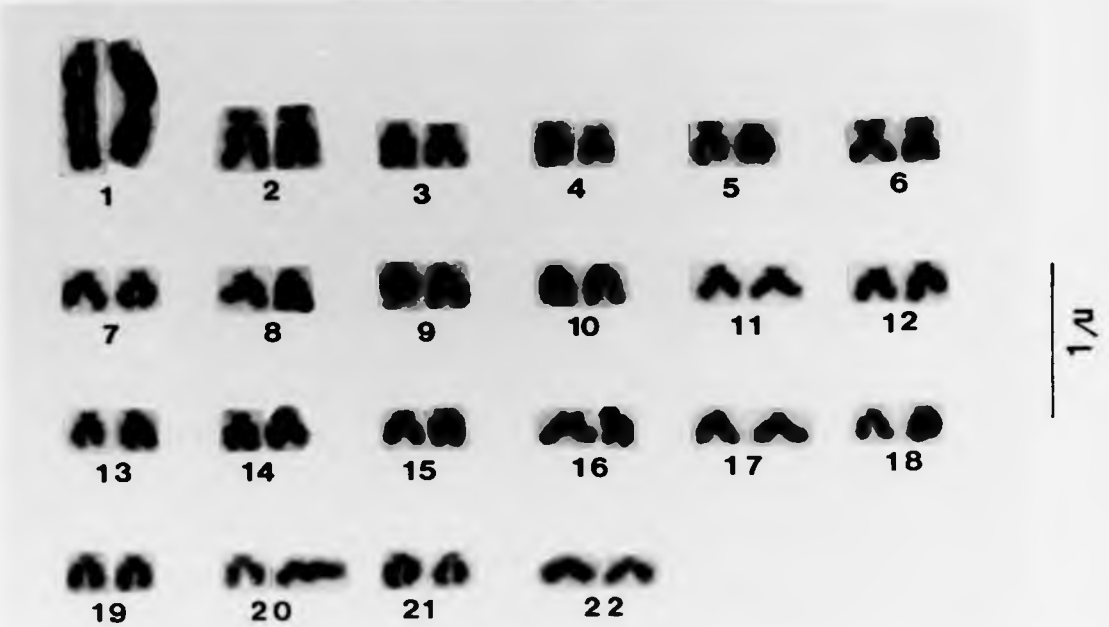

FIG. 3 Representative karyotype of O. niloticus consisting of a single m(No. 6), 9 sm(Nos. 3-5,7,8,11,12,14 and 15), 5 sm-st(Nos. 10,13,16,18 and 19) and 7 st chromosome pairs.

FIG. 4 Representative karyotype of O. aureus consisting of 7 sm(Nos. 3,6,7,9,12,14 and 18), 8 sm-st(Nos. 4,5,8, 10,11,13,17 and 19) and 7 st chromosome pairs.

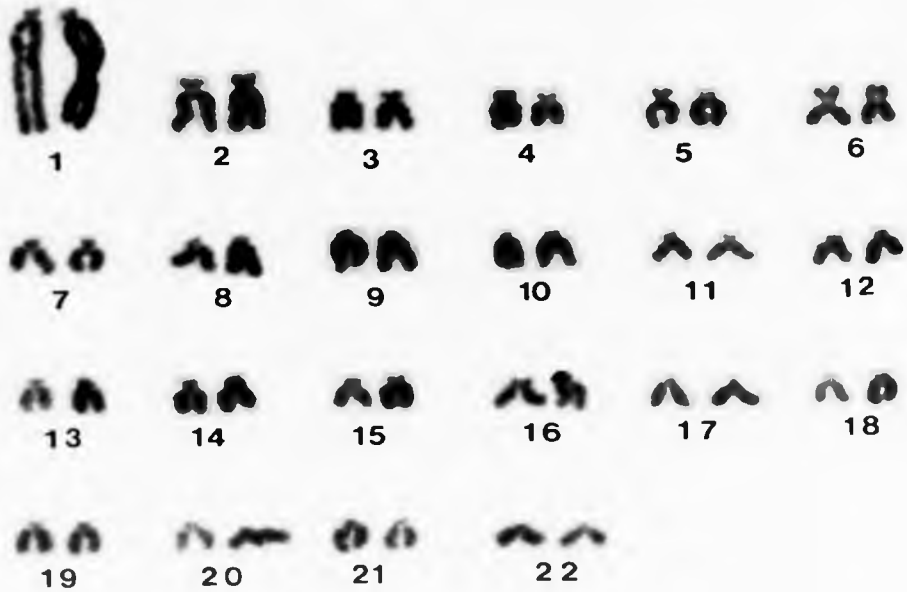

FIG. 3 Representative karyotype of O. niloticus consisting of a single m(No. 6), 9 sm(Nos. 3-5,7,8,11,12,14 and 15), 5 sm-st(Nos. 10,13,16,18 and 19) and 7 st chromosome pairs.

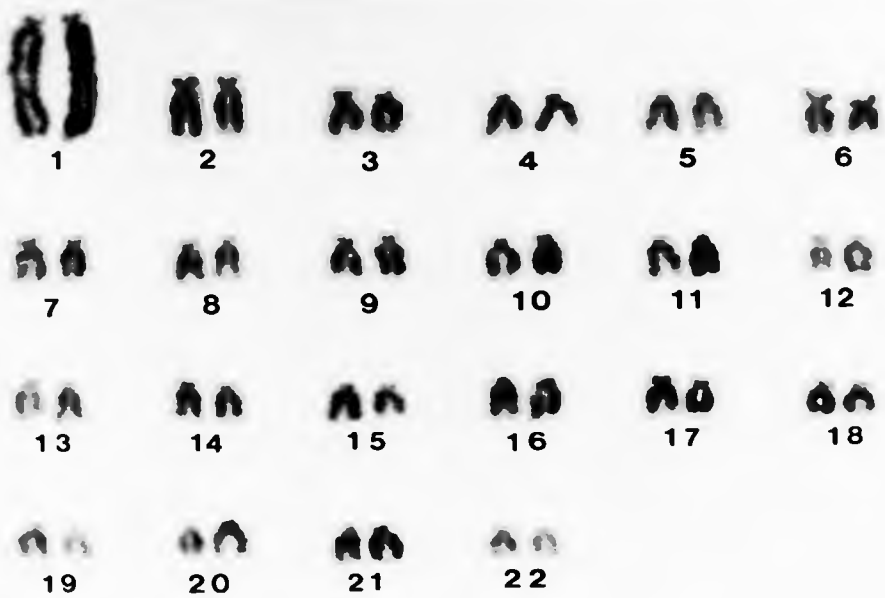

FIG. 4 Representative karyotype of O. aureus consisting of 7 sm(Nos. 3,6,7,9,12,14 and 18), 8 sm-st(Nos. 4,5,8, 10,11,13,17 and 19) and 7 st chromosome pairs.

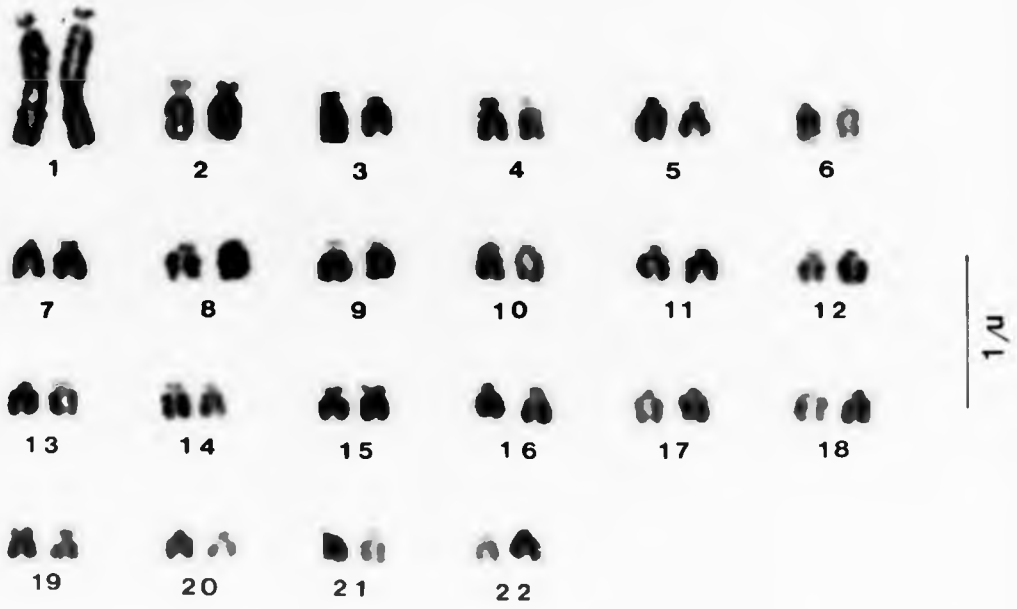

FIG. 5 Representative karyotype of O. macrochir consisting of 5 sm(Nos. 2,4,11,15 and 19), 9 sm-st(Nos. 5-9,13,14, 16 and 20) and 8 st chromosome pairs.

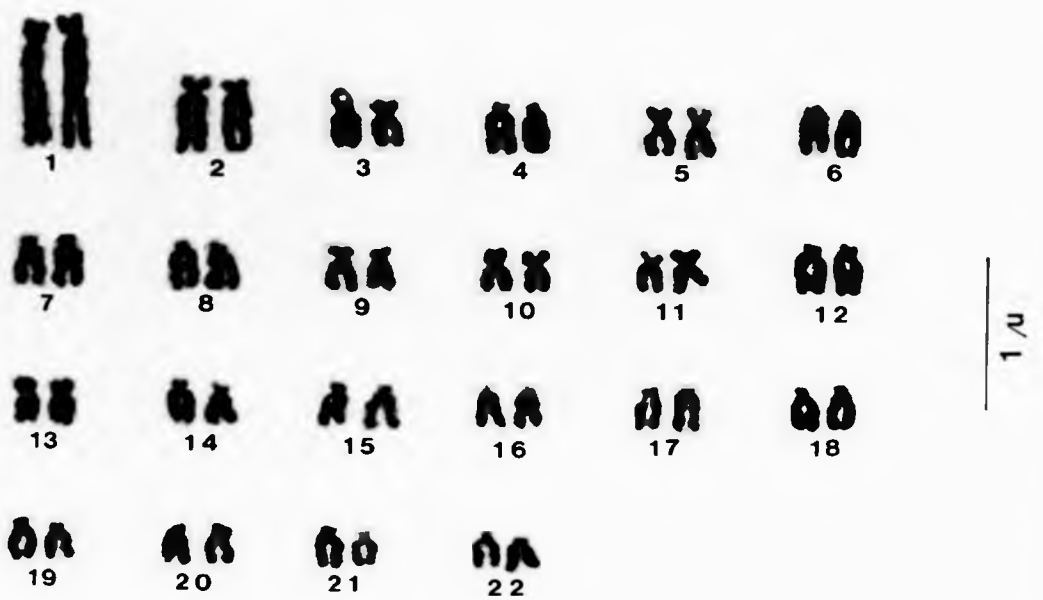

FIG. 6 Representative karyotype of T. zillii consisting of 2 m(Nos. 5 and 10), 9 sm(Nos. 2,3,7,9,11,13-15 and 18), 4 sm-st(Nos. 4,6,17 and 19) and 7 st chromosome pairs.

FIG. 5 Representative karyotype of O. macrochir consisting of 5 sm(Nos. 2,4,11,15 and 19), 9 sm-st(Nos. 5-9,13,14, 16 and 20) and 8 st chromosome pairs.

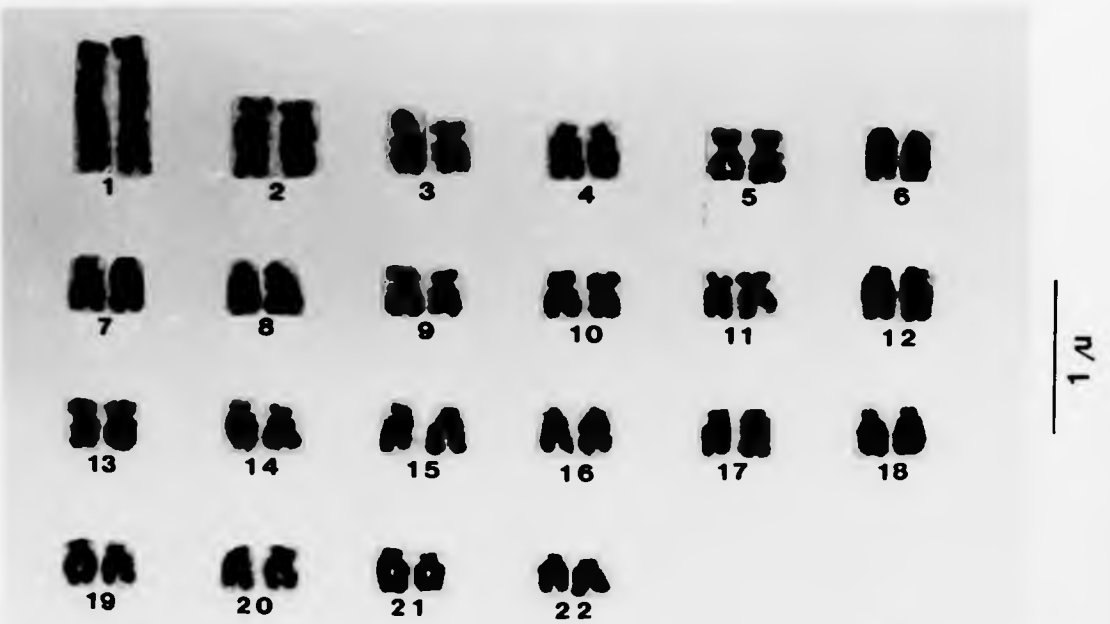

FIG. 6 Representative karyotype of T. zillii consisting of 2 m(Nos. 5 and 10), 9 sm(Nos. 2,3,7,9,11,13-15 and 18), 4 sm-st(Nos. 4,6,17 and 19) and 7 st chromosome pairs.

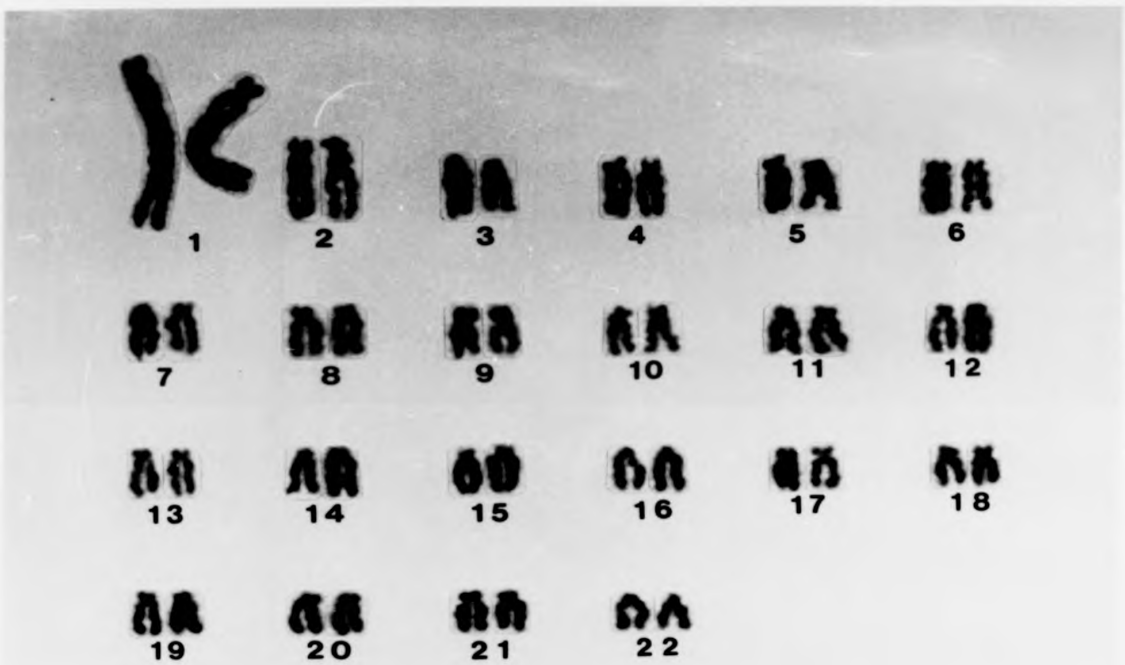

FIG. 7 Representative karyotype of S. galilaeus consisting of 2 sm (Nos. 9 and 17), 4 sm-st (Nos. 3,8,10 and 13) and 16 st chromosome pairs.

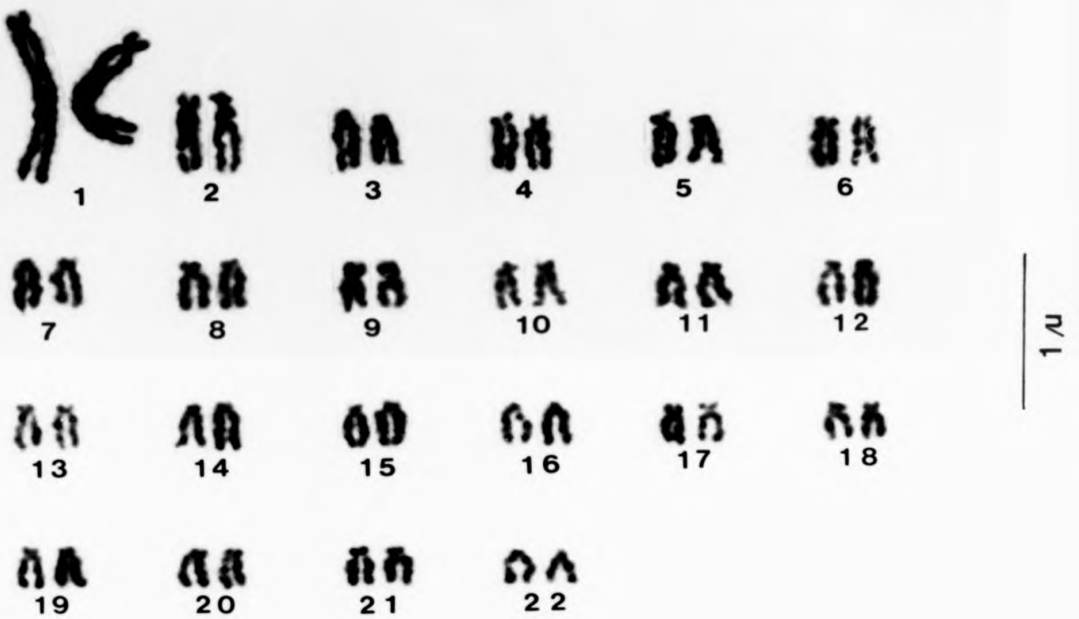

FIG. 7 Representative karyotype of S. galilaeus consisting of 2 sm (Nos. 9 and 17), 4 sm-st (Nos. 3,8,10 and 13) and 16 st chromosome pairs.

TABLE 12. Karyotype Formula and NF in Different Tilapias

Species	Karyotype	NF
O. mossambicus	3 sm + 4 sm-st + 15 st	50
O. spilurus	3 sm + 9 sm-st + 10 st	50
O. niloticus	1 m + 9 sm + 5 sm-st + 7 st	64
O. aureus	7 sm + 8 sm-st + 7 st	58
O. macrochir	5 sm + 9 sm-st + 8 st	54
T. zillii	2 m + 9 sm + 4 sm-st + 7st	66
S. galilaeus	2 sm + 4 sm-st + 16 st	48

Comparative Karyometry

Comparative karyological data in the form of an idiogram is presented in Fig. 8 for all the chromosomes and species. From the figure it is clear that there exists a remarkable constancy of the chromosome sizes of the "corresponding" chromosome in all the seven species. The percentage lengths of the first chromosome appear to vary a little more than the chromosomes Nos. 2-22, all of which have very uniform sizes. Fig. 8 and Tables 5-11 indicate that T. zillii has the lowest length (10.84) whereas the O. macrochir has the highest length (12.65) for chromosome No. 1. Comparison of the 95% confidence limit of the L^R shows that most of the measurements overlap each other. It is also evident from Tables 5-11 that the standard error for the mean length of this chromosome is somewhat higher than that of other chromosomes for every species. Although there are differences in the length, this chromosome can be classified as an st chromosome in all species according to the formulae of Levan et al. (1964).

Inversions

As has already been pointed out the chromosomes show little variation in length between species but the number of m, sm and st chromosomes as well as NF are different for different species (Table 12). This suggests that chromosomal inversion may well have been the cause of these differences between species. The number of inversions involving centromeric shifting is given in Table 13 which is prepared on the basis of a two-way comparison. From the Table it is evident that the maximum number of inversional differences are found between O. niloticus and S. galilaeus (10) whereas the minimum number is found between O. mossambicus and

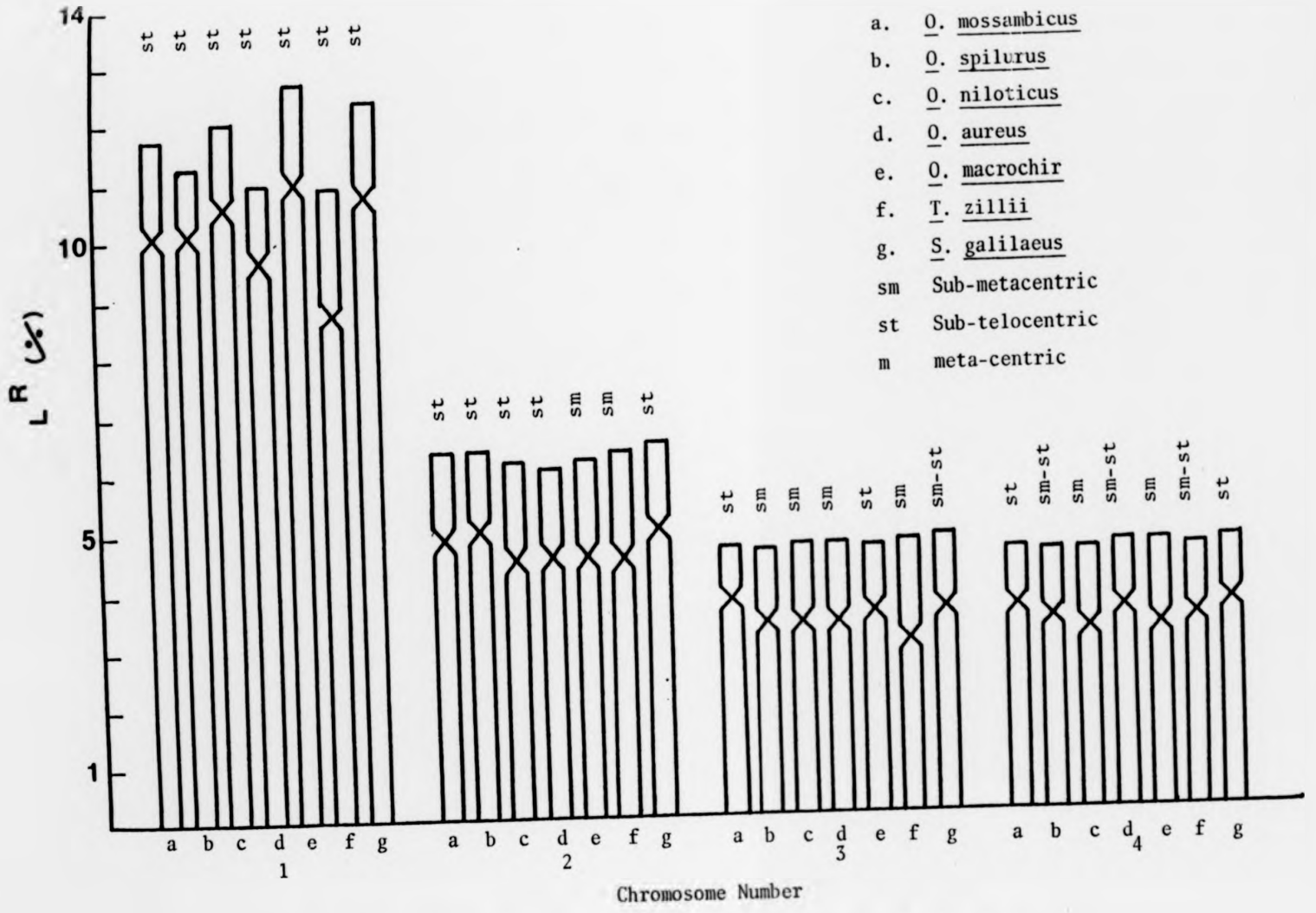

FIG. 8. Comparative idiogram of seven different species of tilapia

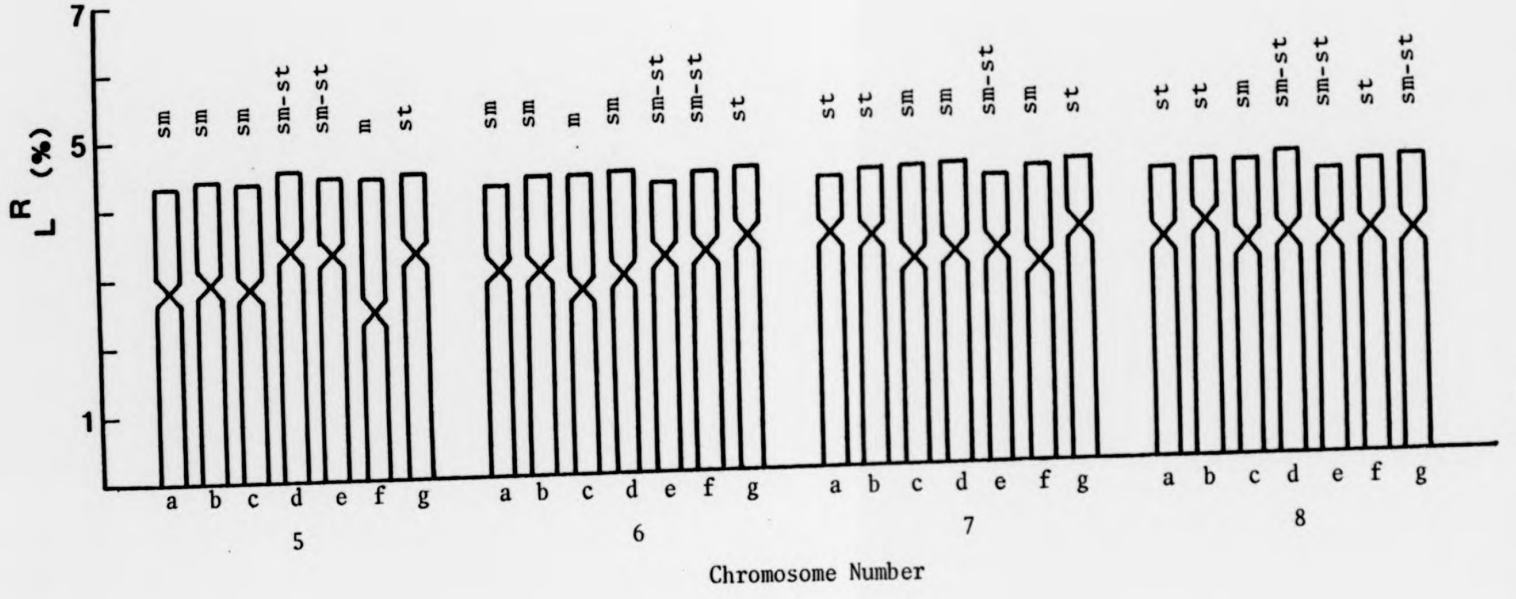

FIG. 8. Comparative idiogram of seven different species of tilapia

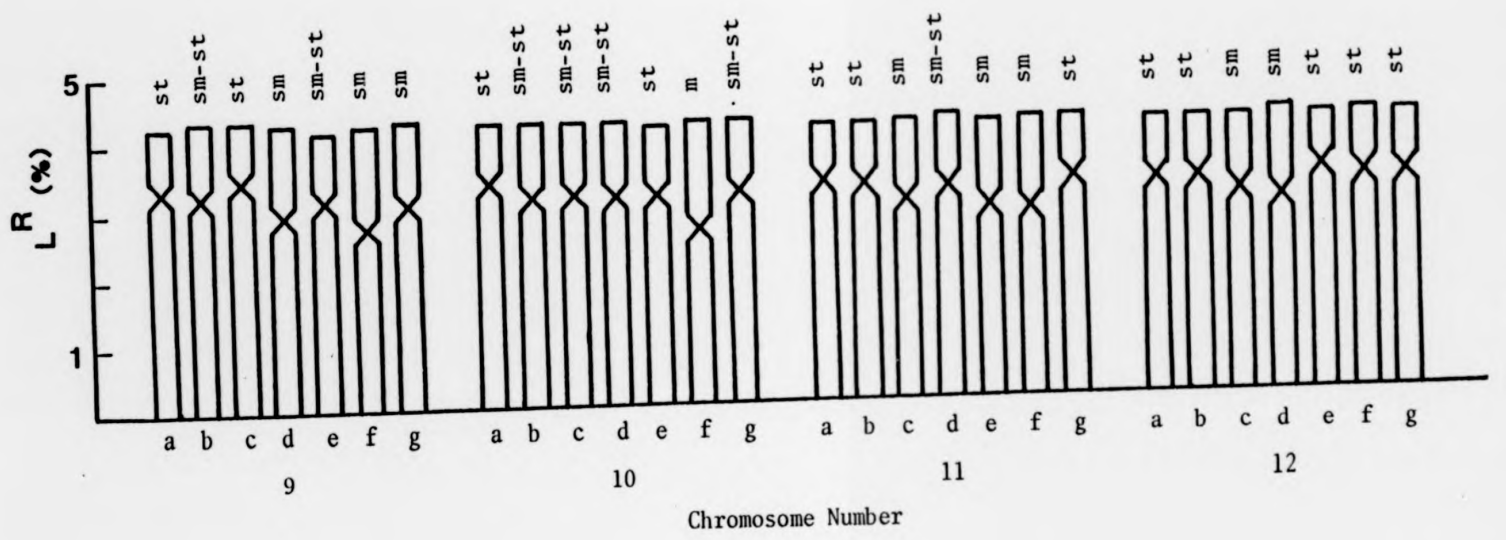

FIG. 8. Comparative idiogram of seven different species of tilapia

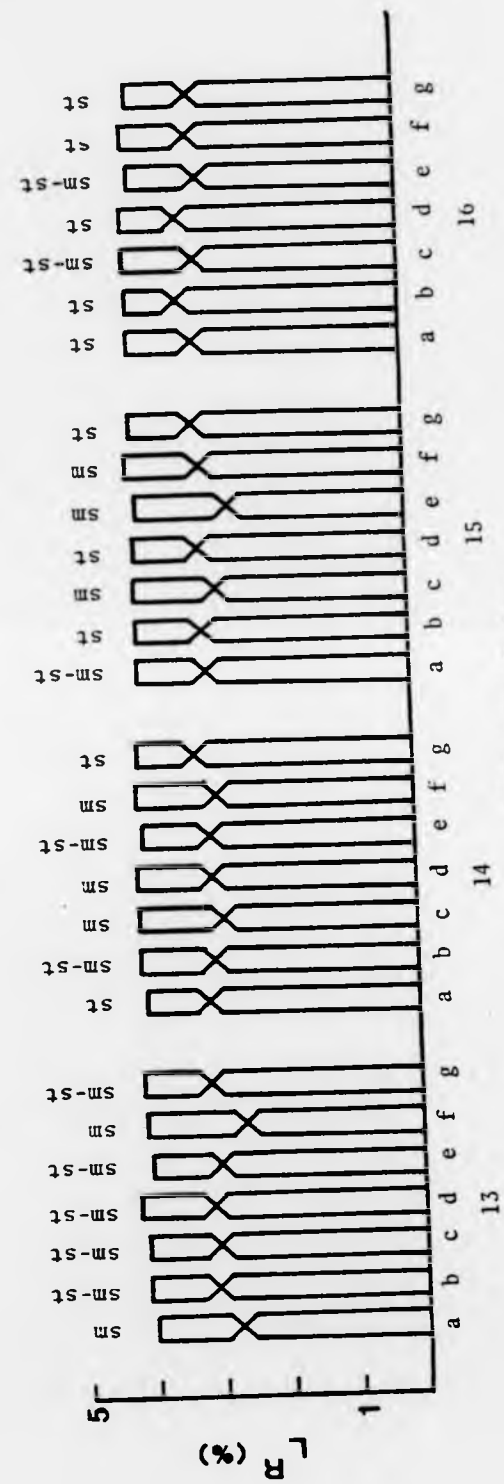

FIG. 8. Comparative idiogram of seven different species of tilapia

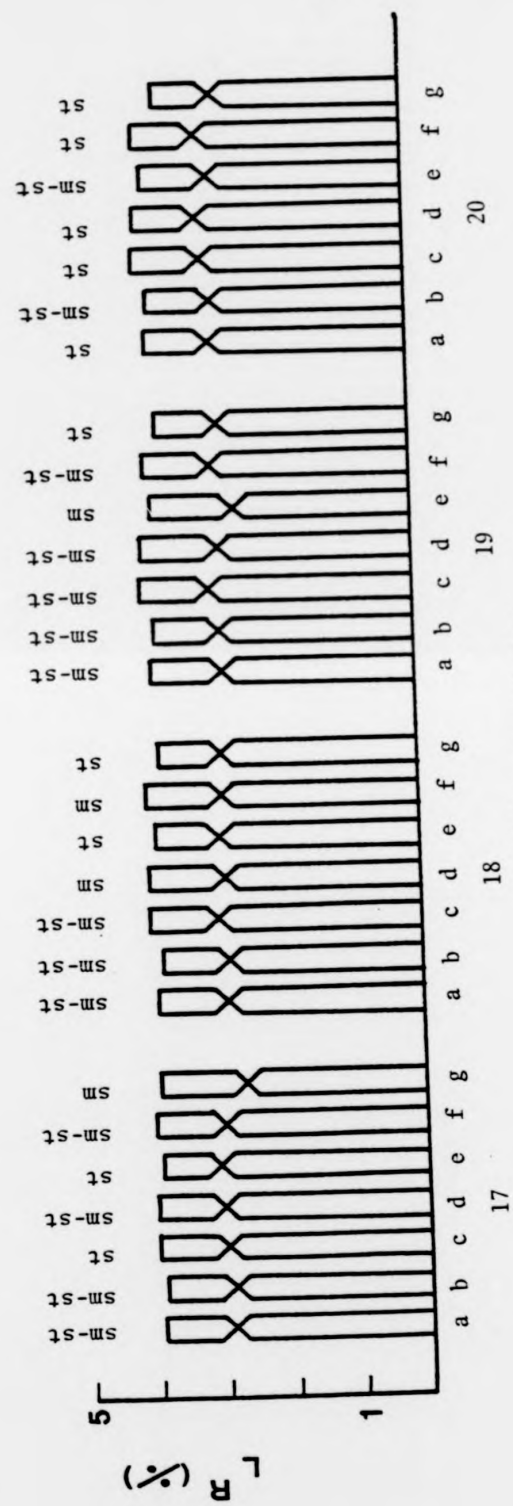

FIG. 8. Comparative idiogram of seven different species of tilapia

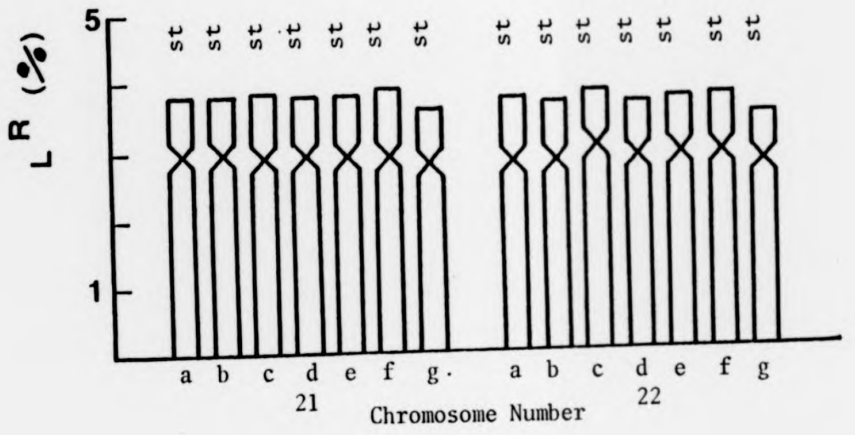

FIG. 8. Comparative idiogram of seven different species of tilapia.

TABLE 13. Total Number of Inversional Differences between Species

	O. mossambicus	O. spilurus	O. niloticus	O. aureus	O. macrochir	T. zillii	S. galilaeus
O. mossambicus		1	8	6	3	8	3
O. spilurus			6	2	4	6	2
O. niloticus				3	4	7	10
O. aureus					5	5	5
O. macrochir						4	6
T. zillii							9
S. galilaeus							

O. spilurus (1). In general the comparison of the T. zillii karyotype to that of other species shows the more inversions. The chromosomes involved in such inversional differences are given in Table 14. In the various species it is found that 16 chromosomes (Nos. 2-12, 14, 15, 17-19) out of 22 are involved in inversion in one or other of the species. The remaining chromosomes (Nos. 1, 13, 16, 20, 21 and 22) are the same in all the species.

C-banding

C-banded metaphase plates of each species are given in Figs. 9-15. Centromeric heterochromatin is observed in every species in and around the centromere of all chromosomes except T. zillii where about 10 to 12 chromosomes are found without any C-heterochromatin. In T. zillii it has also been noticed that 6 (m-sm) chromosomes (arrowed) have their short arm completely C-positive (Fig. 14). In all the other species it is also noticed that one pair of short sm chromosomes show an additional intercallary heterochromatin in their long arm near the centromere (arrowed, Figs.9-13 and 15), whereas in T. zillii it is absent. Another interesting point is that in the case of O. mossambicus, the telomeric heterochromatin is found in one pair of st chromosomes (crossed, Fig. 9). It has also been noticed that in the first chromosome pair (longest) of O. niloticus and O. spilurus the staining was somewhat darker in the distal end of their long arm after C-banding. But such staining seems to be an artefact as the intensity of staining increases with staining time.

Meiotic Chromosomes

Meiotic chromosomes prepared from testes cells of different species are

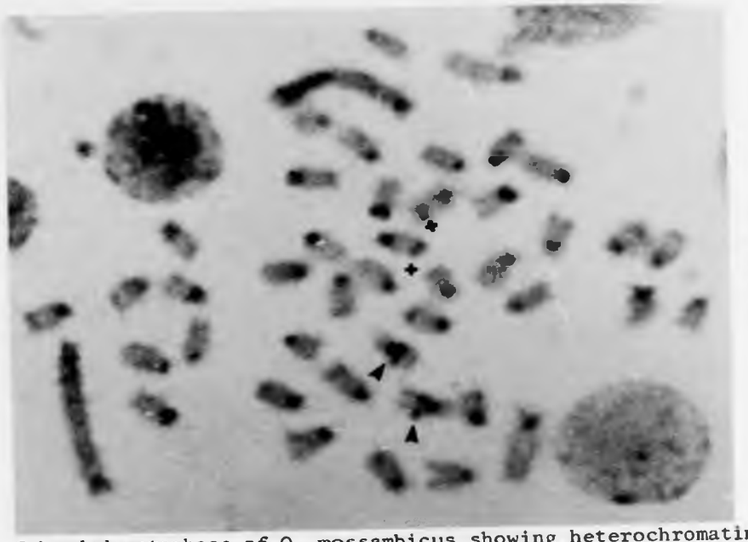

FIG. 9 C-banded metaphase of O. mossambicus showing heterochromatin localization around the centromere. Two small sm chromosomes have an additional intercalary band (arrow head) and two small st chromosomes have telomeric heterochromatin (X).

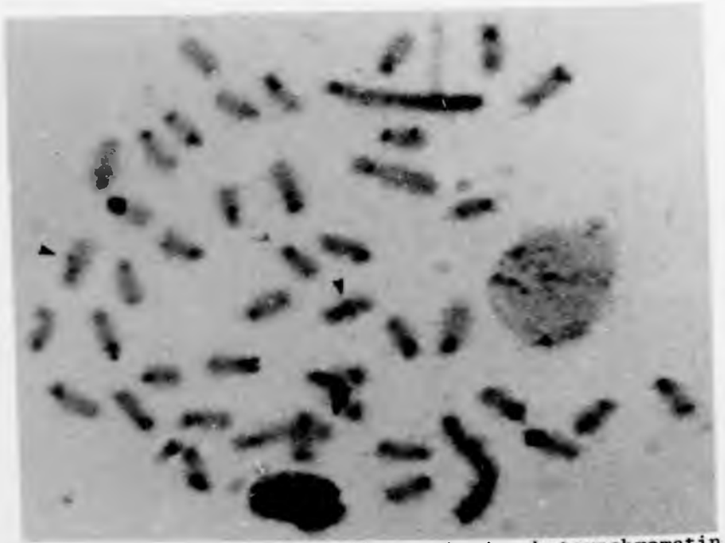

FIG. 10 C-banded metaphase of O. spilurus showing heterochromatin localization around the centromere. Two small sm chromosomes have an additional intercalary band in their long arm (arrow head).

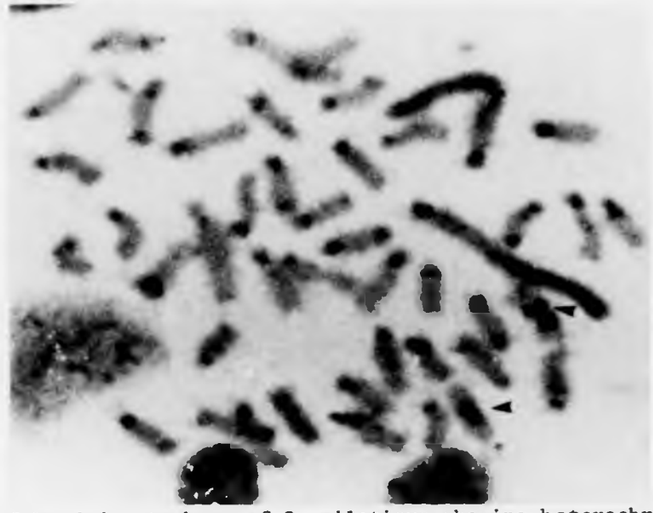

FIG. 11 C-banded metaphase of O. niloticus showing heterochromatin localization around the centromere. Two small sm chromostomes have an additional intercalary band in their long arm (arrow head).

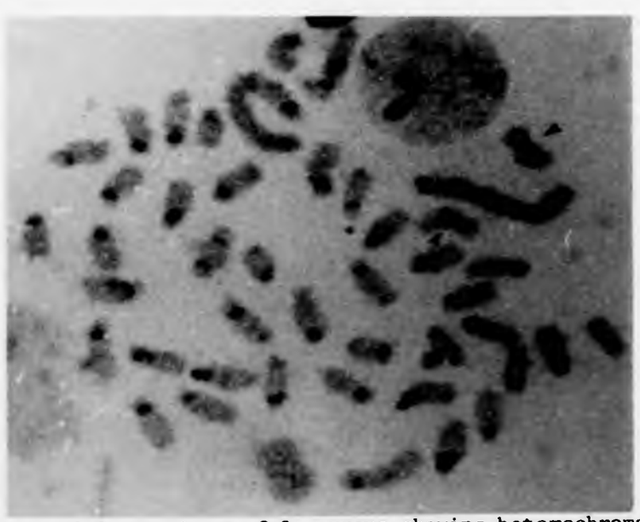

FIG. 12 C-banded metaphase of O. aureus showing heterochromatin localization around the centromere. Two small sm chromosomes have an additional intercalary band in their long arm (arrow head).

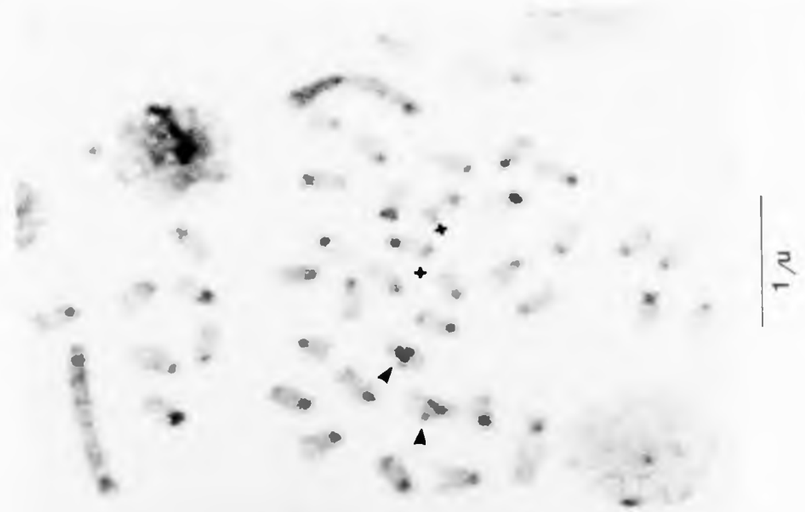

FIG. 9 C-banded metaphase of O. mossambicus showing heterochromatin localization around the centromere. Two small sm chromosomes have an additional intercalary band (arrow head) and two small st chromosomes have telomeric heterochromatin (X).

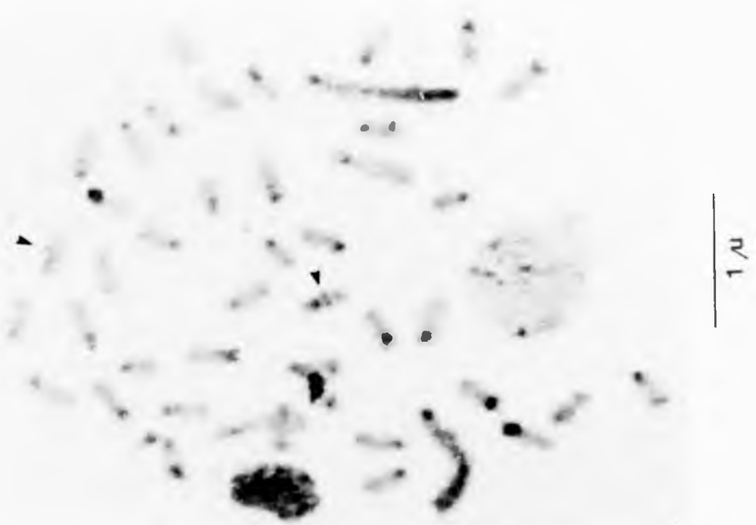

FIG. 10 C-banded metaphase of O. spilurus showing heterochromatin localization around the centromere. Two small sm chromosomes have an additional intercalary band in their long arm (arrow head).

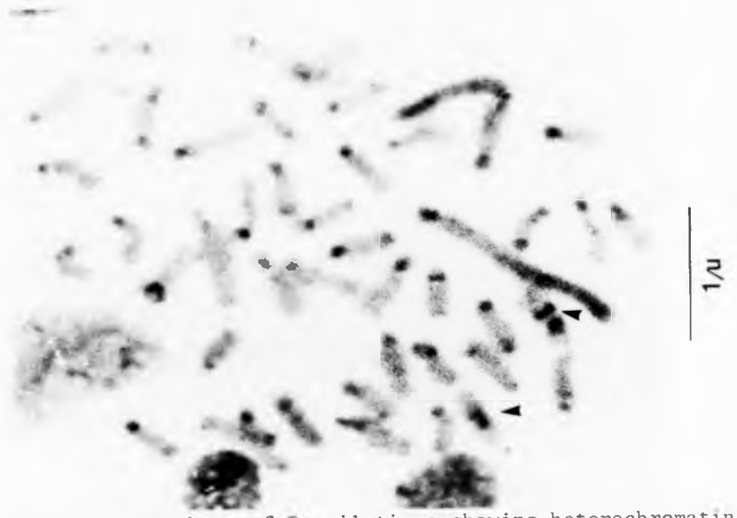

FIG. 11 C-banded metaphase of O. niloticus showing heterochromatin localization around the centromere. Two small sm chromostomes have an additional intercalary band in their long arm (arrow head).

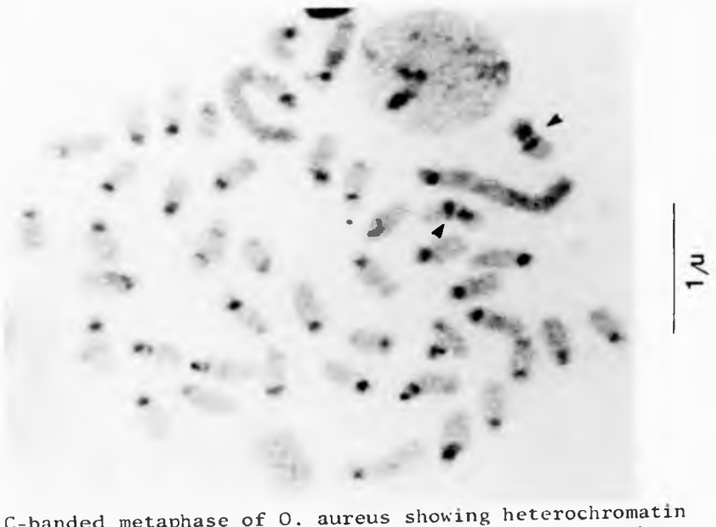

FIG. 12 C-banded metaphase of O. aureus showing heterochromatin localization around the centromere. Two small sm chromosomes have an additional intercalary band in their long arm (arrow head).

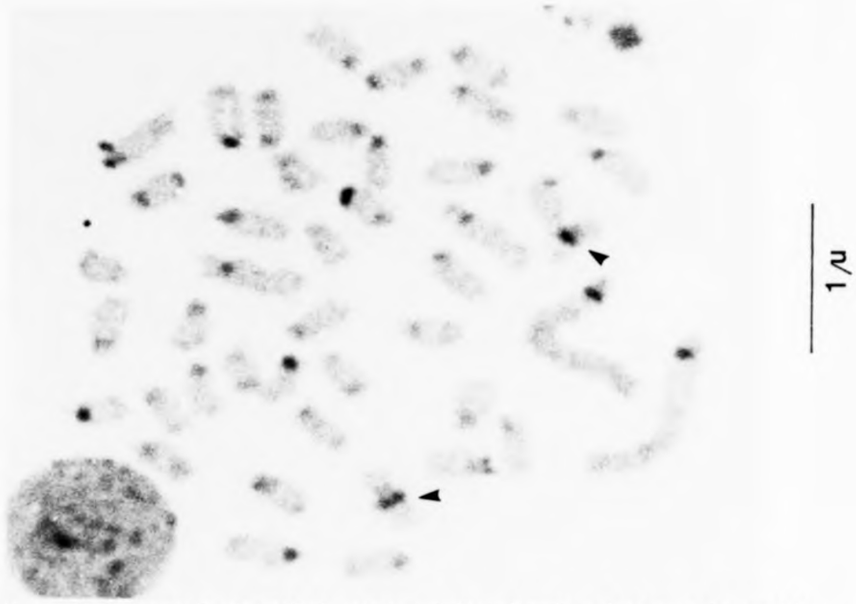

FIG. 13 C-banded metaphase of O. macrochir showing heterochromatin localization around the centromere. Two small sm chromosomes have an additional intercalary band in their long arm (arrow head).

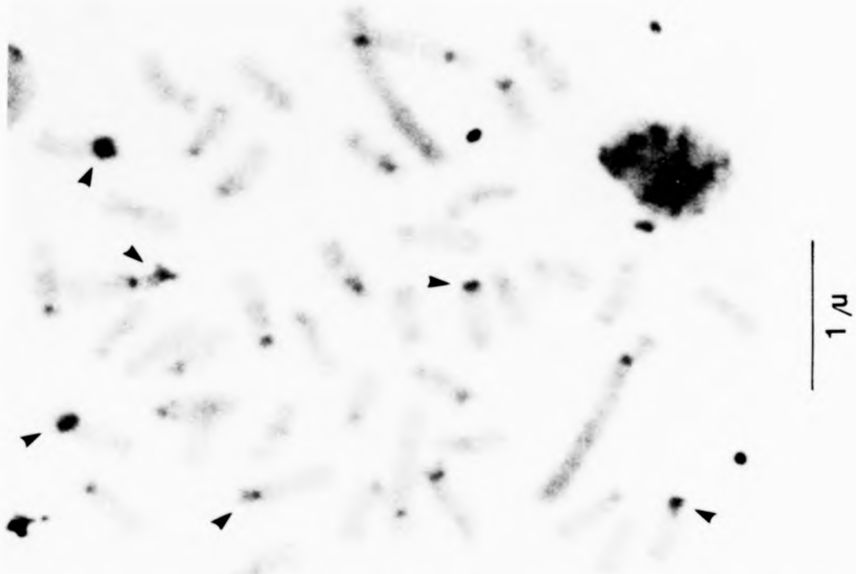

FIG. 14 C-banded metaphase of T. zillii. 6 chromosomes (arrow head) show complete C-positive short arms. Note 10 chromosomes do not have any heterochromatin.

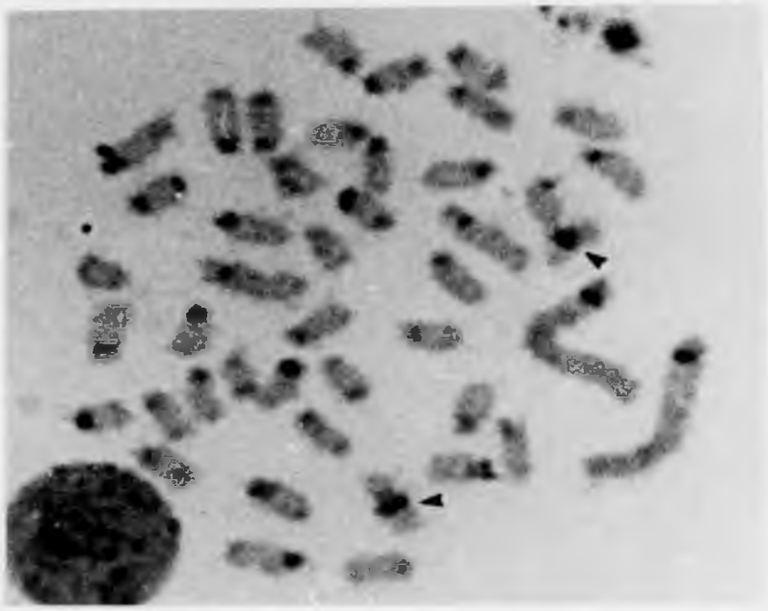

FIG. 13 C-banded metaphase of O. macrochir showing heterochromatin localization around the centromere. Two small sm chromosomes have an additional intercalary band in their long arm (arrow head).

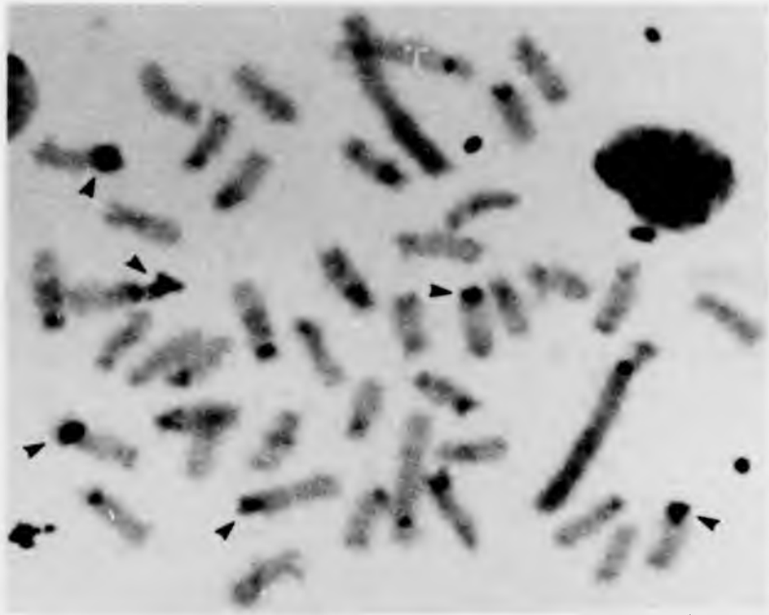

FIG. 14 C-banded metaphase of T. zillii. 6 chromosomes (arrow head) show complete C-positive short arms. Note 10 chromosomes do not have any heterochromatin.

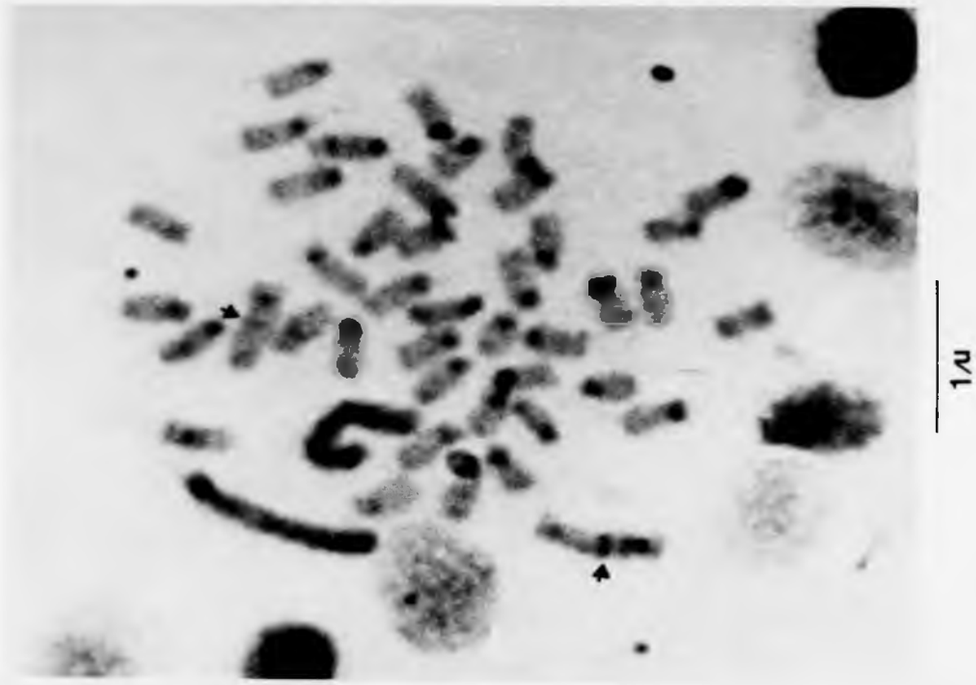

FIG. 15 C-banded metaphase of S. galilaeus showing heterochromatin localization around the centromere. Two sm chromosomes have an additional intercalary band in their long arm (arrow head).

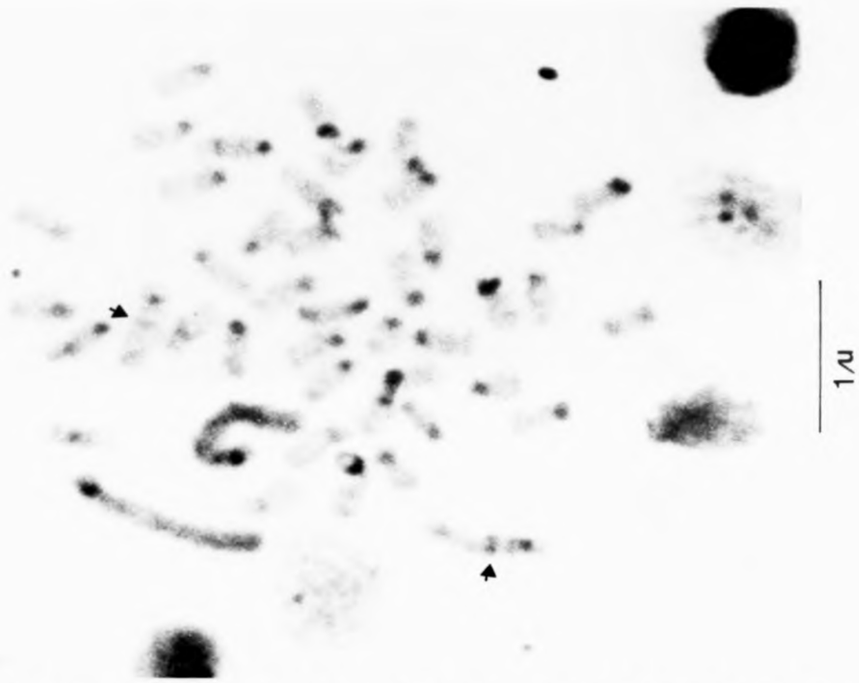

FIG. 15 C-banded metaphase of S. galilaeus showing heterochromatin localization around the centromere. Two sm chromosomes have an additional intercalary band in their long arm (arrow head).

TABLE 14. Inversional Differences Between Pairs of Tilapia sp and Chromosomes Involved

Species Compared	No. of inversions in which they vary	Chromosomes Involved
O. mossambicus and O. spilurus	1	3
O. mossambicus and O. niloticus	8	3,4,6,7,8,11,12 and 14
O. mossambicus and O. aureus	6	3,7,9,12,14 and 18
O. mossambicus and O. macrochir	3	2,4 and 11
O. mossambicus and T. zillii	8	2,3,5,7,9,10,11 and 14
O. mossambicus and S. galilaeus	3	5,6 and 9
O. spilurus and O. niloticus	6	6,7,8,11,12 and 15
O. spilurus and O. aureus	2	7 and 12
O. spilurus and O. macrochir	4	2,3,11 and 15
O. spilurus and T. zillii	6	2,5,7,10,11 and 15
O. spilurus and S. galilaeus	2	5 and 6
O. niloticus and O. aureus	3	6,9 and 15
O. niloticus and O. macrochir	4	2,3,6 and 12
O. niloticus and T. zillii	7	2,5,6,8,9,10 and 12
O. niloticus and S. galilaeus	10	4,5,6,7,9,11,12,14,15 and 17
O. aureus and O. macrochir	5	2,3,12,15 and 18
O. aureus and T. zillii	5	2,5,10,12 and 15
O. aureus and S. galilaeus	5	6,7,12,14 and 18
O. macrochir and T. zillii	4	3,5,10 and 18
O. macrochir and S. galilaeus	6	2,4,11,15,17 and 19
T. zillii and S. galilaeus	9	2,5,7,10,11,14,15,17 and 18
TOTAL	107	2,3,4,5,6,7,8,9,10,11,12 14,15,17,18 and 19 = 16 chromosomes

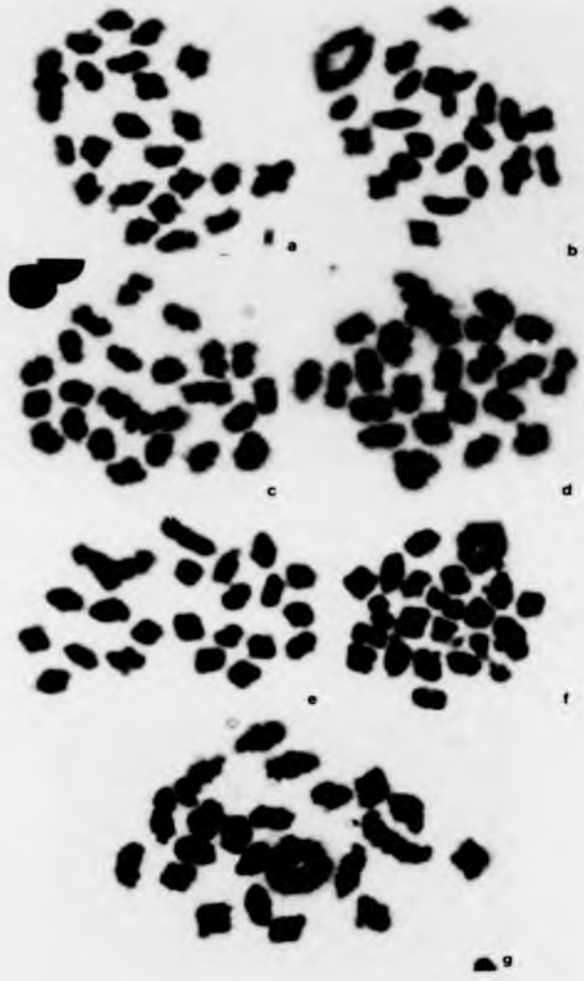

FIG. 16 Meiotic metaphase I of tilapias showing 22 bivalents. a = O. mossambicus, b = O. spilurus, c = O. niloticus, d = O. aureus, e = O. macrochir, f = T. zillii, g = S. galilaeus.

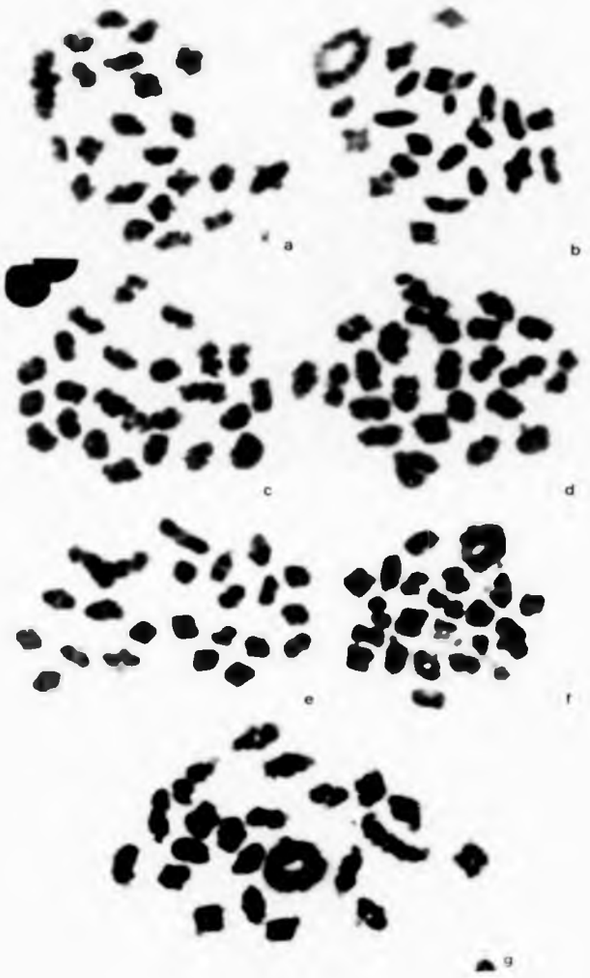

FIG. 16 Meiotic metaphase I of tilapias showing 22 bivalents. a = O. mossambicus, b = O. spilurus, c = O. niloticus, d = O. aureus, e = O. macrochir, f = T. zillii, g = S. galilaeus.

DISCUSSION

Since the original presentation of the DNA values in different animal species by Mirsky and Ris (1951), C-values (Swift, 1950) for about 350 animal species (see Bachmann *et al.*, 1974) have been estimated. From all those results a few generalised conclusions could be drawn:

1) Nuclear DNA amounts vary without any obvious trend in vertebrates. 2) In some of the bony fish families the amount of DNA is fairly constant. 3) More advanced vertebrates have lower DNA values than the more primitive species (Atkin *et al.*, 1965; Hinegardner, 1968; Hinegardner and Rosen, 1972; Ohno, 1974; Szarski, 1974; Hinegardner, 1976). Regarding such generalised conclusions Bachmann *et al.* (1974) wrote that

> "for most vertebrate groups not enough species have been examined to allow a statistical evaluation of the uniformity or variability of genome size."

Extensive studies on teleosts show that they have DNA amounts ranging between 0.6 - 8.8pg in their haploid genome (Hinegardner, 1968; Hinegardner and Rosen, 1972; Ohno, 1974). Ohno also mentioned that DNA value variation in the distantly related species is possible but in teleosts sometimes closely related species also show some variation (Wolf *et al.*, 1969). DNA values obtained for the tilapias in the present study are well within the expected range for teleostean fishes. Only a few reports deal specifically with C-values for tilapias. Hinegardner and Rosen (1972) reported that *O. niloticus*, *O. leucosticta* and *T. zillii* all have 1.2pg of DNA. On the other hand Kornfield *et al.* (1979) reported that *O. aureus*, *S. galilaeus* and *T. zillii* have 1.27, 1.08 and 1.18pg of DNA respectively in their haploid genome. From their calculations they postulated that DNA in *O. aureus* is about 15% higher than in *S. galilaeus*.

The DNA values reported here (Table 2) clearly indicate that in every species the amount is lower than the previous reports. The DNA values of O. spilurus and O. macrochir have been reported for the first time. The six species used for DNA value determination can be placed into three groups. O. aureus has a higher DNA amount, similar to that reported by Kornfield et al. (1979), whereas O. macrochir and S. galilaeus have the lowest values and O. mossambicus, O. spilurus and O. niloticus have the intermediate amounts. Statistically these differences are significant. It has been reported by Sherwood and Patton (1982) that the procedure used for the determination of C-values by using conventional Feulgen staining is probably not sensitive enough, hence the conflicting results. As for the present experiments, the DNA values were determined using the same technique for all the species. So the difference found may not be due to some technical shortcomings. It has also been reported by Sherwood and Patton (1982) that in a species the amount of DNA varies considerably and this may have phenotypic and evolutionary significance (Robertson, 1981).

Regarding the DNA value and nuclear area, no correlation has been found, as the nuclear area in all these species is the same. Similar results have been reported by Kornfield et al. (1979). On the other hand Szarski (1974) in a review has mentioned that there exists a positive correlation between nuclear DNA and nuclear area. The lack of any correlation between DNA amount and nuclear area in tilapias, indicates that possibly the increase or decrease of such a small amount of DNA in the nuclei does not reflect on their area. It also indicates that in tilapia the duplication of the whole genome leading to polyploidization of the nuclear DNA as a whole plays no significant role in the variation of the DNA amount.

Chromosome numbers in all the seven species studied are the same (2n = 44 and n = 22). Conflicting reports have been found in the literature on the chromosome number of these species (Table 1). Jalabert et al., (1971) and Nijjhar et al. (1983) reported 2n = 44 in O. niloticus from Nile Volta Basin and Ghana resp., whereas Badr and El-Dib (1977) reported 2n = 40 in the same species from Egypt. Our sample also comes from Egypt where we found 2n = 44 for the same species. A similar type of contradiction has been reported for T. zillii where Badr and El-Dib (1977) reported 2n = 38, whereas Kornfield et al. (1979) reported 2n = 44 for the same species. The differences in the chromosome number might be due to:

1) technical shortcomings. 2) misidentification of the species, and 3) the presence of a chromosome number polymorphism.

It seems from the photograph provided by Badr and El-Dib (1977) that the possibility of technical shortcoming is the most likely explanation. The chromosome number reported by other workers (Table 1) agrees well with the results of the present study. Identification of tilapia species is known to have problems but McAndrew and Majumdar (1983) using electrophoretic markers have resolved that problem, at least for the 9 species mentioned in their paper. The other possibility, a chromosome polymorphism cannot be ruled out as it has been found in other fish species (e.g. Cyprinus carpio, 2n = 104 by Makino, 1939; Ohno et al., 1967; 2n = 100 by Ojima and Hitotsumachi, 1967; Raicu et al., 1972; Ojima and Takai, 1981; Blaxhall, 1983c; 2n = 98 by Szollar and Hobor, 1972). All three genera within the tilapiines have some species which differ from the general 2n = 44. In the Oreochromis all the species studied have 2n = 44 except O. grahami where 2n = 48, n = 24 reported by Post (1965),

Denton (1973) and Park (1974) (see Table 1). In the case of Sarotherodon, Jakaowska (1950) reported that n = 16 in S. melanotheron and other workers reported 2n = 44 for S. galilaeus (Badr and El-Dib, 1977; Kornfield et al., 1979; present study). Thompson (1981) on the other hand reported that in the Tilapia, T. mariae had a 2n = 40 and T. sparrmanii 2n = 42. So it is not possible to even generalise that within a genera the chromosome number remains constant.

In the description of any karyotype, chromosome classification plays a major role. Levan et al. (1964) mentioned conflicting results on the same species because of nomenclature difficulties (White, 1973). They also proposed a system where they have tried to formulate some generalization of the nomenclature on the basis of the centromeric position of the chromosome. In the literature most of the authors followed the classification of Levan et al. (1964) to describe tilapia karyotypes.

Comparison of the results of the present investigation and published results (Table 1) on different species raises some interesting points. In O. mossambicus the karyotype described by Natarajan and Subrahmanyam (1968) consists of 22 metacentrics. From the photograph presented in their paper it seems that the 'V' shaped chromosomes have been considered as metacentrics. Unfortunately in the C-metaphase plate the single armed chromosomes (st, t and T of Levan et al., 1964) look like a 'V' as the centromere cannot split (White, 1973). On the other hand karyotypic data reported by several authors (Prasad and Manna, 1976; Krishnaja and Rege, 1980; Thompson, 1981) on O. mossambicus indicate that it consists of mostly st chromosomes. Thompson (1981) also reported the occasional occurrence of 3 pairs of sm chromosomes, because of which the NF varies between 44-50, whereas in the present experiments three pairs of sm

chromosomes have been found consistently in the karyotype of the same species. So it seems that the identification of metacentrics instead of acrocentrics by Natarajan and Subrahmanyam (1968) leads to the conflicting results (Prasad and Manna, 1976). It is also evident that the chromosomes of this species do not show much variation in different localities as studied by several authors, indicating the possibility of the non polymorphic nature of the karyotype.

In O. aureus Kornfield et al. (1979) reported 5 pairs of sm and 17 pairs of st chromosomes with a total of NF = 54, whereas Thompson (1981) mentioned 3 pairs of sm(?) and the remaining 19 pairs of st chromosomes. Here too he (loc. cit.) has calculated NF = 44-50. In contrast the results of the present investigation on the same species indicate that the karyotype consists of 7 pairs of sm and 15 pairs of st (including sm-st borderline cases) chromosomes with a total of NF = 58.

Similarly in T. zillii in the present study the karyotype consists of 9 pairs of sm, 2 pairs of m and the remaining 11 pairs of st chromosomes leading to an NF of 66, whereas Kornfield et al. (1979) reported only 5 pairs of sm and no m chromosomes.

On the other hand in S. galilaeus the NF is 48 and the karyotype consists of only 2 pairs of sm chromosomes in the present study. Kornfield et al. (1979) again reported NF = 54 for this species with 5 pairs of sm and 17 pairs of st chromosomes.

Regarding the chromosomes of O. niloticus, O. spilurus and O. macrochir, no comparable reports can be found in the literature and the latter two species have been reported karyotypically for the first time. The

karyotype of the former species reported by Badr and El-Dib (1977) appears controversial, even regarding its 2n number which again may be due to some polymorphism in the chromosome number although the different stocks of O. niloticus used by us and by the above mentioned authors are from the same place.

Classification of chromosomes according to centromeric position (Levan et al., 1964) should be used cautiously as some of the measurements may lie very much at the border between the sm and st or sm and m, etc. groups. In the present investigation therefore, the borderline cases between sm and st are included in the st group which in turn reduces the NF number (According to Levan et al., 1964, adopted from Matthey, 1945, the M, m and sm chromosomes are biarmed whereas st, t and T are single armed). To justify such groupings, calculations of the 95% confidence limit on the mean centromeric index of each chromosome have been made. The results show a wide range and overlapping in the case of sm-st borderline cases. This clearly indicates that localization of the centromere is rather difficult to pinpoint accurately in the karyotype of these fishes. This is probably one of the many shortcomings of the fish chromosome methodology. By comparing the results reported by other workers to that of the present study it can be seen that the technique of chromosome preparation used here greatly enhanced the resolution of biarmed chromosomes, the direct result of which is the higher NF number in every species except in S. galilaeus.

From the analysis of the karyotypes and counting the NF of different species of tilapias it seems that different species have varied numbers of m, sm and st chromosomes. Sarotherodon galilaeus has the least number of sm chromosomes, whereas the Oreochromis species have both sm and m

chromosomes and the Tilapia zillii has more m chromosomes than the other two groups. By comparing the L^R of individual chromosomes (expressed as percent haploid genome length) between species it seems that the length varies little except with chromosome number one which is the longest in the whole karyotype. This in turn indicates that translocation including fusion or fission (Robertsonian) of the whole chromosome arm probably played no significant role in the evolution of the karyotypes of these species. However, reciprocal translocation of short regions of two chromosomes (non homologous) cannot be identified in the length measurement data, but in the meiotic metaphase I (or late stages of prophase) it would have been possible to identify such translocations as has been seen in other species (see White, 1973). Unfortunately in the metaphase I stage of tilapia species no such translocation configuration has been found which indicates that such translocation did not play a great role in their karyotypic evolution. In closely related species if the chromosome number is the same and the arm number (NF) varies it might be due to the shifting of centromeric positions. As already mentioned, the chromosome arm counting was done in general on the basis of the centromeric position expressed as the centromeric index. If the centromeric index was above 25% then that chromosome is taken as two-armed and if it was below 25% then that chromosome is single armed (Levan et al., 1964). One possible way of such centromeric shifting is probably by an inversion involving the centromere or a pericentric inversion. White (1973) wrote that

> "pericentric inversions.....change the arm-ratio (centromeric index) of the chromosome, unless the two breaks are precisely equidistant from the centromere or from the chromosome ends, so that they are, for the most part, detectable in somatic divisions as well as at meiosis."

In tilapia all the species studied show the same 2n number but they have different NF values (Table 12). So it seems rational to believe that karyotypic evolution in this group is associated with inversions including centromeres of the chromosomes. This is indeed supported by Thompson (1981) for this group of fishes, and several authors for other groups of fishes (Chen, 1971; Le Grande, 1975; Avise and Gold, 1977; Gold and Avise, 1977; Gold, 1979). On the contrary, Kornfield et al., (1979) believed that karyotypic change played little part in the evolution and speciation of these fishes. But they also mentioned that the Tilapia species do have more biarmed chromosomes than the Oreochromis and Sarotherodon. From their calculation on NF all the three tilapia species have the same number (54) and probably because of that reason they placed little importance on centromeric shifting and karyotype evolution. The controversy between their results and this study is probably because of the technical aspects of chromosome preparation. It has been observed that chromosome preparation in fishes is rather tricky as the higher concentration and longer treatment time of colchicine can cause chromosomes to look like small dots. Such ill defined morphology for analysis will obviously have an effect on the classification of the chromosomes.

It was mentioned earlier that the length of different chromosomes varies little in all species (except the No. 1 chromosome), and comparison of chromosomes on the basis of I^C will be useful to identify the inversional difference between the species. Quantification of inversions are made on comparative idiograms (Fig. 8) and are presented in Table 14. But the calculation is rather tentative as individual chromosomes (except Nos. 1 and 2) in a karyotype could not be identified. So quantitation of

inversions may be an under- or over-estimation. From such calculations it is evident that direct comparisons of a species in turn with others show that there is both intergeneric and intrageneric variation for these inversions. Comparison between O. mossambicus vs. O. aureus has 3 inversional differences, whereas O. mossambicus vs. O. niloticus has 8 inversional differences. On the other hand, comparisons between T. zillii show more inversions when compared with both the Oreochromis and Sarotherodon species. The highest inversion has been recorded between S. galilaeus and O. niloticus. The question remains as to the significance of such differences. As previously noted, the individual chromosomes could not be identified so precise interrelationships between genera or a species is not possible. In the case of the mammalian system, identification of individual chromosomes has been done by G- or Q-banding which permits the identification of inversional differences between closely related species (Baker and Bickham, 1980; Rumpler et al., 1983). A similar phenomenon has been extensively reviewed by White (1969, 1973) for other groups of animals and their role in speciation. In the case of fish the G-banding reported (Blaxhall, 1983c) is not yet suitable for such work (Hartley and Horne, 1984) as most of the banding success is restricted to the localization of centromeric/constitutive heterochromatin (Abe and Muramoto, 1974; Zenzes and Voiculescu, 1975; Thorgaard, 1976; Majumdar, 1979; Park and Grimm, 1981). So precise cytotaxonomic relationships between genera/species in tilapia will only be possible when individual chromosomes can be identified.

Another important aspect in the C-value and chromosome morphology as mentioned earlier, is that different species show different amounts of DNA; could this be related to the chromosome length? The comparison of L^R

of chromosomes in different species does not show any significant difference and so no correlation could be found in chromosome length and DNA values. The DNA value and chromosome arm number has a negative correlation in the genus Thomomys shown by Sherwood and Patton (1982), whereas positive correlation has been found in Blennius with C-value and total chromosome length (Cano et al., 1982). Comparing the tilapia data in the same way no correlation could be found. The possibility of a quantitative difference of repetitive DNA related to the variation of total amount of DNA in the nuclei of a species cannot be ruled out as shown in Plethodon by Mizuno and Macgregor (1974). Quantitative DNA value differences in closely related species has been mentioned by several workers (Wolf et al., 1969; Ohno, 1970, 1974; Park and Kang, 1976). A plausible explanation for such differences has been reviewed by Cavalier-Smith (1982) who stressed that higher amounts of DNA may be due to the "housekeeping function". Ohno (1970, 1974) on the other hand mentioned that the higher DNA value may help in keeping a higher number of silent genes which might escape from selective pressure and may become the raw material for evolution. Hence a lower DNA amount is characteristic for the specialized species. Sumner and Buckland (1976) stressed that the C-value difference may be due to some other type of short repetitive DNA which remains dispersed throughout the genome.

Several workers pointed out that C-heterochromatin plays a significant role in the karyotype evolution of closely related species (Pathak et al., 1973; Mizuno and Macgregor, 1974; Hatch et al., 1976 Sen and Sharma, 1980, 1983; Patton and Sherwood, 1982). C-banding studies reported here in tilapia indicate that C-heterochromatin distribution in various species is different. As the amount of C-heterochromatin has

not been quantified it is not yet possible to correlate such results with karyotype evolution. But one point to be mentioned is that Tilapia zillii definitely has a higher amount of C-heterochromatin as 6 chromosomes show completely positive reactions in their short arm. The closely related species viz. O. mossambicus and O. spilurus (McAndrew and Majumdar, 1984) could be distinguished on the basis of a pair of chromosomes which show telomeric heterochromatin in the former species. This is probably one of the many differences waiting to be identified in these species of tilapia. As the work on tilapia at the molecular level has not yet been started seriously there may be much hidden genetic variation which escapes the gross morphology at chromosomal, biochemical (Kornfield et al., 1979; McAndrew and Majumdar, 1983, 1984) and phenotypic (Trewavas, 1982b, 1983) level.

In fish chromosomal heterogamety as a sex specific pair is reported by several authors (see Ohno, 1974; Gold, 1979). In tilapia only one report claims the presence of sex chromosomes which is by Nijjhar et al. (1983) They found that in O. niloticus, O. multifasciatus and T. busumana the 2n = 44 and n = 22. All these species have a pair of long chromosomes (marker). In males this pair does not show any size differences (both L^R and I^C) whereas in the female one of the homologues is smaller and its I^C value also differs from the other, in all these species. The authors do not provide any photograph or quantitative measurements for these karyotypes. Also the number of metaphase plates observed in two species (O. niloticus and O. multifasciatus) was quite low. It has been mentioned by several authors (Prasad and Manna, 1976; Kornfield et al., 1979; present investigation) that the longest (marker) chromosome pair occasionally does show size differences in both the sexes. None of the authors

recorded the difference in I^C values between the homologues of this pair of chromosomes. Using hybridization (interspecific) and sex reversal studies in these fishes arguments have been forwarded that both types of sex chromosomes (XX♀:XY♂; WZ♀:ZZ♂) might be present. (see Chapter II). The chromosomal work done so far in these species does not support such a mechanism of sex determination on the basis of chromosome morphology. The work by Nijjhar et al. (1983) on this aspect is doubtful. In Xiphophorus sp and Lebistes sp although genetically XX:XY or WZ:ZZ mechanisms have been reported but chromosomal studies still do not support such predictions (see Kallman, 1975; Yamamoto, 1975). It has also been reported that chromosomal polymorphism and translocations (Robertsonian) play a great role in the change of chromosome morphology even in the same individual, population and species (Ohno, 1974; Thorgaard, 1976; Gold, 1979; Phillips and Zajicek, 1982; Hartley and Horne, 1982, 1984). For the confirmation of cytological heterogamety three criteria have been proposed by Ebeling and Chen (1970) which are as follows:

1) the invariant occurrence of a heteromorphic chromosome pair in one sex in all the mitotic cells;

2) the typical behaviour - usually an end-to-end association of a single bivalent at meiosis;

3) the presence of two different haploid karyotypes at meiosis II, each possessing one of the heteromorphic chromosome pairs.

The work of Nijjhar et al. (1983) does not fulfil any of these criteria. Foresti et al. (1983) using the silver staining method for the localization of end-to-end association in T. rendalli meiocytes have shown the

absence of sex specific heterochromosome pair. From the above discussion it seems that confirmation of the presence of sex chromosomes in tilapia is rather difficult, and more emphasis should be directed towards technical improvement on the methodology and systematic studies on different populations.

It is generally assumed that mouth-brooding species (Oreochromis) have evolved from substrate spawning ancestors (Tilapia). Sarotherodon species exhibit intermediate characteristics between substrate spawners and mouth-brooders (Peters and Berns, 1978, 1982). But Peters and Berns (1982) believe that mouth-brooding has occurred a number of times from substrate spawners, possibly from different ancestors and at different times. Trewavas (1980) on the other hand believes that Tilapia gave rise to a mouth-brooding branch which quickly divided into Sarotherodon and Oreochromis. Using the karyotype, DNA value and C-banding data it is not possible to verify any of these hypotheses at the present moment. But one point is clear that Tilapia have more biarmed chromosomes than the other genera and also more inversional differences are found when species of two genera are compared. Whether such a phenomenon is a generalised characteristic is yet to be proven by studying many more species in each genera.

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CHAPTER IV

COMPARATIVE GROWTH STUDIES ON DIFFERENT TILAPIINES AND THE EFFECT OF TWO ANABOLIC STEROID HORMONES

INTRODUCTION

Tilapias are of great potential importance in aquaculture in the tropics and subtropics encompassing many of the areas suffering from a lack of animal protein (Hickling, 1963). To meet such demand, tilapias especially O. mossambicus were transplanted from Africa in the early forties to other tropical countries (Chimits, 1957). Balarin and Hatton (1979) mentioned that tilapias are now cultured in Japan, the Indian sub continent, Middle East, Far East, Germany, France, Russia, some parts of USA, Mexico, Brazil, Columbia and Nicaragua. Unfortunately proper investigations have not yet been undertaken concerning the culture suitability of the various species. Chimits (1957) wrote that,

> "much publicity has been given to the transplantation of O. mossambicus and to its cultivation in various countries; unfortunately it has been stated directly and conveyed by implication that this species is a wonder-fish and suitable for all situations and all purposes."

No thorough studies have so far been done which support the suitability of O. mossambicus for all purposes. The persistence of this false impression is dangerous for two major reasons: a) it may lead to failure of programs of inland fisheries development, b) it may cause neglect of consideration of the suitability of other species.

A large number of tilapia species have been cultured in ponds. There are about 16 important species recorded by Huet (1970) and Wohlfarth and Hulata (1983). The list given by Balarin and Hatton (1979) includes 23 species which have been tried as cultured species at some stage in different countries. Guerrero (1982) mentioned about 20 cultivable species. Of these only two Tilapia (T. zillii and T. rendalli) and three

Oreochromis (O. mossambicus; O. aureus; O. niloticus) species have been widely used (Hepher and Pruginin, 1982). Pullin (1983) listed 9 commercially important species of tilapia. They are the following: O. mossambicus, O. hornorum, O. aureus, O. niloticus, O. macrochir, O. spilurus, Sarotherodon galilaeus, Tilapia zillii and T. rendalli. Of the Oreochromis species mentioned, O. hornorum is used mainly to produce monosex hybrids especially in Brazil (Lovshin and Da Silva, 1975), O. spilurus is mostly cultured in Kenya, O. aureus and O. niloticus are cultured in Israel. The most widely used species is O. mossambicus. According to FAO (1978) the production of this species was 19,500 tons in Indonesia and 12,000 tons in Papua New Guinea. Tilapia species are used mainly for their herbivorous habit or preference for submerged vegetation which is abundant in tropical conditions (Chimits, 1957; Balarin and Hatton, 1979).

Growth capacity is obviously a major economic characteristic for culture. Most of the growth comparisons are made on maximum size obtained in natural waterbodies (Fryer and Iles, 1972). Growth data on different tilapia from natural waterbodies have recently been reviewed by Lowe-McConnell (1982). She mentioned that O. niloticus grows to a larger size than S. galilaeus in the same water. These two species also grow faster than T. zillii. On the other hand O. aureus grows better than T. zillii, but slower than S. galilaeus. So these species can be ranked as follows: O. niloticus > S. galilaeus > O. aureus > T. zillii. Payne and Collinson (1983) mentioned that O. aureus and O. niloticus grow at a comparable rate up to one year of age in Lake Mariout (Egypt). When they mature, because of the restricted breeding season for O. niloticus it grows faster than O. aureus. In both the Barotse and Kafue flood plain O. andersonii grows better than T. rendalli followed by O.

macrochir (Duerre, 1969; Kapetsky, 1974; Dudley, 1974). Bruton and Allanson (1974) mentioned that the growth rate of O. mossambicus in Lake Sibaya (South Africa) is lower and falls off more rapidly than in Plover Cove reservoir (Hong Kong) (Hodgkiss and Man, 1977). The relative growth rates of young and older fish may differ in different lakes e.g. T. rendalli from Lake Kariba have slower growth in the first year than in the Kafue flood plain. But by the 4th year the Lake Kariba population had caught up in weight with the Kafue population (Kapetsky, 1974).

In a pond culture system Yashouv (1958) showed that O. aureus grew better than S. galilaeus. Yashouv and Halevey (1971) noticed a growth advantage of O. vulcani over O. aureus. No significant growth rate difference was found by Pruginin et al. (1975) between O. aureus and O. niloticus. Bowman (1977) reported that O. aureus grew faster than O. mossambicus.

Coche (1982) reviewed the cage culture of tilapias. He mentioned that in an extensive culture system (without any supplementary food) the ranking in performance between tilapia species was as follows: O. aureus > O. niloticus > O. mossambicus, whereas in a semi-intensive system the following was true: T. rendalli > O. mossambicus and in an intensive system: O. aureus > O. niloticus. Pullin (1983) stated that O. niloticus is the best species as far as growth is concerned, from a worldwide survey made by ICLARM. But he did not provide any experimental results in support of his conclusion.

The wide range of growth performance in tilapia is probably caused by the diversity of habitat that tilapia are found in and their adaptation

to a very great range of ecological and physical parameters, which allows them to survive in sub-optimal conditions (see Balarin and Hatton, 1979; Philippart and Ruwet, 1982; Lowe-McConnell, 1982; Wohlfarth and Hulata, 1983). The species, their distribution, and ecology have been recently compiled by Trewavas (1983). This major work shows that tilapia have penetrated the majority of tropical aquatic environments, even hot soda springs.

Tilapia as a group also display a range of feeding behaviour. The most commonly cultured species are opportunistic omnivores but other species specialize on phytoplankton, zooplankton, or aquatic macrophytes. However a recent review by Philippart and Ruwet (1982) shows that even within a species there is a great diversity of feeding behaviour which is controlled by the ecology of the water body and competition from other species.

In terms of selecting fish for aquaculture the enormous range of variation "tied up" in the various species means that it should be possible to select a species for any given set of fish farm conditions. However, very few studies have been undertaken by fish culturists to select a species best suited to their particular needs.

This point was made very strongly at the recent International Symposium on Tilapia in Aquaculture (Israel, 1983) which called for many more studies to be undertaken in a variety of aquatic conditions to compare and evaluate genetic differences in performances in a wide range of tilapia species.

Although tilapia have so many beneficial characteristics for culture, the major drawback is the high fecundity and early maturity in some species (Lowe-McConnell, 1955; Fryer and Iles, 1972). This has adverse effects on the production, as overpopulation causes stunting and ultimately results in unmarketable small fish (Hickling, 1960; 1968). Several methods have so far been tried to reduce or eliminate reproduction. One of the widely used methods is the sex-reversal method using different steroids. On the basis of studies completed by several workers (review by Guerrero, 1982) a model breeding scheme has been proposed to produce monosex population after hormone treatment and sex reversal (Shelton et al., 1978; Jensen and Shelton, 1979). The model they proposed is based on a simple chromosomal sex determination mechanism. Unfortunately sex determination in tilapia is rather more complicated (Wohlfarth and Hulata, 1983; Chapter II). It has already been mentioned that male tilapia grow faster than female so in a total male population of a species after hormone treatment the growth will be higher. Yet it is not known at which particular time such differential growth is initiated (Chapter V). Hormonal and non hormonal growth promoters have been used in husbandry of mammals and birds. No serious attempt has so far been made in fish (Donaldson et al., 1979). In tilapia hormones have been used to change the sex rather than for their growth promoting effects (Guerrero, 1982).

Yashouv and Eckstein (1965) reported that O. aureus fry when maintained in water containing male hormone had their growth increased. On the other hand Hutchison and Campbell (1964) mentioned that treatment of ethylestrenol has no effect on growth of T. rendalli. Payne (1975) believes that testosterone may be used effectively in increasing tilapia food conversion ratios. In O. aureus Guerrero (1975) reported that

1-dihydrotestosterone (DHT), 17α ethynyltestosterone (ET) and 17 methyltestosterone (MT) treatment at a rate of 15, 30 or 60ppm caused an increase in growth rate. He treated the fry with hormone impregnated food for 3 weeks. After the completion of the hormone treatment the fish were grown for an additional 120 days without the hormone additive. Although an improvement for growth was observed for all the groups, statistically significant growth differences were found for DHT-30, ET-30, MT-30 and MT-60 treatments over the control. From his results no such correlation has been found between sex ratio change towards male and higher growth rate. Anderson and Smitherman (1978) compared growth of sex reversed males (17α ethynyltestosterone treated) and normal males of O. aureus and O. niloticus. They found that O. aureus normal males grew better than all other groups and O. niloticus sex reversed individuals had the lowest growth rate. In their studies they used hormone treated food at a level of 55ppm, and fed the fry for 36 days. Growth studies were done in the following year in earthen ponds. Tayamen and Shelton (1978) mentioned that the androgen treatment (ethynyltestosterone 30, 60; methyltestosterone 30, 60) caused enhanced growth rate when compared to the control and the estrogen treatments (diethylstilbestrol 25, 100; estrone 100, 200) in O. niloticus. They treated the fry for 25, 35 and 59 days with food containing the hormones. But they have not made any statistical analysis on their growth studies. Jensen and Shelton (1979) used estriol, estrone and estradiol treatments on O. aureus fry for 3 and 5 weeks at the following concentrations: 30, 60 and 120ppm. Growth studies show that the hormone treatment does not cause any appreciable change in growth rate, except in estradiol-120 where the growth is slightly lowered. They also have not done any statistical analysis. Here also no correlation has been found with sex

ratio and growth rate. (Estradiol 120, sex ratio 1:1, growth rate slightly lowered, Jensen and Shelton, 1979).

All these studies show an indication of the possibility of using steroid hormones as growth promoters in fish. But in the case of tilapia, hormonal studies have been directed more to the production of males or females (monosex). Donaldson et al. (1979) summarised by suggesting that the use of hormones could reduce the unit cost of fish production in intensive culture system. This can be done through a) shortening the time for releasing the fish into natural waters (trout and salmon industry), b) increasing the growth rate, c) improving food conversion.

It seems that the use of hormone treatment will become a widespread husbandry practice because of the advantages of monosex populations and possible growth promotion.

The few experiments in the literature show a variety of response to hormone treatment and it very well may be that hormone treatment may change the relative values of the different species when compared to untreated stock under a given set of environmental conditions.

In this chapter results of comparative growth studies in six commercially important tilapia species have been presented, as well as the effects of two hormones (17α methyltestosterone and 17β estradiol) on the growth of three Oreochromis (O. mossambicus, O. niloticus, O. aureus) species. All these studies were performed in a closed, warm water recirculating system where no natural food was available, which gave precise measurement of the growth performance on a specified dietary regime. This study

has also been designed to see whether sex steroids can change the growth potency of individual species via enhancing growth or due to a change in the food conversion rate.

MATERIALS AND METHODS

The following species have been used in the growth studies: Oreochromis mossambicus, O. aureus, O. niloticus, O. spilurus, O. macrochir and Sarotherodon galilaeus. The origin of these species is given in Chapter II. The purity of the species was checked through electrophoretic studies by McAndrew and Majumdar (1983). The brood stocks (about 30 pairs) were maintained separately in the warm water ($28^{o} \pm 1^{o}C$) recirculating system to avoid contamination. The fish were fed with trout pellet (Ewos-Baker, UK) twice daily. To avoid the deleterious effects of excretory products a steady flow of fresh water was given through the system. The temperature of the whole water body was maintained by a thermostatically controlled immersion heater (2KW). Photoperiodism of 14hrs light and 10hrs dark was maintained by an electronic timer.

Fry production

The ripe females and males in the ratio of 2:1 were put in glass aquaria (500 litres) connected with the recirculatory system. A gravel bed in the aquarium was used mainly to help the fish with nest building. Once the female picked up the fertilized eggs into her mouth, the male fish was removed. The fry when released by the female were collected by a fine meshed net. To get heterogeneity, fry from different parents were put together. In the case of S. galilaeus, the fry were collected from both parents and were grown on as those of the other species. The fry were fed with ground trout pellet (<200 micron) until they reached about 2-3gms body weight. They were grown in small batteries of aquaria connected to a recirculating system.

Description of the System

The recirculated warm water system used for the growth studies is shown in Fig. 1. It was a two-tier system consisting of 24 tanks in rows of 6. The capacity of an individual tank was 60 litres. The tanks were connected with inflow and outflow pipes. The outflow waters drain through stand pipes fitted in the middle of the tank. All the stand pipes of individual tanks were connected to a pipe which was connected to a 25 gallon settling tank. A series of settling tanks of different sizes were used to get rid of suspended solids. The settling tanks were fitted with plastic egg boxes which acted as filter barriers. The clean water was collected ultimately in a 25 gallon tank which in turn was connected to a 1.0 HP pump. The pump forced the water into two upflow filter tanks, which were half filled with coarse gravel. The water flowed into a header tank fitted with a heater, aerators and the supply to the tanks. The oxygen level was kept at at least 90% saturation by directly connecting the individual tanks with forced air from a central air supply. The temperature of the water in the system was maintained by an immersion heater (3.0 KW) put into the header tank). The water temperature was maintained at $28^{o}C \pm 1^{o}C$ by a thermostat regulator. To reduce the metabolite level, 25% of the total water of the system was replaced by fresh warm water every week.

Growth Trial. Normal:

50 fry weighing 2-3gms each were stocked in each tank. For each species two replicates were used and randomly placed in different tanks to nullify the tank effect. All the comparative studies were done simultaneously. The fishes were provided with food (trout pellet No.4, broken, Ewos-Baker, UK) at a rate of 5% of their total body weight twice daily, seven days

FIG. 1 Recirculated warm water system used for the growth studies

FIG. 1 Recirculated warm water system used for the growth studies

a week. Every 10 days the weight of the fish was taken using a Metler balance and the food ration was adjusted. After 40 days the experiment was terminated.

Hormone :

a) Preparation of food containing hormone

The two hormones used were 17α methyl testosterone and 17β estradiol (Sigma Chemicals). The final concentration of the hormones in the food was 40ppm or 40mg/Kg of food. The hormone crystals were dissolved in 95% ethanol and the solution was then sprayed on to the micronised food (No. 4, trout pellet, Ewos-Baker, UK) and was thoroughly mixed. After spraying, the food was dried at 37° - 40°C for 3-4 hours. The procedure described was modified from Guerrero (1975). The hormone treated food was stored at -20°C in airtight boxes.

b) Feeding regime

The fry were collected as described earlier, and only those which still had their yolk sacs were used in this study. The fry were kept in an aquarium which was fitted with a 200W water heater and an air supply. Everyday waste material was removed by siphoning the bottom of the aquarium. The fry were given excess food containing hormone at roughly 25% of their body weight (Johnstone et al., 1983). The food was given 3-4 times daily, seven days a week. The treatment was continued for 40 days. The temperature was maintained at 28°C ± 1°C.

c) Growth trial

After the hormone treatment, the fry were transferred to the recirculating system and were grown to 2-3gms in weight with trout pellets. As the fish

attained the desired size, they were transferred to the experimental system described earlier. 50 fishes were put in each tank. 2 replicates of each treatment and species were used for the comparative growth studies. For this study three species were used, viz. O. mossambicus, O. niloticus and O. aureus. The fish were fed in the same way as the other trial.

Statistical Test:

Statistical analysis was done by using the multiple range method of Duncan (1955) for initial mean weight, final mean weight, specific growth rate and food conversion ratio. The significance was taken at 1% level. The specific growth rate (SGR) was calculated as follows:

$$SGR = \frac{\log_e \text{Final Weight} - \log_e \text{Initial Weight}}{\text{Total experimental time in days}} \times 100$$

The food conversion ratio (FCR) was expressed as the ratio of amount of total food consumed to total weight gain.

RESULTS

Growth Studies in Different Species

(a) Growth

Growth data is presented for the six different species in Table 1. The data include the mean weight of fish for the total 40 days of the experimental period at 10 day intervals. Fig. 2 is the graphical representation of the average weight of each species for the same period. It seems from the pattern of graphs that all the species showed a steady state increase of body weight throughout the period. It is also clear that O. niloticus reached the highest weight and O. aureus the lowest. The growth rate has been expressed as percentage increase in body weight (Table 1). From the values obtained it is clear that O. niloticus grew best with a 393% body weight increase. From these results the species can be ranked as follows: O. niloticus > O. mossambicus > O. macrochir > O. spilurus > S. galilaeus > O. aureus. Using the multiple range test of Duncan (1955) it was possible to test statistical difference between the species for initial and final weight given in Table 1. It can be seen that the initial weight of all the species was not significantly different. Using the same treatment for the final weight the species could be grouped in four categories. The first included O. aureus and S. galilaeus with mean final weight of 7.67 and 8.08gms respectively. The second group contained only O. spilurus (final weight 10.35gms). The third group included O. macrochir and O. mossambicus with mean final weight of 12.17 and 13.30gms respectively. The fourth group contained only one species, i.e. O. niloticus (final weight 15.53gms). Here again the O. niloticus showed better performance than the other species compared, and O. aureus was lowest in its growth performance.

TABLE 1. Growth Data on Different Species of Tilapia

Species	Initial Weight(gm)	Days 10	20	30	40	SGR	FCR	% Increase
O. mossambicus	2.78[a] ± 0.18	4.31 ± 0.18	6.47 ± 0.14	9.56 ± 0.23	13.30[c] ± 0.24	3.91[b] ± 0.05	1.10[a] ± 0.003	378.42
O. spilurus	2.64[a] ± 0.13	4.24 ± 0.14	6.07 ± 0.21	8.43 ± 0.16	10.35[b] ± 0.27	3.41[b] ± 0.07	1.39[ab] ± 0.03	292.04
O. macrochir	3.01[a] ± 0.06	4.55 ± 0.06	6.69 ± 0.08	8.57 ± 0.12	12.17[c] ± 0.17	3.49[b] ± 0.04	1.24[ab] ± 0.04	304.32
O. aureus	2.75[a] ± 0.05	3.99 ± 0.12	5.41 ± 0.05	6.18 ± 0.11	7.67[a] ± 0.15	2.56[a] ± 0.01	1.86[c] ± 0.01	178.91
O. niloticus	3.15[a] ± 0.09	4.75 ± 0.16	6.76 ± 0.18	10.43 ± 0.19	15.53[d] ± 0.14	3.99[b] ± 0.11	1.01[a] ± 0.05	393.01
S. galilaeus	2.84[a] ± 0.10	3.74 ± 0.11	5.08 ± 0.11	6.40 ± 0.08	8.08[a] ± 0.09	2.61[a] ± 0.16	1.74[bc] ± 0.17	184.51

Mean ± standard error, Figures with common superscripts in the same column are not significantly different. ($P > 0.01$)

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S. galilaeus	2.84[a] ± 0.10	3.74 ± 0.11	5.08 ± 0.11	6.40 ± 0.08	8.08[a] ± 0.09	2.61[a] ± 0.16	1.74[bc] ± 0.17	184.51

Mean ± standard error, Figures with common superscripts in the same column are not significantly different. ($P > 0.01$)

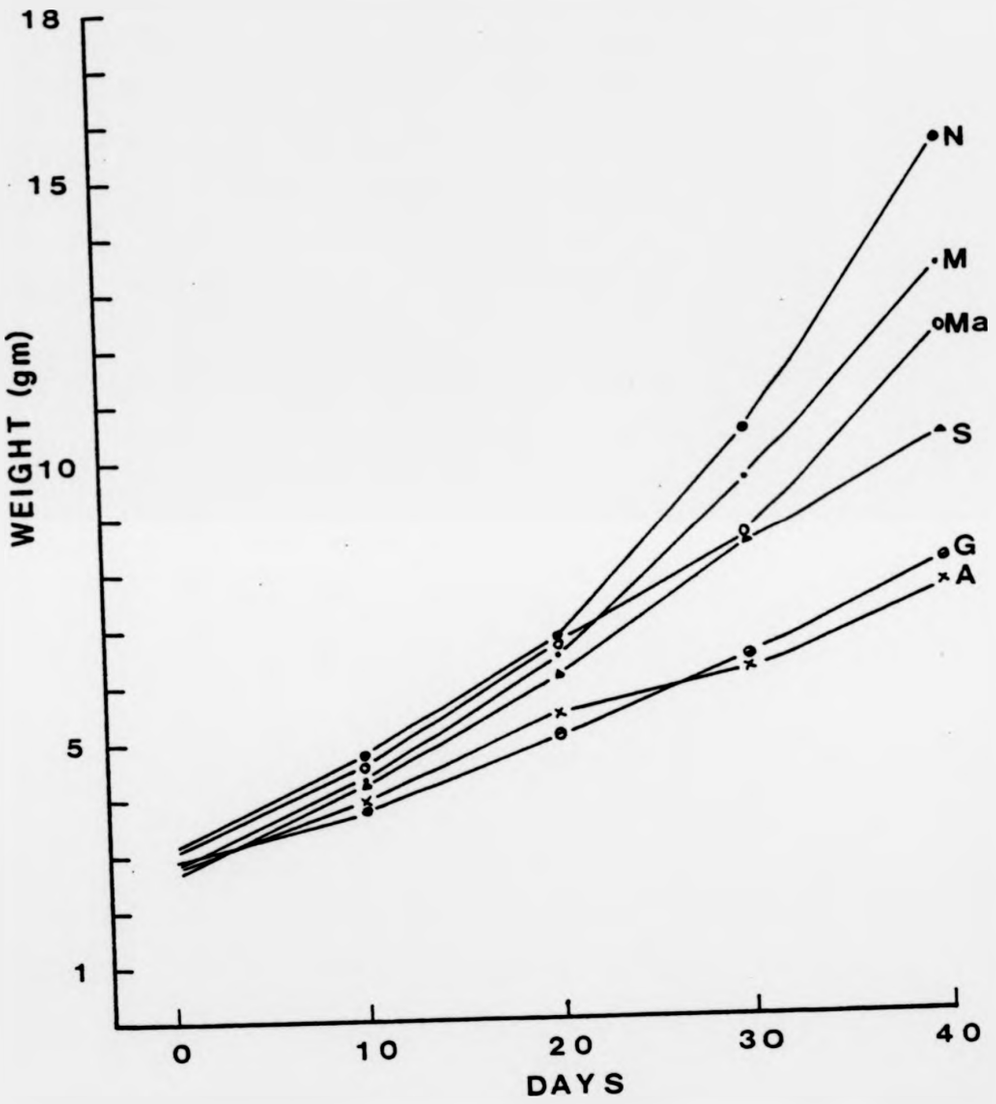

FIG. 2. Comparison of growth in six species of tilapias. N = O. niloticus, M = O. mossambicus, Ma = O. macrochir, S = O. spilurus, G = Sarotherodon galilaeus, A = O. aureus.

(b) Specific Growth Rate (SGR)

Specific growth rate was calculated between final and initial mean weight for the duration of 40 days of the experimental period. The values obtained for the different species varied from 2.56 (O. aureus) to 3.99 (O. niloticus). Using the same statistics as that used for the body weight, the species can be grouped into two groups: 1) O. aureus and S. galilaeus with SGR of 2.56 and 2.61 respectively (the lower group), 2) the higher group includes O. spilurus (3.41), O. macrochir (3.49), O. mossambicus (3.91) and O. niloticus (3.99). It seems from these results that there are distinct differences in growth performance between these species especially O. aureus and S. galilaeus which are slower growing than the other species in the experimental conditions and feeding regime provided.

(c) Food Conversion Ratio (FCR)

Food conversion ratios varied between 1.01 (O. niloticus) to 1.86 (O. aureus). The grouping of species on the basis of FCR values becomes complicated because the values for the different species overlap and are statistically nonsignificant. Here again O. aureus may be marked out along with the S. galilaeus (1.74) whereas the other species were clustered together. In other words the FCR does not differ much for these species.

Effects of Hormones on Growth

(a) Growth

Table 2 includes the growth data after 40 days of hormone treatment from first day of feeding. The statistical analysis was done on initial and final weight, where the initial weight for both the groups was the same.

TABLE 2. Comparative Growth Data on three Oreochromis species showing the effect of Different Hormone Treatments over an Untreated Control Group

Species	Initial Weight(gm)	Days 10	20	30	40	SGR	FCR	% Increase
				Testosterone				
O. mossambicus	2.71[a] ± 0.07	4.47 ± 0.09	6.52 ± 0.09	9.86 ± 0.17	15.26[d] ± 0.12	4.32[d] ± 0.02	0.94[a] ± 0.005	463.09
O. niloticus	3.07[a] ± 0.08	5.17 ± 0.09	7.95 ± 0.12	12.32 ± 0.29	19.53[e] ± 0.21	4.63[d] ± 0.09	0.86[a] ± 0.003	536.15
O. aureus	2.66[a] ± 0.08	3.82 ± 0.06	5.49 ± 0.09	7.69 ± 0.15	10.57[b] ± 0.32	3.45[c] ± 0.13	1.25[a] ± 0.05	297.37
				Estradiol				
O. mossambicus	3.03[a] ± 0.13	4.51 ± 0.12	6.36 ± 0.11	8.40 ± 0.09	11.32[b] ± 0.10	3.29[c] ± 0.16	1.34[a] ± 0.06	273.59
O. niloticus	2.77[a] ± 0.12	3.94 ± 0.11	6.47 ± 0.17	10.63 ± 0.19	15.90[d] ± 0.33	4.36[d] ± 0.11	0.91[a] ± 0.01	474.01
O. aureus	3.00[a] ± 0.07	3.98 ± 0.03	4.39 ± 0.09	5.22 ± 0.04	6.11[a] ± 0.06	1.78[a] ± 0.12	2.69[c] ± 0.18	103.67

Mean ± standard error, Figures with common superscripts in the same column are not significantly different. ($P > 0.01$)

Species	Initial Weight(gm)	Days 10	20	30	40	SGR	FCR	% Increase
				No Hormone				
O. mossambicus	2.78[a] ± 0.18	4.31 ± 0.18	6.47 ± 0.23	9.56 ± 0.23	13.30[c] ± 0.24	3.91[cd] ± 0.05	1.10[a] ± 0.003	378.42
O. niloticus	3.15[a] ± 0.09	4.75 ± 0.16	6.76 ± 0.18	10.43 ± 0.19	15.53[d] ± 0.14	3.99[cd] ± 0.11	1.01[a] ± 0.05	393.01
O. aureus	2.75[a] ± 0.05	3.99 ± 0.12	5.41 ± 0.05	6.18 ± 0.11	7.67[a] ± 0.15	2.56[b] ± 0.01	1.86[b] ± 0.01	178.91

Mean ± standard error, Figures with common superscripts in the same column are not significantly different. ($P > 0.01$)

This table also includes data on the untreated controls for the three species. Fig. 3 is the graphical representation of the comparative growth trial between three species after two different hormone treatments (viz. testosterone and estradiol). It is clear that better growth performance has been achieved after the testosterone treatment, compared to the estradiol treatment. The ranking could be as follows: O. niloticus > O. mossambicus > O. aureus for both the hormone treatments. The same conclusion could be drawn after comparing the percent weight increase. Statistically significant differences were found for every species as well as between every treatment. It is interesting to note that the growth performance of O. niloticus after estradiol treatment is more or less similar to testosterone treatment in O. mossambicus. As mentioned earlier the initial weight of each group was the same but the final weight differed. The range of variation is between 6.11gms (estradiol treatment in O. aureus) to 19.53gms (testosterone treatment in O. niloticus).

(b) Specific Growth Rate (SGR)

After hormone treatment the SGR values have increased considerably in the testosterone treated group compared to the estradiol group. Both the treatments have the same SGR values for O. niloticus which is similar to the testosterone treatment in O. mossambicus. The SGR for O. aureus after the testosterone treatment (3.45) is similar to the estradiol treatment in O. mossambicus (3.29).

(c) Food Conversion Ratio (FCR)

The FCR values after the various treatments were more or less the same as SGR findings with the exception of O. aureus where after estradiol treatment the FCR was recorded as 2.69. The range of variation in the

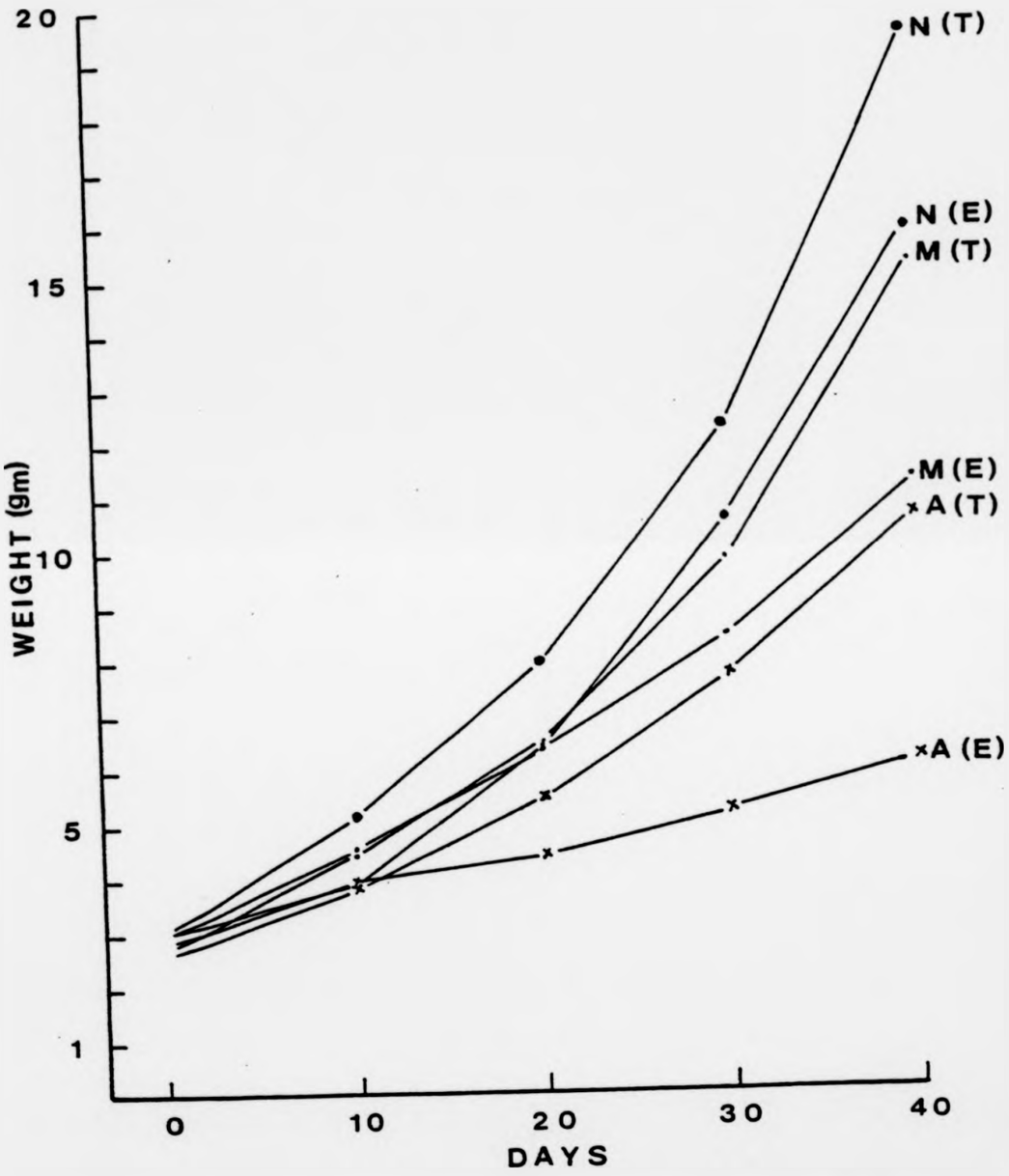

FIG. 3. Comparison of growth between three species of Oreochromis after two hormone treatments. N = O. niloticus, M = O. mossambicus, A = O. aureus, (T) = Testosterone, (E) = Estradiol.

other species is between 0.86 for O. niloticus (testosterone treatment) and 1.34 for O. mossambicus (estradiol treatment).

Comparison of Growth Between Testosterone Treated Group and Untreated Group

(a) Growth

The testosterone treatment has caused enhanced growth rate in all the species when they are compared to their untreated group (Fig. 4 and Table 2).

(b) Specific Growth Rate (SGR)

Comparison of SGR values show that testosterone treatment has increased it, in all the species compared with the untreated group.

(c) Food Conversion Ratio (FCR)

Significant differences were observed in FCR values between the untreated O. aureus and the other groups considered. As a whole testosterone reduces the FCR values for all the species.

Comparison of Growth Between Estradiol Treated Group and Untreated Group

(a) Growth

Comparative growth data has been presented in Fig. 5 and Table 2 for estradiol treated and untreated groups of every species. Except for O. niloticus, estradiol has lowered the growth in other species. In O. niloticus the treatment has resulted in higher growth as is indicated in percent weight increase data. In the treated group it is 474% whereas in the untreated group the value is 393%. The poorer growth

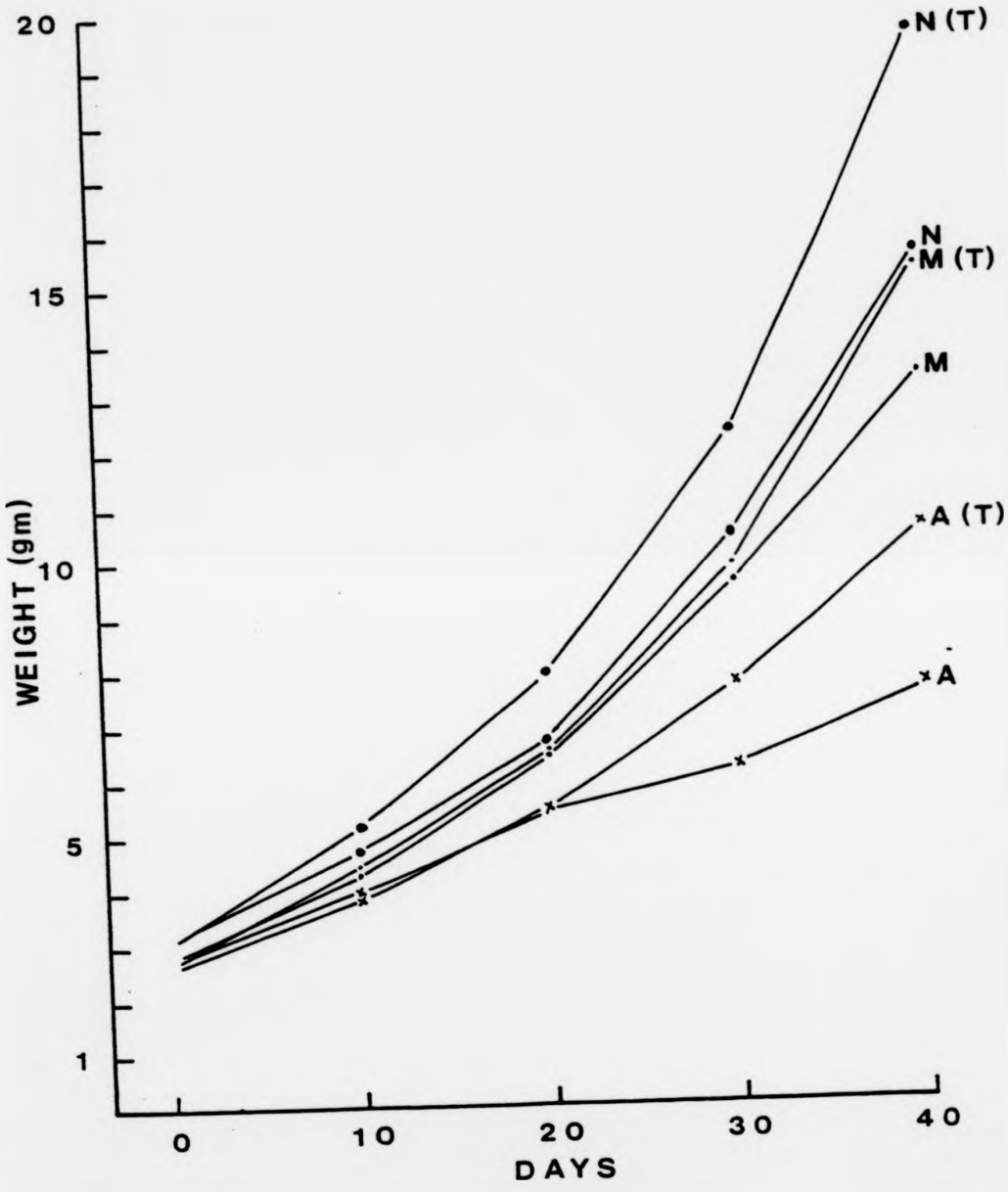

FIG. 4. Comparison of growth between testosterone treated group (T) and the control group in three Oreochromis species. N = O. niloticus, M = O. mossambicus, A = O. aureus.

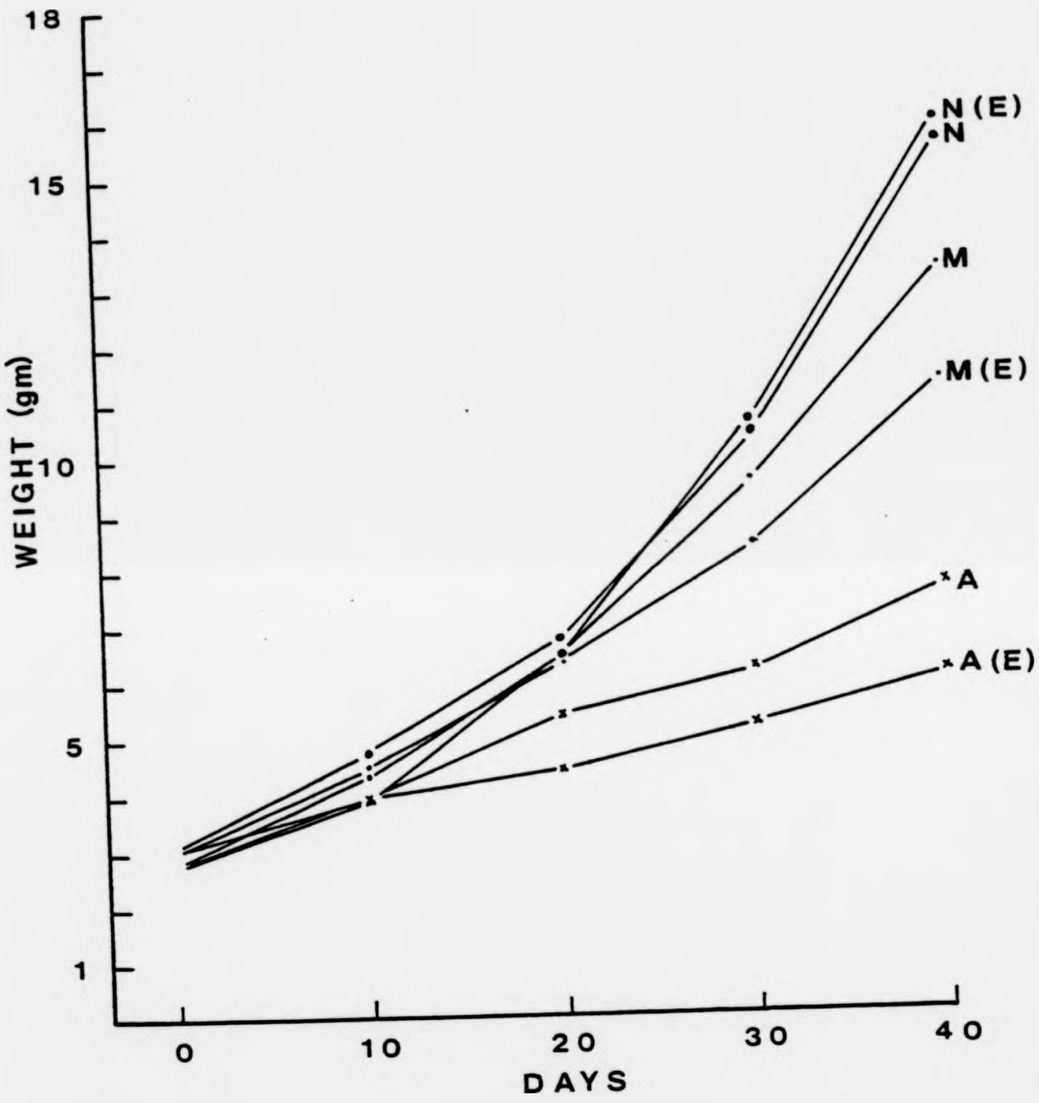

FIG. 5. Comparison of growth between estradiol treated group (E) and the control group in three Oreochromis species. N = O. niloticus, M = O. mossambicus, A = O. aureus.

effect is evident in O. mossambicus, where the treated group has 273% increase of body weight compared to 378% increase in untreated group. The same is true for O. aureus. Although the present weight increase in O. niloticus is higher after estradiol treatment the final weight difference between treated and untreated group is not statistically significant. A significant weight difference has been recorded for O. mossambicus (treated versus untreated).

(b) Specific Growth Rate (SGR)

SGR values were lowered after estradiol treatment in all species except in O. niloticus where it is slightly higher but statistically insignificant. In the case of O. aureus the difference is considerably lower (1.78 compared to 2.56 in untreated group).

(c) Food Conversion Ratio (FCR)

FCR values after estradiol treatment do not seem to change much except in O. aureus where it has increased dramatically from 1.86 (untreated) to 2.69 (treated).

Data on Sex Ratio After Hormone Treatment

Table 3 is the compilation of the sex ratio data after hormone treatment for the three species of Oreochromis. Identification of the sex has been done by looking directly at the gonads. After testosterone treatment the sex ratios observed were as follows: 100% males in O. mossambicus; 83% males in O. niloticus and 65% males in O. aureus. In about 1.48% of the total population of O. niloticus even after looking at the gonads it was not possible to identify the sex. On the other hand in the estradiol treated group the sex ratio data was as follows:

TABLE 3. Sex Ratio Data after Hormone Treatment in three Oreochromis species.

SPECIES	Testosterone				Estradiol				Control		
	Male %	Female %	Unidentified %	TOTAL	Male %	Female %	Unidentified %	TOTAL	Male %	Female %	TOTAL
O. mossambicus	100	-	-	150	4.44	95.56	-	150	54	46	150
O. niloticus	82.97	15.55	1.48	150	41.08	56.60	2.32	150	56	44	150
O. aureus	65.04	34.96	-	150	6.14	79.83	14.03	150	51	49	150

96% females in O. mossambicus; 56.6% females in O. niloticus and 79.83% females in O. aureus. In this group 2.32% and 14.03% of the specimens in O. niloticus and O. aureus respectively, the sex could not be identified. On the other hand in the untreated group the sex ratio was 1:1.

DISCUSSION

The final weight in a growth trial is a good criterion for comparison providing the initial weights are the same. From the results it appeared that the six species studied may be ranked into four groups (Table 1). It is clear that O. niloticus is the best species and O. aureus is worst as far as the growth is concerned under the experimental conditions. This study minimized the risk that growth differences were due to differences in the physiochemical conditions of water, initial size, age, density and primary production, as all these factors were kept constant for every species.

The other criteria used to evaluate the growth potential in the present experiments are the specific growth rate (SGR) and food conversion ratio (FCR). Comparison of SGR values between the species is only effective where the growth rate is linear against time. In tilapia it has been reported that the growth is linear in fish up to approximately 200gms (Fryer and Iles, 1972; Balarin and Hatton, 1979).

The SGR is also dependent on the composition of the feed stuff. Jauncey (1982) has worked on a similar trial in O. mossambicus designed to evaluate the level of dietary protein required for optimum growth. From his experiment he suggested that for O. mossambicus 40% protein was the optimal level. In the present experiments the protein content of the diet was 48% and the SGR obtained was 3.91 which is comparable to that of Jauncey (1982) where 48% protein gave 3.49 (SGR). Comparison of SGR values for other species is not possible as no reports have been found in the literature comparable to the experimental conditions used here.

Food conversion ratio (FCR) is the indicator of food consumption and body weight increase. Any species with lower FCR will be beneficial to aquaculturists as food is the major economic factor in fish production. The comparison of FCR between different species again shows that O. niloticus perform best (FCR 1.01) and O. aureus (FCR 1.86) is at the bottom of the table followed by S. galilaeus (FCR 1.74). It has been reported by Coche (1978; 1979) that the FCR of fish in cage culture is determined by the interactions between fish, i.e. size, density, the food quality (protein source, composition, etc.) and rearing environment. He also pointed out that

> "to maximize the feeding efficiency, every one of these factors should be maintained at its optimum level for the particular species being cultured."

A wide variability of FCR for different food stuffs in O. niloticus is reported by Hepher and Pruginin (1982). Guerrero (1980) reported that FCR for the same species varies between 2.7 and 2.5 for moist and dry pelleted food respectively, where the composition and amount of food (4% of the body weight) are fed. The subject has been extensively reviewed by Coche (1982) and he stated that the O. niloticus monosex male culture studies performed in different parts of the world gave different values, and the FCR value reduces with the amount of higher protein in the diet. In Belgium, Philippart et al. (1979) has shown that with a feeding rate of 3% to 7% of the body weight and feeding with 46% protein in the diet the FCR varies between 1.1 to 2.0, which is quite similar to the results obtained in the present experiments. In the case of O. mossambicus the FCR is reported to be 4.0 by Pantastico and Baldia (1979) from the Philippines where the feeding rate was 10% of the body weight but the amount of protein in the diet was not mentioned. On the

other hand the FCR value obtained is quite low (1.10) for this species in the present experiments but the feeding schedule was only 5% of the body weight with 48% protein. In the case of O. aureus, Suwanasart (1972) reported the variation of FCR when floating and sinking pellets were used for a growth trial (food with 40% protein and feeding rate of 3% of the body weight). Coche (1982) in his review stated that the same amount of protein in the food and a similar feeding rate also gave variable FCR in O. aureus (1.1 to 6.3), whereas Jordan and Pagán (1973) reported that the FCR varies between 0.91 - 0.95 when food containing 36% protein is fed at a rate of 5% of the body weight. The FCR values obtained in the present trial for this species is somewhat higher.

It has been mentioned earlier that SGR and FCR depend much on the composition of the food (Jauncey, 1982). A differential response in growth rate to the same food and environment in different species, may be due to differential nutritional requirements or the inherent genetic differences in the species themselves. To evaluate the nutritional requirements further trials need to be performed but the present work indicates that at 48% protein level and at 5% of the body weight per day as the feeding rate O. niloticus grows better than other species and O. aureus is the slowest growing species.

Much attention has been recently focused on developing new methods for more efficient cultivation of fish, because of the ever increasing cost of fish hatchery and aquaculture operations. The cost of such operations could be reduced by introducing a species with a) faster growth rate and b) by improving the food conversion ratio (Simpson, 1976; Donaldson et al., 1979). It is well known that to develop a fast growing strain, it takes years of experimentation through selection which costs

money and needs expert personnel. On the other hand if one can somehow trigger the growth and food conversion ratio by certain means one can achieve the goal in a shorter time.

In the live stock industry it has been reported by Anon (1972) that as many as 75% of all cattle reared for meat production in USA had diethylstilbesterol (DES) administered. This has been done as a feed supplement or as pellet implants. The animals where DES is used are found to attain the desired marketable size 35 days earlier than the untreated group.

Although the effects of androgens and estrogens are not fully understood, it is known that these hormones generally have an anabolic or growth promoting effect either on sex related organs or on the total organism (see Donaldson et al., 1979).

In the case of fish the anabolic effects of androgens and related substances have been tested in eight salmonid and seven non salmonid species. Effects of testosterone and related substances on growth in tilapia have been restricted to only three species, e.g. O. mossambicus (Guerrero, 1976; 1979); O. niloticus (Katz et al., 1976; Tayamen and Shelton, 1978) and O. aureus (Guerrero, 1975). All these workers mention that after hormone treatment for certain period in the early life of the fish it causes enhancement of growth rate, in the form of weight increase over the untreated group.

The present experimental results clearly show that the final weight was increased for every species after testosterone treatment. For comparison we have used a non hormone treated group as a control. Comparing the

results, the ranking in growth performance could be as follows: O. niloticus > O. mossambicus > O. aureus. This ranking is also the same for the controls. But the percentage increase after hormone treatment is higher in every species over the control, and the ratio is not the same for all the species, i.e. the increment over the control was 143%, 84% and 118% for O. niloticus, O. mossambicus and O. aureus respectively. The specific growth also increased considerably. Statistical analysis shows that SGR for O. niloticus and O. mossambicus does not increase significantly but In O. aureus it is significantly higher than the control. Comparison of FCR after hormone treatment gives interesting results, a) the FCR in all the species becomes closer to each other and statistically insignificant, b) comparison of the control and the treated group shows in O. aureus, the FCR is reduced in the treated group, whereas in the two other species it remains the same. Although statistically nonsignificant the values (FCR) for the latter two species go below one. In O. niloticus it is 0.86 after hormone treatment and in the control it is 1.01.

It is evident from the results that 17α methyltestosterone causes enhanced growth rate and reduction in FCR in the three tilapia species studied. Interestingly with every criterion considered separately O. aureus shows better performance over the control. This obviously raises the question whether hormone treatment has altered the genetic makeup at least in function as far as the growth is concerned. The effects of anabolic agents is generally defined as an increase in nitrogen retention by two means: a) increase food intake, b) improved food utilization (Donaldson et al., 1979). In the present experiments the food given was 5% of the fish's body weight in every case, so the question of higher

food intake seems to be not critical. It might possibly be that larger fish in the same tank took more food than the smaller ones, but from the standard error calculation, it suggests that the size variation within a tank was also not different from the control groups. It seems that the weight gain after hormone treatment is likely to be due to improved food utilization, which reflects in the FCR calculation. But we need to have proof to substantiate our prediction through analysis of flesh. It has been reported for Oncorhynchus kisutch by Fagerlund and McBride (1975) that after 17α methyltestosterone (10mg/Kg), the amount of flesh and lipid content is reduced. In the case of steelhead trout (Salmo gairdneri) and pink salmon (Oncorhynchus gorbuscua) the lipid content is increased and the moisture content is decreased at a lower dose of hormone (0.2 - 10mg/Kg) but the final weight remains higher than the control (Fagerlund and McBride, 1977). But it is still not known whether these hormones have any effect on lipid biosynthesis (Donaldson et al., 1979).

In another report Yamazaki (1976) noted that in Carassius auratus, the 17α methyltestosterone (1-30mg/Kg) treated group had food consumption proportional to the final body weight. On the contrary we have given the same amount of food and have obtained higher body weights after hormone treatment. The same has been reported by McBride and Fagerlund (1976) in the case of Coho salmon (Oncorhynchus kisutch).

In the case of fish most of the reports describe that estrogen treatment has a negative effect on growth (Donaldson et al., 1979). Cowey and Sargent (1972) reported that diethylstilbisterol (DES) has a growth promoting effect on Pleuronectes platessa at a dose between 0.6 - 1.2 mg/Kg (hormone/food) whereas at a higher dose (2.4mg/Kg) it has a

negative effect on growth (Cowey et al., 1973). In tilapia growth rates after estrogen treatment have been reported for two species. Tayamen and Shelton (1978) reported that DES treatment (25mg/Kg) in O. niloticus causes increased growth rate but estrone (100-200mg/Kg) treatment has no effect on growth. Jensen and Shelton (1979) reported that estriol, estrone, and estradiol (30-120mg/Kg) treatment has no effect on growth in O. aureus.

In the present experiments, after 17β estradiol treatment in three different species of tilapia, the results are somewhat different. Growth rate has been significantly retarded in O. mossambicus after hormone treatment, whereas in O. niloticus and O. aureus it causes no significant alteration.

Comparison shows that after estradiol treatment the SGR has been reduced significantly in O. aureus compared to the control, whereas in O. niloticus the SGR is a little higher than the control. Basically higher FCR values have been found for O. aureus after estradiol treatment, whereas in the two other species the FCR does not change much.

From the results it seems that the estradiol treatment has in general a lowering effect on growth rate which is more pronounced in O. aureus when comparing the SGR and FCR values with the control. It has been reported for guppy and platyfish that estradiol treatment has increased weight gain in males only (Berkowitz, 1938; Cohen, 1945). The males in these species are smaller than the females, and growth inhibiting effects for females using androgens have also been reported (Cohen, 1945; Clemens et al., 1966). It seems from these studies that in the guppy (Lebistes sp.) and platyfish (Xiphophorus sp.) the effect of hormones

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are sex specific. The subject has also been reviewed extensively by Donaldson et al. (1979). In mammals estrogen has a growth retarding effect but exceptions do occur, such as in ruminants where both androgen and estrogen have a growth stimulating effect (see Donaldson et al., 1979). It seems most likely from the discussion that estrogen has a species specific growth effect. Our results also support the above assumption.

One of the reasons for the differences in growth after hormone treatment, might be due to the sex alteration effect of hormones in tilapia (reviewed by Guerrero, 1982). It has also been reported by Fryer and Iles (1972), and Lowe-McConnell (1982) that the growth rate in male and female tilapia might have a genetic basis. There is certainly a marked amount of sexual dimorphism in size particularly in the "mossambicus" group. The males growing much larger after sexual maturity. On the other hand O. niloticus show more or less the same size even after maturity (Lowe-McConnell, 1982). Sex ratio data after testosterone treatment in the present experiments show differential response among the species. Only in O. mossambicus 100% male population is recorded. In O. niloticus and O. aureus the percentage of males are 83% and 65% respectively, whereas in the controls the sex ratio is 50:50. To explain the possibility of sex influence on growth, it should be expected that O. mossambicus, testosterone treated group should come on top of the list. On the contrary the mixed sex O. niloticus shows the highest growth rate after hormone treatment. Similar arguments hold for estradiol treatment, where the following sex ratios were observed: 95%, 56% and 80% females for O. mossambicus, O. niloticus and O. aureus respectively. The growth rate data show that O. aureus is the lowest in the scale (on the basis of SGR and FCR).

From the results it seems that by hormone treatment, FCR can be reduced and a growth rate increase could be achieved in O. mossambicus, O. niloticus and O. aureus. These three species are the most widely cultured (Hepher and Pruginin, 1982) and hormone treatment especially testosterone has a real potential in practical tilapia aquaculture. Although hormone treatment increased the growth rate, the growth pattern in these three species did not alter very much when compared to trials of non-hormone treated groups. The extent of such an increase due to the functional modification of the gene function is yet to be determined.

The major problem with hormone treatment is that if the hormones are deposited in the body of the fish it might be a health hazard for human consumption. It has been reported by Fagerlund and McBride (1978) in Oncorhynchus kisutch that 10 days after withdrawal of hormone treatment the concentration of hormone present in the treated fish is less than 1ng/g (hormone/tissue). In the case of Salmo gairdneri and O. mossambicus Johnstone et al. (1983) have used ^{3}H testosterone to calculate the hormone clearance time in these fishes. They reported that 100hrs after the withdrawal of the hormone, the radioactivity in both the species goes below 1% level in all the tissues. They reported that the liver is the major organ responsible for the clearance of the hormones. These results indicate that the hormone clearance time is quite short compared to the time taken to grow the fish up to marketable size. In the present series of experiments, the fishes were provided the diet containing hormone at a very early stage of life and for only 40 days. After the treatment the fish are grown using the food without any hormone, for the rest of their lives. So the hormone level, when the fish attain marketable size, is expected to remain well below the prescribed risk level for human consumption (Johnstone et al., 1983).

Summarising the results on growth studies on different tilapia species it seems that under the experimental conditions, they show species specificity in their growth rate as judged by SGR and FCR. It is known that growth is a complicated phenomenon not only governed by the genotype but also of the manifestation of the environmental conditions (Moav et al., 1975; Wohlfarth et al., 1975, 1983). Although ranking in the present study show O. niloticus is the "best" species, this holds true at the moment only for the conditions specified, e.g. amount of food, protein content of food, temperature, oxygen level etc. So for choosing a particular species for its suitability in certain water bodies further growth trials are required using much wider criteria. Besides, it is also known that different species have preference for different ecological niches and food (Philippart and Ruwet, 1982; Wohlfarth and Hulata, 1983) so this might definitely affect the growth studies in a defined condition as has been done here. So to find out which particular species is suitable for the particular situation, comparative growth trials have to be performed in those conditions. After knowing the suitability of that species the selection work can be initiated to improve upon the strain concerned. It is also borne in mind that culture condition and husbandry technique also plays a major role in changing the growth rate which is indicated in the studies using hormone.

Hormone treatment is more promising for increasing growth rate through reduction of FCR and increase in SGR. This might be a temporary measure for increased fish production. This study also shows that hormone treatments do not alter the overall ranking of the performance of the species. To obtain a permanent solution for increased production, selection studies using growth as the major criteria are to be done for every species. The

success of selection depends on the availability of variation in the population for the character to be selected. From the literature on tilapia growth studies, a wide range of SGR and FCR values are reported (Hepher and Pruginin, 1982; Coche, 1982) in every species. If it is believed that this variation is the result of environment-genotype interactions for different species there remains the possibility of successful selection work on growth rate in tilapias (Gjedrem, 1983).

CHAPTER V

GROWTH PERFORMANCE IN INTERSPECIFIC HYBRIDS OF THE GENUS *OREOCHROMIS*

INTRODUCTION

Hybridization in fish has been described as "heterospecific insemination" by Chevassus (1983). The majority of the intra- and interspecific vertebrate hybrids have been reported from fish (Schwartz, 1972; Dangel et al., 1973; Chevassus, 1979). In plants and farm animals selection and hybridization have played important roles in domestication, increasing yield, survival rate and improving product quality (see Gjedrem, 1983). A proper evaluation of the potential interest of hybrids in aquaculture requires, primarily a good description of their characteristics. Their value may in fact result from one or from an adequate combination of several of these characters. In a broad sense the potential of hybridization in aquaculture lies in five main areas (Chevassus, 1983). These are:

(a) parthenogenetic development
(b) viability of hybrids
(c) growth potential of hybrids
(d) reproduction
(e) hybridization and sex ratio.

Chevassus (1983) cited many examples within these categories and their possible utilization in aquaculture.

In tilapia, hybridization has been utilized for over two decades by different workers in attempts to produce all-male hybrid fry (see Balarin and Hatton, 1979; Wohlfarth and Hulata, 1983; Chapter II of this thesis). Hybridization has therefore mainly been used to control unwanted reproduction of tilapia in pond culture (Guerrero, 1982; Wohlfarth and Hulata, 1983). Despite the large number of hybrid combinations, no serious comparative studies, either between different hybrids, or between a hybrid and its parental components have been made. Only

a few hybrid combinations have been used for commercial purposes in different countries: Brazil (O. niloticus female x O. hornorum male), Israel (O. niloticus female x O. aureus male), Taiwan (O. niloticus female x O. hornorum male). Of these only Brazil has recorded a production of 60 tons of hybrid tilapia in 1979 (see Lovshin, 1982). But there is no indication of any benefit over normal mixed sex culture populations.

It has been reported by several authors that males in some species of tilapia grow faster than the females and that this has a genetic basis and is not just a function of the differences in energy demands for reproduction between the sexes (Fryer and Iles, 1972; Shelton et al., 1978; Lowe-McConnell, 1982). The implication is that an all-male or near all-male population in the F1 hybrid leads to the assumption that hybridization will directly result in higher growth rates. The other possible effect of hybridization is hybrid vigour (Skinner, 1938) which might also be reflected in the growth rate. Only a few reports compare the performance of the hybrid against its parental species.

Pruginin (1967) reported that all-male hybrids produced by the crossing of O. niloticus female and O. hornorum male grew 30% faster than mixed sex fingerlings of O. niloticus, and 40% faster than mixed fingerlings of O. hornorum. Lovshin et al. (1977) demonstrated that although the all-male hybrids of the same cross show 18% higher growth than the male of O. niloticus, there was no statistically significant difference in their growth rate. Dunseth (1977) compared the growth rate of O. niloticus x O. hornorum all-male hybrids and males of O. niloticus and O. aureus grown in polyculture systems. There was no statistical difference found in the growth rates of the three male tilapias.

However, the tilapia hybrid grew 7% faster than the O. niloticus male. Fram and Pagan Font (1978) mentioned that hybrids of O. niloticus x O. hornorum grew better than mixed sex O. niloticus populations.

In another cross Yashouv and Halevey (1967) did not find a significant difference in growth between O. niloticus x O. aureus hybrids and pure O. aureus. On the other hand Chervinski (1967) noted significantly higher growth in the hybrids originating from the cross between O. niloticus x O. aureus and its reciprocal cross than from a mixed sex O. aureus population. Pruginin et al. (1975) were unable to demonstrate the difference in growth rate between the hybrids of O. niloticus x O. aureus, their reciprocal cross and the parental species.

Hickling (1968) noted that hybrids of O. mossambicus x O. hornorum grew faster than their parental species.

Avault and Shell (1968) reported that the growth rate was higher in both combinations of hybrids between O. aureus and O. mossambicus than their parents.

Kuo (1969) compared the growth of hybrids produced by the crossings of O. mossambicus x O. niloticus, their reciprocal cross, and the parental species. In the two hybrid combinations the growth rate was faster than the parental species.

Pruginin et al. (1975) compared the growth rate of O. vulcani x O. aureus hybrids and their parental lines. They found no significant growth rate difference in all three groups.

Information on the comparative growth of different combinations of hybrids is even more limited (Wohlfarth and Hulata, 1983). Pruginin

et al. (1975) found no significant difference between growth of the all-male hybrids of O. niloticus x O. hornorum and O. niloticus x O. aureus. Hulata and Wohlfarth (mentioned in Wohlfarth and Hulata, 1983) compared the growth of the following hybrids in a polyculture system. These were: O. niloticus x O. hornorum all-male hybrids, O. mossambicus x O. hornorum all-male hybrids, and O. niloticus x O. aureus (70% males). Growth rates of the hybrids of O. niloticus x O. aureus and O. niloticus x O. hornorum were similar and faster than that of the O. mossambicus x O. hornorum hybrids.

The results from the literature are equivocal and the benefits to be gained by using all-male hybrid fry over well managed populations of the faster growing of the two parental species are still to be proven. The majority of the reports in the literature are results from commercial production and do not have the controls and standardized conditions necessary for this type of comparative trial. Another reason for such contradictory reports may be due to the use of different stocks by the various workers which have a different potential for growth rate.

Results will be presented in this chapter on the growth studies performed on nine combinations of interspecific hybrids within the genus Oreochromis. These hybrids were obtained by crossing of the pure species which are considered as commercially important (Pullin, 1983; Chapter IV). Comparative growth studies were performed under strictly controlled environmental conditions in a recirculated water system. All the growth studies on pure species (results presented in Chapter IV) and hybrids were replicated and fed a strictly controlled ration of pelletised food so that growth criteria such as food conversion ratio (FCR) and specific growth rate (SGR) could be calculated.

It was hoped that close control of all environmental and husbandry conditions would allow an accurate assessment of the relative value of the hybrids growth performance over its parental components in a given set of conditions. It was also realised that the conditions in this experiment were very artificial and bear little resemblance to normal culture conditions, particularly as the experiment finished before the onset of maturity in many of the species used.

MATERIALS AND METHODS

The following are the interspecific hybrids used for the growth studies: (1) O. mossambicus x O. aureus, (2) O. niloticus x O. aureus, (3) O. aureus x O. niloticus, (4) O. mossambicus x O. niloticus, (5) O. niloticus x O. mossambicus, (6) O. mossambicus x O. spilurus, (7) O. spilurus x O. mossambicus, (8) O. spilurus x O. macrochir, and (9) O. spilurus x O. niloticus. The convention of standard genetic practice with the female component first has been used in the description.

The detailed procedure of hybridization has been given in Chapter II. The fry obtained were grown in small batteries of tanks connected to a recirculated system, until they were ready for the commencement of growth trials.

The description of the tank system where all the growth trials were performed is given in Chapter IV.

The growth study was continued for 40 days so that water quality did not become a limiting factor. Fishes were bulk weighed at 10 day intervals. Experimental fishes were introduced at a stocking density of 50 fish per 60 litre tank. At least two replicate growth studies were performed concurrently for each of the parental species and hybrid crosses. In some of the crosses more replicate studies were performed to determine any variability in growth pattern of the same hybrids from different parental pairs.

The standard Ewos-Baker (UK) trout grower pelleted food (No. 4) was given twice daily at a feeding rate of 5% of the total body weight of

the fish, and was adjusted accordingly at 10 day intervals after weighing the fish.

Specific growth rate (SGR) and food conversion ratio (FCR) were calculated for the growth trials performed. The difficulties in obtaining fry of the same size meant that the initial weights for all the fish, between the different trials could not be kept the same, therefore, the growth rate was also calculated using a regression equation (mean weight vs. time). In order to see the effects of the initial weight on the growth rate, comparison of regression coefficient values were tested for significant differences by using the F-test (Sokal and Rohlf, 1969, Appendix I). Analysis of variance was applied for SGR and FCR values. As the replicate in every hybrid cross was not equal, the individual mean values were compared using the t-test for ranking.

RESULTS

Growth data for the different hybrid crosses are given in Table 1. The graphical representations are made using the regression equation for each growth trial (Figs. 1-9).

1. O. mossambicus x O. aureus

In this cross 4 separate growth trials were performed originating from two different crosses. Comparison of growth between and within groups, on the basis of regression coefficients using the F test showed no significant difference. Although the initial weights in each group were not equal, the regression analysis showed that the difference in final weights was proportional to the initial weight, giving more or less the same regression coefficient. In this cross the regression coefficient varied between 0.228 (No. 4) and 0.299 (No. 3).

2. O. niloticus x O. aureus

A total of eight growth trials were performed. The fry were obtained from 4 different crosses. The initial weight here varied between 4.43gms (No. 6) and 7.96gms (No. 11) whereas the regression coefficient varied between 0.232 to 0.335 respectively. The regression coefficient values did not differ significantly between separate trials.

3. O. aureus x O. niloticus

Four different growth trials from two separate crosses were performed for this hybrid combination. The regression coefficient varied between 0.478 (No. 14) and 0.635 (No. 16) which is non-significant statistically.

TABLE 1. Growth Data and Regression Equation for all the trials on Different Hybrid Combinations

No.	Hybrid ♀ x ♂	Initial Weight(gm)	Days 10	20	30	40	Regression Equation
1[a]	O. mossambicus O. aureus	3.28 ± 0.04	5.20 ± 0.08	7.79 ± 0.09	10.40 ± 0.03	13.83 ± 0.11	Y = 2.84 + 0.263X
2[a]	"	3.43 ± 0.04	5.12 ± 0.05	7.42 ± 0.18	10.27 ± 0.03	13.76 ± 0.09	Y = 2.84 + 0.258X
3[b]	"	4.06 ± 0.05	6.16 ± 0.05	9.28 ± 0.21	12.07 ± 0.09	16.05 ± 0.15	Y = 3.55 + 0.299X
4[b]	"	4.10 ± 0.03	5.86 ± 0.02	7.91 ± 0.10	10.72 ± 0.26	13.09 ± 0.08	Y = 3.77 + 0.228X
5[a]	O. niloticus O. aureus	4.52 ± 0.10	6.52 ± 0.11	8.54 ± 0.13	12.46 ± 0.12	14.07 ± 0.15	Y = 4.21 + 0.250X
6[a]	"	4.43 ± 0.13	6.16 ± 0.11	7.84 ± 0.23	10.94 ± 0.14	13.63 ± 0.19	Y = 3.96 + 0.232X

No.	Hybrid ♀ x ♂	Initial Weight(gm)	Days 10	20	30	40	Regression Equation
7[b]	O. niloticus O. aureus	5.80 ± 0.14	7.81 ± 0.17	9.90 ± 0.13	13.57 ± 0.15	16.37 ± 0.06	Y = 5.31 + 0.269X
8[b]	"	5.71 ± 0.18	6.62 ± 0.16	8.66 ± 0.09	11.94 ± 0.18	14.94 ± 0.27	Y = 4.82 + 0.238X
9[c]	"	6.26 ± 0.13	8.81 ± 0.17	10.40 ± 0.17	13.57 ± 0.10	16.60 ± 0.12	Y = 6.04 + 0.254X
10[c]	"	5.88 ± 0.05	7.41 ± 0.19	9.20 ± 0.11	13.03 ± 0.11	15.99 ± 0.30	Y = 5.13 + 0.258X
11[d]	"	7.96 ± 0.25	9.31 ± 0.21	13.10 ± 0.25	16.93 ± 0.19	20.92 ± 0.16	Y = 6.94 + 0.335X
12[d]	"	6.37 ± 0.15	8.79 ± 0.14	10.61 ± 0.21	15.26 ± 0.22	18.24 ± 0.23	Y = 5.81 + 0.302X

No.	Hybrid ♀ x ♂	Initial Weight(gm)	Days 10	20	30	40	Regression Equation
13[a]	O. aureus O. niloticus	7.03 ± 0.06	10.85 ± 0.12	16.06 ± 0.19	22.04 ± 0.10	25.73 ± 0.17	Y = 6.62 + 0.486X
14[a]	"	7.50 ± 0.18	12.13 ± 0.02	17.96 ± 0.22	22.47 ± 0.10	26.60 ± 0.14	Y = 7.90 + 0.478X
15[b]	"	7.22 ± 0.08	10.65 ± 0.05	15.91 ± 0.17	22.72 ± 0.18	28.98 ± 0.14	Y = 5.98 + 0.556X
16[b]	"	7.32 ± 0.12	11.14 ± 0.26	17.08 ± 0.19	25.73 ± 0.16	31.79 ± 0.25	Y = 5.91 + 0.635X
17[a]	O. mossambicus O. niloticus	10.22 ± 0.17	13.25 ± 0.17	18.43 ± 0.26	26.73 ± 0.16	30.12 ± 0.09	Y = 9.09 + 0.533X
18[a]	"	10.83 ± 0.13	13.32 ± 0.11	20.58 ± 0.18	28.57 ± 0.20	31.27 ± 0.19	Y = 9.69 + 0.561X

No.	Hybrid ♀ x ♂	Initial Weight(gm)	Days 10	20	30	40	Regression Equation
19[b]	O. mossambicus O. niloticus	6.85 ± 0.16	9.73 ± 0.24	13.59 ± 0.15	21.16 ± 0.21	25.84 ± 0.21	Y = 5.55 + 0.494X
20[b]	"	6.52 ± 0.17	9.42 ± 0.04	12.65 ± 0.15	20.90 ± 0.14	26.69 ± 0.23	Y = 4.87 + 0.518X
21[a]	O. niloticus O. mossambicus	3.40 ± 0.14	4.80 ± 0.13	6.32 ± 0.14	9.33 ± 0.27	13.47 ± 0.13	Y = 2.53 + 0.247X
22[a]	"	2.84 ± 0.17	3.92 ± 0.07	6.15 ± 0.19	8.77 ± 0.19	12.83 ± 0.19	Y = 1.94 + 0.248X
23[a]	O. mossambicus O. spilurus	2.88 ± 0.15	3.97 ± 0.15	5.67 ± 0.12	7.97 ± 0.18	10.86 ± 0.21	Y = 2.28 + 0.200X
24[b]	"	3.00 ± 0.09	3.84 ± 0.10	5.46 ± 0.11	7.31 ± 0.20	9.64 ± 0.21	Y = 2.50 + 0.168X

No.	Hybrid ♀ x ♂	Initial Weight(gm)	Days 10	20	30	40	Regression Equation
25[a]	O. spilurus O. mossambicus	3.09 ± 0.16	4.22 ± 0.05	5.98 ± 0.08	8.03 ± 0.10	10.78 ± 0.14	Y = 2.58 + 0.192X
26[b]	"	3.71 ± 0.14	4.85 ± 0.16	6.14 ± 0.19	7.61 ± 0.13	9.72 ± 0.15	Y = 3.45 + 0.148X
27[a]	O. spilurus O. macrochir	4.84 ± 0.15	6.21 ± 0.11	8.83 ± 0.08	12.79 ± 0.19	18.22 ± 0.15	Y = 3.51 + 0.333X
28[b]	"	4.59 ± 0.12	5.87 ± 0.13	8.69 ± 0.13	11.88 ± 0.11	15.78 ± 0.18	Y = 3.68 + 0.284X
29[a]	O. spilurus O. niloticus	2.92 ± 0.30	4.84 ± 0.15	7.96 ± 0.10	11.57 ± 0.34	17.62 ± 0.21	Y = 1.76 + 0.361X
30[b]	"	2.30 ± 0.19	3.57 ± 0.17	5.54 ± 0.11	9.23 ± 0.18	13.00 ± 0.20	Y = 1.32 + 0.271X

The same superscript in any given cross indicates the same parental pair

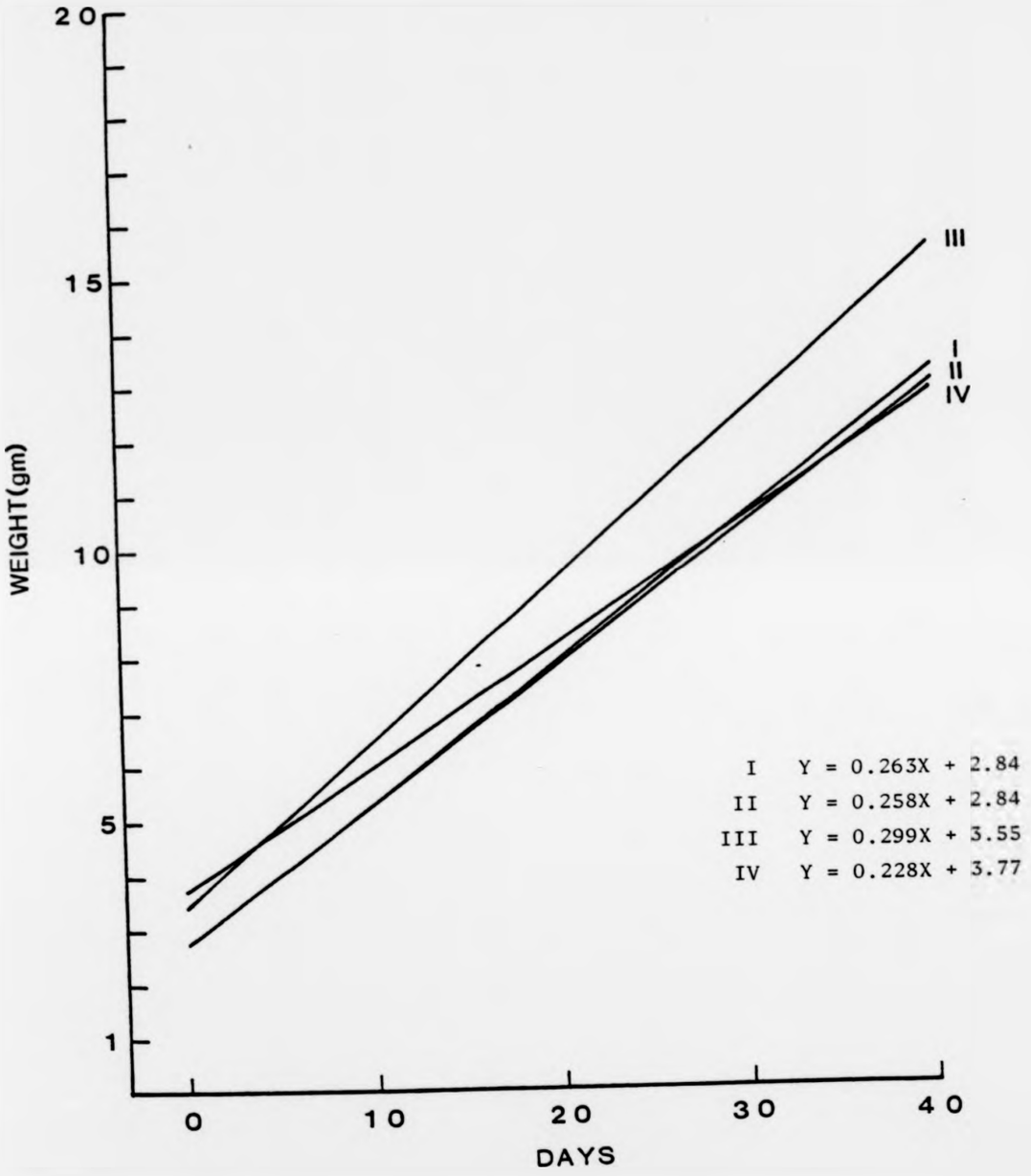

FIG. 1. Regression lines for the growth comparisons between different trials of the hybrid O. mossambicus female x O. aureus male.

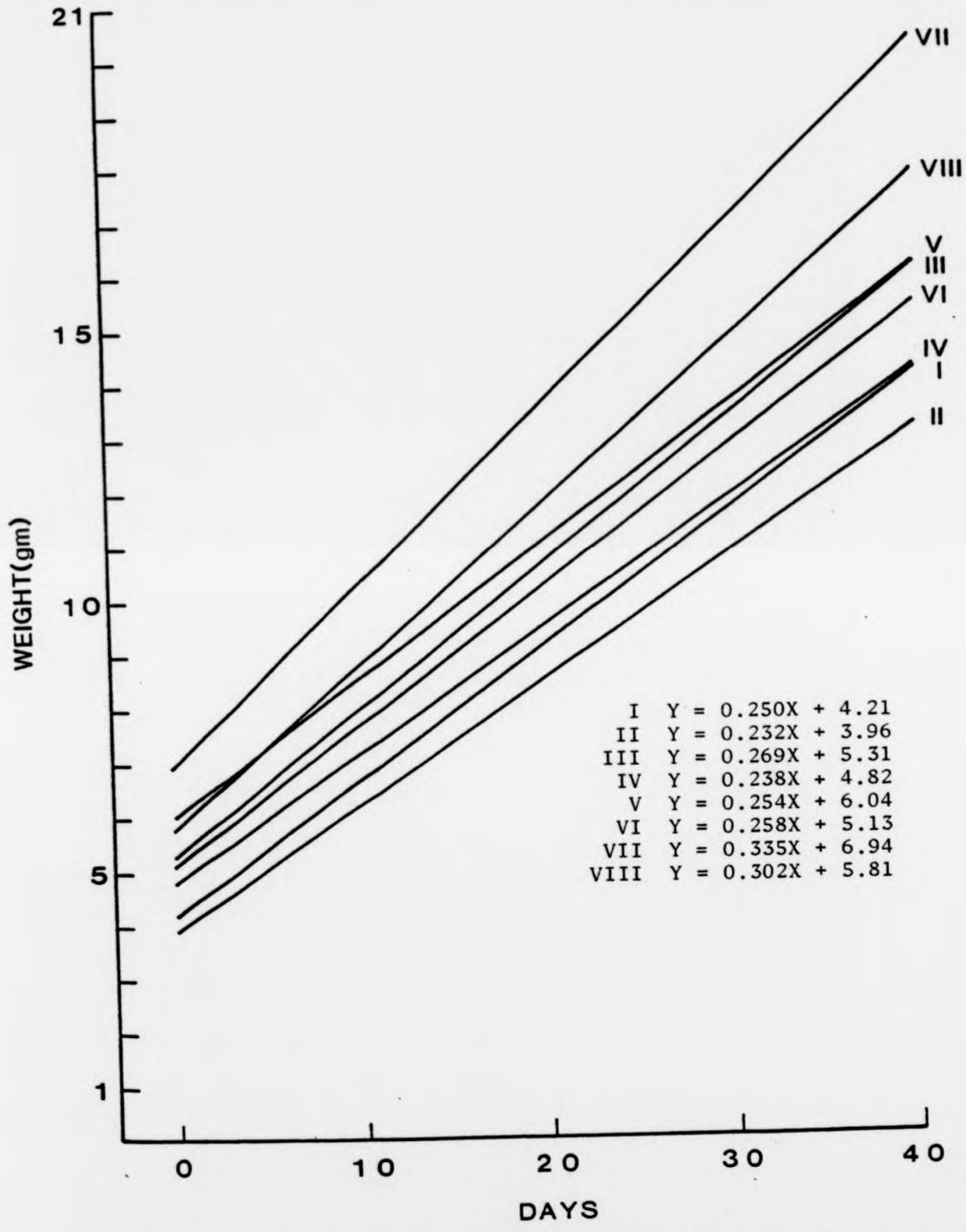

FIG. 2. Regression lines for the growth comparisons between different trials of the hybrid O. niloticus female x O. aureus male.

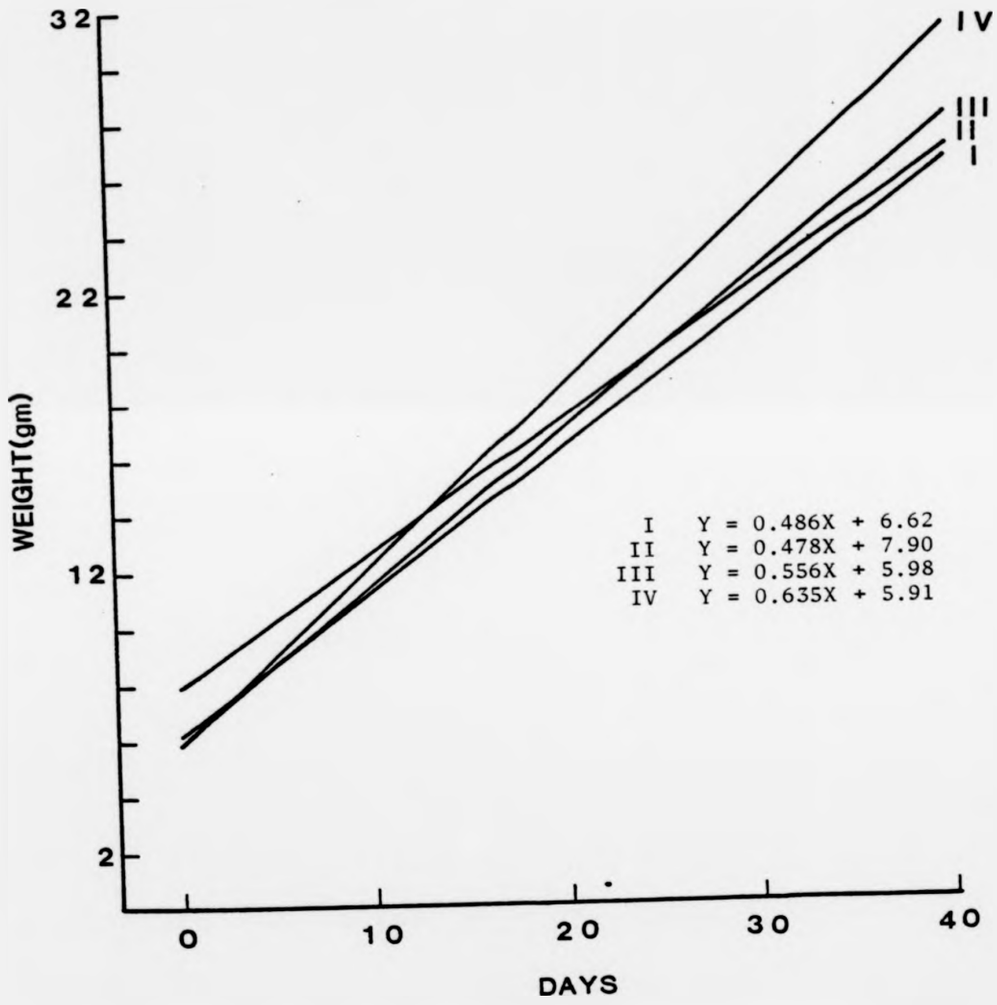

FIG. 3. Regression lines for the growth comparisons between different trials of the hybrid O. aureus female x O. niloticus male.

4. O. mossambicus x O. niloticus

From two separate crosses, four different replicate growth trials were performed for this cross. The initial weights in different replicates varied between 6.52gms (No. 20) and 10.83gms (No. 18). Although some variations were observed for the regression coefficients in these four trials the differences were not statistically significant.

5. O. niloticus x O. mossambicus

Two different growth trials were performed from the same parental cross. The initial weights and regression coefficients were not significantly different between the two trials.

6. O. mossambicus x O. spilurus

Two different growth trials were performed for this cross from two different sets of parents. The growth rate in the form of a regression coefficient when compared does not show any significant difference.

7. O. spilurus x O. mossambicus

The regression coefficient values in these two trials do not vary significantly. The fish for these trials came from two separate crosses.

8. O. spilurus x O. macrochir

Two separate growth trials were performed for this cross. The fry for these trials came from two separate crosses. The regression coefficient varied between 0.284 (No. 28) and 0.333 (No. 27), which is statistically non-significant.

4. O. mossambicus x O. niloticus

From two separate crosses, four different replicate growth trials were performed for this cross. The initial weights in different replicates varied between 6.52gms (No. 20) and 10.83gms (No. 18). Although some variations were observed for the regression coefficients in these four trials the differences were not statistically significant.

5. O. niloticus x O. mossambicus

Two different growth trials were performed from the same parental cross. The initial weights and regression coefficients were not significantly different between the two trials.

6. O. mossambicus x O. spilurus

Two different growth trials were performed for this cross from two different sets of parents. The growth rate in the form of a regression coefficient when compared does not show any significant difference.

7. O. spilurus x O. mossambicus

The regression coefficient values in these two trials do not vary significantly. The fish for these trials came from two separate crosses.

8. O. spilurus x O. macrochir

Two separate growth trials were performed for this cross. The fry for these trials came from two separate crosses. The regression coefficient varied between 0.284 (No. 28) and 0.333 (No. 27), which is statistically non-significant.

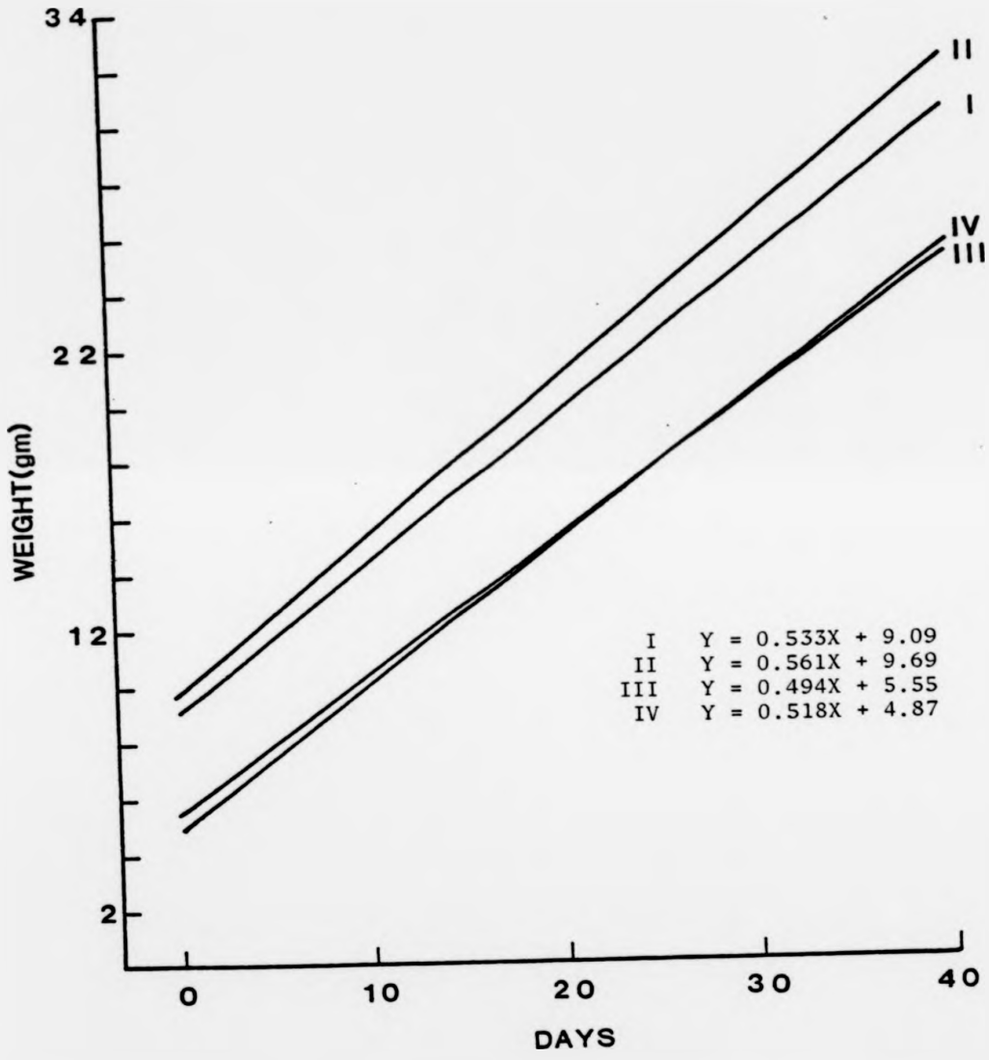

FIG. 4. Regression lines for the growth comparisons between different trials of the hybrid O. mossambicus female x O. niloticus male.

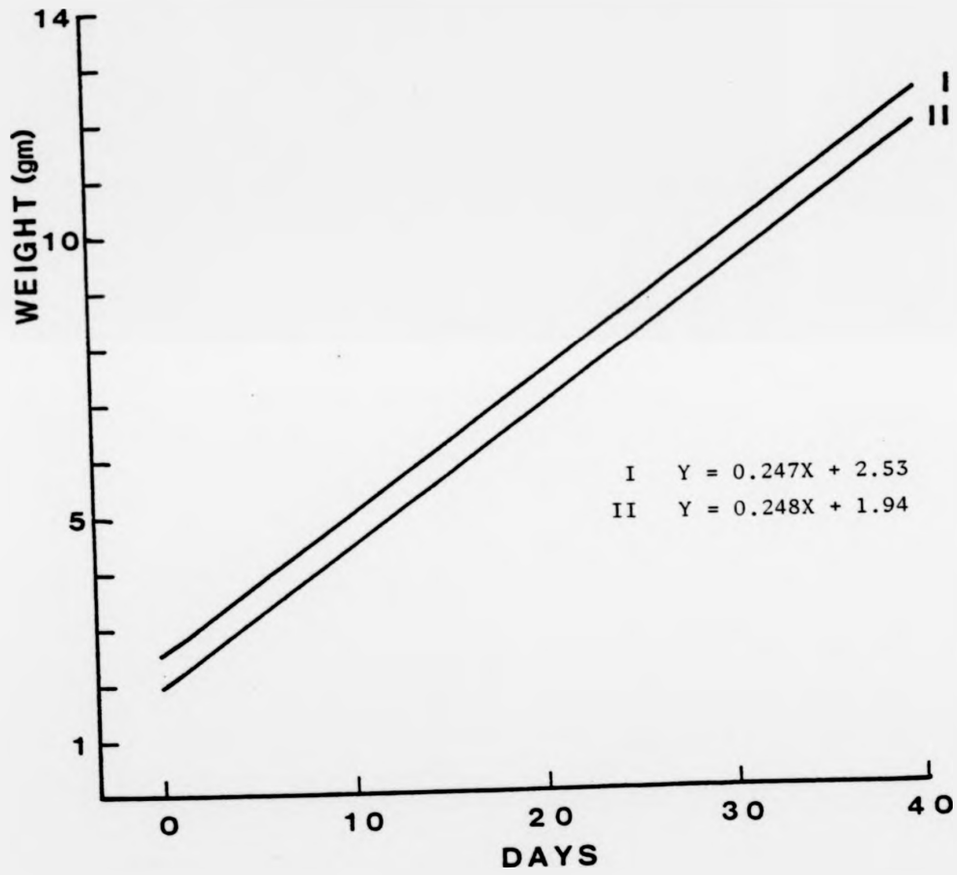

FIG. 5. Regression lines for the growth comparisons between different trials of the hybrid O. niloticus female x O. mossambicus male.

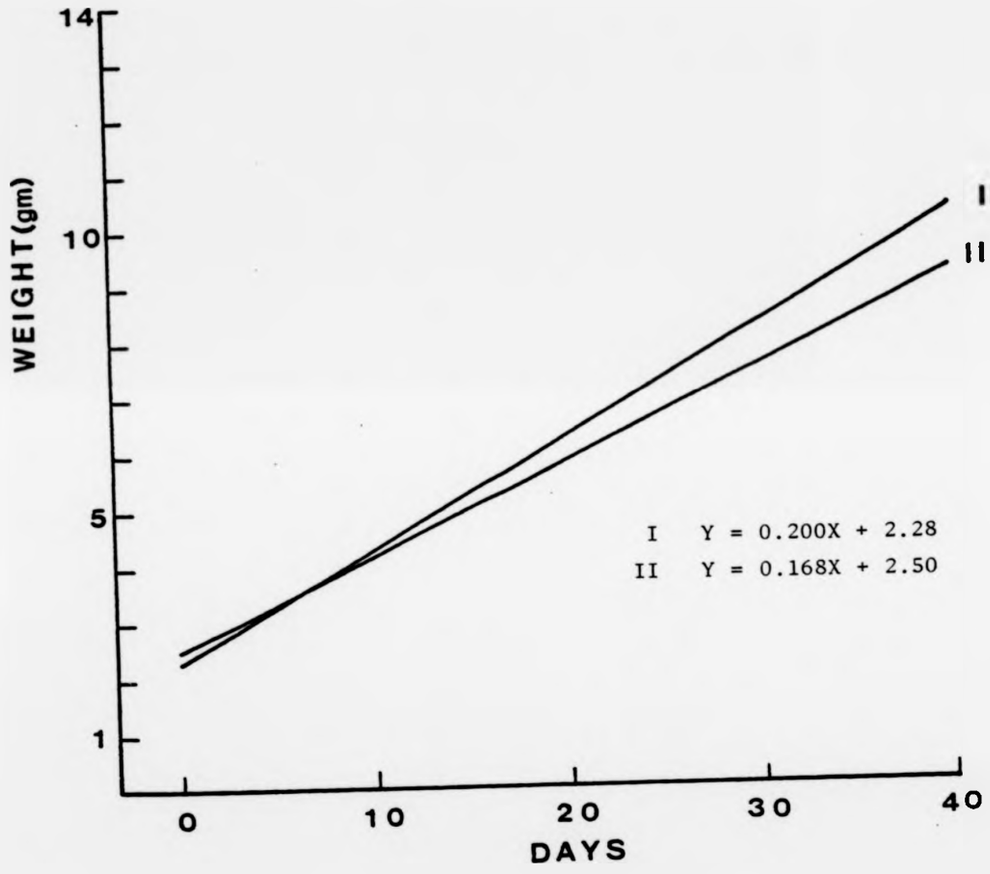

FIG. 6. Regression lines for the growth comparisons between different trials of the hybrid O. mossambicus female x O. spilurus male.

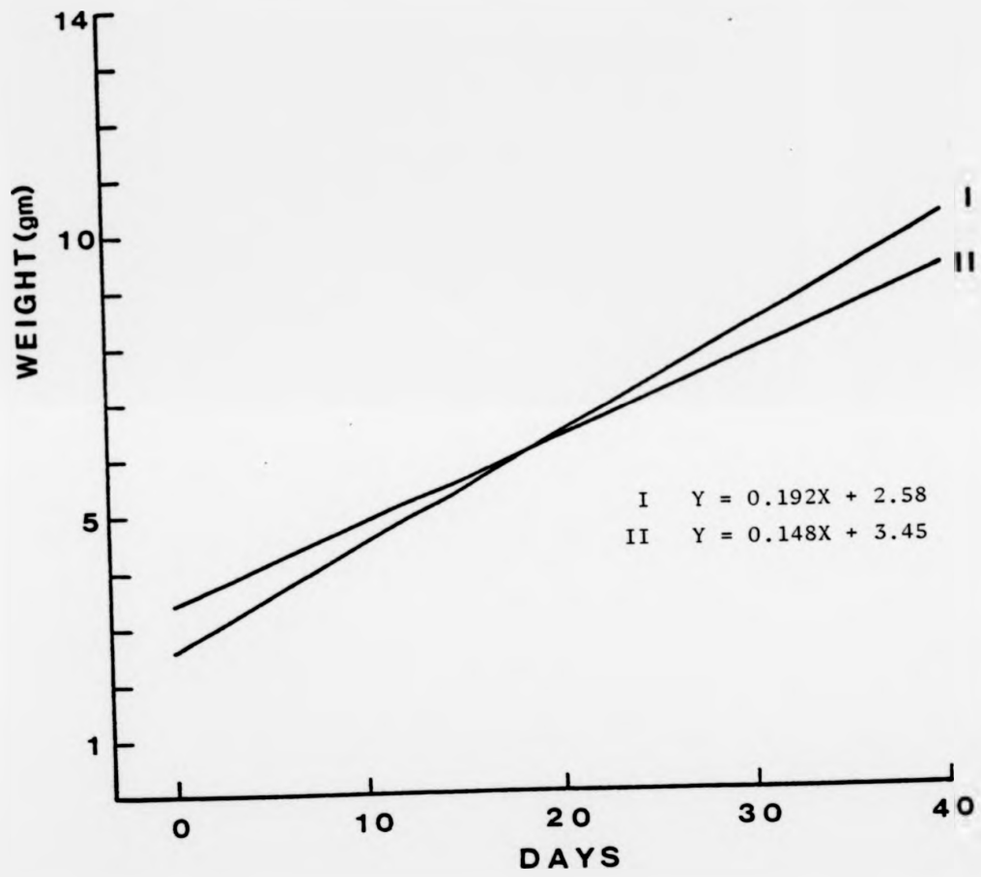

FIG. 7. Regression lines for the growth comparisons between different trials of the hybrid O. spilurus female x O. mossambicus male.

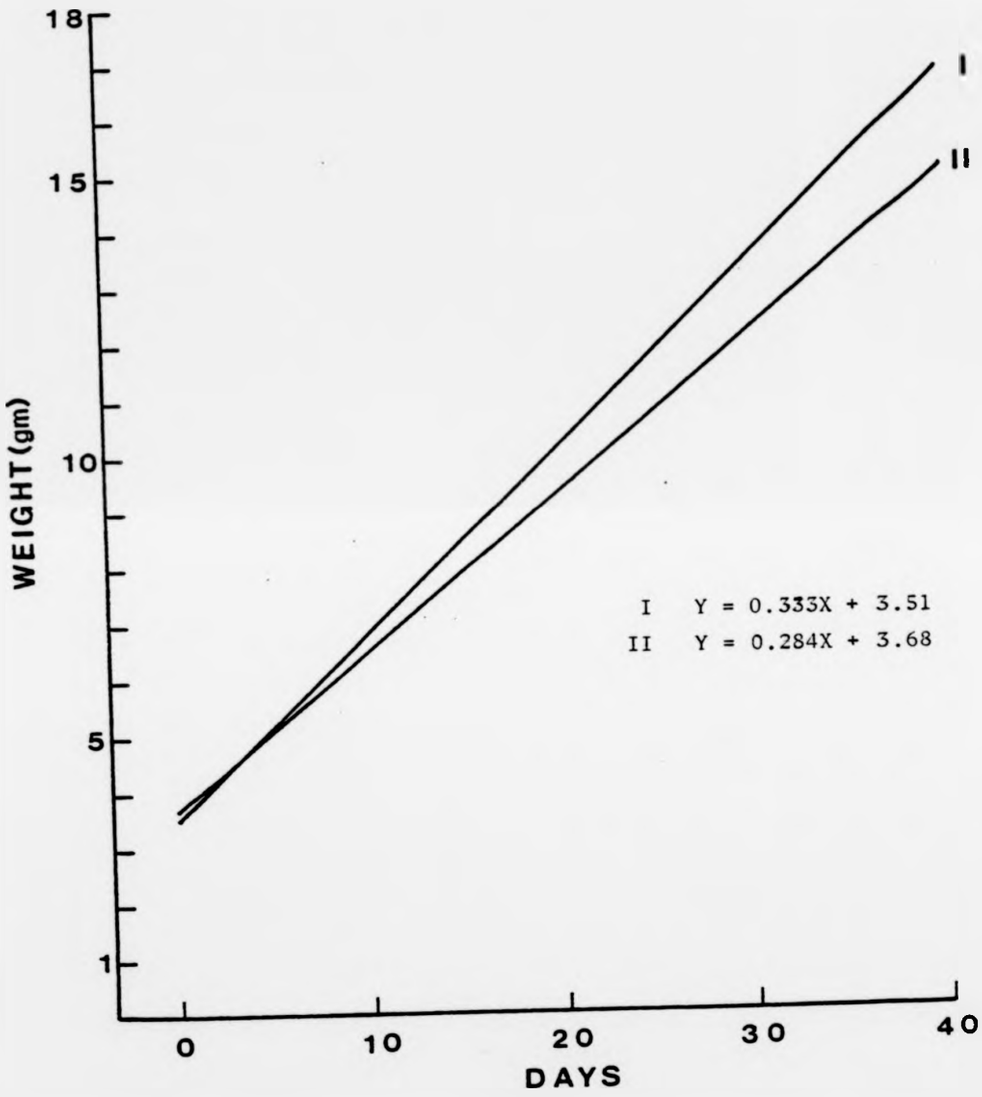

FIG. 8. Regression lines for the growth comparisons between different trials of the hybrid O. spilurus female x O. macrochir male.

9. O. spilurus x O. niloticus

The regression coefficient varied between 0.271 (No. 30) and 0.361 (No. 29) although the initial weights were 2.30gms and 2.92gms respectively for the two crosses. The difference in regression coefficients is not statistically significant.

The results of comparison of regression coefficients between the replicates of each hybrid is given in Table 2.

Specific Growth Rate (SGR)

The mean SGR values for different crosses are given in Table 3. The lowest and highest values were obtained for O. spilurus x O. mossambicus (2.77) and O. spilurus x O. niloticus (4.41) respectively. An analysis of variance test with SGR values shows a significant difference at the 1% level. For ranking of all these values, individual t-tests were performed, as the replicate in each cross could not be kept equal and comparable where Duncan's (1955) multiple range test would have been appropriate. From the ranking, three different groupings became apparent. The first group includes cross Nos. 2, 4 and 7 with the smallest SGR values. The highest SGR was obtained from cross No. 9 which is the sole representative in the second group. The third group includes the rest of the crosses (Nos. 1, 3, 5, 6 and 8) with SGR values intermediate between the other two groups. Interestingly the SGR significantly differs between O. niloticus x O. aureus (2.58) and its reciprocal cross (3.39).

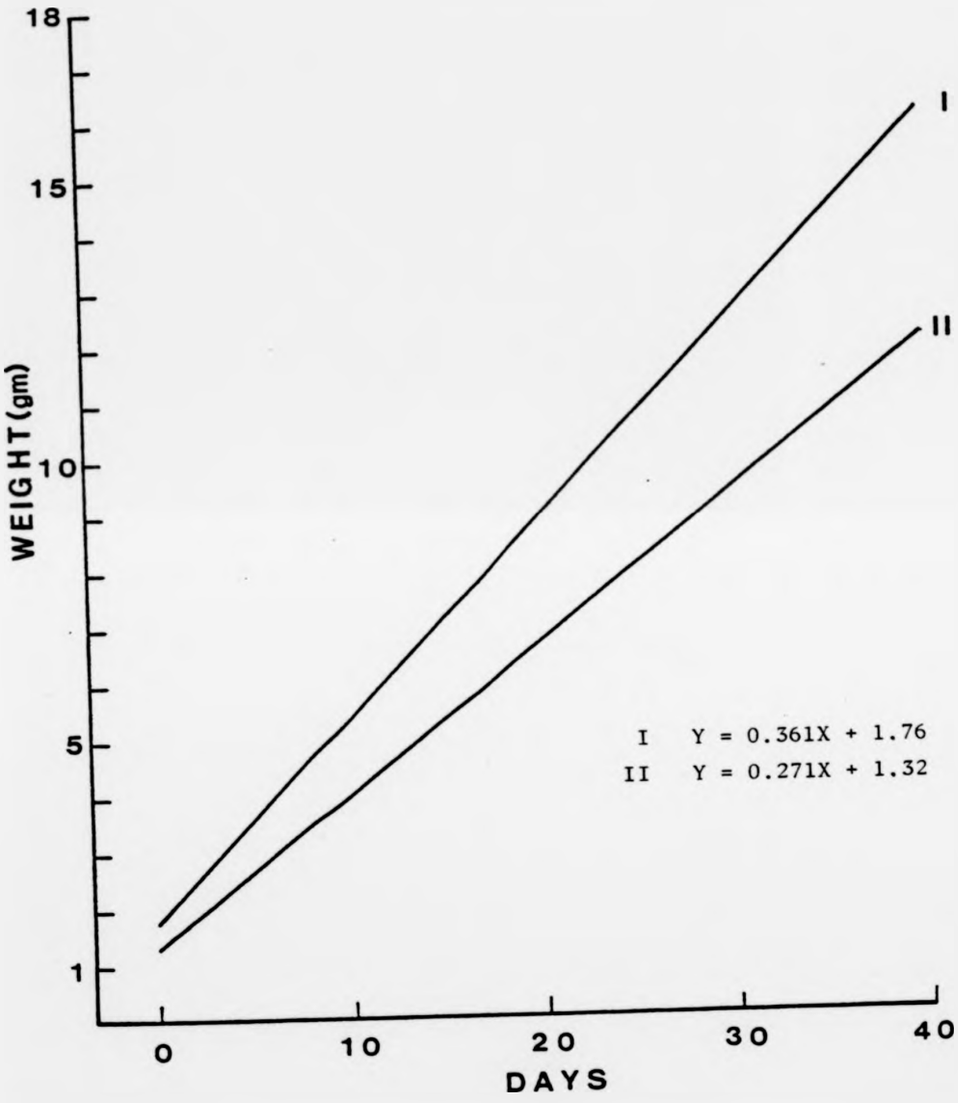

FIG. 9. Regression lines for the growth comparisons between different trials of the hybrid O. spilurus female x O. niloticus male.

TABLE 2. Shows the F-test Results to test for Significant Differences in the Regression Lines within each of the Hybrid Crosses

	MxA I	MxA II	MxA III	MxA IV	NxA I	NxA II	NxA III	NxA IV	NxA V	NxA VI	NxA VII	NxA VIII	AxN I	AxN II	AxN III	AxN IV
M x A I		0.039	2.352	3.450												
M x A II			2.300	1.683												
M x A III				11.336												
M x A IV																
N x A I						0.857	0.494	0.123	0.024	0.057	5.715	2.464				
N x A II							2.062	0.032	0.828	0.649	8.853	4.754				
N x A III								0.857	0.372	0.114	3.574	1.036				
N x A IV									0.245	0.267	5.685	2.753				
N x A V										0.016	5.760	2.371				
N x A VI											3.715	1.327				
N x A VII												0.686				
N x A VIII																
A x N I														0.090	2.538	8.462
A x N II															3.657	10.495
A x N III																1.743
A x N IV																

M = O. mossambicus, A = O. aureus, N = O. niloticus

	MxN I	MxN II	MxN III	MxN IV	NxM I	NxM II	MxS I	MxS II	SxM I	SxM II	SxMA I	SxMA II	SxN I	SxN II
M x N I		0.134	0.331	0.036										
M x N II			0.080	0.258										
M x N III				0.097										
M x N IV														
N x M I						0.004								
N x M II														
M x S I								1.422						
M x S II														
S x M I										4.657				
S x M II														
S x MA I												0.823		
S x MA II														
S x N I														2.674
S x N II														

M = O. mossambicus , A = O. aureus, N = O. niloticus, S = O. spilurus, MA = O. macrochir

TABLE 3. Specific Growth Rate (SGR) and Food Conversion Ratio (FCR) in Different Hybrids

No.	Hybrid	SGR Mean ± SE	FCR Mean ± SE
1	O. mossambicus x O. aureus	3.35 ± 0.13^{b}	1.36 ± 0.06^{b}
2	O. niloticus x O. aureus	2.58 ± 0.05^{a}	1.75 ± 0.02^{c}
3	O. aureus x O. niloticus	3.39 ± 0.09^{b}	1.40 ± 0.06^{b}
4	O. mossambicus x O. niloticus	3.05 ± 0.18^{ab}	1.52 ± 0.11^{bc}
5	O. niloticus x O. mossambicus	3.61 ± 0.12^{b}	1.14 ± 0.03^{b}
6	O. mossambicus x O. spilurus	3.12 ± 0.13^{b}	1.38 ± 0.06^{bc}
7	O. spilurus x O. mossambicus	2.77 ± 0.25^{ab}	1.62 ± 0.16^{bc}
8	O. spilurus x O. macrochir	3.20 ± 0.07^{b}	1.30 ± 0.05^{b}
9	O. spilurus x O. niloticus	4.41 ± 0.05^{c}	0.95 ± 0.01^{a}

Figures with common superscripts in the same column are not statistically significant ($P > 0.01$).

Food Conversion Ratio (FCR)

FCR is also given in Table 3 for all the hybrid combinations studied. Ranking of the different hybrid combinations on the basis of FCR gave: O. spilurus x O. niloticus < O. mossambicus x O. aureus; O. aureus x O. niloticus; O. mossambicus x O. spilurus and its reciprocal cross; O. spilurus x O. macrochir and its reciprocal cross < O. niloticus x O. aureus. The FCR values again differed significantly in the reciprocal cross of O. niloticus female x O. aureus male indicating the possibility of a sex related influence.

From the comparison of both SGR and FCR it seems that the O. spilurus x O. niloticus cross gives the best performance. The worst performance was recorded in O. niloticus x O. aureus as far as the growth is concerned in the stipulated condition of culture. The other crosses were shown to be intermediate between the above mentioned crosses.

Comparison of SGR and FCR Between Parents and Their Hybrids

Comparison of SGR and FCR between the parental species and their hybrids are given in Figs. 10 and 11. All the SGR and FCR values for the hybrids remained intermediate to their parents except O. spilurus x O. niloticus, where the SGR was higher than both the parents. The FCR on the other hand was slightly lower than both the parents. To evaluate the statistical significance a t-test of individual parent and hybrids was performed which is given in Table 4. The results of this test can be summarised as follows:

1) O. mossambicus x O. aureus hybrid had a significantly higher SGR and lower FCR value compared with O. aureus, whereas no significant

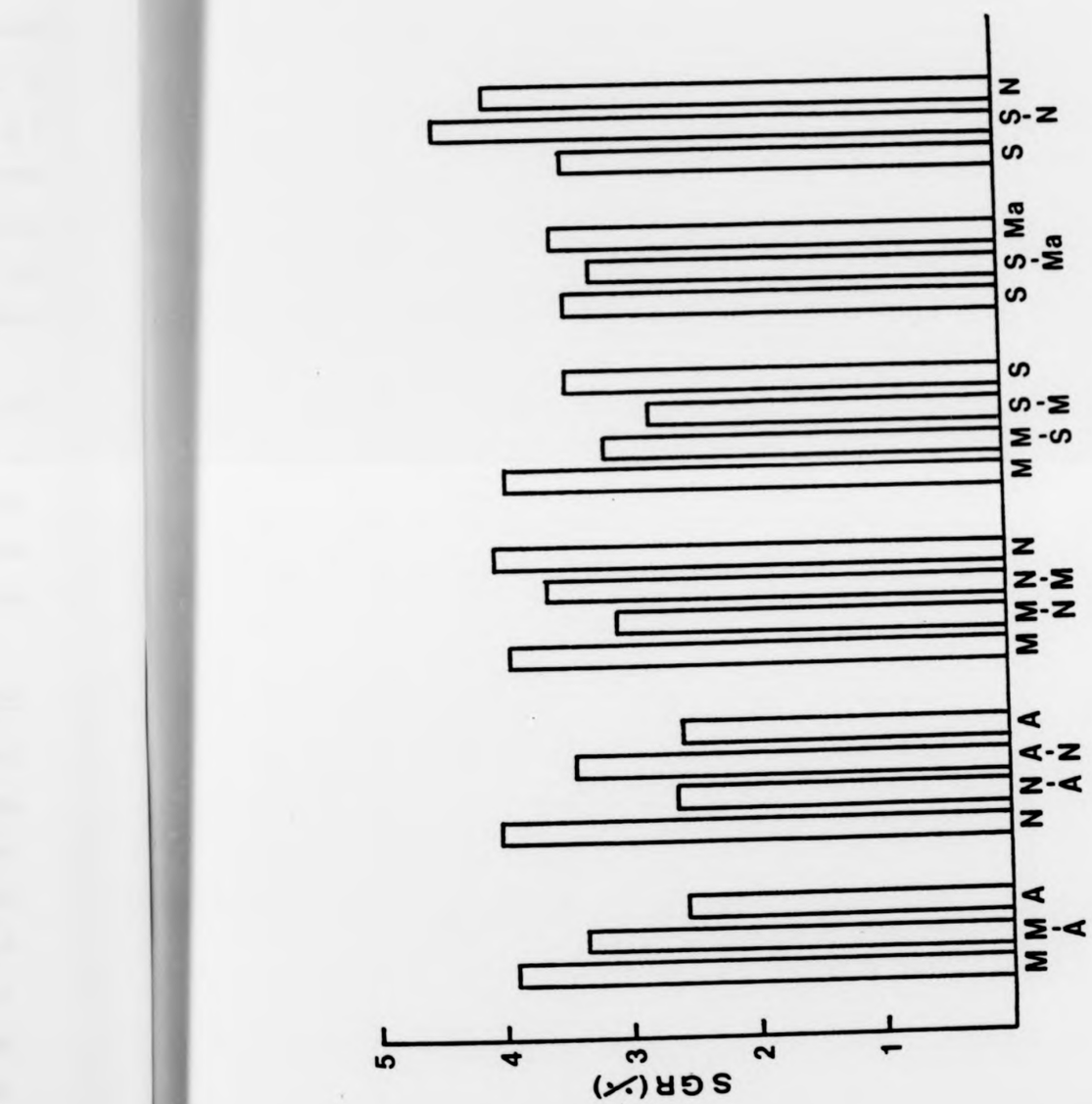

FIG. 10. Comparison of Specific Growth Rate (SGR) between parents and their hybrids. M = O. mossambicus, A = O. aureus, N = O. niloticus, S = O. spilurus, Ma = O. macrochir.

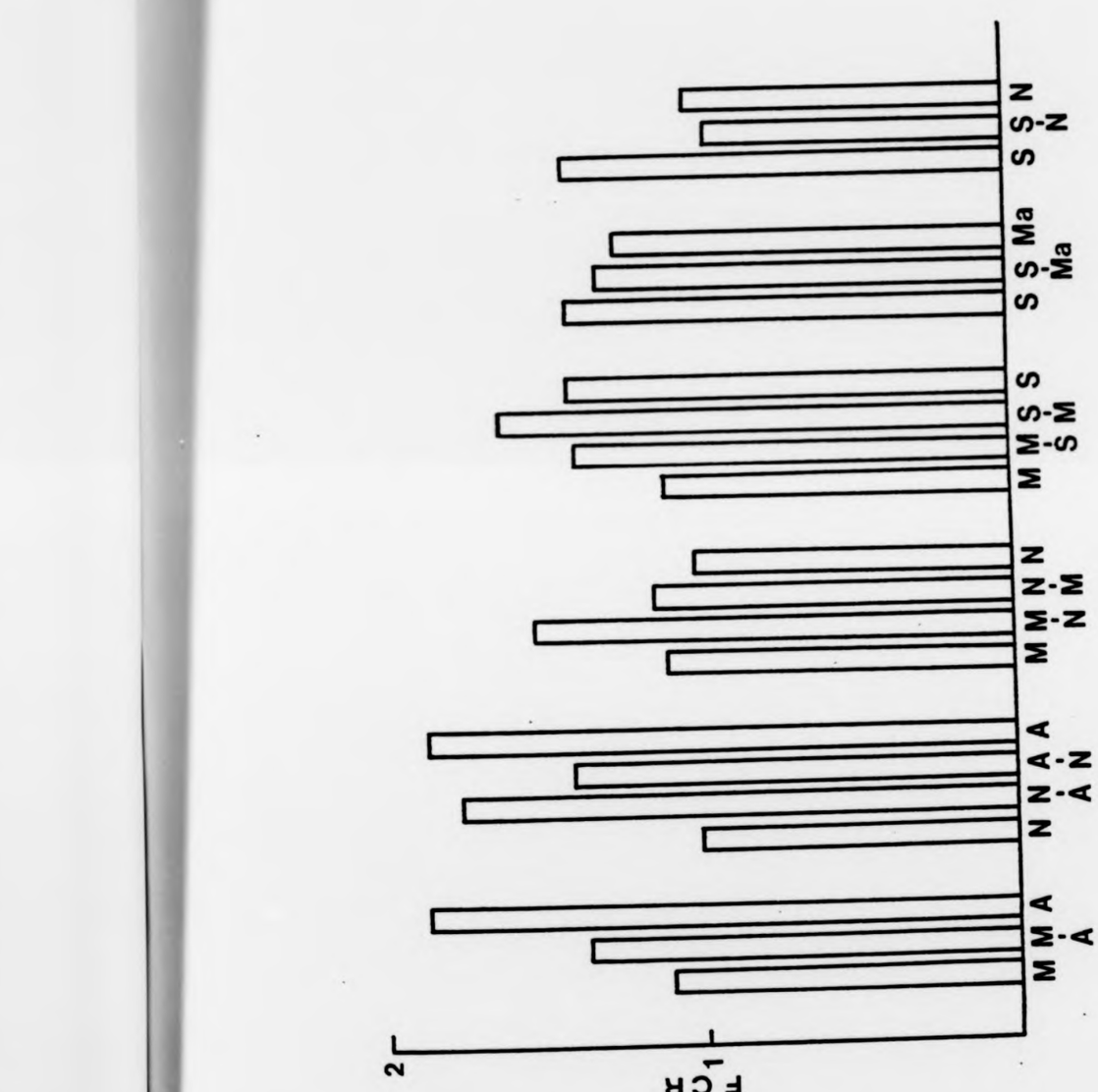

FIG. 11. Comparison of Food Conversion Ratio (FCR) between parents and their hybrids. M = O. mossambicus, A = O. aureus, N = O. niloticus, S = O. spilurus, Ma = O. macrochir.

TABLE 4. 't' Values Obtained by Comparing SGR and FCR between Parental Species and their Hybrids

Hybrid		Parents	
O. mossambicus x O. aureus (4)		O.mossambicus(2)	O.aureus(2)
	SGR	4.02	6.06*
	FCR	4.33	8.22*
O. niloticus x O. aureus (8)		O.niloticus(2)	O.aureus(2)
	SGR	11.67*	0.39
	FCR	13.74*	4.92*
O. aureus x O. niloticus (4)	SGR	4.22	9.16*
	FCR	4.99*	7.56*
O. mossambicus x O. niloticus (4)		O.mossambicus(2)	O.niloticus(2)
	SGR	4.60	4.45
	FCR	3.81	4.22
O. niloticus x O. mossambicus (2)	SGR	2.31	2.33
	FCR	4.66	4.74
O. mossambicus x O. spilurus (2)		O.mossambicus(2)	O.spilurus(2)
	SGR	5.67	1.96
	FCR	4.66	0.15
O. spilurus x O. mossambicus (2)	SGR	4.47	4.33
	FCR	3.24	1.41
O. spilurus x O. macrochir (2)		O.spilurus(2)	O.macrochir(2)
	SGR	2.12	0.99
	FCR	1.54	0.94
O. spilurus x O. niloticus (2)		O.spilurus(2)	O.niloticus(2)
	SGR	11.62*	3.47
	FCR	13.91*	1.17

* $P < 0.01$

difference was found with O. mossambicus. The ranking between these is as follows: O. mossambicus ≥ O. mossambicus x O. aureus > O. aureus.

2) O. niloticus x O. aureus. The SGR and FCR values were significantly different when compared to the values of SGR and FCR obtained for O. niloticus whereas only the FCR was significantly different from the FCR obtained for O. aureus. In the reciprocal cross both the SGR and FCR were significantly different from O. aureus. The ranking of parents and hybrids may be as follows: O. niloticus > O. aureus x O. niloticus > O. niloticus x O. aureus > O. aureus.

3) In O. mossambicus x O. niloticus and its reciprocal cross no significant difference was found between their parents as far as the SGR and FCR values were concerned.

4) O. mossambicus x O. spilurus. No significant difference was found in the SGR and FCR values compared with its parents. Similar results were found for the reciprocal cross.

5) O. spilurus x O. macrochir. The SGR and FCR values remained similar in this cross as compared to their parents.

6) O. spilurus x O. niloticus. The SGR and FCR values obtained for the hybrid were significantly different from values obtained for O. spilurus. The ranking is as follows: O. niloticus ≥ O. spilurus x O. niloticus > O. spilurus.

DISCUSSION

In the previous chapter (Chapter IV) it has been mentioned that in any comparative growth experiments the final weight after the experimental period can be considered as one of the best criteria for the evaluation of the growth potency of any species. The assumption of such a criterion strictly depends on the initial weight, which should be the same for every species at the beginning of the experiments. The reason for such consideration is explained exhaustively by Wohlfarth and Moav (1972) in their classical paper on common carp (Cyprinus carpio). They have shown that growth rate depends on the initial weight of the fish. They have formulated an equation for correcting the initial weight bias for growth rate between strains where initial weight was unequal. Such criteria have been used by several workers (Moav et al., 1975, 1978; Wohlfarth et al., 1975, 1983; Ayles and Baker, 1983) in comparing growth rates of fish in different experimental conditions. All these studies were mainly confined to the different strains of common carp (Cyprinus carpio) and rainbow trout (Salmo gairdneri). In tilapia most of the experimental growth studies have used fish of equal size at the beginning of the experiments (Anderson and Smitherman, 1978; Collis and Smitherman, 1978; Hepher and Pruginin, 1982; Coche, 1982; Jauncey, 1982; Appler and Jauncey, 1983). Fram and Pagan Font (1978) on the other hand used different sizes of fish in their growth studies. To analyse their data they used the extrapolation method of Swingle (1965) who mentioned that condition factor (C) = $(\text{weight} \times 10^5)/(\text{length}^3)$. This relationship is based on the length and weight relationship in fish and has been used by Hopkins (1977) in O. aureus, and Silvera (1978) in O. niloticus for the determination of age. But this relationship

is calculated from the larger size fishes which could not be used in the present experimental series.

In the present series of experiments on the growth studies of different hybrids it was impossible to obtain fish of the same initial weight. Therefore, other criteria such as SGR and FCR have been used to calculate the growth rate. The SGR is strictly dependent on the linearity of the growth against time. To prove the linearity of growth in the replicate trials with different initial weights linear regressions on trials of the same species or hybrids have been calculated individually. The linear regressions in the form of regression coefficients have been compared by the standard F-test (Sokal and Rohlf, 1969) between different trials of the same hybrids with different initial weights. Table 2 is the compilation of all the results of such comparisons. It is clear that none of the regression coefficient values in the replicate studies show any significant differences. This clearly indicates that the initial size of fish although different in the same cross does not affect the growth relationship up to the size difference used in the present studies. This also proves that different parents have no significant effect on the growth of the hybrids in this study as long as they are in the same combination. Wohlfarth and Moav (1972) working on C. carpio found a different relationship which is not in agreement with the present results. They mentioned that

> "the coefficient of regression of weight gain on initial weight increases with increased weight gain but decreases with increased initial weight."

One of the reasons for such a difference may be due to the difference in size groups used in the two experiments (present study 2-10gms;

Wohlfarth and Moav (1972) 10-40gms), and the differences in growth patterns between tilapia and carp.

From the above discussion it is clear that as the replicate experiments do not show any difference in growth rate, comparison between hybrids of different combinations could be made on the basis of SGR and FCR. Comparative data on SGR and FCR (Table 3) clearly indicate that O. spilurus x O. niloticus has the best values, therefore, indicating its superiority in growth performance over the other hybrid combinations at this stage of the growth curve. However, the relative value of the different hybrids may vary after maturity. This study is the only one where so many hybrids of tilapia have been compared under one set of conditions. No other comparable reports have been found in the literature. It is obvious that other studies could be undertaken which cover the whole growth cycle of many more species and hybrids under a whole range of culture and environmental conditions.

Hybridization has been extensively used by plant and animal breeders because the progeny of inter-strain crosses often out perform the parental lines for any given character. Such improvements in the quantitative characters in the hybrids are often described as hybrid vigour or heterosis (Falconer, 1964). Dobzhansky (1952) on the other hand used another term "luxuriance" for the hybrid vigour in the inter-specific hybrids. He also mentioned that whether heterosis and luxuriance are really different phenomena remains to be determined and

> "at present these words may only be regarded as vehicles of a certain working hypothesis which may or may not be verified by further work."
>
> (Dobzhansky, 1955)

Regarding the cause of hybrid vigour in the inter-strain crosses it is often explained by the increased genetic variation in the hybrid compared to the inbred parental strains (Falconer, 1964). On the other hand

> "in many instances it is rather hard to see why parents of the luxuriant hybrids should be regarded as inbred, or how they could have acquired coadapted gene complexes."
>
> (Dobzhansky, 1955)

Recently a number of workers (Pruginin et al., 1975; Chevassus, 1983; Smitherman et al., 1983) used the term heterosis to explain the hybrid vigour in interspecific hybrids. Regarding the controversy over the use of these two terms Dobzhansky (1955) wrote that

> "definitions have their important uses, but they should be framed and modified to help rather than to hinder, analysis of the phenomena of nature which we attempt to understand and to explain."

Leaving aside the cause of heterosis or luxuriance the hybrid vigour or heterosis can in general be expressed as the difference between the F1 progeny and the mid parent values using some measurable criteria such as growth rate and it depends for its occurrence on the dominance of the gene(s) responsible for the characters under observation. Loci without dominance cause neither inbreeding depression nor heterosis. If the two populations which are crossed to obtain the F1 do not differ in gene frequency for these characters, there will be no heterosis. On the other hand heterosis depends on the total effect of the genotype. If some loci are dominant in one direction and some in the other, their effect will tend to cancel each other, and no heterosis may be observed in spite of the dominance at individual loci. So the occurrence of heterosis depends on directional dominance (Falconer, 1964).

The effect of individual gene components on the expression of a phenotypic effect on the F1 has been mentioned by several authors (Bucio-Alanis, 1966; Bucio-Alanis and Hill, 1966; Perkins and Jinks, 1968; Bucio-Alanis et al., 1969; Knight, 1971). According to the model, heterosis can be expressed on the basis of the potence ratio (D/A) (Bucio-Alanis, 1966; Bucio-Alanis et al., 1969). The 'D' represents the deviation of the cross bred from the mid parent value (mean of Parent I plus Parent II divided by 2) and 'A' half the difference between the parents. The relative magnitude of these components determines the following:

(a) heterosis where $D > A$, (b) complete dominance where $D = A$, and (c) incomplete dominance where $D < A$. Heterosis is then considered to occur only when hybrids exceed the best parent for any given character (Ayles and Baker, 1983). But this does not take into account any statistical significance between such values. The use of such criteria for expressing the levels of heterosis in certain hybrids has been made in the crosses between strains of common carp and rainbow trout (Moav et al., 1975; Wohlfarth et al., 1975; Ayles et al., 1979; Ayles and Baker, 1983).

In the tilapia hybrids under investigation use of the potence ratio data shows that one cross (O. spilurus x O. niloticus) out of nine shows heterosis when the specific growth rate (Table 5, Fig. 10) is taken as the phenotypic character in question. In the O. mossambicus x O. aureus and the O. aureus x O. niloticus crosses D is positive whereas in the other crosses D has negative values. According to the potence ratio where $D < A$ this indicates incomplete dominance. A negative value represents the incomplete dominance of the slow growth rate over the

TABLE 5. Potence Ratio based on the SGR for the Hybrids and their Mid parent Values

No.	Hybrid	Mid parent	A	D	D/A
1	O. mossambicus x O. aureus	3.235	0.675	0.115	0.170
2	O. niloticus x O. aureus	3.275	0.715	-0.695	-0.972
3	O. aureus x O. niloticus	3.275	0.715	0.115	0.160
4	O. mossambicus x O. niloticus	3.950	0.040	-0.900	-22.500
5	O. niloticus x O. mossambicus	3.950	0.040	-0.340	-8.500
6	O. mossambicus x O. spilurus	3.660	0.250	-0.540	-2.160
7	O. spilurus x O. mossambicus	3.660	0.250	-0.890	-3.560
8	O. spilurus x O. macrochir	3.450	0.040	-0.250	-6.250
9	O. spilurus x O. niloticus	3.70	0.290	0.710	2.448

higher growth rate of the respective species. For calculation of the mid parent value the data from Chapter IV have been used for the pure species.

For easier comparison of the food utilization and also to get positive values the food efficiency rate (FER) was calculated. This is the ratio of total weight gain and total food used, which is just opposite to the food conversion ratio (FCR) as mentioned in Chapter IV. The FER values are given in Table 6 and Fig. 12 along with their parents. Using the potence ratio, heterosis has been found in the case of the O. spilurus x O. niloticus hybrid. The cross between O. mossambicus x O. aureus and O. spilurus x O. macrochir showed a positive value, whereas the rest of the hybrids gave negative values. This again indicates incomplete dominance. The negative value is the evidence of the dominance of a low FER over high FER in different species.

It is interesting to note that the comparison of SGR between hybrids of certain species pairs and their reciprocal crosses gave a different potence ratio. These were O. niloticus x O. aureus, O. mossambicus x O. niloticus and O. mossambicus x O. spilurus. In the case of O. niloticus x O. aureus it is negative whereas in the reciprocal cross it is positive. In O. mossambicus x O. niloticus and its reciprocal cross the potence ratio is negative but the former has a higher value than the latter. In O. mossambicus x O. spilurus and its reciprocal cross again the potence ratio is negative but the latter has a higher value than the former. Comparison of the FER gave the following results: (a) the O. niloticus x O. aureus and its reciprocal cross both had negative values, but the former was higher than the latter, (b) O. mossambicus x O. niloticus had a higher value than the reciprocal

TABLE 6. Potence Ratio based on the FER for the Hybrids and their Mid parent Values

No.	Hybrid	Mid parent	A	D	D/A
1	O. mossambicus x O. aureus	0.725	0.185	0.015	0.081
2	O. niloticus x O. aureus	0.765	0.225	-0.195	-0.866
3	O. aureus x O. niloticus	0.765	0.225	-0.045	-0.200
4	O. mossambicus x O. niloticus	0.950	0.040	-0.280	-7.000
5	O. niloticus x O. mossambicus	0.950	0.040	-0.070	-1.750
6	O. mossambicus x O. spilurus	0.815	0.095	-0.085	-0.894
7	O. spilurus x O. mossambicus	0.815	0.095	-0.185	-1.947
8	O. spilurus x O. macrochir	0.760	0.040	0.010	0.250
9	O. spilurus x O. niloticus	0.855	0.135	0.205	1.518

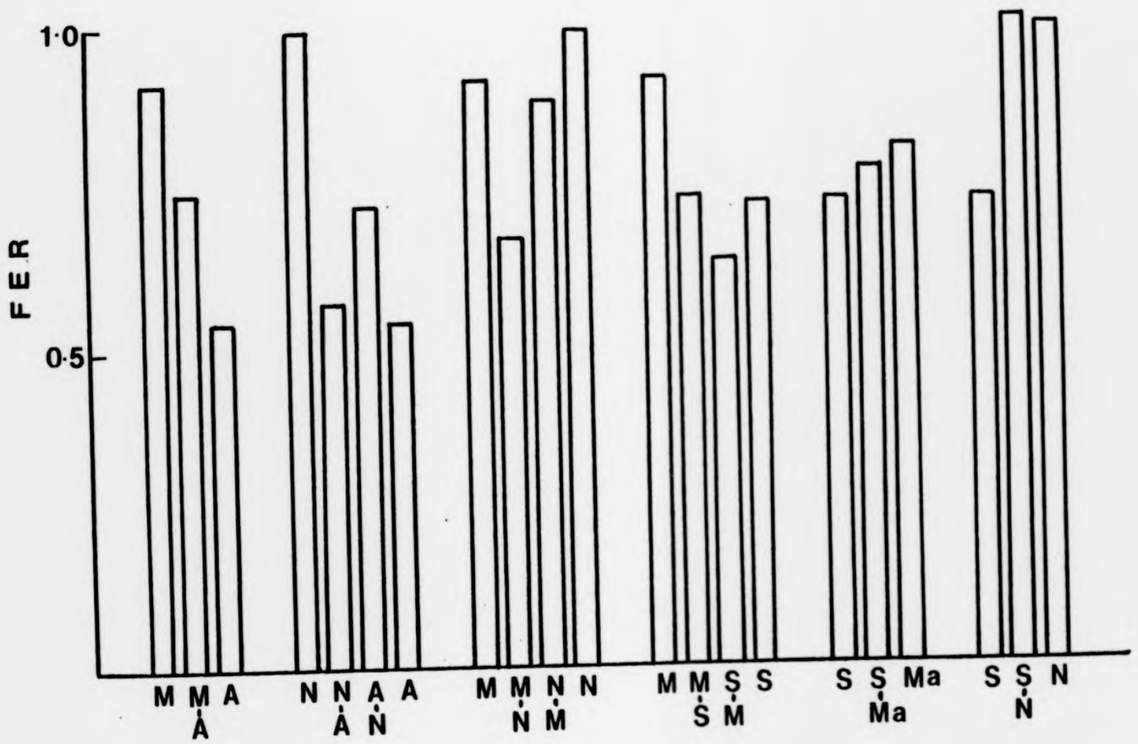

FIG. 12. Comparison of Food Efficiency Ratio (FER) between parents and their hybrids. M = O. mossambicus, A = O. aureus, N = O. niloticus, S = O. spilurus, Ma = O. macrochir.

cross but both had negative values, (c) O. mossambicus x O. spilurus had a lower value than the reciprocal cross but both had negative values.

Refstie and Gjedrem (1975) reported crosses between char (Salvelinus alpinus) and brown trout (Salmo trutta) and its reciprocal cross and between char (Salvelinus alpinus) and sea trout (Salmo trutta) and its reciprocal cross, and showed that they had significantly different growth rates. They inferred from their results that this might be due to the interaction of dam and sire species. The same inference might hold true for tilapia. In tilapia it has been known that the male grows faster than the female in some species (e.g. O. mossambicus, O. spilurus, Fryer and Iles, 1972). It is yet not known whether such a high growth rate is initiated at a very early stage of life.

Analysis of the sex ratio data and growth rate data in this study suggests that there is no evidence for enhanced male growth rates within this size group of tilapia. In O. niloticus x O. aureus the mean sex ratio from different crosses yields 79% males whereas in the reciprocal cross the value is about 63% males (see Chapter II for sex ratio data), but a higher growth is observed in the latter cross. In O. mossambicus x O. niloticus the male percentage is 47% and in the reciprocal cross it is only 6% but the SGR is higher in the latter group. Similar results have been observed in the case of O. mossambicus x O. spilurus (34% males) and its reciprocal cross (35% males). It is rather logical to assume that if the male growth is faster at the early stage (growth studies performed here on 2-10gms size individuals), there should be some positive correlation with sex ratio (towards higher percentage males). In practice just the opposite results were obtained,

indicating the possibility of some dam and sire interaction between the parental species affecting the growth rate difference in the reciprocal crosses and indicating also the possibility that there might be growth superiority in male tilapia after the maturation at a later stage of life.

Reports on the heterosis in tilapia hybrids have many contradictions (see Introduction). Pruginin et al. (1975) mentioned that three main factors are responsible for such contradictions.

(1) All the tests for detecting growth rate differences were done in separate ponds. These tests are insensitive as the variance components "between ponds" and "pond genotype interaction" are compounded with the "error" variance of the differences between the tested groups (Buck et al., 1970; Wohlfarth and Moav, 1972).

(2) Dominance including heterosis is a variable function of the environment. It has been pointed out that genetic differences between strains can be related to improvement of the environment. So the D and A value would be expected to increase as the environment improved (Bucio-Alanis, 1966; Bucio-Alanis et al., 1967; Knight, 1973; Moav et al., 1975; Wohlfarth et al., 1975, 1983; Ayles et al., 1979; Ayles and Baker, 1983).

(3) Tilapia hybridization tests were based on small numbers of parents. Intraspecific variation could also contribute significantly to the conflicting results.

The experimental design used in the present trials reduced the risks of unwanted environmental variation, in that all the experiments were

performed under identical environmental and husbandry conditions and each experiment was replicated so that any environmental effect could be assessed.

Gustafsson (1952) classified the cases of heterosis into three groups: 1) somatic heterosis, 2) reproductive heterosis, and 3) adaptive heterosis. Somatic heterosis as the name implies means that the hybrid will be more superior for somatic growth than its parents. The results on tilapia hybrids in the present series of experiments on growth shows that hybrids are rarely faster growing than their parents except in the O. spilurus x O. niloticus cross. Reproductive heterosis can be arbitrarily explained in two ways. From the population point of view if the hybrid shows a higher progeny number or reproductive fitness than its parents it will be of real benefit. On the other hand heterosis from the fish culturist point of view would be to have less reproductive efficiency, e.g. a lower number of progeny or even sterility. In the case of tilapia, hybridization has been aimed primarily to control population by producing monosex populations (Wohlfarth and Hulata, 1983) as these species are prolific breeders (Fryer and Iles, 1972) and such over population causes stunting in growth (Hickling, 1960, 1968). It has already been pointed out that male tilapia grow faster than the female, so it will naturally be beneficial to the growth if the hybrid combination has a higher percentage of males (Fryer and Iles, 1972; Lovshin and Da Silva, 1975; Anderson and Smitherman, 1978; Lowe-McConnell, 1982; Lovshin, 1982). The present study, however, shows some advantage of higher male frequency in the hybrids but no correlation has been found with their growth rate. The reason for this might be because the growth advantage of male tilapia does not probably start at the stage

when the growth trials had been completed. So to get the benefit of higher male percentage and growth rate, growth trials have to be performed which will indicate what percentage of male is beneficial over a 1:1 sex ratio in the population as far as the growth is concerned. It is also desirable to know when exactly the male superiority in growth rate starts. Regarding the adaptive heterosis it is expected that hybrids will be better adapted in the environments or wider spectrum of environment than their parents. It has been mentioned that tilapias in general show wider preference of niche and food habit, and it has also been pointed out that some species (e.g. O. mossambicus, O. aureus, T. zillii) can adapt quickly to new environments and food habit (Balarin and Hatton, 1979; Philippart and Ruwet, 1982, Lowe-McConnell, 1982; Wohlfarth and Hulata, 1983). So combining a wider genome in the hybrids might have beneficial effects. Unfortunately the present study has not been designed to find out the adaptive nature of hybrids, it remains an open field worth exploitation.

It has been shown by several workers (Moav et al., 1975; Wohlfarth et al., 1975, 1983) that heterosis (somatic) does change as a function of genotype - environment function, so the different tilapia hybrids might show heterosis in different environments and the ranking could be changed drastically. The possible future for hybridization may well be in the field of producing better adapted "strains" for fish culture which bring together the wider environmental tolerance and feeding habit into a single individual and to hopefully broaden the range of tilapia into new environments which are presently hostile or marginal to them.

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APPENDIX I

Computational Formula for F-test Between Two Regression Lines

(Sokal and Rohlf, 1969)

$$Fs = \frac{(b_1 - b_2)^2}{\frac{\Sigma x_1^2 + \Sigma x_2^2}{(\Sigma x_1^2)(\Sigma x_2^2)}} \quad s^{-2}_{Y.X}$$

Where b_1 = regression coefficient of trial I

b_2 = regression coefficient of trial II

x_1 = deviation of X axis (time) from mean for trial I

x_2 = deviation of X axis (time) from mean for trial II

$$s^{-2}_{Y.X} = \frac{(\Sigma d^2_{Y.X})_1 + (\Sigma d^2_{Y.X})_2}{V_2}$$

where $(d^2_{Y.X})_1$ = Unexplained sum of squares for trial I

$(d^2_{Y.X})_2$ = Unexplained sum of squares for trial II

$$V_2 = n_1 + n_2 - 4$$

Observed Values for Fs were checked for statistical significance ($P = 0.01$) in the F-table.

CHAPTER VI

SUMMARY AND CONCLUDING REMARKS

The results of the present investigation can be summarised as follows:

1. The wide variation in the sex ratio observed in the interspecific hybridization experiments in this study appear to be caused by genetic rather than environmental or experimental factors.

2. Experiments using pure species of tilapia for hybridization reveal that the sex ratio in the F1 hybrids shows wide range of variation even within the same cross.

3. Intraspecific crosses generally resulted in sex ratios of 1:1 but in O. spilurus there was a bias towards females whereas in O. aureus the bias was towards males.

4. In O. mossambicus crosses of the same male with different females resulted in variation in the sex ratio.

5. Summarising all the results found in the literature as well as the present study on hybridization it is difficult to justify the concept of sex determination on the basis of sex chromosomes and autosomal influence.

6. To explain the result so far obtained from hybridization studies a system of polygenic inheritance for sex determination is proposed.

7. The chromosome preparation by in vivo phytohaemagglutinin (PHA) injection and colchicine treatment resulted in satisfactory chromosome plates.

8. In all the species the chromosome number (2n = 44, n = 22) was found to be the same.

9. No sex specific heteromorphic chromosome pair was found in any species by morphological or banding procedure.

10. The chromosome formula for O. mossambicus is 3 sm + 4 sm-st + 15 st with NF = 50.

11. The chromosome formula for O. spilurus is 3 sm + 9 sm-st + 10 st with NF = 50.

12. The chromosome formula for O. niloticus is 1 m + 9 sm + 5 sm-st + 7 st with NF = 64.

13. The chromosome formula for O. aureus is 7 sm + 8 sm-st + 7 st with NF = 58.

14. The chromosome formula for O. macrochir is 5 sm + 9 sm-st + 8 st with NF = 54.

15. The chromosome formula for T. zillii is 2 m + 9 sm + 4 sm-st + 7 st with NF = 66.

16. The chromosome formula for S. galilaeus is 2 sm + 4 sm-st + 16 st with NF = 48.

17. On the basis of the morphology of individual chromosomes it can be ascertained that gross translocation or deletion did not play a major role in the evolution of karyotype of these species.

18. The differences in the NF but the same diploid number in all these species indicates that inversions involving centromere shifting played a role in the evolution of karyotypes of tilapias.

19. Comparison of inversional difference between two species at a time indicates that T. zillii has more inversional difference compared to all the other species.

20. Inversional differences can be found in 16 out of the 22 chromosomes in one or other species. The other six chromosomes (Nos. 1,13,16, 20,21 and 22) are identical in all the species studied.

21. C-banding analysis indicates that more C-heterochromatin is found in T. zillii than all the other species.

22. Using C-banding closely related species, viz. O. mossambicus and O. spilurus can be distinguished by the presence of telomeric heterochromatin in one pair of st chromosomes in O. mossambicus which is lacking in O. spilurus.

23. The DNA value calculated after Feulgen cytospectrometry of blood cell nuclei indicates that the amount of DNA present is not the same in all the species.

24. O. macrochir and S. galilaeus have the same amount of DNA in their somatic nuclei (0.84pg).

25. In O. mossambicus, O. spilurus and O. niloticus the C-values are 1.0, 0.95 and 0.95pg respectively which are statistically not significantly different.

26. O. aureus has the highest amount of DNA in their nuclei (1.21pg).

27. In all these species the nuclear area was found to be the same.

28. On the basis of comparative growth performance studies the species could be ranked as follows:
O. niloticus > O. mossambicus > O. macrochir > O. spilurus > S. galilaeus > O. aureus.

29. The ranking of the species using the SGR is as follows: O. aureus, S. galilaeus < O. spilurus, O. macrochir < O. mossambicus, O. niloticus.

30. According to the FCR O. niloticus is the best food converter whereas O. aureus is the worst in its performance.

31. 17α methyltestosterone treatment in three species, viz. O. niloticus, O. mossambicus and O. aureus increased their body weight over untreated controls.

32. Though the weight increment was highest in O. niloticus the overall ranking of the species remained the same as that of the control, i.e. O. niloticus > O. mossambicus > O. aureus.

33. In every species testosterone enhanced the SGR and reduced the FCR.

34. 17β estradiol treatment in the above mentioned three species showed some differential effect on their growth.

35. In O. mossambicus and O. aureus estradiol reduced the body weight whereas in O. niloticus it increased a little compared to the control.

36. The SGR increased in O. niloticus after estradiol treatment whereas it was reduced in the case of O. mossambicus and O. aureus.

37. The FCR in the O. aureus increased considerably after estradiol treatment.

38. Testosterone treatment caused 100% males in the O. mossambicus whereas estradiol treatment produced 96% females.

39. In O. niloticus testosterone and estradiol treatment produced 83% males and 57% females respectively.

40. Testosterone and estradiol treatment in O. aureus produced 65% males and 80% females respectively.

41. The following hybrids were tested for their growth performance: (Female x Male). O. mossambicus x O. aureus, O. niloticus x O. aureus, O. aureus x O. niloticus, O. mossambicus x O. niloticus, O. niloticus x O. mossambicus, O. mossambicus x O. spilurus, O. spilurus x O. mossambicus, O. spilurus x O. macrochir and O. spilurus x O. niloticus.

42. The growth performance was evaluated using SGR, FCR and the FER rather than final weight, as the initial weight of the fish in every case could not be kept the same because of technical difficulties.

43. Using all these criteria "heterosis" was found in only one combination of hybrids, i.e. O. spilurus x O. niloticus.

44. The use of the "potence ratio" data supports the above conclusion.

45. Use of the "potence ratio" and "mid-parent value" indicates that in most cases the slower growing parent dominated the faster growing parent.

46. Comparison of growth rate in the hybrids resulted in the following ranking: O. niloticus x O. aureus, O. mossambicus x O. niloticus, O. spilurus x O. mossambicus < O. mossambicus x O. aureus, O. aureus x O. niloticus, O. niloticus x O. mossambicus, O. mossambicus x O. spilurus, O. spilurus x O. macrochir < O. spilurus x O. niloticus.

47. The reciprocal crosses between O. niloticus x O. aureus, O. mossambicus x O. niloticus, and O. spilurus x O. mossambicus show growth differences indicating the possibility of a sex specific growth effect.

48. No correlation has been found between higher male percentages in the hybrid progeny and higher growth rate.

The present work on the interspecific hybridization in tilapias demonstrates that the variation in the sex ratio data in any hybrid combinations is wide. The previous notion of species identification and the experimental error of such variation in the proportion of male and female in the hybrid could be ruled out as the experiments have been done using pure species and in more controlled conditions. From the results so far obtained the

sex determination in these species seems to be governed by a number of genes distributed all over the genome rather than by a few sex chromosomes and autosomes. Two factors support such a hypothesis; the absence of visible sex chromosomes, and the production of progeny with varying sex ratios by the same male crossed with different females. The final proof of polygenic sex determination might come through: 1) selection of lines with higher or lower male percentages, 2) individual pairmating for a number of spawnings to calculate the heritability of sex ratios and to find out the total genetic component involved in the sex determination, 3) the identification of sex specific phenotypic or biochemical markers and their utilization in the breeding studies. 4) The other approach might be the use of the sex specific DNA markers and tracing their inheritance pattern in the subsequent generations. Use of such a sex specific marker (BKM) by Singh and Jones (1982, Cell, 28: 205-216) has resolved the problem of the classical sex reversal mutant (Sxr) in mice (Mus musculus).

The work on the chromosomes of these species indicates that chromosome morphology is distinct for every species. The difference although present in their C-value is not as distinct as the chromosome morphology. The use of chromosome morphology and banding analysis could be used in the identification of species and their hybrids, in addition to morphological and biochemical markers. The inversional difference predicted in the present study between different species could only be confirmed if the longitudinal differentiation of the chromosomes became possible. This would also help to identify phylogenetic interrelationships in the tilapias. For cytotaxonomic evaluation many more species should be included.

Growth studies on the pure species indicate species specificity. But the growth is influenced by both genotype and external factors. The comparative growth trials in this study have been undertaken in relatively artificial conditions compared to normal farm practices. Much more emphasis should be given to comparative growth trials in a wider range of species and environmental conditions particularly under commercial farm conditions. This should help to reduce the risk of indiscriminate transplantation of a species; leading to the failure of aquaculture programmes. On the other hand as a temporary measure, manipulation of growth performance could be done by the use of hormones, especially testosterone which should give additional advantages as well as monosex populations.

As hybrids do not show much superiority in the growth over their parents except in O. spilurus x O. niloticus, comparative growth trials should always be given to evaluate the heterosis for any combination of hybrids. Although in the present trials the hybrids do not show the heterosis, it might be possible that the conditions used were not suitable for the expression of their full performance potential. So growth trials using a wider range of environmental conditions and food (protein component, protein source, etc.) should be given which will indicate the possible utilization of a specific hybrid for the exploitation of specific environmental conditions. The other criterion of sex specific growth performances could not be confirmed in the present study. It might be possible that the growth trials were too short to show sex specific growth enhancement as this may not start at such an early age and may only occur after maturation. To get a better understanding of such a phenomenon growth studies should be continued for a longer period even after the fish has attained gonadal maturity.

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