Simposio Centroamericano de Acuacultura

Memoria: Sesiones de Tilapia

Proceedings: Tilapia Sessions


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Tegucigalpa, Honduras 22-24 Agosto de 2001

6to Simposio Centroamericano de Acuacultura

Memoria: Sesiones de Tilapia

Proceedings: Tilapia Sessions

Daniel E. Meyer
Editor

Fotos en la portada son todas de Honduras:
1. Estación Acuícola de Zamorano
2. Canales de producción, Aquacorporación
3. Jaulas con tilapia, Lago de Yojoa
4. Ejemplares de la tilapia del Nilo
5. Ejemplar de la tilapia roja

Cover photos are all from Honduras:
1. Zamorano Aquaculture Station
2. Production raceway, Aquacorporation
3. Cages with tilapia in Lake Yojoa
4. Two Nile tilapia
5. A red tilapia

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6to Simposio Centroamericano de Acuacultura

Program for the tilapia sessions
(Programa de las sesiones sobre tilapia)

Contents (Contenido):

Sixth Central American Aquaculture Symposium:

Proceedings of the Tilapia Sessions

Introduction

Welcome to the ***Sixth Central American Aquaculture Symposium***! We hope that the technical presentations and other activities planned for this week, will contribute to enhancing your knowledge of aquaculture and provide you with ample opportunities to interact with others attending this meeting.

This publication, the proceedings for the tilapia sessions, has been organized and printed with financial support from the Pond Dynamics/Aquaculture Collaborative Research Support Program (PD/A CRSP), which in turn is funded primarily by USAID, Washington, DC, USA. Our objective has been to provide all attendees with the appropriate written materials, to complement the oral presentations on tilapia culture to be given as part of the symposium.

I want to thank each of the authors of these papers for their cooperation and hard work. I would also like to thank my secretary at Zamorano, Juana Espinosa de Ayestas, for her dedication and patience, to work with me through the process of compiling and editing these documents.

I accept full responsibility for any errors or omissions in this document. Additional copies of the proceedings can be obtained by contacting me at my address in Honduras, or at the Zamorano booth in the commercial exhibition that is part of this symposium.

Sincerely;

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August 2001

Tilapia Genetics in Asia[1]

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Abstract

This paper presents and discusses major issues in the application of genetics to tilapia, with emphasis on commercially important species used in aquaculture. The paper presents past and recent advances in the development and application of genetics based technologies to tilapia, from an applied perspective describing the existing and potential impacts on tilapia culture. Following on from a discussion of tilapia genetic resources and the impacts of domestication processes, the major emphasis of the paper is on the progress in the application of selection, hybridisation, sex control, chromosome set manipulation and transgenic technologies. The uptake and impact of these technologies is discussed mainly in the context of Asian aquaculture, where more than 85% of cultured tilapia are produced.

Many of the tilapia genetic resources used in aquaculture today are little different from, or in many cases inferior to, wild caught stocks. Domesticated stocks have suffered from the consequences of poor broodstock management including inbreeding, genetic drift, unconscious negative selection and hybrid introgression.

However, as the species has gained in importance as an international aquaculture commodity, so has there been a considerable increase in research effort to improve tilapia stocks, particularly over the past ten years. In terms of real applications and their uptake in aquaculture, recent advances in selective breeding and sex control technologies are having the greatest impact. A large volume of research work on hybridisation and chromosome set manipulations,

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[1] This article is modified from one that is to be published by INFOFISH in the proceedings of Tilapia 2001, May 28-30, 2000, Kuala Lumpur, Malaysia

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whilst providing useful research tools, is having minimal impact upon aquaculture production. There have been a number of important recent advances in the application of transgenesis, which have been demonstrated to bring about substantial improvements in growth rates and yields, under experimental conditions. However, there are some technical and numerous socio-political and environmental constraints to be overcome before this technology can be widely adopted for aquaculture although it is likely that there will be isolated cases were transgenics might be approved for aquaculture in the near term.

Future prospects are promising with the advent of molecular technologies including the identification of quantitative trait loci (QTL) and their application in marker assisted selection programmes that can enhance traditional breeding programmes, and it appears likely that significant further production gains will be achieved in the near future. There is thus considerable optimism that tilapia farmers will be among the first aquaculturists to benefit from the widespread applications of genetics based technology in tropical fish species.

Tilapia culture in Asia

Asian tilapia production dominates world production statistics with FAO data for 1999 (FAO, 2001) indicating that more approximately 82% of the world's production of tilapia comes from Asia (although only 70% of total value). Statistics provided from China indicate production levels in excess of 560,000 MT in 1999 indicating a growth rate in production of 400% per annum in the preceding decade (FAO, 2001). Other major producers in Asia include Thailand, the Philippines and Indonesia (**Error! Reference source not found.**). The bulk of tilapia produced in Asia is for domestic consumption although Taiwan, China and Indonesia do export significant quantities, supplying 28%, 24% and 3% respectively of tilapia imports into the major import market of the U.S.A. in 2000 (Fitzsimmons, 2000). Tilapia production is predominantly in freshwater ponds although there is some production in brackish water ponds and in cages in some locations. Tilapia are either cultured in monoculture or often in polyculture, mainly with carp species. Culture systems can be stand alone, as predominates in the Philippines for example, or integrated with other parts of farming systems, as commonly seen in Vietnam and parts of China There is some intensive production of tilapia, particularly in Taiwan. Unfortunately, whilst China dominates Asian tilapia production there is little available literature describing tilapia production systems in the country.

Genetic resources for aquaculture

Although there are a number of tilapia species of commercial interest for culture in Asia (see Table 1) *Oreochromis niloticus*, *O. mossambicus* and their various hybrids dominate in aquaculture. Recent growth in tilapia production from aquaculture has been in the culture of *O. niloticus* (**Error! Reference source not found.**), which represents the most important tilapia species for freshwater aquaculture today. These figures should however, be considered with some caution and species wise production is often inaccurately recorded. The

introduction of *O. niloticus* has commonly been preceded by the introduction of *O. mossambicus.* There have been several attempts to characterize and document indigenous genetic resources in Africa (Trewavas, 1983, Pullin *et al.*, 1986 and Pullin *et al.*, 1997) and to record the sequence of introductions and transfers of various species outside of their natural range (Pullin and Capili, 1988, Welcomme, 1988 and Agustin, 1999). Further to this, there have been numerous reintroductions of tilapias for aquaculture in the 1990s, especially in Asia and the Americas, to the extent that it would now be extremely difficult to document these adequately. However, the majority of tilapia cultured throughout the world (predominantly in Asia) is derived from the original introductions, in the 1950s for *O. mossambicus* and in the 1960s and early 1970s for *O. niloticus*.

Genetic basis of introductions for aquaculture

The original introduction of *O. mossambicus* to Asia can be traced to the discovery of five individuals (3♂and 2♀) in Indonesia in the 1950s (Agustin, 1999). These individuals were bred (possible only a single pair mating!) and their progeny formed the basis of the aquaculture and feral stocks throughout Asia and probably further a field than this. Although it is still actively and deliberately cultured in a few areas, most commonly these are in brackish water areas or in regions where alternative species are not available. In general *O. mossambicus* is no longer popular for culture due to its poor growth performance (stunting is very common), early sexual maturation and high levels of recruitment under culture and it is regarded as a pest species in many countries. In a study of feral populations, Agustin (1999) used biochemical and molecular markers to characterize populations from a number of Asian countries and compared these to samples from a number of indigenous populations in southern Africa. She concluded that all the Asian populations most likely came from a single source population in southern Africa, most probably via Indonesia. She further determined that a number of rare alleles were missing in the feral Asian populations. In a study using five microsatellite DNA markers, she identified a six-fold reduction in allelic diversity and a two to three fold reduction in heterozygosity levels. Furthermore only one mitochondrial DNA haplotype was found in Asian stocks, compared to nine that were identified in indigenous African stocks. Thus, it is apparent that there have been profound bottleneck effects on these stocks, which may account for the relatively poor performance of domesticated stocks of this species in aquaculture. Furthermore, it is evident, that whilst the total size of the Asian populations may now exceed that of the indigenous African populations, little if any genetic variation has been regenerated through mutation or more recent introductions.

The introduction of *Oreochromis niloticus* to Asia was traced by Pullin and Capili, (1988) to the transfer of approximately 120 fish from Egypt to Japan in 1962 and a shipment of 50 fish from Sudan to China in 1978. The majority of *O. niloticus* cultured in Asia today originate from these two introductions although this situation is rapidly changing following numerous reintroductions over the past 10-15 years. Asian stocks have also been used to found stocks in other countries including the Americas.

A substantial culture industry, producing in excess of 50,000 metric tones *per annum* (with some estimates being closer to 100,000) has built up in Thailand based on the introduction from Japan of 50 fish in 1965. It is not firmly established how many of these fish were actually used to produce seed which formed the basis of the current industry but it seems likely that the effective population size (N_e) was not much greater than 30. However, compared to the apparent poor and declining culture performance of *O. mossambicus*, the culture performance of *O. niloticus* has been more robust with few reports of declining performance that could be associated with inbreeding depression. This may be a result of the slightly larger genetic base for the introduction of this species and inherently greater levels of genetic variation in *O. niloticus*. It is evident, nevertheless, that the majority of cultured tilapia possess performance characteristics that are little different or in some cases worse than their wild relatives.

Strain comparisons

It is currently accepted that the Nile tilapia *O. niloticus* is the best species for culture in the majority of inland, warm water aquaculture systems ranging from extensive, low input pond culture through to intensive recirculating systems. A number of studies in recent years have demonstrated that there are large differences in the relative culture performance of different populations and strains of tilapia across a range of different environments. In animal and plant breeding the term "strain" is normally applied to intra-specific sub-populations exhibiting distinctive traits, which are normally homozygous (i.e. true breeding). However, in fish and particularly in tilapia strains or isolates are normally loosely designated according to their location or origin and commonly have no distinctive traits, which can lead to considerable confusion.

In the most comprehensive study of its kind, (Eknath *et al.*, 1993) compared the performance of eight "strains" of *O. niloticus*, four Asian strains and four strains collected from the wild in Africa, across 13 different farm environments. This study demonstrated that, with the exception of a strain from Ghana, the wild caught "strains" had better culture performance than the domesticated stocks, with strains from Egypt and Kenya having the best performance. However, it should be noted here that previous genetic studies had indicated that the domesticated Philippine stocks of *O. niloticus* were introgressed with the slower growing *O. mossambicus*, which may have impacted their growth performance (Macaranas *et al.*, 1986). A further study on stocks established as pure *O. niloticus* was conducted by Capili, (1995). She compared the growth performance of 11 strains of tilapia from various African origins and found similar results in that the strains of Egyptian origin had the fastest growth rates, although the performance of three Kenya strains was relatively poor. In a further study,

Capili (1995) also determined that the degree of sexual dimorphism varied between strains; with the Kenyan strains have the greatest difference in size between males and females. Oldorf *et al.*, (1989) also noted significant differences in the rate of sexual maturation between strains.
In growth trials of tilapia species and strains, changes in ranking of strains between environments are common, indicating significant genotype x environment interactions in many cases (Dahilig, 1992, Elghobashy *et al.* 2000, Capili, 1995, Romana-Eguia and Doyle, 1992). However, in the Eknath *et al.*, (1993) study, the analysis of the performance of the strains across environments led to the conclusion that the relative importance of genotype environment interaction was low compared to that of strain and sex differences. It was as result of this finding that the researchers elected to go ahead with a large centralized breeding programme on the assumption that the improved fish would be superior across a wide range of culture systems.

Implications of domestication

The effects of domestication which can include loss of genetic variation through genetic drift and inbreeding and unconscious selection, are felt less in tilapia than in some other species. This is largely due to the fact that individual tilapia have relatively low fecundity, necessitating the maintenance of large populations of brood stock, probably increasing the effective populations sizes and reducing the probability of mating close relatives, which would result in inbreeding. Furthermore, since brood stock are normally spawned randomly, the effects of unconscious selection are less than they might be in other species such as carp. However, genetic bottlenecks are common and this can result significant changes in gene frequencies through genetic drift. Also, due to its relatively short generation time (6-12 months) any selection forces acting on domesticated stocks can change their phenotypic characteristics over relatively short periods of time. It is thus likely that local adaptation of strains does occur in some environments with resulting superior performance of these local adapted strains when evaluated in the particular environment under which they were domesticated. Another risk with the domestication of the tilapia, a species group in which many hybrid crosses produce fertile offspring, is that of the breakdown of species barriers through hybrid introgression. This was shown to have occurred on a wide scale among Philippine tilapia stocks (Macaranas *et al.* 1986).

As more populations come under domestication, and attempts at genetic improvement move forward, more distinct strains of tilapia will be developed. It is important that these strains be adequately characterized and documented. At present the only documentation of tilapia strains is within FishBase, a relational database being developed and maintained by ICLARM in cooperation with FAO (Froese and Pauly, 2000).

Options for genetic improvement

With the exception of some ornamental fish, common carp and salmonids, there has been relatively little application of genetic enhancement technologies to fish compared to that which has been achieved in other forms of agriculture. Given its relatively recent history of domestication and adoption for aquaculture, this is certainly true of the tilapia and it is only within the past 10 years that significant attempts have been made to improve cultured stocks. In common with many fish species a number of approaches to improvement are available which are not possible in higher organisms. In the case of tilapia, its large effective population sizes, short generation time, ease of handling, stress and disease resistance and ease of both "natural" and artificial reproduction, make it highly suited to the application of a number of genetic enhancement technologies. With tilapia apparently destined to become a major international commodity, the development of these technologies has accelerated in recent years.

Qualitative traits

Qualitative traits are those which fall into discrete categories (i.e. are not continuously distributed) and their inheritance can usually be understood on the basis of basic or Mendelian genetics. New qualitative phenotypes arise from mutations. In tilapia the main qualitative traits that have been studied are body shape and colour traits. The inheritance of a number of body shape traits has been well described by Tave (1992). For aquaculture purposes body shape changes induced by mutation are invariably deleterious and thus their inheritance is of little interest to aquaculturists other than to know how to eliminate them if they arise in cultured stocks. Colour varieties, particularly the red tilapias, are however of considerable commercial interest, often securing a higher market price than the normal wild type coloration. There are a number of variants of red tilapia that have arisen independently in several different stocks and several of these variants, with variable expression of colour phenotypes, are being used in aquaculture around the world. It is probable that the red colour first arose in *O. mossambicus* and was transferred to other stocks through hybridization with *O. niloticus* and in some cases also *O. u. hornorum*. The situation regarding the precise origins of some of the red tilapias used in aquaculture today (e.g. Philippine, Thai, Florida and Taiwanese) is now somewhat confusing. However, it is clear that the nature of the inheritance of the various colour patterns in these strains is complex (see reviews by Wohlfarth, 1990 and McAndrew and Wohlfarth, in press). There are no known examples of a true breeding and homogenous red tilapia. The expression of the red phenotypes is highly heterogeneous and red tilapia can exhibit varying amounts of red, pink, orange and white pigmentation, often combined with degrees of black blotching. There is no doubt that there would be a considerable commercial demand for a true breeding homogenous red tilapia as many producers of red tilapia today have to grade their fish according to the colour and for different markets, reducing potential revenue and increasing labour costs. Efforts to select for homogeneity of colour have made some progress but would appear unlikely to yield true breeding homogenous phenotypes in the near future.

There are a number of colour variants within pure species in which the inheritance is more easily understood and in which true breeding lines may be established. These include the red in *O. mossambicus* and "blond" and "pearl" in *O. niloticus* (Wohlfarth, *et al.* 1990, Scott *et al.*, 1987, McAndrew *et al.*, 1988) but commercial demand for these colour varieties has yet to develop.

Selective breeding

Tave (1988) reviewed studies on quantitative characters in tilapia up to that date, concluding that moderate heritabilities (0.15 – 0.5) are common for a number of commercially important traits, indicating significant contributions of additive genetic variance to these traits. However, several early attempts to apply selective breeding to commercial stocks were disappointing indicating low heritabilities and producing low response to selection (Table 3). Since this time there have been a number of important attempts to apply techniques of traditional selective breeding to tilapia stocks as summarized in Table 3. Most studies have produced significant response to selection, usually for traits associated with growth rate. However, most of these published studies have been on an experimental basis and few of the benefits of selection have been passed on to the industry. A major exception is the Genetically Improved Farmed Tilapia (GIFT) programme coordinated by ICLARM in the Philippines. This comprehensive and well-funded selection programme has demonstrated significant responses to selection over multiple generations. Using a combined selection methodology on a synthetic base population developed from newly introduced strains from Africa and domesticated Asian strains, this programme achieved genetic gains averaging 13% over five generations providing an estimated cumulative increase of 85% in growth rate compared to the base population from which is was selected (Eknath and Acosta, 1998). Whilst the genetic gains are significant and clearly demonstrate benefits of well organized breeding programs, the accumulated response to selection appears not to be fully expressed in all culture environments and difficulties in identifying adequate controls have created difficulties in accurately assessing genetic gains.

Since 1997, the benefits of this programme in the form of the GIFT tilapia have been widely disseminated into the tilapia culture industry in the Philippines. The GIFT strain has also been introduced to a number of other countries in the region for dissemination and as the base population for the establishment of a number of national tilapia breeding programmes.

A number of issues have been highlighted in terms of sustainability of selection programmes and it is evident that unless they receive long-term financial support (e.g. Government) for qualified personnel and running costs, it will be necessary to generate income via the dissemination process. This can have profound effects on the uptake and sustainability of genetic gains and on the social and economic impact of selection programmes on their intended beneficiaries.

Molecular techniques for enhanced selective breeding

There has been considerable effort in recent years directed at the construction of linkage maps of the tilapia genome using a range of different genetic markers particularly microsatellite DNA markers and amplified fragment length polymorphisms – AFLP (Lee and Kocher, 1996, Kocher *et al.* 1998). This mapping effort opens up the potential for identifying quantitative trait loci (QTL), gene loci that directly influence a trait, which can be identified through combining gene mapping, breeding and trait evaluation. One example of the identification of such a trait is the association between alleles at a microsatellite locus and cold tolerance in an F_2 hybrid of *O. mossambicus* and *O. aureus* (Hallerman *et al.*, personal communication). The identification of QTL associated with commercial important traits such as disease resistance, environmental tolerances, sex or even growth rate will create the possibility to carry out marker-assisted selection (MAS) for targeted traits. In MAS, DNA markers that are closely linked to one or more QTLs can be used to increase the response to selection in a population, increasing the efficiency of selective breeding programmes.

Hybridization and crossbreeding

Whilst the majority of research and development work on selective breeding of tilapia is relatively recent, the main early emphasis on the study of quantitative traits was through hybridization and crossbreeding. As is indicated in Table 1, tilapia species differ remarkably from one another in many traits of commercial importance. Differences between strains, within species, are less but can be significant as indicated above. These intra-strain differences have only be evaluated to any degree in the commercially important *O. niloticus.*

Hybrids between tilapia species and even genera and have occurred in wild and feral populations, especially where translocations of species into new environments have occurred. There have also been numerous deliberate hybridizations between species (reviewed by Lovshin, 1982 and Schwartz, 1983) and between genera (reviewed by Rana *et al.*, 1996). More than 60 different hybrids have been produced between and among the *Oreochromis, Sarotherodon* and *Tilapia* with the majority being between *Oreochromis* species.

F_1 hybrids are commonly produced with specific objectives in mind, usually in the hope of observing heterosis (hybrid vigour) for commercially important traits or to produce a particular desirable phenotypic feature (such as colour or environmental tolerances) in the hybrid or its subsequent generations.

Virtually all reports of hybridization show that hybrids within and between the tilapia genera are viable indicating the speciation within the tilapiine fishes may be relatively recent. Furthermore, there are no reports of sterility, commonly found in hybrids of other species groups, among the tilapia hybrids. Despite the

large number of reports of various hybridizations, there are few, if any, published studies that clearly demonstrate heterosis for any commercially important trait. In the vast majority of hybrids, the traits studied were intermediate between those of the parental species (McAndrew and Majumdar, 1988).

Oreochromis hybrids are characterized by a surplus of males and the occurrence of all-male broods is relatively common and this is where the major interest in hybridization lies. The first report of monosex hybrids created significant interest with the potential for mass production of all-male progeny to prevent the serious problem of unwanted reproduction in aquaculture. However, in most cases, sex ratios differ between reciprocal crosses and there are few hybrid combinations which consistently give monosex progeny, with perhaps those using male *O. urolepis hornorum* being the most reliable. Table 4 summarizes the hybrid combinations known to produce monosex male progeny. Attempts to commercialize monosex hybrids, usually with the *O. niloticus* x *O. aureus* cross or using male *O. urolepis hornorum* with females from *O. niloticus* or *O. mossambicus,* have been disappointing with females usually occurring in previously all male broods. Failure to sustain production of all-male tilapia hybrids is most likely due to insufficient care in keeping brood stock segregated by sex and species, and in preventing introduction of hybrids into the brood stock ponds (Wohlfarth, 1994). Improvements in brood stock management may enable more effective utilization of monosex hybrids. However, with *O. niloticus* accepted as the best commercial species for the majority of tropical freshwater aquaculture environments, dilution of the *O. niloticus* genome with other species tends to reduce the performance potential in aquaculture, compared to pure *O. niloticus* except under specific circumstances, for example the benefits of cold tolerance of hybrids involves *O. aureus* in over wintering fish in seasonal sub-tropical climates.

Despite the lack of clearly demonstrated benefits, in terms of enhancement of commercially important traits, \there is a significant commercial production of hybrids in some parts of Asia, most notably the production of *O. niloticus* x *O. aureus* F1 hybrids in Taiwan and parts of China. It is not clear whether these hybrids are produced primarily due to the high proportions of males or for their enhanced cold tolerance compared to pure *O. niloticus*, or possible for a combination of these and other factors. Also most red tilapia in commercial production have hybrid ancestry and are usually cultured for their marketability and/or their enhanced saline tolerance compared to pure *O. niloticus*.

With the failure of hybridization to effectively solve the problem of early sexual maturation, unwanted reproduction and overpopulation in tilapia culture, alternative technologies were sought. One popular alternative is hormonal sex reversal but this technique has a number of important technical, environment and social constraints. An alternative genetics based solution has been sought, founded on the current knowledge of the mechanisms of inheritance of sex in tilapia.

Sex control

Due to commercial interest in monosex populations for aquaculture a considerable amount of research has been conducted on the genetics of sex determination in tilapia (see review by Trombka and Avtalion, 1993). A number of theories have been proposed on the genetics of sex determination in tilapia ranging from a single gene model through to polygenic inheritance. The current consensus is that, in the commercially important *Oreochromis* species, sex determination is "predominantly" monofactorial, being controlled by sex chromosomes or primary sex determining gene(s). Research using sex reversal, progeny testing and chromosome set manipulation has revealed two alternative "sex chromosome" models. In *O. niloticus* the female is homogametic XX, the male being heterogametic XY (Mair *et al.*, 1991a) whilst in the closely related *O. aureus* the alternative model of heterogametic WZ females and homogametic ZZ males applies (Mair *et al.*, 1991b). There is also substantial evidence for effects of one or more autosomal genes on sex ratio together with an increasingly well documented effect of temperature. The role of temperature in influencing sex differentiation is becoming increasingly evident and should be considered when evaluating all past research on sex ratios. Elevated temperatures (~36°C) during the period of sex differentiation have been shown to increase the proportions of males in putative monosex female *O. niloticus* and, to a lesser degree, to increase the proportion of females in putative all male progeny (Baroiller *et al.*, 1996, Abucay *et al.*, 1999 and Baras *et al.*, 2001). Similarly high temperatures have been shown to push sex ratios in both directions in *O. aureus* (Mair *et al.*, 1990, Desprez and Melard, 1998, Baras *et al.* 2000). The only recorded effect of low temperatures was to increase the proportion of males in *O. mossambicus* (Mair *et al.*, 1990).

Based on the theory of predominantly monofactorial sex determination, it has proved possible to manipulate sex ratio using a combination of sex reversal and progeny testing to identify sex genotypes. In a major breeding program in *O. niloticus*, Mair *et al.*, (1997) were able to mass-produce novel YY "supermales". When crossed to normal females (XX) these YY males have the unique property of siring only male progeny. These progeny are termed genetically male tilapia (GMT) and are normal (XY) genetic males (although some can "naturally" revert to female, giving GMT an average sex ratio of >95% male). The hormone treatments used as part of the process to produce YY males are two generations removed from the fish that are marketed to the consumer so neither the GMT or their YY male parents are hormone treated in any way. This makes the technology more user and environmentally friendly than the alternative of direct hormonal sex reversal. Furthermore, the technology can be applied in a range of hatchery systems simply by replacing brood fish with YY males although good brood stock management is required to prevent contamination.

On-station and on-farm trials indicated substantial increases in production (40% increases in yields) using GMT compared to normal mixed sex tilapia. Since the development of the original intra-strain GMT in an Egyptian strain of *O. niloticus* trials

of various crossbred GMT have produced significantly faster growth rates. Recently trials involving GMT, created by crossing the original YY male to a selected female line, produced further gains in growth rate, compared to both original and crossbred GMT (Table 5). The selected female line was developed through three generations of within family selection for growth rate and selection for combining ability in GMT sex ratio, applied to a synthetic base population consisting of five fast growing strains of *O. niloticus*. The outputs of this is technology in the form of GMT and GMT producing brood stock are now being widely disseminated in the Philippines and Thailand through a network of accredited hatcheries. The technologies has also been adopted in up to 20 other countries worldwide.

A similar although slightly simpler breeding programme has been developed to produce monosex male *O. aureus* through the production of sex reversed ZZ females (Mair *et al.*, 1991b and Melard, 1995).

Chromosome set-manipulation

A number of chromosome set manipulations are possible including gynogenesis, androgenesis, production of clones, triploidy and tetraploidy (see review by Mair, 1993). Gynogenesis and androgenesis are forms of uniparental inheritance, induced by fertilizing one gamete with another in which the DNA has been denatured by irradiation, to produce a haploid zygote which can then be diploidized by application of physical shock such as temperature or pressure. Gynogenesis, where eggs are fertilized with denatured, UV irradiated, sperm, has been a useful research tool for studying the inheritance of sex, among other things. Where the diploid state is restored by disrupting first mitosis (as for mitotic gynogenesis and androgenesis) using physical shocks, the resulting individual is completely homozygous due to the retention of two sets of homologous chromosomes (i.e. 100% inbred). Induction and survival rate of these homozygous fish are very low but all genes that are deleterious or lethal in their homozygous state will be selected out in the process. A second generation of gynogenesis or androgenesis will produce genetically identical individuals or homozygous clones. Crosses between homozygous gynogenetic or androgenetic fish will produce heterozygous clones. Both homozygous and heterozygous clones have been produced in tilapia (Hussain *et al.*, 1998 and Jenneckens *et al.* 1999). There is the potential that heterosis may be realized in the crosses between these highly inbred clonal lines but evidence for this has yet to be found. The clonal lines, however, have considerable potential in research as internal controls in communally stocked growth trials and through the elimination of genetic variance when studying the effects of other variables such as nutrition, sex determination or disease resistance.

Triploidy has been induced in a number of tilapia species through the application of physical shock to eggs fertilized with normal sperm, at the stage of second meiosis, inducing retention of the second polar body (see review by Mair, 1993). Triploids are sterile and as such have some commercial potential in addressing

the constraint of early sexual maturation and unwanted reproduction. In growth trials of induced triploids Brämick *et al.*, (1996) demonstrated that, post-sexual maturation, growth of sterile triploids was significantly faster than diploids in a pond environment. Final yields of triploid fish after 25 weeks of grow-out were 56 to 123% greater than for diploids although these results were confounded by the presence of recruits following reproduction in ponds stocked with normal diploid fish. In a more recent study on triploid *O. aureus* (Byamungu *et al.* 2001) little difference was observed in the relative growth performance of diploids and triploids under normal feeding regimes, but triploids were found to have significantly higher yields under restricted feeding regimes.

Despite these promising findings the potential for the application of triploidy in tilapia culture is very limited due to the requirement of artificial fertilization in order to apply physical shocks at precise intervals after fertilization. Due to the relatively low fecundity of individual fish and the difficulty in collecting ovulating eggs in this multiple spawning species, artificial fertilization in tilapia is impractical on a commercial scale. It may be possible to mass-produce triploids in matings of diploid and tetraploid fish. Tetraploidy has been induced in tilapia, albeit at very low rates of induction (Mair, 1993) but the majority of tetraploid embryos have been characteristically deformed and inviable. A recent study produced a high incidence of tetraploidy (80%) in *O. niloticus* (El-Gamal *et al.*, 1999) although the viability beyond the early fry stages was not determined. Only a few viable and fertile tetraploids would be required to produce tetraploid lines, which would enable large-scale production of triploids in diploid x tetraploid matings.

Transgenesis

Transgenesis is one of the most promising technologies for generating relatively rapid genetic improvements. Transgenesis involves the introduction of an exogenous genes into a new organism to confer novel phenotypic characteristics on that organism. Typically, the desired genes are first identified and or constructed, commonly combining coding and promoter sequences from different donor sources, and then cloned. Multiple copies of the transgene are then introduced to the fertilized eggs, commonly by microinjection or electroporation. At a later stage of development, cells of the organism are tested to determine whether copies of the transgene has become incorporated into the genome and whether this incorporation is in all cells or only in the cells of some tissues (i.e. a mosaic). After incorporation is determined the organism can be evaluated to determine if the transgene product is being expressed and in what amounts. The next stage of development is to determine whether the transgene is inherited and expressed in the next generation via the germ cells. Inheritance of the transgene is required in order to develop true breeding lines of the transgenic organism. Several studies have made progress in developing methodologies to introduce and ensure or monitor incorporation, expression and inheritance of transgenes in tilapias (Brem *et al.*, 1988, Indig & Moav, 1988, Maclean *et al.* 1992, Alam *et al.*

1996, Rahman *et al.* 1997). However, only two major published programmes have produced true breeding, enhanced transgenic lines.

The first of these programmes has focused on the introduction of a tilapia growth hormone cDNA into a hybrid tilapia (originating *from O. urolepis hornorum* and *O. aureus* crosses) in Cuba (Martinez *et al.*, 1999). This study has demonstrated that the transgene construct (incorporating a human cytomegalovirus regulatory sequence) has been incorporated, expressed and transmitted through four generations. Growth performance trials of the homozygous and hemizygous transgenic fish, communally stocked in ponds with non-transgenic fish, for a three-month grow-out period, were conducted. Transgenic fish were significantly larger (up to 82% with an average of 55%) than non-transgenic fish with indications of a transgene-dosage effect. The results indicate stable germ line transformation in this fast-growing transgenic tilapia line and it seems likely that this transgenic strain will be adopted for aquaculture in Cuba.

The second published study in the U.K. involves the introduction of an all-fish construct of a chinook salmon growth hormone gene with an ocean pout antifreeze regulatory sequence, into *O. niloticus* (Rahman and Maclean, 1999). The initial transmission rate from G0 to G1 generation was observed to be less than 10% in these lines indicating a mosaic distribution of the transgene in the germ cells. However, transmission rates from the first to the second generation were found to follow the expected Mendelian ratios. The chinook salmon growth hormone was produced in several generations of the transgenic tilapia indicating expression and transmission of the gene. This expression of the transgene resulted in dramatic growth enhancement with the average weight of the transgenic fish being three to four times that of their non-transgenic siblings and with equivalent food conversion efficiencies (Rahman and Maclean, 1999, Rahman *et al.*, in press).

Transgenesis would appear to offer very considerable potential for enhancement of yield in tilapia. However, the rate of genetic change in transgenics is such that their phenotypic and behavioural properties cannot easily be predicted and the introduction of these fish for commercial aquaculture faces many constraints. The risks to the environment posed by the uncontrolled introduction of transgenic fish needs to be adequately assessed and many governments are currently adopting cautious policies with regard to their introductions. Many of these constraints may be overcome if guaranteed sterile transgenic tilapia can be produced. This could be achieved through efficient methods of triploidization or, in the medium to longer term, through disruption of the physiological pathways of reproduction via the introduction of new transgenes such as anti sense constructs, which are currently under development. Consumer response to genetically modified fish (GMOs) in some countries may be very negative to the extent that adoption by farmers may involve significant economic risks. This negative response may be lessened with regard to transgenic fish developed using con-specific gene constructs, which is now the trend in research.

At the time of writing, negative reaction to the concept of genetically modified fish among the popular media across much of the world, particularly in Europe, is so strong, it seems unlikely that such fish would be approved for production or consumption in the developed world in the near future. The risk-benefit ratio is very different in developing countries where food security can be a major issue and possibly we will see transgenic tilapia produced first in these countries.

Conclusions and Future prospects

It is evident that significant progress has been made in the last decade in the application of genetic techniques to tilapia. The immediate applications of some current technologies such as hybridization, crossbreeding and chromosome set manipulation would appear to have limited potential for significant production gains. Substantial benefits in terms of growth rates and improved yields under culture have been demonstrated from breeding programmes for selection and sex control. The results of the successful applications of these breeding programmes need to be introduced to aquaculture through technically and economically sustainable dissemination programmes.

Transgenesis would appear to offer great potential for genetic enhancement of tilapia under culture provided that the remaining technical constraints can be overcome and that an appropriate legislative environment can be created following the satisfactory completion of appropriate environmental and health risk assessments.

Future development in the application of genetics are likely to include:

- The use of hybrid introgression to breed desirable characteristics of some species/strains (such as saline and cold tolerance) into other faster growing species/strains.
- The application of different genetic improvement technologies (such as selection and sex control) into combined breeding programmes.
- The application of molecular markers to enhance selective breeding programmes through marker assisted selection.
- The application of bioinformatics to enhance breeding programmes, enable strain labeling (e.g. for protection of IPR or breeding rights) and for disease diagnostics.
- The application of sex specific markers, possibly combined with gynogenesis, to increase the efficiency of sex control breeding programmes (such as the YY male technology) and increase sex ratios up to 100% male.
- The development of new strains of transgenic tilapia, most likely incorporating cloned tilapia genes identified through on-going gene mapping programmes.

Whilst it can be said that the levels of genetic improvement present in aquaculture species is some distance behind those developed for other agricultural species, at the current pace of development and with the options available in manipulating fish genomes, the gap may narrow appreciably in the

coming years. The ease of handling and domestication of tilapia together with its reproductive characteristics and relatively short generation time, make this an ideal "model" species for research. When considered in the light of the rapidly growing worldwide commercial importance of tilapia as a cultured species, it appears likely that this will be the tropical species in which we see the most rapid developments of genetic technologies.

Table 1 Summary of major characteristics of commercially important tilapias

Common name	Species	Characteristics
Nile tilapia	*Oreochromis niloticus*	Commonly the fastest growing of the tilapias in freshwater. Breeds readily in many types of hatchery system. Caudal fin bars a distinctive feature A number of colour varieties exist including "red" and "blond".
Mozambique tilapia *or* Black tilapia	*Oreochromis mossambicus*	High fecundity, overpopulation and stunting common. High saline tolerance and well adapted to brackish water. Normally black coloration but red varieties exist.
Blue tilapia	*Oreochromis aureus*	Often sympatric with *O. niloticus* but usually slower growth. Cold tolerant and used in hybridization for production of monosex. Blue colour, no other colour varieties known
None	*Oreochromis urolepis hornorum*	Used mainly for production *of* monosex hybrids. Similar characteristics to *O. mossambicus* although less fecund
None	*Oreochromis spilurus sp.*	Salt tolerant, used in seawater cage culture.
Zill's tilapia or redbelly tilapia	*Tilapia zillii*	Substrate spawner, tolerant of high salinities. Feeds on macrophytes
Redbreast tilapia	*Tilapia rendalli*	Substrate spawner. Feeds on macrophytes
Gallilee tilapia	*Sarotherodon galileus*	Paternal mouthbrooder, saline tolerant. Slow growth
Red tilapia	*Hybrid origins*	Commonly derived from crosses between *O. mossambicus* and *O. niloticus* but some also thought to include introgression *from O. urolepis hornorum* or *O. aureus.* Red colour seldom fixed with red/pink and black blotching common. Saline tolerant but sometimes exhibit low fecundity.

Table 2 Various measures of genetic variability based on data from five microsatellite loci (N = 28-45 fish per sample) in feral populations of *O. mossambicus* from Asia compared to wild caught fish from Southeastern Africa (Source: Agustin, 1999).

Population	Mean no. of alleles per locus	% polymorphic loci	Mean observed heterozygosity
Feral			
Malaysia	1.80	60	0.27
Fiji	2.20	80	0.40
Australia	1.80	80	0.28
Wild			
Bangula	14.20	100	0.86
Elephant Marsh	11.20	100	0.87

Table 3 Summary of the progress in the application of traditional methods of selective breeding to tilapia

Species	Trait	Method	Progress	Source
O. niloticus	Growth rate	Mass selection	No response to selection was detected after 2 generations.	(Hulata *et al.*, 1986)
O. niloticus		Early growth	Estimates of heritability were low producing negative realized heritability	Teichert-Coddington and Smitherman (1988)
O. niloticus	Age at maturation	Within family selection	Fish selected for early maturation matured 11-14 days earlier after one generation of selection. Inconsistent results produced in 2nd generation	(Uraiwan, 1988)
O. niloticus	Growth rate	Mass selection	No response was produced after one generation of selection	(Huang and Liao, 1990)
O. niloticus	Growth rate	Within-family selection	Average response of 3% per generation over 8 generations (recent gains have been greater, Bolivar pers. comm.)	(Bolivar *et al.*, 1994)
O. niloticus	Growth rate	Combined selection	A genetic gain of 12-17% per generation has been recorded over 5 generations of selection	(Eknath & Acosta, 1998)
O. niloticus	Late sexual maturation	Family selection	Significant responses to selection were observed for stage of maturation (-29%) in females and GSI (-39%) in males after two generations	(Hörstgen-Schwark and Langholz, 1998)
O. aureus	Growth rate	Mass selection	High line fish were 49% heavier and 10% longer than random bred controls after one generation of selection. Low line fish were 52% lighter and 21% shorter than controls.	(Bondari *et al.*, 1983)
O. aureus O. niloticus & hybrids	Cold tolerance	Mass selection	Realized heritabilities ranged from –1 to +1 indicating problems with methodology	(Behrends *et al.*, 1996)
Red tilapia	Growth rate	Mass selection	Realized h^2 of 0.32 and 0.37 for weight and length respectively after 5 generations	(Jarimopas, 1990)
Red tilapia	Weight	Mass selection	Results confounded by correlated response. Realized heritability ranged from –0.75 - +1.0	(Behrends *et al.*, 1988)

Table 4 Summary of different hybrid combinations that have been known to produce monosex male progeny

Female parent	Male parent	Note
O. niloticus	*O. aureus*	Applied commercially but results inconsistent
O. niloticus	*O. macrochir*	
O. niloticus	*O. urolepis hornorum*	Majority of broods are all-male Some commercial application
O. niloticus	*O. variablis*	All progenies were monosex
O. mossambicus	*O. aureus*	
O. mossambicus	*O. urolepis hornorum*	All progenies were monosex
O. spilurus niger	*O. macrochir*	
O. spilurus niger	*O. urolepis hornorum*	All progenies were monosex
O. aureus	*O. urolepis hornorum*	
T. zillii	*O. andersonii*	All progenies were monosex

Table 5 Summary of progress in the development of "new" genetically male tilapia (GMT) since its original development and release for culture in 1995 (Mair *et al.* unpublished data)

Basis of genetic improvement	Release no & date	Growth performance
Original GMT (developed in an Egyptian strain)	Release 1.0 (1995)	30-35% faster growing than mixed sex tilapia of Philippine strains
Best crossbred GMT	Release 2.1 (Philippines – 1999) Release 2.2 (U.S.A. - 2000)	Rel. 2.1 15-25% faster growing than the "original" GMT (Rel. 1) in the Philippines
GMT from selected female line	Release 3 (2001)	7.5 – 17.5% faster growing than best crossbred GMT (Rel. 2.1)
GMT from crossbred YY male x selected female line	Release 4 (2002?)	Preliminary data indicates 5-15% advantage over Rel. 3. Still under development

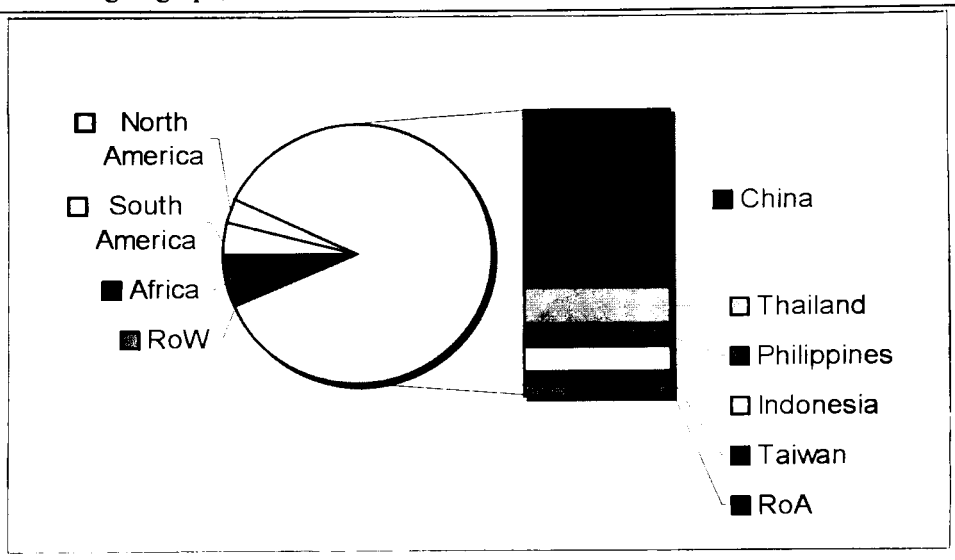

Figure 1 Pie chart illustrating the dominance of Asia in worldwide tilapia production in 1998 (Data Source: FAO 2000)

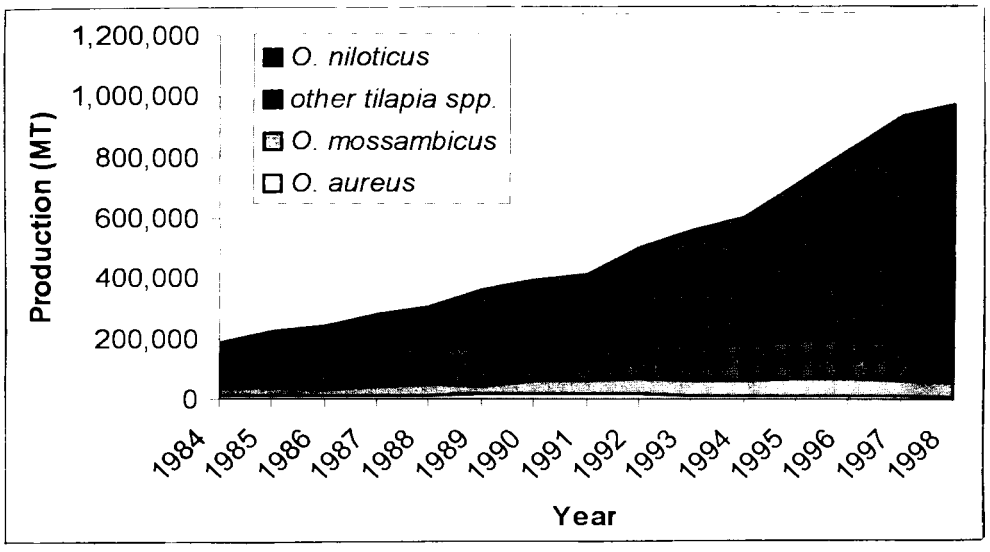

Figure 2 Worldwide tilapia production according to species demonstrating that expansion in production in recent years has come from the expansion in culture of *Oreochromis niloticus* (Data source: FAO, 2000).

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Sex Reversal: the directed control of gonadal development in tilapia

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Abstract

Tilapia are becoming the most widely produced species of freshwater fish in the world. They can be produced in a variety of settings using a range of nutrient inputs. Males are the preferred sex to culture as they grow faster and divert less energy into reproduction. Males can be obtained using a variety of procedures but the most practical is through controlling gonadal development. Recently hatched tilapia fry have gonads that have not differentiated into ovaries or testes. It is possible to give such fish an exogenous source of hormone (androgen or estrogen) to control the development of the gonad. Fry less than 12 mm long can be harvested by seining along the edge of a spawning pond or from specialized spawning ponds where the pond is drained and harvested after 16-21 days. Proper size fish can also be obtained through a more intensive management approach where eggs are collected from the mouths of incubating females.
Most commonly used approach to obtain male tilapia is to feed fry for 28 days or less a feed containing the androgen methyltestosterone. When fed properly the frequency of females in the population can be reduced to less than 5%. The short treatment duration very early in the fish's life history and rapid metabolism of metyltestosterone helps insure that tilapia are free of MT before fish reach the consumer. The production techniques associated with sex reversal are efficient and straight forward enough so that sex reversal has become the commercial procedure of choice to produce male tilapia fingerlings and has been a significant factor in the rapid growth of the tilapia industry

Introduction

An aquaculturist is always looking as to how a product can be produced more economically and brought to the market sooner. The growth rate of the animal being cultured is one of the primary factors affecting the costs of production and how soon it reaches market size. Tilapia are a fast growing fish capable of going from egg to 1 kilo in a year under optimum conditions. Tilapia respond well to a variety of management practices and nutrient inputs making them a popular fish to culture. However, one of the primary disadvantages of tilapia is that they reach maturity early and are capable of reproducing before reaching a marketable size. Most species of tilapia under favorable growth conditions will reach maturity within 6 to 8 months of birth. When mature fish are present in ponds they will reproduce and expend energy on reproduction that otherwise could be directed to growth.

They will compete with their offspring for food, resulting in less food available, slower growth and typically unmarketable fish. Unless reproduction is controlled more than 75% of the fish biomass may be too small for public acceptance.

Growth rates vary among species of fish and even among the sexes of the same species. The sexual dimorphism in growth can be significant. In Nile tilapia the growth rate of males continues to accelerate after they are more than 100 g. in weight. The growth rate of females often begins to slow once sexually maturity is reaches and they start to reproduce (Figure 1). Female tilapia are diverting energy from growth into egg development, forming new eggs to replace those lost each time it reproduced. When reproducing, feed intake by Nile tilapia is limited due to the oral incubation of eggs. This diversion of energy to reproduction and limited feed intake can result in males being twice the size of females of the same age.

For commercial tilapia culture, the issue is not only how to control reproduction during the production phase but also how to have only males for production. Adding a predator can control reproduction, but that does little to address the issue of the slower growth of female tilapia. To obtain maximum growth males are needed. Males can be hand selected but this is an inefficient system prone to errors where half the fingerling production has to be discarded. The genetics of tilapia are such that selective crosses using a male of one species crossed with a female of another has resulted in all male hybrids. This approach was at one time was a common method to produce males but was largely replaced by the mid-1980's. Difficulties in maintaining two pure lines of brood fish and keeping them separate, and the space required contributed to the decline. In addition, the genetics of sex does not appear to be as straightforward as once thought, often less than 100% male progeny would be obtained in certain crosses even if pure lines were maintained.

The most widely used technique to obtain male tilapia is commonly referred to as sex reversal. However this term requires explanation and might be better described as directing of gonadal development. The term sex reversal as used in this paper refers to the addition of exogenous steroids to override the genetically based control of gonadal development to produce a fish that is functionally the desired sex without altering the genetics of the fish. The production techniques associated with sex reversal are efficient and straight forward enough so that sex reversal has become the commercial procedure of choice to produce male tilapia fingerlings and has been a significant factor in the rapid growth of the tilapia industry.

Why Sex Reversal works

The sex of fish is not permanently set at hatch and can be altered by a number of factors. At hatch distinct ovarian or testicular tissues are not present. The gonads develop from primordial germ cells with female differentiation occurring before male differentiation. The point in time when differentiation begins differs among the different fish species. In tilapia and trout this is early in the life history while in grass carp and paddlefish it is months later. In newly hatched *O. niloticus* (Alvendia-Casauay and Carino 1988) and *O. mossambicus* (Nakamura and Takahashi 1985) primordial germ cells are found at the dorsal root of developing mesentery in the mesoderm, ventral to the gut and in the endoderm cells of gut. The germ cells

eventually migrate to the gonadal region. Paired gonadal analgens are observed 9 to 10 days post hatching. The appearance of ovocoel and testocoel, indications of sex differentiation to female and male takes place at 16 to 20 days post hatching in *O. mossambicus* (Nakamura and Takahashi 1985) and perhaps as late as 30 to 33 days post hatching in *O. niloticus* (Alvendia-Casauay and Carino 1988).

Hines et al (1999) found that in the first 7 days post fertilization when no gonadal structures are yet present, the level of androgen in tilapia begins to decline and when indifferent germ cells appear androgen and estrogen levels are lowest. From day-15 to 29 days post-fertilization, they found visible but undifferentiated gonadal tissue. It is in this period when tilapia are vulnerable to exogenous steroids. We are fortunate in that tilapia fry are actively feeding at that point and an exogenous steroid can be added to the diet to influence gonadal development. If tilapia are given exogenous steroids at the proper concentration and frequency from before the start of gonadal differentiation through when it is complete, this will override the genetic control of gonadal differentiation and monosex populations can be obtained.

The exact mechanisms and chemical pathways that control gonadal development in tilapia are not clear. Natural steroid production is not evident in tilapia until differentiation begins (Baroiller et al., 1988) suggesting that some precursor or other compound is involved in directing gonadal differentiation. However, from the numerous studies with tilapia, it is clear that, if exogenous steroids are given before the start of gonadal differentiation and administered past differentiation, it is possible to alter the sex ratio. An understanding of the mechanics of exogenous direction of gonadal development in tilapia is further complicated by the success that has been obtained by short-term immersions in steroid solutions even though the treatment ended well before gonadal differentiation is completed (Contreras et al 1997).

Types of Steroids

Steroids are a group of lipids with several unique properties affecting animal growth and development. Steroids are called androgens if they are able to induce male characteristics and estrogens if they induce female characteristics. Androgens have two physiological actions: (1) androgenic activity, promoting the development of male sex characteristics and (2) anabolic activity, stimulating protein biosynthesis. Androgens can be classified into two groups: androstane derivatives, having both androgenic and anabolic properties, and 19-nor-androstane derivatives that have anabolic properties but only weakly androgenic ones (*Camerino* and Sciaky, 1975). From a sex reversal perspective, androstane derivatives are of more value because of their potential to direct the sexual development of fish into males. When evaluating a steroid for sex reversal by oral administration, three main criteria for selection should be considered: metabolic half-life, androgenic or estrogenic strength and solubility in water. Testosterone is the principal androgen secreted by the testis and the main androgenic steroid in the plasma of human males (Murad and Haynes, 1985). It is often used as the standard to evaluate the androgenic properties of a steroid. It is ineffective when given orally and has a short duration when given by injection due to rapid hepatic metabolism. Synthetic androgens are preferred over natural ones because some

can be administered orally and withstand catabolism in the gut. The chemical structure, bonds and attached groups determine the effectiveness (Brueggemeier, 1986). Introduction of a 3-ketone function or a 3a-OH group or reduction of the 4,5-double bond enhances androgenic activity. Alkylation of the 17a-position or the 1a-position allows for oral activity.

Masculinization

A number of synthetic androgens either applied as a bath or a feed additive have altered the sex ratio of tilapia. Clemens and Inslee (1968) produced all male populations of *Oreochromis mossambicus* incorporating 17-a methyltestosterone into the diet at 10 to 40 mg/kg. Methyltestosterone (MT) has since become the most commonly used synthetic androgen to alter the sex ratio of fish. It has proven to be effective in a number of different species of tilapia and under a variety of management scenarios. Other synthetic androgens have been incorporated into the diet of tilapia for sex reversal are given in Table 1.

A less widely used approach for producing male populations is through the use of non-steroidal compounds that interfere with steroid binding or metabolism. In the sequence of events associated with gonadal differentiation endogenous androgens are aromatized into estrogen by an aromatase enzyme. It is possible to block this action by the addition of aromatase inhibitor. Kwon et al (2000) treated a genetically female population of *O. niloticus* fry with the aromatase inhibitor Fadrozole at 200 to 500 mg/kg diet and obtained 92.5 to 96.0% males. Guiguen et al (1999) was able to skew the sex ratio of an all-female *O. niloticus* population to 75.3% male using the aromatase inhibitor 1,4, 6-androstatriene-3-17-dione at 150 mg/kg of diet. Blocking of estrogen binding sites is another approach to production of males. Tamoxifen, an anti-estrogen when given as a feed additive to tilapia at 100 mg/kg of diet produced an all-male population (Hines and Watts, 1995).

Feminization

Female tilapia are not preferred for culture but feminization of genetically male Nile tilapia *O. niloticus* offers the possibility of all male tilapia through a YY breeding program. Likewise of interest is the feminization of a homogametic male *O. aureus* to produce functional females for mating with normal male *O. aureus* to produce all male offspring.

Estrogens are those agents that induce feminization. Estrone and 17□-estradiol are two natural steroidal estrogens found in the ovary of tilapia (Katz et al. 1971). Synthetic estrogens are more potent than natural estrogens when given orally. This greater activity is due to their stability in the digestive tract and the liver (White et al. 1973). The most commonly used synthetic estrogens for sex reversal are the non-steroidal estrogens, ethynylestradiol (EE) and diethylstibestrol (DES). DES is more potent and once was used as a growth promotant in livestock until banded by the U.S. Food and Drug Administration in 1979. Both are carcinogens.

The effectiveness of DES and EE to feminize may be dependent on the species of tilapia and the management conditions. Hopkins et al. (1979) fed 100 mg DES/kg diet to *O. aureus* fry for 5 weeks and produced 64% females. Rosentein and Hulata (1993) obtained 98% and 100% females in two sets of *O. aureus* fed DES at 100 mg/kg for 30 days. Scott et al. (1989) fed two sets of genetically all male *O.*

niloticus fry DES at 100 mg/kg and obtained 52% females in one set and 84% in the other. EE was given at 100 mg/kg to *O. aureus* for 40 days by Melard (1995) to obtain a 94% female population. Potts and Phelps (1995) fed *O. niloticus* fry EE at 100 mg/kg and obtained a 65% female population. Toxicity is an issue in estrogen treatments. Eckstein and Spira (1965) reported high mortality of *O. aureus* fry when given stilbestrol diphosphate baths at 400 to 1000 ug/L.

Fry Production

For successful sex reversal it is critical that the treatment begin with fish of an age where gonadal differentiation has not begun. Recently hatched fry less than 12 mm in total length are needed. They can be obtained by seining along the edge of spawning pond in the early morning for fry that tend to gather along the edge of a pond or tank. Large quantities of fry can be obtained from specialized spawning ponds where the pond is drained and harvested after 16-21 days. Proper size fish can also be obtained through a more intensive management approach where eggs are collected from the mouths of incubating females. The eggs are incubated in a hatchery and a more uniform age and size of fry obtained. The selection of a fry production technique is influenced by various factors including the number of fry needed at one time, labor availability, water resources, and facilities availability.

Partial harvests

Earthen ponds are stocked with up to 2000 to 3000 kg of brooders/ha at a sex ratio 2-3 females/male. Brooders are fed during the spawning period at approximately 1 to 2% body weight per day and the pond may be fertilized. Some brooders spawn within a few days after stocking and swimup fry can be expected within 10 to 15 days after brood stocking. With once-weekly fry collections, Verdegem and McGinty (1989) obtained an average of 153,100 fry/ha per week (2.2 fry/m^2/d) over a 116-day period. Little (1989) averaged 2.5 sex reversible size fry/m^2/d from ponds stocked with *O. niloticus* harvested every 5 days, six times/day, and 1.5 fry/m^2/d of sex reversible size fry when harvested three times/day every 5 days.

How long a spawning pond can be kept in production depends on how successful partial harvests are. Ideally all fry are harvested before they reach a larger size. Those that escape capture soon prey on subsequent swim-up fry. Macintosh and De Silva (1984) found that even within fry of the same age, cannibalism contributed up to 35% of total fry mortality. Some fry will escape even with careful seining resulting in a progressive decrease in fry harvested due to cannibalism. Little (1989) found that the number of oversize fry harvested could be kept to a minimum (0.015 fry/m^2/d) if the pond was harvested six times/d every five days. Such careful seining is often not practical and it is best to not leave a spawning pond in production for more than 8-10 weeks before making a complete harvest.

Partial harvesting of ponds to produce tilapia fry may be acceptable for locations where the production season is year-round and large quantities are not required at any one time. Fry yields from a pond are variable day-to-day therefore several ponds are needed to produce a constant production of fry. The technique is labor intensive but does not require highly skilled labor.

Complete harvest of fry from ponds

Complete harvest of fry can be made in a spawning pond with a catch basin or from a fine mesh net enclosure (hapa) stocked with brood fish. Spawning ponds are generally no larger than 2000 m^2 and are designed to drain completely into a catch basin that is 10 m^2 or larger (at least 1% of pond area). The catch basin should be 30 to 40 cm deep with a firm bottom, ideally concrete. The spawning pond is prepared by lining the catch basin with large mesh netting that is about 20% larger than the catch basin. This net is used to remove brooders from the pond at harvest without removing the fry. The pond must be completely dry before restocking or if puddles remain they are poisoned with chlorine or other toxicant to insure no fry remain from the last production cycle to cannibalize fry produced in the subsequent cycle. Tilapia fry can remain alive in small puddles for days if a special effort is not made to eliminate them.

Brooders are stocked at a sex ratio of 1 male: 1.5 to 2 females, adding a total weight of fish up to 5000 kg/ha. Brooders are fed at approximately 1% body weight/day and the pond is not usually fertilized. The fish are allowed to spawn over a 2 to 4 week period before the pond is harvested. The timing of the harvest is important to achieve maximum fry yields. Not all females will spawn at the same time but there will be a peak in the spawning activity and a point in time where there is a maximum number of fish of the desired size. If a pond is harvested too soon, part of the reproduction will be eggs or sac fry that are generally lost when the brooders are removed. If the pond is harvested too late, a portion of the fish will have started gonadal differentiation and cannot be sex reversed effectively. Green and Teichert-Coddington (1993) developed an equation to time the fry harvest to obtain the maximum number of fry of a sex reversible size. They found that a 195 to 220 degree-day (temperature°C x no. days) period was optimum for best production of fry suitable for sex reversal.

Once the appropriate degree-days have been reached, fry are harvested by draining the pond into the catch basin early in the day. A screen with a fine mesh and large surface area is placed over the drain to prevent fry from being lost or impinged. Popma and Green (1990) recommend approximately 0.5 to 0.8 m^2 of screen area to drain a 500-m^2 pond over a 5 to 10 h period. Brooders are removed from the catch basin by lifting the netting previously placed in the basin. The brooders may be placed directly into another spawning pond or be separated by sex and be held for a few days in a recovery tank.

Fry are captured from the catch basin using fine mesh hand nets. It is important to be organized and efficient during fry collection. Dissolved oxygen concentration in the catch basin often declines rapidly bringing the fry to the surface. Adequate labor should be on hand to catch all the fry and move them into fresh water in a few minutes. Tilapia fry are not as hardy as adults and extra care is needed to insure that healthy fry are harvested. Special care should be taken to prevent excessive turbidity in the catch basin. Fry should not be held in collecting buckets for more that a few minutes before transfer into clean water.

By following such a degree-hour guide, Green and Teichert-Coddington (1992) obtained 1,500 to 2,500 sex reversible size fry/kg of female brooder stocked with only a minimum of over sized fish. The spawning pond was then prepared again and a new cycle of fry production begun. By making complete harvests and scheduling the timing of the harvest, it is possible to obtain 7.5 to 10 fry/m^2/d, not counting down time between cycles. This method has the advantage of producing large numbers of fry at one time and giving the opportunity to rotate sets of brood stock. However, this approach requires an adequate water supply so the ponds can be drained and refilled frequently, and if the fry are not handled carefully during harvest a high mortality may occur. This approach also requires that the pond be held out of production for a period to insure no fry remain from the previous production cycle.

Spawning in Net Enclosures

The use of fine-mesh cages or net enclosures (hapas) is another alternative for producing fry for sex reversal. Hapas have the advantage in that they can be placed in existing bodies of water where other fish species are present and do not require that the pond be drained before the fry can be harvested. The downtime between reproductive cycles is minimum. A complete harvest of spawning hapas also allows the collection of eggs or sac fry that may have been lost using techniques discussed earlier.

Spawning hapas are typically rectangular in shape, ranging in size from 2 to >500 m^2 and are constructed with 1.6 mm mesh netting (Figure. 2). The hapas are designed to allow the fish to be crowded to one end for collection. Once crowded together brooders can be removed and females examined for eggs or sac fry and any free-swimming fry in the hapa can be removed. Brooders are generally stocked at one male: <2 females at a density of 4 to 5 fish/m^2 of hapa or 0.2 to 0.6 kg/m^2 of hapa.

Sex reversal is most successful when the initial age and size of fry being treated is tightly controlled. One advantage of hapas is that they can conveniently be harvested every 5 to 10 days to obtain fertilized eggs. By using hapas, females can be collected with a minimum of disturbance and each fish can be examined to determine which one is holding eggs. The eggs are rinsed from the mouth and the female returned to the hapa to spawn or placed in a conditioning hapa. As eggs are found in the mouth, the approximate age can be estimated by their color. Younger eggs are light yellow and older eggs a dark orange or brown. As the eggs are collected those of similar age can be pooled for incubation.

Using an incubator system as described by MacIntosh and Little (1995) the sinking eggs of tilapia can be rolled vigorously in a round-bottomed incubator with a downward flow. A high hatch rate can be expected when older eggs are collected and incubated, younger eggs are more difficult to incubate. Fry that are collected right after they swim up and out of the incubator are ideal for sex reversal. They are young and of a uniform size.

Seed production/hapa can be improved particularly when the spawning units are harvested frequently and brood stock are replaced each cycle. The advantage of brood stock rotation is that the reproductive cycle of the brood females is more synchronized, permitting a higher percentage of females to spawn during the next cycle. Two or three sets of female brooders are maintained, one actively spawning and another one or two sets where the females have been separated from the males and are being fed to recover lost energy associated with spawning or from any physical damage. When harvesting every 10 days without brood stock replacement, seed production averaged 106 seed/kg female/d but with female replacement increased seed production to 274 seed/kg/d. (Little et al. 1993). Broodstock replacement can double seed production, but this practice is more labor-intensive and requires additional facilities for brood stock maintenance. A disadvantage of weekly seed collection is that incubation facilities are needed, but short production cycles reduce fouling of nets (if air dried for a couple days between cycles), increase fry production per female brooder, and give uniformly smaller/younger fry. Extended spawning cycles of 21 days for fry production in hapas along with a similar period of brood recovery did not improve seed production in hapas (Lovshin and Ibrahim 1988).

Grading

For sex reversal to be effective fish must be of the proper size. Fry collected soon after swimup from an incubator are generally <9 mm and do not have to be graded before being used for sex reversal treatment. Fry that are collected from ponds or hapas may be of mixed sizes and should be graded to eliminate fish >12-mm. A grader is a mesh container where fish are added, the small fish are able to swim through the mesh into a receiving hapa or tank and larger fish are retained in the grader (Figure. 3). Popma and Green (1990) described a grader made of 3.2 mm mesh metal hardware cloth or plastic suitable for separating tilapia fry. They suggested that a grader with a 1-m^2 working area is adequate to grade 50,000 fry. Grader selectivity should be verified to confirm that 85-90% of the 12 mm fish are able to swim through the grader and no more than 5% of the 14 mm fish are able to swim through. If necessary the mesh size of the grader can be reduced by carefully applying paint to the mesh.

Sex Reversal of Tilapia with Hormone-Treated Feed.

Treatment Setting

During sex reversal all fry must receive a daily intake of hormone from the period before gonadal differentiation has begun until it is complete. This requires that the fish be held in a setting where they will receive an adequate quantity of feed containing the hormone. The early investigations into sex reversal using hormone treated feed were conducted in aquaria or troughs receiving clear water (Clemens and Inslee 1968; Guerrero 1975; Tayamen and Shelton 1978). Tanks with flowing water have been successfully to produce commercial quantities of sex reversed fry (Rothbard et al. 1983; Guerrero and Guerrero 1988). Indoor tanks often are not as suitable as outdoor tanks due to greater disease related mortality.

Initial concerns that tilapia must consume no natural food during hormone treatment proved to be unfounded. Buddle (1984) compared the use of indoor tanks with clear water and outdoor tanks and hapas in static water ponds as treatment units for tilapia sex reversal. He obtained 96- 98% males from those held in hapas or treated in indoor or outdoor tanks. Chambers (1984) working with *O. niloticus* obtained 98.5% males and a 95% survival using hapas placed in a fertile earthen pond or fertile static water outdoor tanks.

When hapas are used to hold fry for sex reversal, they are stocked at densities of 3,000 to 5,000/m^2 of hapa (Popma and Green, 1990) or 12 fry/L (MacIntosh and Little, 1994). The size of the hapa and the number needed should be proportionate to the quantity of fry available on a given day. Hapas with a water surface area of 2 to 5 m^2 and with a water depth of 50-60 cm are convenient for management. The mesh size should be no larger than 1.6 mm but this small mesh will foul during the treatment period. Attention should be given preventing the hapas to become fouled to the point where dissolved oxygen becomes low within the hapa. To help insure overall water quality remains high, 100-200 m^2 of pond area should be allowed for every 10 to 15 m^2 of hapas.

It has been possible to sex reverse fry stocked free into static or flowing water tanks or earthen ponds. Phelps and Cerezo (1992) stocked *O. niloticus* fry into static 20 m^2 outdoor concrete tanks at 150/m^2 and fed a MT-treated feed for 28 days and obtained a 98.3% male population which averaged 1.86 g at the end of the treatment period. Stocking fry directly into earthen ponds as also been effective. Phelps et al. (1995) obtained > 96% males when *O. niloticus* fry were stocked at 200 to 260/m^2 into 215-m^2 earthen ponds and fed MT-treated feed for 28 days. In a second trial, fish were stocked at only 75/m^2, the percentage of males was 91.3%. Many producers in Colombia successfully sex reverse red tilapia in shallow 15-30-m^2 outdoor tanks, stocking fry at 1000-2000/m^2 and exchanging water at a rate of 4-7 times daily (Popma and Phelps, 1998).

Stocking of Fry

Fry are most commonly stocked at densities of 3000-4000/m^2 of hapa, or flowing water tank. Vera Cruz and Mair (1994) compared stocking densities of 1000, 3000, and 5000/ m^2 of hapa using *O. niloticus* and found best sex reversal at 3000 and 5000/m^2 but lower survival at 5000/m^2. High densities help insure an active feeding response needed so all fish are consuming feed. Pandian and Vardaraj (1987) observed that fry could establish a hierarchy in feeding order resulting in small fish not consuming adequate quantities of hormone treated feed for successful sex reversal. This is more common at lower stocking densities.

Fry are first graded if necessary, and counted for stocking. An efficient method is to enumerate the fry by visual comparison. As a standard, fry are counted individually into a bucket or pan, adding enough fish that will give a uniform distribution throughout the container. A second bucket or pan of the same color and size is prepared adding water to the same depth and fry are added until the fish density appears the same. Commonly a 5 gal bucket would be filled with 2"-4" of water and

a standard prepared using 1000 fry per bucket. When enumerating by visual estimation, care should be given to keeping healthy fish in the standard container and replacing them if they become stressed. If another lot of fish is to be counted that might be of a different size, then a new standard should be prepared. It is important to try and avoid having aquatic insects or plant material mixed with the fish being estimated.

Fry can also be enumerated efficiently by weight when a balance capable of weighing 0.1 g is available. A known number of fish is weighed in water and a larger quantity of fish is weighted in water and the number extrapolated. Care should be given to not transfer additional water to the weighing container when the fish are weighed. Sex reversible fry should average 10 to 30 mg at the start of treatment.

Feed Preparation

A highly palatable feed is needed to obtain an active feed response and effective sex reversal. Commercial fish diets for young fish are suitable. They are generally >40% protein, complete in vitamins and minerals with fish oil added to increase the palatability. Effective diets can be prepared using rice bran or finely ground poultry or hog diet and increasing the percent protein by adding fishmeal. The feed ingredients should be reground, mixed and passed through a 0.6-mm mesh screen before use. Vitamins and minerals can be added especially if fry have limited access to natural food, using premixes available for other livestock. The feed particle size should be the equivalent of a no. 00 or 0 crumble of a commercial feed (0.42 to 0.59 mm) for the first week of feeding. A no. 1 crumble (0.59 to 0.84 mm) may be given in the second or third week of feeding.

Steroids are not water-soluble and are added to the diet by dissolving an appropriate quantity of hormone in alcohol, or fish or vegetable oil to prepare a stock solution. Androgens such as methyltestosterone (MT) dissolve readily in ethanol and a stock solution using 95% to 100% pure ethanol can be prepared at a strength of 6 g/L. Ten ml of stock solution added to a carrier and mixed with 1 kg of diet would be adequate to prepare a diet to obtain 60 mg MT/kg of diet. Lesser strength ethanol or isopropyl alcohol, or vegetable oil may be used as a carrier. However, weaker alcohols will add greater quantities of water to the feed. This additional moisture must be allowed to evaporate off or the feed may become moldy. The prepared feed should not be more than 10% moisture. Excess oil can contribute to rancidity and to hormone loss if oil floats off the feed when fed to fish.

The quantity of carrier is dependent on the type of carrier and the mode of application. When small quantities of feed are prepared, it is convenient to use 200 ml of an alcohol carrier and the appropriate quantity of stock solution per kg of feed. The solution is poured over the feed and thoroughly mixed until all the feed is moist. The hormone can also be applied using a lesser volume of carrier solution by spraying the solution over the feed. Both alcohol and oil have been used as carriers when applied as a spray (McAndrews and Majumdar 1989, Killian and Kohler 1991, Galvez et al. 1995). When small quantities of feed are prepared, the

feed is spread into a thin layer, sprayed, mixed and sprayed again. Large quantities of feed can be sprayed in a mixer over a period of time to insure all the feed is exposed to the solution. The moist feed is air dried out of direct sunlight or stirred in the mixer until dry then stored under dark, dry conditions.

Androgens will breakdown when exposed to sunlight or high temperatures. The pure hormone, any stock solution, and treated feed should be stored in the dark at room temperature or less. Varadaraj et al. (1994) compared storage conditions of MT stock solutions and treated feed and the impact on efficacy of sex reversal of *O. mossambicus*. They found that when either the stock solution or feed was exposed to light the efficacy of treatment was significantly reduced. Smith and Phelps (2001) held a feed containing 60 mg MT/kg in the dark in a freezer, then at various times in a 4°C refrigerator or ambient temperature (28°C±1.5) before being fed to *O. niloticus* fry. They obtained populations of >98% male using feed stored under any condition tested including feed held under refrigeration 60 days and an additional 26 days at ambient temperature. When first prepared the feed was analyzed and found to contain 60.4 mg MT/kg and when analyzed after being held in the refrigerator 60 days and an additional 26 days at ambient temperature, the MT concentration was 54.8 mg. When stored under the most harsh conditions the feed which contained 15 % fat showed a sight degree of rancidity but that did not appear to effect palatability or the effectiveness of the sex reversal treatment. Teichert-Coddington et al. (2000) stored a commercial tilapia diet treated to have an initial concentration of 60 mg MT/kg at several temperatures. They found that the MT concentration was 56.9 mg/kg for feed held frozen at –18 C for six months. After they held the feed at 5 C for two months, the concentration was 59.3 mg/kg, and when the MT-treated feed was held at 30 C for four weeks, the concentration was 58.0 mg/kg.
Ideally a freshly prepared feed would be available and used rapidly. Storing the feed in the dark either frozen or under refrigeration will help insure that MT concentrations will remain high however extended storage may affect palatability.

Feeding
Young tilapia fry grow very rapidly and depending on water temperature, consume 20% or more of their body weight/day at the start of hormone treatment. Such a rapid growth requires the quantity of feed being added be increased daily. The quantity of feed needed can be determined from a feeding chart based on anticipated growth and making weekly corrections of the assumed growth rate. Because of the low average weights involved, it can be difficult to obtain accurate average weights in the field. A practical approach is to extrapolate average weight by measuring length and using a length: weight formula to calculate average weight. A sample of at least 50 fish from each lot to be treated should be measured to the nearest mm. It is best that the fish be densely crowded when collecting the sample. This initial length can be used in the following formula to estimate the average weight:

$Weight_{(g)}$ of 1000 fry = 0.02 $L(mm)^3$, where L is the mean total length.

Feeding tables can be prepared based on known mean lengths and anticipated increases in length. Anticipated growth is best estimated based on the results from previous sets of fish. A general guide for anticipated growth would be:

Size range of fry (mm)	Expected growth, mm/day
8-12	0.25 - 0.5
12-17	0.50 - 0.75
17-25	0.75 -1.25

Table 2 is an example of a portion of a feeding table where the length was known on day one at stocking and on day 8 based on a sample measured to the nearest mm. During treatment, the fish should be sampled weekly to determine the mean length and recalculate growth rate. A new growth rate for the upcoming week is estimated based on the growth rate during the last week.

Feeding can be done by hand or by an automatic feeder. The fish should be fed three or more times per day for best growth. Bocek et al. (1992) found that effective sex reversal could be obtained when fish were fed twice daily, 5 days per week but best growth was obtained when fed daily 4 times/d. When automatic feeders are used, the daily diet should be divided into 4-5 portions so large quantities will be released at each feeding. When small quantities of feed are released uniformly throughout the day, the larger tilapia dominate the area around the feeder and consume most of the feed, resulting in considerable size variation and often poor sex reversal.

The length of the feeding period should be adequate to allow all fish to complete gonadal differentiation during the treatment period. This is typically 21 to 28 days but factors such as temperature and food availability need to be considered. Mbarereehe (1992) found that at 18°-22°C, a 40-day treatment period resulted in 95% males but a 20 day treatment gave only 69% males. Pandian and Varadaraj (1988) treated 10-day old *O. mossambicus* with MT for 11 days obtained 100% males; in another set they treated 13 day old fry for 13 days and obtained 69% males. Duration of treatment should be related to initial size and growth conditions. As a general rule, fish should receive at least 14 days of hormone treatment before reaching 18 mm. If growth is slower the duration of treatment should be extended until all fish reach this size or a total treatment period of 28 days is exceeded. If growth is too fast, it may be necessary to reduce the quantity or quality of diet to reduce the growth rate.

Evaluation of Treatment Efficacy

Treatment efficacy should be based on a detailed examination of the gonads of a representative sample of fish. Tilapia can be sexed with reasonable certainty based on the appearance of the genital papilla if they have not been hormone treated. But for hormone treated fish the nature of the gonad does not always correspond with the shape of the papilla. Phelps et al. (1993) followed a MT treatment protocol that gave only a partial sex reversal. They carefully examined

the papillae of 270 *O. niloticus* for the opening of an oviduct and based on the appearance of the papilla, 82% were male. Examination of gonads of the fish with "male" papilla revealed that only 60% had gonads that were all testicular tissue, while 29% were intersex, and 11% had normal ovaries. Likewise, 12% of the fish with female papilla had gonads comprised only of testicular tissue.

The most secure way to evaluate treatment efficacy is by a direct examination of the gonads. For gonadal examination, dissecting equipment is needed along with a microscope, slides and a stain. Guerrero and Shelton (1974) describe a gonadal squash technique using an acetocarmine stain. Other effective stains include fast green and hemotoxin. Fish should be preserved a minimum of 10 days in 10% formalin before gonadal examination. The gonadal tissue of fish preserved for less than 10 days remain elastic and often breaks when being removed. Fish are dissected by making a cut near the anus to below the base of the pectoral fin. The entire gonad, located on the dorsal portion of the peritoneal lining, should be removed carefully beginning ventrally and going forward. For efficient use of supplies, 4 to 5 sets of gonads are placed on a microscope slide and each given a drop of dye. Another slide is placed on top and the gonads are gently rolled or squashed. When larger fish are examined an obvious ovary with readily apparent eggs may be seen in the body cavity, but on occasions, the gonad may also contain testicular tissue and should be examined microscopically. Thick gonads may need to be sliced longitudinally before they can be examined properly. The entire length of gonad should be examined to see if it contains only one type of gonadal tissue.

In a gonadal squash of an ovary, eggs of various sizes will be evident throughout the gonad (Figure. 4). It should be possible to focus up and down on an egg and see the nucleus as well. Testicular tissue is not as obvious to identify. Lobes of the testis will be apparent but other structures are not as distinct (Figure. 5). Connective tissue, oviduct or sperm duct may also appear on the slide.
Gonads may be found that contain both ovarian and testicular tissue (ovotestes). Such intersex fish are found at a low frequency in normal, non-hormone treated tilapia (Clark and Grier 1985, Okoko 1996), most commonly where small portions of ovary are interspersed within the testis. Intersex fish contain a variety of patterns of ovarian and testicular tissue dispersal in terms of the percentage of the gonad that is of a given tissue type and where such tissues are located. Okoko (1996) found some fish with gonads that were anteriorly ovarian and posteriorly testicular, others showed a pattern of longitudinal bands of eggs in predominately testicular tissue.

The reproductive viability of intersex fish is difficult to evaluate. The intersex condition is known only after the fact, when the fish has been sacrificed and the gonad removed. On occasions, bloated hormone-treated adults are found with the anterior portion of the gonad being putrefied ovary with no oviduct evident. Clark and Grier (1985) reproduced several apparently male *O. aureus* and found that three were intersex fish with nonvitellogenic oocytes (25 to 75 mm) within the testicular tissue.

Sampling error resulting from an inadequate sample size or bias can contribute to a misinterpretation of efficacy. When producing on a commercial scale, a minimum of 300 fish should be collected at the end of the hormone treatment period by crowding the fish together and collecting a random sample. These fish should be grown out to 5 cm or more with a non-hormone treated feed in water free of hormone and preserved in 10% formalin. A representative sample of 100 or more fish should be selected for gonadal examination. The sample must represent the length-frequency distribution of the population. Popma (1987) found for Nile tilapia which were 11 to 17+ mm at the end of the treatment period, the smaller fish had a greater percentage of females. Hiott (1990) found that when fish were grown to 4 to 11 cm following MT-treatment, females were more common among the larger fish.

The minimum acceptable percentage male after sex reversal depends on the culture technique, and acceptable market size. When androgen treatments are effective, the percentage females should be less than 5%. The impact of a few females can be significant if a larger market weight is required. Lovshin et al. (1990) found that even 2.5% females in a tilapia population depressed growth within 4 months when no attempt was made to control recruitment. Reproduction was 58% of the weight harvested after 9 month of culture in ponds when females were 2.5% of the initial stock. Recruitment is most serious after the offspring from the few females begin to spawn, typically 5 to 7 months after initial stocking of 20- to 30-g "sex reversed" fish.

In outdoor ponds, production of fish large enough to yield 5-7 oz fillets requires a 2-phase growout or predators being added to control recruitment. Several species of predators including Guapote tigre (*Cichlasoma managuense*), Largemouth bass, (*Micropterus salmodies)*, and tucunare (*Cichla ocellaris*) have been used effectively to control tilapia reproduction in " all" male tilapia ponds (Dunseth and Bayne 1978; Green et al. 1994; McGinty 1983; McGinty 1985; Pike 1983). Although tilapia can reproduce in recirculating systems, predators are seldom used to control recruitment. Females can be removed to some extent by grading. Pruginen and Shell (1962) were able to grade *0. niloticus* weighing 13 to 17.9 g and separate males with 88+% effectiveness.

Health and Environmental Considerations

Sex reversal of tilapia should consider food safety and environmental issues associated with the use of steroids. It is the obligation of the producer to insure that the public receives the highest quantity fish products possible produced using techniques that have minimal negative effects on the environment. In the US the use of drugs in aquaculture is regulated by the Food and Drug Administration (FDA). As a relatively new industry compared to other forms of livestock husbandry, only a few drugs have been approved by FDA for use in aquaculture. Efforts are now underway to obtain approval for the most commonly used steroid, methyltestosterone. There are numerous scientific studies regarding MT to support the approval process. Other androgens that have been used for sex reversal have

a smaller database and will require considerably more investigation to provide the data needed for the drug approval process.

The short treatment duration and rapid metabolism of MT help insure that tilapia are free of MT before fish reach the consumer. Digested MT is rapidly metabolized and excreted. Curtis et al. (1991) fed tilapia fry for 30 days a feed containing radioactively labeled MT. Ten days after the 21-d treatment only a trace of MT could be found. In a study by Goudie et al. (1986), the head and viscera were found to contained >90% of the radio-labeled MT, and after 21 days post-treatment <1% remained. Johnstone et al. (1983) found >95% of the radio-labeled MT in the viscera and no radioactivity could be found 50 h post-treatment. This rapid metabolism and excretion of MT by a fish treated early in its life history, combined with the extended period needed to produce a marketable size fish results in a safe consumer product.

Detailed studies on the environmental fate of androgens are not available but under certain conditions may produce secondary effects. MT is susceptible to breakdown when exposed to light or high temperatures (AHFS Drug Information 97, 1997). Both fungi and bacteria can metabolize exogenous steroids. Many different steroid metabolic reactions, including metabolism of MT, are possible in bacteria (Schubert et al. 1972; Jankov 1977), as well as metabolism of steroids to CO_2 and H_2O (Sandor and Mehdi 1979). In an outdoor pond where fry are treated in hapas the combination of light, temperature and microbial degradation should result in a rapid break down of MT. Phelps et al (2000) studied the fate of MT when given to tilapia fry in outdoor hapas. They found MT levels in water from within the treatment hapa to be in general similar to pre-treatment levels of pond water. Likewise levels in the soil were similar pre and post-treatment.
When water is reused or recirculated, residual levels of MT may be adequate to affect sex ratios. Abucay et al. (1997) found that reusing water that had held tilapia fry during a 25-d MT treatment could alter sex ratios. When a second group of fish were stocked into such water and given a non-hormone treated feed, the sex ratio was skewed. They also found that when "all" female fry were stocked into a cage in an aquarium and MT-treated feed added to the bottom of the aquarium where the fish had no access to it, the sex ratio became skewed to males. In recirculating systems where MT has been given daily, MT may remain in the water column long enough to influence sex ratios (Gomelsky et al. 1994). It is clear that unmetabolized MT and metabolites of MT can accumulate in water of recirculating systems or perhaps static water not exposed to direct sunlight. The degree of accumulation appears to depend on the frequency and dose of MT administered to the target fish. Effects of the excreted metabolites and unmetabolized MT on non-target fish held in the same system could range from elevated serum MT levels to altered sex ratios. However, microbial degradation of androgen in biofilters of recirculating systems appears to occur quickly when androgen is applied in low doses or infrequently. In an outdoor setting the degradation may be more rapid and the effect on non-target organisms less.

Conclusions

Production of male tilapia through the use of androgens is very effective. It does not require that a portion of the production be discarded as in manual selection, or that two separate stocks of fish be maintained as in hybridization. There are several seed production techniques adaptable to most scales of production. The relative ease and predictability of tilapia sex reversal has been a major factor in the rapid growth of the commercial tilapia industry.

Although a variety of hormones have been used for sex reversal, methyltestosterone is the most commonly used androgen. Dose rate and treatment durations vary depending on the environment and the experience of the producer. Tilapia fry <12 mm should be treated for at least 14 days before reaching 18 mm and, if growth is slower the duration of treatment should be extended until all fish reach this size or a total treatment period of 28 days is exceeded. A dose rate of 30 to 60 mg of MT/kg of diet fed at an initial rate of 20% body weight/day should result in successful treatment. The efficacy of treatment should be based on gonadal examinations.

As aquaculture continues to supply an increasing portion of the world's fisheries products, tilapia culture will play a more important role. Sex reversal will remain an important for reproduction control in tilapia.

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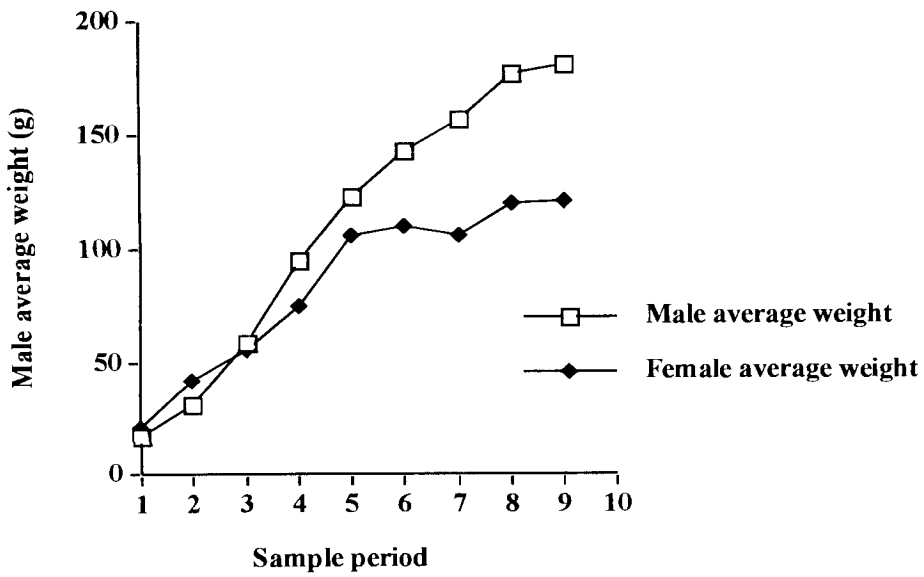
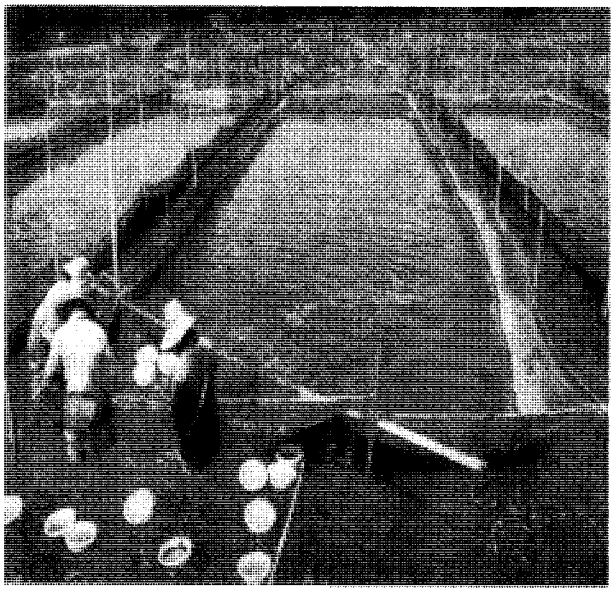


Figure 1. Male and female average weights for *Oreochomis niloticus* when grown separately where the fish were sampled bi-weekly from June 21 to September 30 in Auburn Alabama. Data from Hanson, 1984



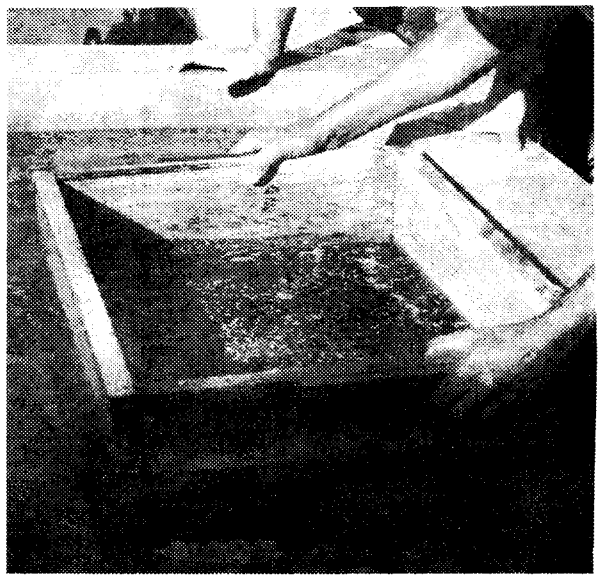


Figure 2. Spawning hapa where tilapia brooders are harvested and eggs collected from the mouths of the females for incubation in a hatchery.

Figure 3. A grader designed to retain tilapia fry too large for effective sex reversal but allowing suitable size fry to swim through.

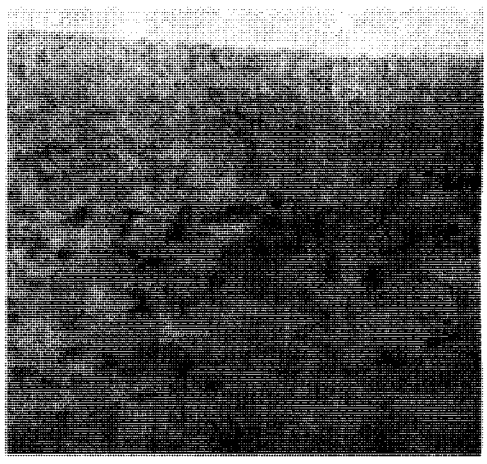


Figure 4. A portion of ovary from a fingerling tilapia when examined as a gonadal squash under the microscope.

Figure 5. A gonadal squash of testes from a fingerling tilapia when examined m

Table 1. Other synthetic androgens that have been incorporated into the diet of tilapia for sex reversal.

Androgen	Tilapia sp.	Dose Rate	Efficiacy	Author
1-dehydrotestosterone	O. aureus	15 mg/kg	69% male	Guerrero 1975
" "	" "	30 mg/kg	59% male	" " "
" "	" "	60 mg/kg	44% male	" " "
ethynyltestosterone	O. aureus	15 mg/kg	85% male	Guerrero 1975
" "	" "	30 mg/kg	98% male	" " "
" "	" "	60 mg/kg	100% male	" " "
fluoxymesterone	O. niloticus	1 mg/kg	87.3% male	Phelps et al 1992
" "	" "	5 mg/kg	100% male	" " "
" "	" "	25 mg/kg	100% male	" " "
mestanolone	O. niloticus	5 mg/kg	99.5% male	Soto 1992
" "	" "	10 mg/kg	97.0% male	" "
" "	" "	20 mg/kg	99.0% male	" "
mibolerone	O. mossambicus	1.5 mg/kg	84% male	Guerrero and Guerrero 1993
" "	" "	1.75 mg/kg	88.0% male	" "
" "	" "	2.0 mg/kg	94.0% male	" "
19-norethisterone acetate	O. mossambicus	1mg/kg	52% male	Varadaraj 1990
trenbolone acetate	O. aureus	25 mg/kg	98.3% male	Galvez et al 1996
" "	" "	50 mg/kg	99.3% male	" " "
" "	" "	100 mg/kg	99.0% male	" " "

Table 2. Feeding table for 1000 tilapia fry, assuming no mortality, fed at 20% body weight during week 1 and 15% during week 2. Fish are sampled weekly to determine an accurate length.

Week	Day	Daily growth (mm/d)	Fish length (mm)	Wt (g) 1000 fish	Feed rate % body wt/d	Daily Diet (g/d)
1	1	sample	11.0	26.6	20.0	5.3
1	2	0.3	11.3	28.9	20.0	5.8
1	3	0.3	11.6	31.2	20.0	6.2
1	4	0.3	11.9	33.7	20.0	6.7
1	5	0.3	12.2	36.3	20.0	7.2
1	6	0.3	12.5	39.1	20.0	7.8
1	7	0.3	12.8	41.9	20.0	8.4
2	8	sample	12.9	42.9	15.0	6.4
2	9	0.7	13.6	50.3	15.0	7.5
2	10	0.7	14.3	58.5	15.0	8.8
2	11	0.7	15.0	67.5	15.0	10.1
2	12	0.7	15.7	77.4	15.0	11.6

Nutrition and feeding of tilapia

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Abstract

According to the most recent estimates, world production of cultured tilapia (*Oreochromis sp.*) is in excess of 1 million metric tons. Tilapia are cultured in a great variety of aquatic environments and with many different management protocols.

The management of modern commercial tilapia production systems is an aquatic analog to North American feedlots used for beef production. The fish are held in cages and raceways at stocking densities that can exceed 100 fish/m^3. The fish are fed to satiation several times each day using specially formulated feeds, and then promptly sacrificed and filleted, for shipment to market.

Modern manufactured fish feeds are not well assimilated by tilapia. Typically only a small fraction (<30%) of the total content of N and P in the diet is incorporated into the fish's biomass (= growth). The remaining amounts of each macronutrient are never ingested (feed not consumed), excreted into the pond water, lost as part of fecal material, or used for maintenance.

There are several commercial farms in Central America that are successfully growing tilapia to export fresh fillets to North America. Additional farms are coming online in the region. In Honduras the demand for tilapia has increased in the past few years and several farms are focusing on local markets. No matter where they are sold, the purchase of feed for fattening these fish is the largest cost in the production budget for commercial tilapia farmers regionally.

Small-scale tilapia culture has not prospered locally. Fish culture is not a part of traditional agriculture in Central America. Local NGO run extension programs have had limited success in assisting rural farmers in growing tilapia. One important problem is the lack of knowledge in the proper management of costly inputs such as fish feed.

How farmers manage the feeding of their fish is often the key to success, or reason for the failure, of a particular farm. This paper discusses some of the biological aspects of tilapia in relation to its nutritional needs and practical feeding of fish in the culture environment.

Introduction

There have been several recent reviews of the nutrition and feeding of tilapia in commercial operations (Diana 1997; Lim 1997; Ulloa 1995). This discussion will focus on the biology of the Nile tilapia and its nutrition and feeding in managed systems. For this paper an intensive production system is defined as a management scheme resulting in a net production exceeding 3000Kg/ha/year in live weight of tilapia.

Anatomy of the tilapia digestive tract

Tilapia are categorized as herbivorous fish. They have special adaptations to separate algae and other particulate matter from water for ingestion. As with almost all animal species, tilapia are not strictly herbivorous in their feeding habits. The consumption of zooplankton, aquatic insects and other animals has been documented for tilapia. Several researchers have reported cannibalism among cultured tilapia. Tilapia will re-ingest fecal material in situations of food scarcity.

Tilapia have small pre-maxillary and pharyngeal teeth to scrape algae (periphyton) from submerged surfaces, and to physically macerate food particles, respectively, prior to digestion. These food items, primarily organisms that form part of the phyto- and zooplankton in pond waters, are highly nutritious and contain elevated levels of crude protein, on a dry weight basis.

There is controversy as to whether tilapia have a true stomach. There is a general consensus that the anterior portion of their digestive system secretes acid (HCl) that results in very low pH (~ 1.0). A low pH environment contributes to the breakdown of algal and bacterial cell wall structure.

There is no obvious anatomical stomach in tilapias. This means that tilapia cannot consume large amounts of food in a short time period, as do carnivorous species. In natural settings these fish are actively feeding during most of daylight hours. In the culture environment, tilapia increase consumption of feed in relation to the number of portions offered daily (Figure 1) (Meyer and Camaaño 1999).

We fed fish once, twice and four times daily to satiation to observe differences in feed consumption in relation to feeding frequency. In the experiment, fish fed four times a day, on an average, consumed twice as much feed as fish fed only once per day (Figure 2). The fish fed twice a day had an intermediate level of consumption.

Although offering feed in multiple daily portions can result in additional production costs, improved fish growth can be realized by greater consumption of the feed. It is interesting to note that the fish fed four times a day consumed similar amounts

throughout the day (Figure 2). They seemed to become trained to expect and consume food at periodic intervals. A similar trend was observed among the fish fed twice daily. They consumed similar amounts at each feeding opportunity.

As with most herbivores, tilapia have long intestines for efficient digestion and assimilation of nutrients from plant tissues.

Natural foods for tilapia

Due to their primarily herbivorous nature, tilapia can efficiently utilize plant tissues and algae in their diet. *O. niloticus* can assimilate more than 70% of the carbon in several species of cyanobacteria (Philippart and Ruwet 1982), common algae in natural fresh waters with low concentrations of nitrogen.

Tilapia also consume zooplankton and detrital material present in water and pond sediments. Small tilapia (<35g) tend to consume a greater proportion of zooplankton, especially larger species of crustaceans (Diana 1997), than do larger fish. As the fish grows there is an ontological shift to consume increased amounts of phytoplankton and smaller species of zooplankton.

The production of natural food items for tilapia can be enhanced by fertilization of pond waters. The Pond Dynamics/Aquaculture Collaborative Research Support Program (PD/A CRSP) has contributed an enormous amount of information regarding the use of fertilizers, especially organic materials, for increasing the production of tilapia in static water ponds (Green et al. 2000).

The application of chicken litter and other organic fertilizers to pond waters stimulates the proliferation of phyto- and zooplankton species. Nutrients released to the water as dissolved N and P stimulate increased phytoplankton growth. The particulate matter in the organic fertilizer, suspended in the water column, is quickly colonized by aquatic microfauna (mostly bacteria and protozoans) and eventually consumed by the fish. The use of manures to fertilize fishponds results in increased production from the autotrophic and heterotrophic aquatic food chains.

Tilapia fingerlings can be grown to an acceptable size for local markets (~150g live weight) in six months using chicken litter applied at rates up to 1000Kg/ha/ week (Green et al. 2000). The production of tilapia based on organic fertilization of ponds can be a profitable activity for rural farmers.

Feeding of tilapia in intensively managed systems

The rapid growth of any animal depends on a nutritious diet and adequate consumption. Food consumption is related to appetite and satisfying the energetic requirements of the animal. Feeding fish in intensively managed raceways or cages can represent 50% or more of the variable costs of production.

The quality of a particular food depends on its composition, and how the feed is stored and managed in a particular feeding regime. We think of good quality foods as being easily digested and well assimilated by the fish.

The processing and physical presentation of aquaculture feeds is a very important consideration. In Honduras there are several feed mills with the capability of manufacturing extruded pellets (= floating pellets) for tilapia.

Tilapia efficiently digest and utilize the protein, lipid and carbohydrates, in many ingredients commonly used to formulate artificial diets for their culture (Table 1). Tilapia digest carbohydrates well, especially complex carbohydrates such as starch. Soybean meal is an excellent source of protein in diets for tilapia. More research needs to be done to properly substitute plant protein for animal protein in diets for tilapia.

Protein is required by animals for growth and maintenance of body components. The amount of crude protein in diets for fish has a great effect on the feed cost. Feed ingredients that provide primarily protein to the formula are expensive. By lowering the crude protein in diets and maintaining fish growth, we can reduce feed costs and improve profits.

Table 1. Digestibility coefficients for protein, lipid and carbohydrate in several ingredients commonly used to formulate diets for tilapia.

Feed ingredient	Protein	Lipid	Carbo-hydrate	Gross energy
Fish meal	80-90	95-99		80-88
Meat and bone meal	70-80			65-75
Soybean meal	90-99		48-58	68-75
Maiz (uncooked)	80-88	85-94	41-49	50-59
Wheat bran	66-76			

Insufficient protein in the diet results in retarded growth or possibly, loss of weight. The inclusion of excessive amounts of protein in the diet will result in higher feed costs, increased contamination of culture water with nitrogenous wastes, and the inefficient use of amino acids in catabolic processes for energy production.

Most animals, including tilapia, requiere the same 10 essential amino acids in their diet (Table 2). The essential amino acids are those that the animal cannot synthesize in its metabolism and must be ingested as part of the diet. In some instances, the animal can synthesize the essential amino acid, but not at a rate sufficient to meet its metabolic needs.

In fish culture the feed is eventually immersed in water. Upon contact with water, the pellets begin leaching soluble components into the pond. Some of the first nutrients to be lost through leaching include water-soluble protein and vitamins.

Feed pellets can lose more than 25% of their crude protein content during two hours immersion in water. This emphasizes the importance of feeding tilapia a daily amount divided into several portions that are offered to the fish throughout the day. This feeding protocol helps to assure rapid and complete ingestion of the offered amount of feed.

Most researchers recommend crude protein levels in diets for tilapia between 25 and 50%. On commercial tilapia farms, with intensive management of the cultures, artificial diets with crude protein levels above 35% become prohibitively expensive.

Table 2. Essential amino acids for tilapia (Lim 1997).

Amino acid	% dietary protein
Arginine	4.20
Histidine	1.72
Isoleucine	3.11
Leucine	3.39
Lysine	5.12
Methionine	2.68
Phenylalanine	3.75
Threonine	3.75
Tryptophane	1.00
Valine	2.80

Practical diets for tilapia generally contain between 25 and 32% crude protein. In static water with abundant plankton, the amount of crude protein in the feed can be further reduced due to the availability of natural foods as a supplement. These natural foods can make an important contribution of protein, vitamins and minerals, to cultured tilapia. On a dry weight basis plankton is very nutritious for tilapia.

We studied ammonia production in relation to dietary protein levels of 25, 35 and 45% in diets for tilapia (Table 3). As the protein in the diet was increased from 25 to 35% we observed improved weight gain but similar levels of TAN excretion rate by the fish. This indicated an efficient use of dietary protein for growth at both protein levels.

When we compared the growth and excretion rates between tilapia fed the 35 and 45% crude protein diets, their weight gain was similar. However, the fish fed the diet with 45% protein excreted a statistically greater amount of ammonia. The increased excretion of NH_3 indicates that the fish were ingesting excess protein and utilizing a greater amount of the amino acids derived from ingested protein as an energy source, not for growth.

The technique to measure ammonia excretion as a physiological response to protein metabolism requires additional refinement. The procedure could prove to be very valuable as a tool to compare the efficiency of utilization comparing plant and animal protein sources for tilapia diets, for example.

Table 3. Fish growth and ammonia excretion rate in response to dietary protein level for tilapia. Values in columns followed by different letters are statistically different (P = 0.05) (Meyer and Peña 2001).

% Protein in diet	Fish growth (g/fish/day)	TAN excretion (mg/Kg/h)
25	0.68a	17.0a
35	1.35b	19.5a
45	1.48b	25.5b

Conclusions

- The offering of feed to cultured tilapia is the most important and costly activity related to fish production on commercial farms.

- The growth of tilapia is strongly influenced by feed consumption and the nutritional value of the feed.

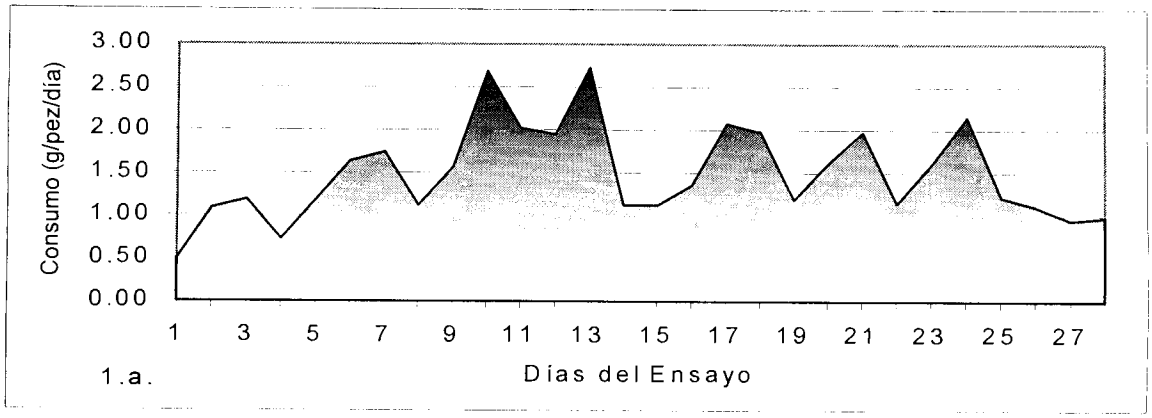
- We should offer feed to tilapia in several portions each day to promote efficient and rapid consumption of the feed pellets by the fish.

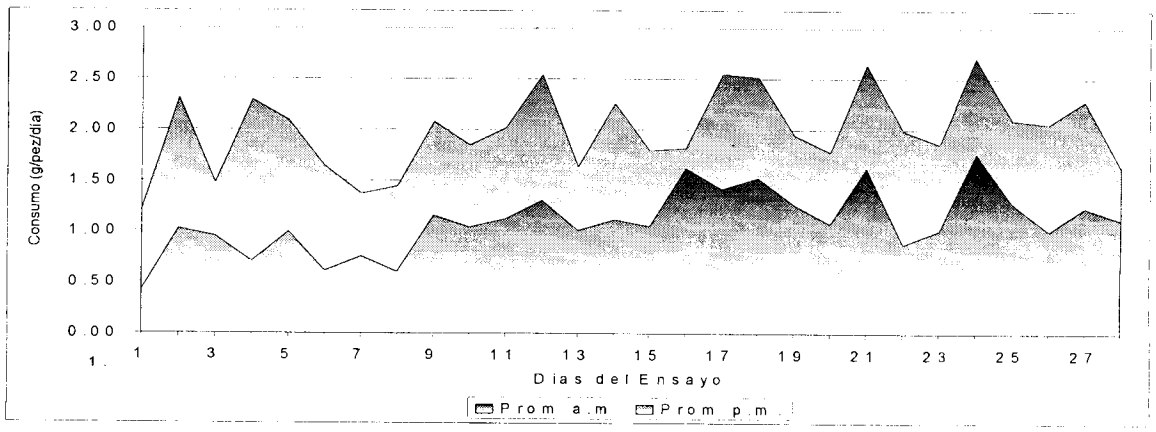
- Floating pellets allow us to better manage the feeding of our cultured tilapia.

- We should carefully monitor protein levels in feeds in relation to feed price and growth rate of our fish.

- Practical diets for tilapia in intensively managed ponds should contain between 25 and 35% crude protein.

- The development of manufactured feeds with improved water stability and containing nutrients more efficiently available to fish should be among the principal objectives of aquaculture nutrition and practical feeding research.





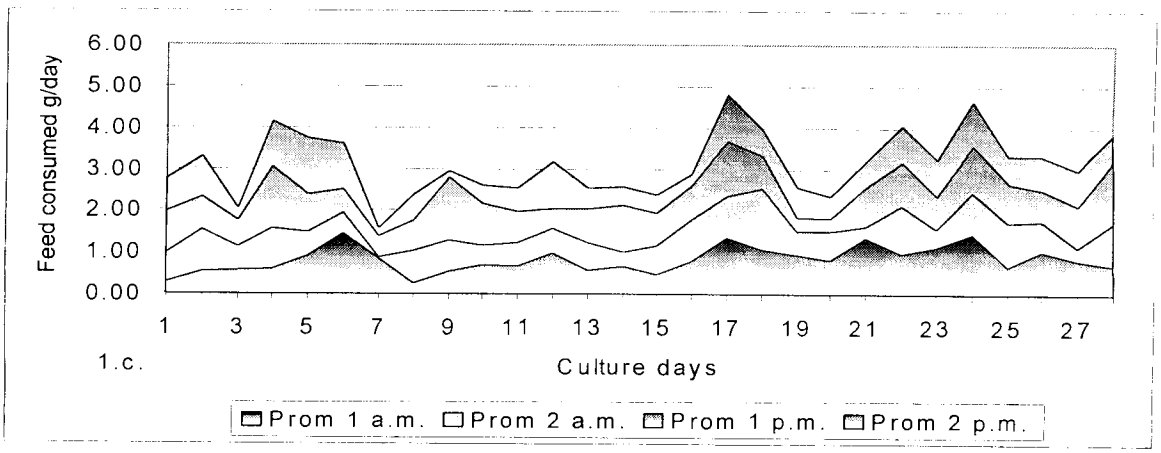


Figure1. Average individual feed consumption by adult tilapia (100g each) fed one, two or four times daily to satiation with a diet containing 30% crude protein, during a 28-day laboratory experiment (Meyer and Camaaño 1999).

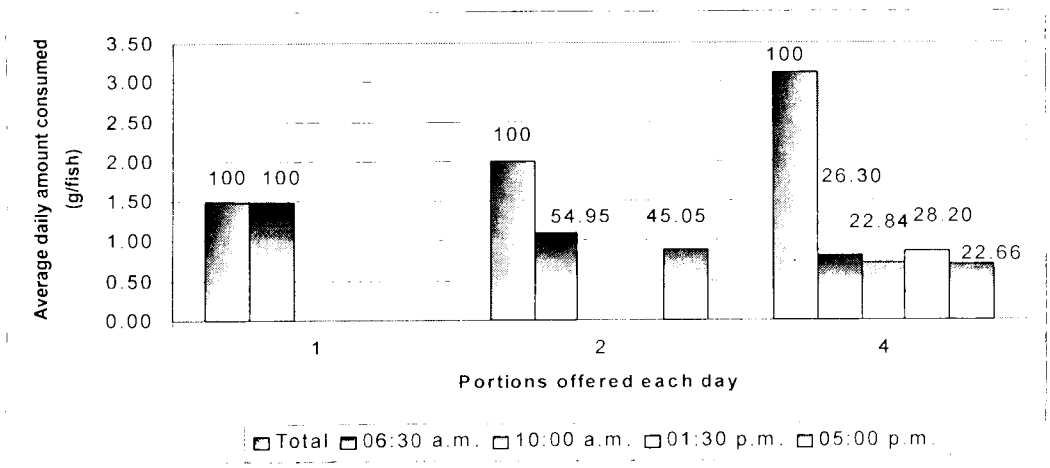


Figure 2. Temporal distribution of the consumption of a 30% crude protein diet by adult tilapia fed to satiation one, two or four times daily under laboratory conditions (Meyer and Camaaño 1997).

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Tilapia genetics: an American perspective

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Abstract

Significant levels of North American tilapia production have developed over the past decade, primarily in response to Asian and Hispanic demand for live fish in large metropolitan areas. Estimates indicate 90 percent or more of domestic production is sold through live markets in U.S. and Canadian cities such as New York, Toronto, Chicago, Los Angeles, Vancouver, and San Francisco. This production is realized in a variety of systems. Tank-based systems are often operated in greenhouses in southern climates and in more heavily-insulated structures in the midwest, northeast, and several Canadian provinces. Tilapia are also produced seasonally in outdoor ponds in a number of states where suitable ter eratures occur, specifically from southern California to Florida and as far northward as Arkansas. Costs of tilapia production must be reduced below current levels in order to expand distribution beyond live markets and compete with imported fillets. The only possible alternative for US producers over the next decade would be to struggle to expand live markets and compete for market share within them. Comparing available strains of tilapia has been problematic for most growers in the US due to the high variability in production systems and management. Outstanding results from one strain in one facility are frequently difficult to repeat in another time or place. In contrast, several large production facilities and hatcheries have reported encouraging results when practicing selection for growth and body conformation within breeding populations. Methodical crossbreeding can also often result in immediate gains simply through alleviating accumulated levels of inbreeding. The use of monosex stocks has been viewed by many US producers as a genetic application that may significantly improve growth rates, and therefore profitability. While sex-reversal is widely recognized, the use of YY male technology is also well established among fingerling producers. The appeal of these methods to US producers is the reduction in unwanted reproduction and the increase (albeit slight in some instances) in growth rate.