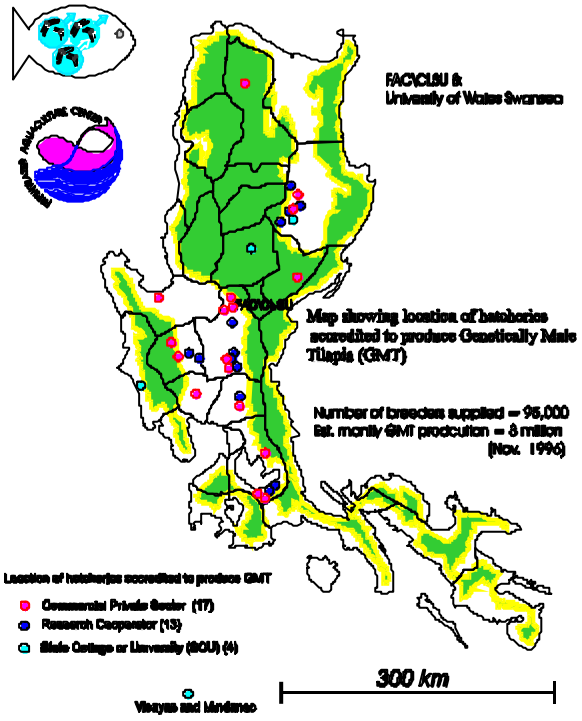
Genetic Manipulations for Improved Tilapia - Technology Adaptation and Development II (R 6070A)

Final Report

April 1, 1994 to March 31, 1997



Graham C. Mair

University of Wales Swansea
Central Luzon State University
Asian Institute of Technology
National Aquaculture Genetics Research Institute, Thailand

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Executive Summary

The research reported here has been conducted as a collaborative project funded solely by a grant (R. 5070A) from the British Overseas Development Administration through the Fish Genetics Programme of its Renewable Natural Resources Aquatic Production Systems Research Programme.

The project has been executed by the School of Biological Sciences of the University of Wales Swansea (UWS), in principal collaboration with the Freshwater Aquaculture Center of the Central Luzon State University (FAC/CLSU) in the Philippines. The research work in Thailand was performed in collaboration with the Agricultural and Aquatic Systems Programme (AASP) of the Asian Institute of Technology (AIT) and the National Aquaculture Genetics Research Institute (NAGRI) of the Thai Department of Fisheries (DOF).

Previous ODA funded projects have resulted in the development of the YY male technology for the mass production of all-male tilapia known as genetically male tilapia (GMT). This technology was developed as a solution to the problem of unwanted reproduction in tilapia culture which has significant negative impact upon yields. It was proposed that the technology could be applied through large scale production of YY male and normal female broodstock which could then be dispersed to hatcheries to be used for the mass production of fast growing GMT. This technology was developed and tested in the Philippines but demand for the products of this technology was such that preliminary investigations had been made into methods for transferring the technology to other countries in the region.

The original purpose of this research (defined as the measurable near term impact of the project) was to "Increase tilapia production through adoption of the YY male technology and the culture of *O. niloticus* in brackishwater".

This overall purpose was further outlined in the following series of objectives:

- Performance testing of GMT and YY males, the main activity being on-station and on-farm trials of GMT producing broodstock.
- Identification of social and economic factors relevant to the uptake of the YY male technology and its products.
- Evaluation of technology transfer to Thailand through the completion of technological development and growth comparison of the different genotype/phenotype combinations.
- Evaluation of salinity tolerance in available strain of *O. niloticus* in brackishwater environments.

The following summarises the progress made towards the proposed outputs of this project:

- *Programme and necessary broodfish for mass production and appropriate dissemination of the YY male technology developed and initiated.*

  Achieved - Based on the results of this project, in particular the evaluation of the performance of YY males in commercial hatchery systems which showed that hatcheries can apply the technology effectively, a dissemination programme was designed and initiated in the Philippines. The programme was initiated earlier than planned and has exceeded expectations to the extent that the technology is already likely to be having a significant impact upon the industry.

- *Feasible and effective methodology for technology transfer of YY male technology developed.*

  Achieved - The research at AIT demonstrated that indirect transfer and the application of the technology anew in the local strain can both be used to transfer the YY male technology, in addition to the simplest form of direct transfer. The growth trials indicated that the crossbred (local female x introduced YY male) and the local (Egypt-AIT strain) GMT exhibit superior growth performance to the introduced GMT in most environments and are likely to become the preferred GMT in Thailand.

- *YY male technology transferred to Thailand.*

  Achieved - The technology was effectively transferred to Thailand, initially by direct transfer (using immediate descendants of fish introduced from the Philippines). The Thai Department of Fisheries has taken a policy decision to disseminating the products of the technology throughout the country. This dissemination has commenced, on a small scale via local fisheries stations.

- *Case studies on socio-economic status of tilapia farmers produced and likely long term impacts of the introduction of YY male technology determined.*

  Partially achieved - Two case studies were completed and the principal factors related to the adoption of technologies were identified. However, without setting up farm trials it proved difficult to accurately predict the likely long term impacts of the introduction of the YY male technology.

- *Brackishwater tolerant strain of O. niloticus identified.*

  Not achieved - with the benefit of hindsight this activity did not integrate well with the principal activities of the project. Only one field trial was completed which did not yield much useful data. This topic should be researched in a single project dedicated to this objective.

- An additional output of the project was the identification of appropriate tagging methods for use in marking YY males used in the dissemination programme.

By the end of the project a full dissemination programme was underway in the Philippines. Over a two year period more than 2.5 million GMT fingerlings had been dispersed to over 170 farms in 20 provinces in the Philippines. Furthermore, a network of hatcheries accredited to produce GMT had been established with an estimated production of 36 million GMT per annum coming from 17 hatcheries. The programme is generating income and is moving towards financial sustainability of research and dissemination activities related to the YY male technology in the Philippines. Initial income was used to produce information materials including a technoguide and a video describing the technology. A smaller but significant dissemination programme was also initiated in Thailand by the Department of Fisheries.

Background

A major constraint to the optimisation of yield in tilapia culture

Tilapia, a tropical fish species originating from Africa, has been introduced around the World and is widely cultured throughout the tropics and sub-tropics. The Philippines is the World's second largest tilapia producer, with production in 1993 recorded as 95,000 MT per annum (FAO, 1995), this almost exclusively for local markets. Tilapia provides a vitally important source of protein, largely in inland, rural areas where tilapia culture presents a sometimes lucrative alternative to more traditional forms of agriculture. The Nile tilapia, *Oreochromis niloticus*, considered the best species for freshwater aquaculture, has many attributes suited to domestication and culture. These include good flesh quality and flavour, a wide tolerance of different environments, resistance to many common fish diseases, and relative ease of reproduction in captivity.

This ease of reproduction actually represents one of the principal problems in the optimisation of yields in tilapia culture, the fish breeds too readily. Energy is diverted from growth, into the behavioural and physiological interactions between the sexes and into the production of gametes. Females also divert energy into maternal care of eggs and embryos which is a contributing factor in smaller sizes of females compared to males. A consequence of this reproduction is overpopulation in discrete culture systems such as ponds and tanks, which, in extreme cases, can result in a cessation of growth altogether if the carrying capacity of the system is exceeded. Harvests commonly include 30-40% of largely unmarketable small fish resulting from unwanted reproduction.

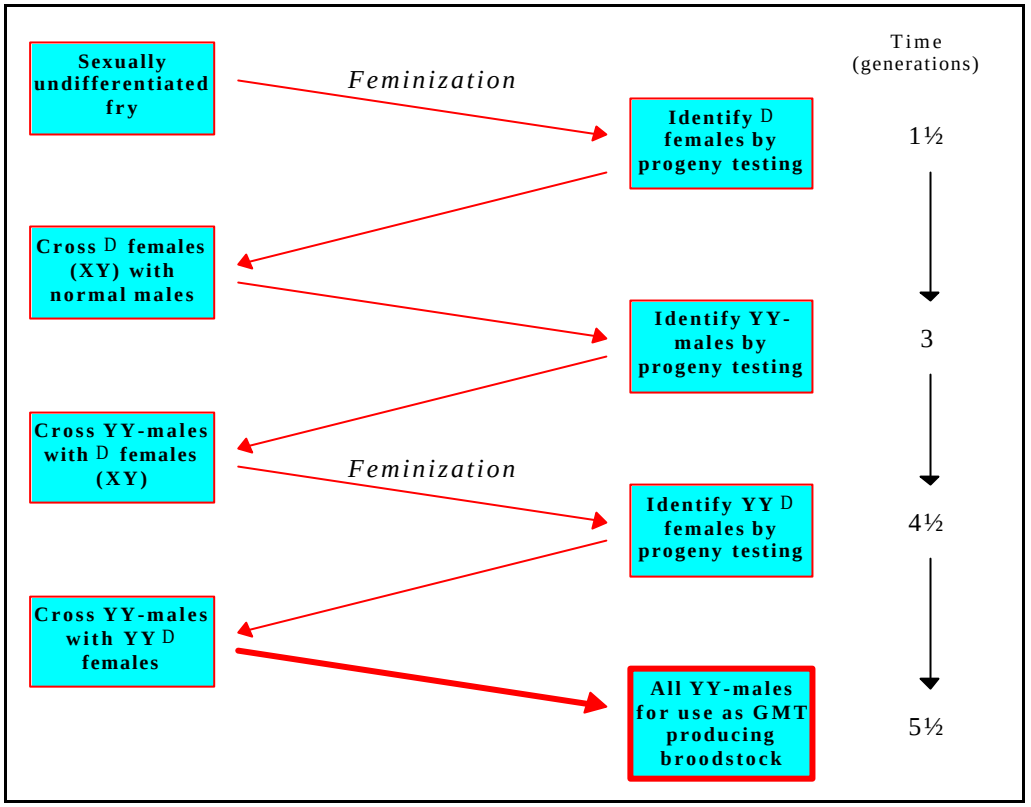
The most effective solution to this problem is to grow only one sex, preferably males as they grow faster and to a larger size. There have been numerous technologies developed for this purpose including hybridisation and hormonal sex reversal but none have achieved this in a consistently effective, affordable, and environmentally sound way.

Research and Development of the YY male technology

Following on from basic laboratory based research on the genetics of sex determination, the University of Wales Swansea (UWS) initiated a collaborative research project with the Freshwater Aquaculture Center (FAC) of the Central Luzon State University (CLSU) in August 1988, based principally in the Philippines. This project, funded by the Overseas Development Administration(R4452) was designed to test the feasibility of a proposed breeding programme for the genetic manipulation of sex determination to mass produce all male tilapia through a combination of hormonal feminization and progeny testing (see Figure 1). Research progressed well with the results indicating that the technology was effective

and feasible and had the potential for application on a commercial scale to the benefit of tilapia farmers in developing countries.

Figure 1 Schematic diagram depicting the model for large scale production of monosex male tilapia.



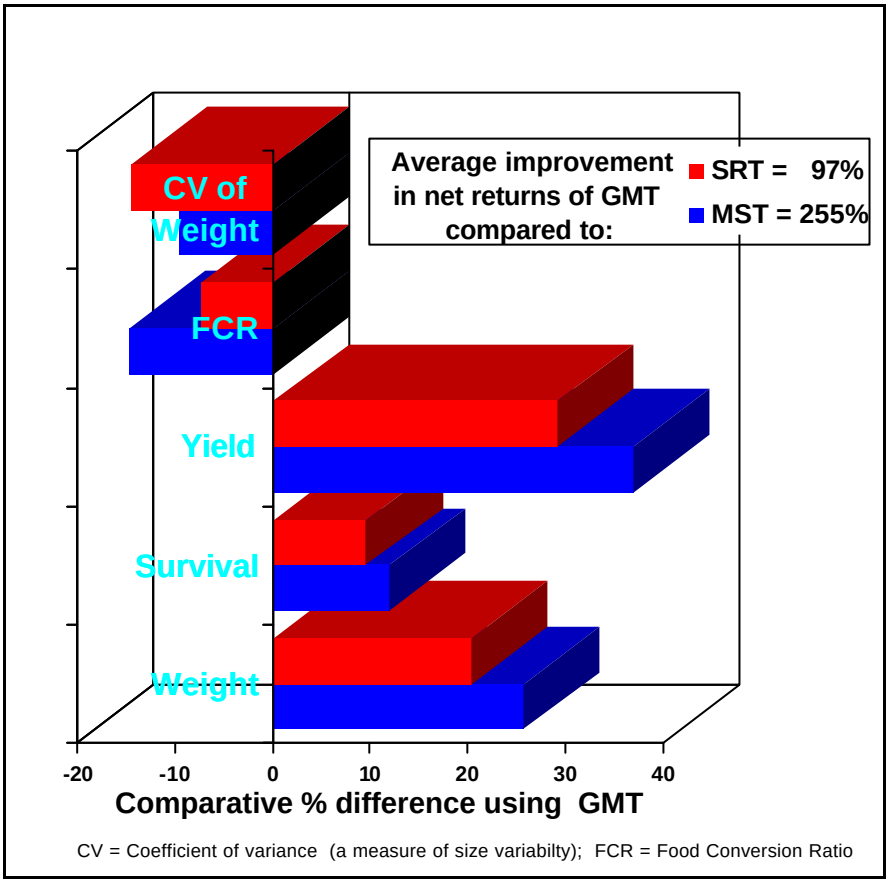
A second follow on project (R4803) was funded, now under the ODA's newly formed Fish Genetics Programme, to continue the research on the technical aspects in the development of the technology, commencing in April 1991. The research was expanded in November of 1992 with the addition of a further project (R.5068A) conducting adaptive research on the field testing and transfer of the YY male technology to Thailand. This latter project known as GMIT-TAD I had two principal objectives:

- To test the feasibility of large scale production of genetically male tilapia (GMT) and determine their potential impact for Philippine tilapia growers through the conduct of a range of on-farm growth performance trials.
- To conduct a feasibility study of the transfer of the YY male technology to other countries in the region through transfer of fish to Thailand and initiation of breeding programmes in collaboration with partner institutes in Thailand.

In relation to the first of these objectives, trials were initiated on 32 different farms throughout the tilapia growing regions of the country, representing the commonly used culture systems in the Philippines. Despite a high failure rate by the end of the project these trials did produce sufficient data for analysis and for firm conclusions to be drawn. The trials

confirmed that GMT has very considerable benefits for aquaculture. GMT produced significant improvements in all commercially important harvest characteristics including growth, survival, yield, food conversion and uniformity of harvest size. These differences were clearly apparent (although not always statistically significant) between GMT and both MST and SRT (see Figure 2). The magnitude of gains shown by the culture of GMT were similar for all environments (Mair, 1995). Collection of economic data showed that net returns varied widely but the pooled average increase in net returns using GMT compared to controls was very consistent across ponds, cages and tanks ranging from 116 to 125%.

Figure 2 Summary of the comparative harvest characteristics of GMT and controls (either mixed sex tilapia - MST or hormonally sex reversed male tilapia - SRT) from 18 completed on-farm trials in a range of Philippine tilapia production systems. Values represent percentage difference of GMT compared to the two types of control fish.



Significant progress was also made regarding the second objective of investigating means for transfer of the technology to Thailand. Genetically manipulated fish were transferred to Thailand from the Philippines and used in the evaluation of three alternative methods of transfer i) direct transfer by producing GMT in the transferred Egypt-Swansea strain; ii) indirect transfer by producing F_1 hybrid (with the option of subsequent back crossing to the Thai strain) GMT between the Egypt-Swansea and Thai strains; and iii) developing YY males anew in the a local strain. Data showed that all of these approaches are possible,

although with respectively increasing investment of resources required. The direct option proved to be the simplest, cheapest and quickest method. Results from the development of indirect approaches revealed that sex ratios of F_1 hybrid GMT and GMT resulting from F_1 hybrid YY males, were lower than predicted based on the results from previous work within the Egypt-Swansea strain (for example hybrid F_1 GMT had mean sex ratios of only 89-93% male). Some progress was made in developing YY males only within the Thai strain but YY males could not be identified within the timescale of this project. Data from preliminary growth trials revealed that monosex fish (as either GMT or SRT) of the Thai strain, and to some extent of the F_1 hybrids, grew significantly faster than those of the Egypt-Swansea strain in Thai environments. It was concluded that indirect transfer via crossbreeding was likely to be the most appropriate mechanism of technology transfer but that further research was required to confirm this and to determine the potential benefits from the transfer of this technology.

Researchable constraints to further development and uptake

The results from the on-farm trials under R.5068A clearly indicated that there are very significant production and economic gains to be made from disseminating the products of the research and development work on the YY male technology. Towards the end of the project a decision was taken by CLSU, with the support of other NARS institutes, to commence dissemination of the products of this technology in the Philippines. However, although the benefits of growing GMT had been clearly established, the characteristics of the YY males used to produce them were not well determined.

From on-station work it was known that they were viable and that they produced very high proportions of males in their progeny. Little was known about the commercially important traits relevant to the use of YY males as broodstock, namely their growth, survival and reproductive capacity. This information was of considerable relevance to the dissemination of the products of the research as GMT could only be dispersed in significant numbers through the distribution of GMT producing broodstock to commercial hatcheries. If the YY males proved to have significant disadvantages in terms of survival, growth and especially reproductive capacity, commercial hatcheries may be unwilling to adopt the technology. Furthermore it is necessary to determine the cost benefit ratios of the production of GMT using YY males in commercial hatcheries and compare these with those of the production of mixed sex or sex reversed male fish, in order to develop appropriate dissemination strategies.

Allied to this specific research, it was also considered important to gather some background information on social and economic factors in Philippine aquaculture and how these pertain to the adoption and impact of new technologies such as the YY male technology. It was

these researchable constraints that provided the basis for the research conducted in the Philippines and reported here.

In Thailand, the work on evaluating the various technology transfer options had progressed well under R.5068A but had not gone far enough to enable a decision to be taken on the best method for technology transfer and the appropriate fish for evaluation and potential dissemination in Thailand. Research under R.5068A indicated that, in the test environment at AIT, the local strain (Egypt-AIT) performed better (in terms of growth and survival) than the introduced strain (Egypt-Swansea). However, the crossbred between the two strains had not been adequately assessed and YY males (and therefore GMT) had not been produced in the local strain so direct comparisons could not be made with the introduced GMT. It was therefore a priority to continue with the development work on the YY male technology in Thailand and to comprehensively evaluate the products of this technology transfer in comparison with existing tilapia in Thailand, in on-station and on-farm growth trials.

Further to the above, but not directly related to the production of monosex male tilapia, there is a rapidly growing interest in the culture of tilapia in brackishwater throughout the region. This demand is large and is associated with the decline in the profitability of the culture of prawns and milkfish, the traditional brackishwater cultured species. It is the policy of the Philippine Government to support the culture of tilapia in brackishwater and the identification of saline tolerant strains or species (G. Morales and S. Aypa, pers. comm.). However, *Oreochromis niloticus*, the most popular species for production in freshwater aquaculture systems due to its faster growth, has a lower tolerance to high salinity than other species of tilapia. There was thus, considerable interest among several sectors of the tilapia culture industry in the Philippines (and in other countries of the region) in the identification or development of a high performing (in terms of growth and survival) tilapia, tolerant of the fluctuating and at times, high salinity, in brackishwater ponds . There was therefore, a demand from both the private sector and among the Philippine NARS to conduct research in this area with the objective of identifying or developing 'brackishwater tilapia'.

Project Purpose

The original purpose of this project (defined as the measurable near term impact of the project), as defined in the logical framework (see Appendix 1) was as follows:

Increase in tilapia production through adoption of the YY male technology and the culture of O. niloticus in brackishwater.

This overall purpose can be further outlined in the following series of objectives.

Performance testing of GMT and YY males:

On-farm trials of GMT, initiated under R.5068A would be completed but the main activity would be the on-station and on-farm evaluation of the performance of YY males. These would include an assessment of all the commercially important traits of the fish. This objective would effectively complete the technical evaluation of the commercial application of the products of the YY male technology. Having demonstrated that the GMT had significant benefits for tilapia growers (with few, if any, technical disadvantages) it was necessary to conduct a similar study of the performance of YY males in commercial hatcheries to ascertain its comparative performance with the fish presently used in these hatcheries and to determine any significant advantages or disadvantages of their use as broodstock. The on-farm study should also compare the costs of production and income generated from GMT to determine the relative profitability of production.

Social and economic factors relevant to the uptake of the technology

It is necessary to study the social and economic issues relevant to the uptake of genetic technologies in tandem with studying the technical aspects. At the start of this project there was a paucity of available information on social and economic issues pertaining to the Philippine tilapia culture industry. Although technologies targeted at solving the problem of unwanted reproduction in tilapia culture, such as sex reversal, have been available to farmers for some considerable time, rates of adoption have been low. There are possible technical reasons for this but there may also be underlying social and economic factors behind this lack of impact. Due to the lack of baseline data other than some micro economic studies of hatcheries and grow-out farms and some marketing surveys (Smith *et al.*, 1985) we wished to conduct an in-depth study of social and economic factors in a community of hatchery operators and a community of tilapia growers. The principal objective of this study, which was primarily constituted by the PhD research of Ruben Sevilleja, the director of FAC/CLSU, was to address the following broad questions and to determine the key social and economic factors pertaining to them:

i. What are the factors which contribute or impede the adoption of tilapia culture technology; and;

ii. What are the economic and social consequences that follow the widespread introduction of the technology, within the context of the agrarian structure in terms of the financial benefits which accrue to the participants.

Further to this we also wished, in a separate study, to survey common tilapia hatchery practices to identify key factors that may influence specifically, the application and subsequent uptake of the YY male technology and its products.

Technology transfer to Thailand

The previous project (R. 5068A) succeeded in transferring genetically manipulated fish to Thailand and made significant progress in evaluating two of the three methods of technology transfer (namely direct and indirect). However, the emphasis on the previous project was in the breeding and technical aspects of technology transfer. The project also did not succeed in developing YY males in the local Egypt-AIT strain for direct comparison with the introduced fish. The priorities in the present project were to complete the technological development through the production of GMT representing the three different methods of technology transfer and to conduct a comprehensive growth comparison of the different genotype\phenotype combinations. The ultimate objective was to collect data to enable informed decisions to be made regarding the options for technology transfer and formulate recommendations concerning the choice of fish for further field testing and ultimately dissemination within Thailand.

Salinity tolerance of *O. niloticus*

The previous ODA funded projects in the Philippines had been responsible for the introduction of a number of strains of *O. niloticus* into the Philippines and these are maintained in a live gene bank at the Freshwater Aquaculture Center of Central Luzon State University. At present in the Philippines, and in other areas of the region, the species of choice for culture in brackishwater is commonly *O. mossambicus*. However, this species has poor growth rates, is less favoured in most markets and has a high fecundity leading to overpopulation. It is thus considered unlikely that this is the best choice of species for brackishwater pond culture. It was thought that there is likely to be genetic variation for salinity tolerance in strains of *O. niloticus* and thus that some strains may be better adapted to culture in brackishwater than others. Typically *O. niloticus* survives well up to 18ppt but if salinities rise above this level, especially if for sustained periods, as is common in many brackishwater fish ponds, survival and growth rate are both adversely affected. The principal objective of the research under this project was to compare some commercially

important characters of several strains of *O. niloticus* in brackishwater environments and hopefully to identify one or more strains that are better adapted to culture in this environment. Identification of such a strain could act as a catalyst for expansion of the culture of tilapia in brackishwaters leading to an increase in tilapia production and more effective utilisation of coastal fish ponds.

Although the stated purpose apparently gives equal weighting to the work on the development of the evaluation of the YY male technology and on the identification of a saline tolerant strain, the latter was always considered secondary to the former.

Project Organisation

Introduction

As it built on previous projects and collaborations, the project had a well developed and established structure as shown in the accompanying organogram (see Figure 3). The project was co-ordinated by Dr. Graham Mair of the University of Wales Swansea who reported directly to the ODA's programme manager. The project conducted research in the Philippines and Thailand and as these activities have minimal overlap, these can be considered separately.

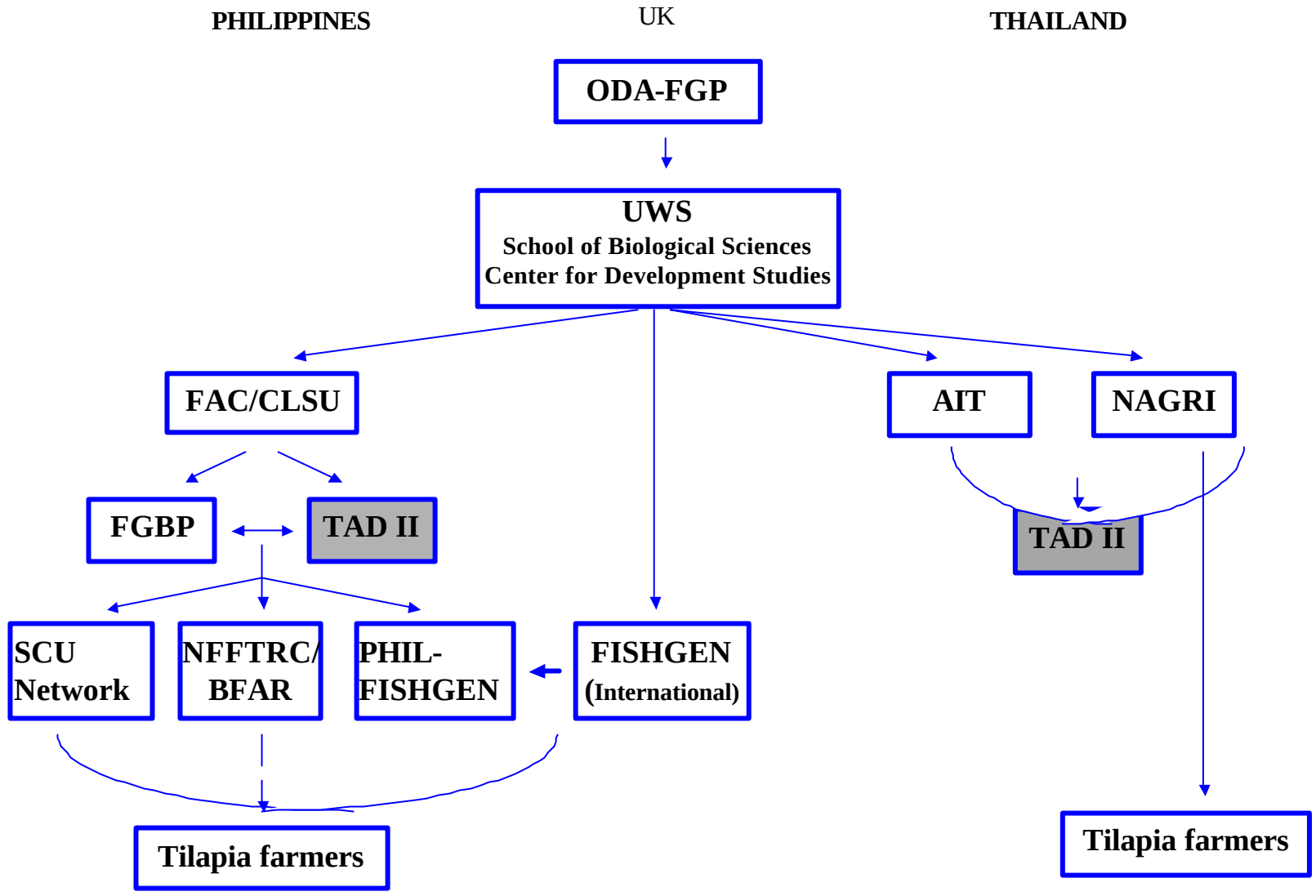
Philippines

Collaboration and institutionalisation

Links were developed at the beginning of the project between the School of Biological Sciences and the Centre for Development Studies (CDS) at UWS. Staff of CDS were initially involved as Ph.D. advisers to Ruben Sevilleja, Director of the principal Philippine collaborating institute, with his work on the socio-economics of genetic improvement technologies in Philippine aquaculture. During the first year of the project Dr. Gerard Clarke from CDS became involved in the project in the context of using the dissemination of GMT as a model for studying the potential impact of the introduction of transgenic tilapia. Dr. Clarke's work contributed significantly to our socio-economic studies.

The principal collaborator in the Philippines was the Freshwater Aquaculture Center (FAC) of Central Luzon State University (CLSU), with whom UWS has collaborated on various aspects of the YY male technology since 1988. With support from UWS, FAC-CLSU formulated its own Fish Genetics and Biotechnology Programme (FGBP) in 1994. This programme effectively institutionalised the staff and activities developed under R. 5068A as described in the final report of this project (Mair, 1995). The principal purpose of the FGBP was to continue research, development, testing and dissemination of the YY male technology. For this CLSU included a line item in its annual budget for 1995 and subsequent years of approximately 1,000,000 pesos (£25,000). This enabled the hiring of all of the field staff and some of the research staff that had been trained under R. 5068A and even included a small number of staff destined to be taken up by this project.

Figure 3 Organogram show the organisational structure of the project (TAD II) and linked activities



Personnel

It was intended, from the financial year 1995\96 to hire three senior research staff, one administrator and one field assistant. As it transpired one of the senior researchers, the administrator and the field assistant were all taken up by CLSU under institutionalisation. In their place we hired two labourers and a temporary post doctoral researcher. A major problem encountered in 1995 was staff shortages. Miss Leah Dahilig, who had worked as a research associate, with principal responsibilities for on-farm research under R. 5068A, resigned at the end of May 1994 in order to take up work in the private sector, despite arrangements for her to register for a Ph.D. with AIT in Thailand. Miss Dahilig's departure caused very significant delays to the on-farm research under this project as it took some time to identify a suitable replacement, Mr. Ernesto Morales who joined the project in October 1994, more than six months after its start.

A further problem was the delay of the return of the assistant project leader, Dr. Josie Capili who completed her Ph.D. at UWS and returned to the Philippines only in August 1994. This brought about delays to other parts of the research programme, particularly the work on salinity tolerance.

Fishgen and Phil-Fishgen

As part of the dissemination activities and with a view to the future financial sustainability of the research activities in the Philippines, 1994 saw the establishment of an income generating project, Phil-Fishgen. This was established as a project of CLSU, as approved by the Board of Regents in August 1994. Phil-Fishgen is affiliated to Fishgen Ltd., a UWS company set up to commercialise the products of research on the YY male technology. The principal activities of Phil-Fishgen were and are to disseminate the products of the research under this project and under R 6058 (namely GMT and GMT producing broodstock), to Filipino fish farmers with emphasis on small farmers. In doing so, income generated is intended to be used to fund future research activities (through the ODA Fish Genetics Programme), to fund dissemination activities and to provide additional financial support to the project staff to ensure their loyalty and reduce the risk of losing highly trained staff to the private sector.

Facilities

Under the project, the development of a commercial scale hatchery at FAC, CLSU was completed but by the end of the project was not fully utilised due to insufficient seed production from the hapa based hatchery. In addition to this a new pond site was developed for semi-intensive pond based hatchery production. The construction of this pond site, consisting of thirty-two 200 m^2 hatchery ponds and two 600 m^2 nursery ponds was completed by mid 1996 under funding from Phil-Fishgen (CLSU contributed for the perimeter fence surrounding this site). In addition to the aforementioned new facilities, renovations were

made to an existing pond site of CLSU which was intended for use in the hapa based production of GMT.

Thailand

Collaboration

UWS collaborated with two institutes in Thailand, the Agriculture and Aquatic Systems Program (AASP) of the Food and Agricultural Engineering Division of the Asian Institute of Technology (AIT) and the Thai Department of Fisheries National Aquaculture Genetics Research Institute (NAGRI). A Vietnamese research associate at AIT, with the assistance of one field worker, worked on the investigation on mechanisms for transfer of the YY male technology to Thailand. At NAGRI, the project has only one staff, who had the role of evaluating the performance of the Egypt-Swansea YY males and GMT in Thai aquaculture. The Department of Fisheries recognised the potential value of the YY male technology and invested its own financial resources and five additional staff into the replication of GMT producing broodstock and of GMT themselves. In addition, the Department of Fisheries has produced information material on the technology and has commenced dissemination of GMT and some GMT producing broodstock. No facilities have been developed specifically for project activities either at AIT or at NAGRI.

Research Activities

Significant progress was made towards most of the objectives of this project although more so in some areas than others. Also significant research was conducted over and above that necessary to achieve the purpose and objectives. There were however some delays in initiating project activities due to late approval of the project (and late transfer of project funds) and the staffing problems that arose due to the departure of a key research staff in the Philippines.

Performance testing of GMT and YY males

Completion of on-farm trials of GMT

Of the 32 on-farm trials comparing the grow-out performance of GMT with that of farmer's normal fish under TAD I (R. 5068A), four remained to be completed at the beginning of this project. Three of these trials failed, largely due to errors by the farmers themselves. In one case the trial was harvested without the researchers being informed, in a second case the fish were accidentally mixed and in the third the control fish were lost when a cage was holed just prior to harvest. The one remaining trial that was completed compared GMT with mixed sex (MST) and sex reversed (SRT) controls in tank based culture at the farm of the Meralco Foundation Inc. The results from this trial, given in the outputs section (see page 41) became available early enough for their inclusion in the overall summary of the results of the on-farm trials in the final report of R. 5068A (Mair, 1995).

On-station evaluation of YY males

The initial plan for this study was to simply compare the reproductive capacity of XY and YY males by direct comparison of the two genotypes, crossed with a common source of females, in a hapa based hatchery. However, due to delays in setting up this experiment is was decided to expand the study to include comparison of sperm quality in an additional experiment.

Sperm count and fertilising abilities of YY males

This study, which was completed by a CLSU BS student Miss Sharon Macabale, compared the fertility of XY and YY males through determining sperm counts for representative fish of each genotype and then comparing fertilisation rates in artificial fertilisation of eggs from common females. Males of both genotypes were matched for size and age and maintained in aquaria under identical feeding and management regimes for two weeks prior to beginning the experiment.

Sperm was then sampled from the fish (25 males for each genotype) by drawing up a fixed volume of sperm in a capillary tube following gentle abdominal pressure taking care to avoid contamination with urine or faecal matter.

An aliquot of the milt sample was first diluted 1:2 with distilled water to activate the sperm which was then observed under the high power objective of the microscope. Sperm was scored subjectively for motility, the sample being classed as non-motile, slightly motile and motile depending on the comparative motion of the sperm.

To determine sperm concentration a further aliquot of 10 µl was placed in an Eppendorf and serially diluted to 1 µl in 100 µl of deactivator solution. A drop of the diluted sperm was pipetted onto a haemacytometer slide and sperm counts made. Sperm concentration was estimated following counts in 10 haemacytometer cells.

Sperm fertility was further assessed by fertilising eggs stripped from ovulating females. Eggs were collected from ripe females picked from a breeding population. Eggs from individual females were divided into two or four batches with half of the eggs being fertilised with fixed volumes of sperm stripped from XY males and half from YY males.

Following fertilisation eggs were incubated in separate downwelling incubators and fertilisation rates were estimated from the proportion of pigmented eggs 48 hours after fertilisation.

The principal hypothesis of this study was that there was no difference in the sperm count, motility and fertilisation rate between XY and YY males.

Comparisons of fry production in XY and YY males

The commencement of this experiment was first delayed by the departure of the research associate assigned to the task and subsequently was further delayed by the lack of availability of suitable broodstock. The design of the experiment was to compare the fry production capacity of XY and YY males crossed to females from a common source. The experiment was designed to directly compare the reproductive capacity of the two male genotypes, while as far as possible eliminating all other variables. Problems arose in producing and maintaining XY and YY male genotypes of approximately the same size and age. The broodstock and the pond and hapa facilities to be used for the trial only became available in October 1996 and the scale of the trial was limited by labour constraints.

This experiment compared the fry production of YY males and XY males of the Egypt-Swansea strain crossed to females taken from a common batch (eliminating maternal genetic variation as a source of variation) in a small, hapa based production system. A total of 120 females and 30 males (1♂:4♀) were used in each treatment split into 3 replicate breeding hapas (10♂ and 40♀ in each).

Only four of collections of fry had been made by the end of the project over a period of three months. Data collected included survival and weight change of the broodstock, number of fry produced (per broodstock package of 1♂ : 4♀ and per unit weight of females) and survival of fry during nursing. The fingerling production in each cycle was treated as a replicate in the t-test analysis testing for differences in the means.

The principal hypothesis of this study was that there is no difference in the reproductive capacity (fry production and survival during nursing, broodstock survival and growth) of XY and YY males.

On-farm evaluation of YY males

For the same reasons as described above (difficulty in recruiting suitable staff and shortage of broodfish) these trials, which represented the major activity of the project, were also slow to get underway and required great attention to logistical detail. The running of the trials themselves was also extremely time consuming requiring frequent visits to each hatchery to ensure their proper conduct.

The objective of this study was to test the performance of the YY males on-farm in terms of fry and fingerling production, ease of management, and economics. These performance parameters were compared with those of the broodstock normally used by the farmer. The GMT producing broodfish and the control broodfish were maintained and bred in as near identical ways as was possible. The original research plan required the identification and initiation of trials in a number of distinct hatchery systems representative of commonly used commercial practices. Based on our knowledge and experience in working with and visiting commercial hatcheries , we identified four types of hatchery system:

Extensive Pond Based (EPB)	Semi-intensive pond based (SIPB)
Intensive tank based (ITB)	Intensive hapa based (IHB)

These four systems can be broadly defined as follows:

Extensive pond based (EPB)

This hatchery system is the most extensive form of fingerling production requiring the least labour. It can be done in a range of different pond sizes but most commonly in ponds ranging from 500 to 5,000 m². The ponds are not specifically designed as hatchery ponds being the same in form as those used for tilapia grow-out. Breeding is done on a long cycle, with the ponds serving as spawning and nursing grounds. After pond preparation the broodstock are stocked, usually at a ratio of 1♂ : 3♀ at one fish per 2-4m². If ponds are well fertilised, feeds are not normally given. There is no regular harvesting of fry or fingerlings but rather periodic harvesting or usually just a single complete harvest by draining of the pond anywhere from 50 to 65 days after the initial stocking of the broodstock. Once the broodfish have been

removed the fingerlings are collected from the pond and sold direct to the buyers. This method has the advantage of simplicity and minimal capital investment in facilities and labour. However, it is also thought to be one of the least productive systems per unit weight of broodfish and per unit area, largely because of cannibalism among fingerlings and fry.

Semi-intensive pond based (SIPB)

This is a system that has increased in popularity in recent years and is operated by large and small scale farmers alike. The system operates a shorter cycle in shallower, usually purpose built, fish ponds. Ideal ponds would be approximately 200 m² in area with a depth of 0.4 to 0.6m. Fry are nursed in similar sized ponds, with nursery ponds required at a ratio of one for every 3 or 4 breeding ponds. Brood fish are managed in a monthly cycle. Following pond preparation, conditioned broodstock are placed in the pond (at one fish per 2m²) and fed with pelleted feeds. As soon as the presence of fry is detected the pond is fertilised with organic fertilizer (usually chicken manure). Collection of fry, from the periphery of the pond, begins the next day and is carried out, usually early in the morning, for the next 5-10 days. After this breeding cycle of 21-26 days, the pond is drained and harvested. Breeders are reconditioned and ponds are prepared for the next breeding cycle. The main advantage of this kind of hatchery system is that it is not too labour intensive and gives higher productivity due to the minimised cannibalism. However it does require more water and wastes fertilised water. Furthermore it requires purpose built ponds and is therefore not suited to farmers who wish to switch from grow-out to hatchery production.

Intensive tank based (ITB)

Under this system which is not very common in the Philippines, fish are bred and fry are nursed in concrete tanks, usually rectangular in shape and ranging from 10- 500 m². Conditioned breeders are stocked in the tanks at approximately four fish per m² with a sex ratio of 1♂: 3:♀. Collection of fry is either done by regular scooping of the schools of fry for up to 21 days after first spawning or by draining and total harvest of the tank approximately 2 weeks after stocking. Fry can also be collected at night by scooping them out as they are attracted by a bright light shone on the water. Fry of no more than four days difference in age are pooled for nursing in additional tanks. Due to the higher stocking densities used, compared to ponds, aeration or flushing with freshwater is sometimes required to maintain water quality. Tank based hatcheries are easy to manage and broodfish easily traced and controlled but the disadvantages are that this kind of system is more labour intensive than pond based production and requires greater capital investment. There is also a greater dependence on artificial feeds and the risk of disease is higher.

Intensive hapa based (IHB)

This is the most recently introduced hatchery method and is growing in popularity, mainly among middle and upper income farmers interested to invest in intensive fingerling production operations. In this method fish are bred and fry are nursed in fine mesh cages known as hapas. These hapas are placed in earthen ponds supported by wooden or bamboo poles. Conditioned broodfish are placed in the hapas which can vary in size from 5 to 100 m^2 at densities up to 10 $fish.m^{-2}$ depending on their size. There are two approaches to hapa based fry production, depending on the efficiency of the incubating system. Where this is not very efficient it is advised to operate a longer cycle and collect predominantly fry rather than eggs. If hapas are harvested after 10-14 days, prior to the likely first release of fry by the spawned females, the majority of the collection will be hatchlings which are relatively easy to incubate. A shorter cycle of 5 to 7 days will result in the collection of predominantly pre-hatch embryos which require incubation in more efficient systems. A well set up incubator system can produce higher rates of survival than is estimated for natural incubation (Mair *et al.* 1993). Eggs or fry are incubated in purpose built incubators or trays respectively, and these are supplied from a clean water source which can be either flow through or recirculating. Upon completion of incubation fry are nursed for up to one month in fine mesh hapas and later in cages prior to dispersal. The advantages of properly run hapa based systems are that they can maximise fry production per unit area and per broodfish and this method can be applied in fishponds formerly used for grow-out. Furthermore this method provides for easy control and maintenance of broodfish, disease is low and if ponds are properly fertilised, feed costs are low. Major disadvantages include the requirement of capital investment in an incubating system and in the hapas themselves and a requirement of a larger labour force to handle the regular activities. An experienced technician is also required to develop the facilities and to co-ordinate the hatchery activities.

More comprehensive descriptions of these hatchery systems can be found in the technoguide produced by the project (see attached) with intensive methods described by Morales (1997) and extensive and semi-intensive methods described by Bartolome (1997).

The objective was to identify three co-operator hatcheries operating each of four types of commercially applied hatchery systems listed above to participate in these trials. The first stage of this study was to visit prospective farmer co-operators to determine their suitability. Some of the factors considered on these visits included the type and scale of the hatchery, availability of facilities, management and technical expertise of its staff, location and the willingness of the operator/owner to collaborate.

It proved a little difficult to identify willing co-operators as hatchery managers were reluctant to risk using relatively untried fish. The last of the hatchery co-operators was not identified

until March 1996. The hatcheries were located throughout the main island of Luzon with concentrations in the major tilapia growing regions (see Figure 4).

Table 1 lists the farms which participated in the trials. It is worth noting that the farms that were persuaded to join the experiment tended to be relatively new to the industry and were owned by middle and upper income farmers. An additional tank based hatchery was added to the list due to the enthusiasm of its owner and doubts over the capability of one of the other tank based co-operators. Further details of the farms and their management and fry production systems can be found in the farm-fact files contained in Appendix 2.

Further delays were encountered in setting up the experiment associated with the problem of matching our GMT producing broodstock with the control. Most of the co-operators obtained their future breeders from the first production of their new sets of breeders. Thus, the strain of origin of the control fish was not clearly identified as many hatcheries were producing inter-strain hybrids (indicating a basic knowledge of some genetic principles). The broodstock packages (1♂: 3♀) were transferred to the hatcheries between January and July 1996, with the number of packages for each farm varying according to the availability of broodfish, the size of production facilities and the requests of the farmers.

Other than taking additional precautions to prevent broodstock contamination, the co-operators were encouraged to produce GMT using their normal hatchery procedures. Trials could not be conducted blind as the farmers needed to know which of the fingerlings were GMT prior to their dispersal. Six of the hatcheries which agreed to co-operate were using sex reversal in attempts to produce high proportions of male fry. In the case of these farms the procedures for rearing and nursing fry were different between the controls and the GMT. Fry produced by the control broodstock were stocked in special facilities for sex reversal through oral application of methyltestosterone. As GMT did not require this treatment they were stocked directly into the nursing facilities. Attempts were made to estimate the additional costs and labour requirements for sex reversal in addition to determining the effects on nursing period and survival.

Each hatchery manager entered into an agreement with the project which covered their rights and obligations during and after the trial which were intended to last for a minimum period of six months. The farmers stood to benefit in terms of provision of free broodfish and the right to continue production as accredited hatcheries, under favourable terms, following completion of the experiment. Farmers were free to set their own costs for GMT sales and the researchers attempted not to influence this decision.

Table 1 Location of Co-operators for the production of GMT.

No.	Farm	Location	No. of Packages	Controls
	Extensive pond based			
1	ABES Farm	Gen. Tinio, N. Ecija	1500	MST
2	BFAR	Muñoz, N. Ecija	1,200	MST
3	J & B Agri Farm	San Manuel, Isabela	300	MST
	Semi-intensive pond based			
4	NAFIDECO Farm	San Mateo, Isabela	400	MST
5	San Isidro Farm	Calauan, Laguna	400	MST
6	B & W Farm	Magalang, Pampanga	1500	SRT
	Tank based			
7	Jonathan Reyes' Farm	Sta. Rosa, N. Ecija	400	SRT
8	J & A Integrated	Arayat, Pampanga	1000	SRT
9	Petines Aqua Farm	Alicia, Isabela	300	MST
10	Reniag Hatchery	Ramon, Isabela	240	MST
	Hapa based			
11	Creekside Farm Inc.	San Leonardo, N. Ecija	276	SRT
12	SEAR	Baliuag, Bulacan	276	SRT
13	SMFI	Calauan, Laguna	500	SRT

Farms were visited on a regular basis during the early periods of the trials in order to ensure that they were being properly conducted and that they were set up in such a way as to collect all the necessary data. As far as possible the farms were visited twice a month with visits preferably coinciding with fry collection schedules. Given the widespread location of the hatcheries this required an arduous travelling schedule.

Data collected included monthly fingerling production (or according to the production cycle); growth performance of breeders; survival of both breeders and fingerlings; and the cost and returns of the production of GMT and control fingerlings. Possibly the most important of these parameter, fingerling production, was expressed using three different measures:

<u>According to the number of broodstock packages (1♂ : 3♀):</u> This is perhaps the most relevant measure as it is the measure used in the dissemination programme to estimate the number of fingerlings that a hatchery should be able to produce. In these trials, the number of broodstock packages available at the time of the first setting of broodstock for the first cycle of production was used. Where the male and female numbers were no longer in a ratio of 1:3, the number of packages was based on one third of the number females rather than the number of males as fingerling production is more closely correlated with female number than it is with male number.

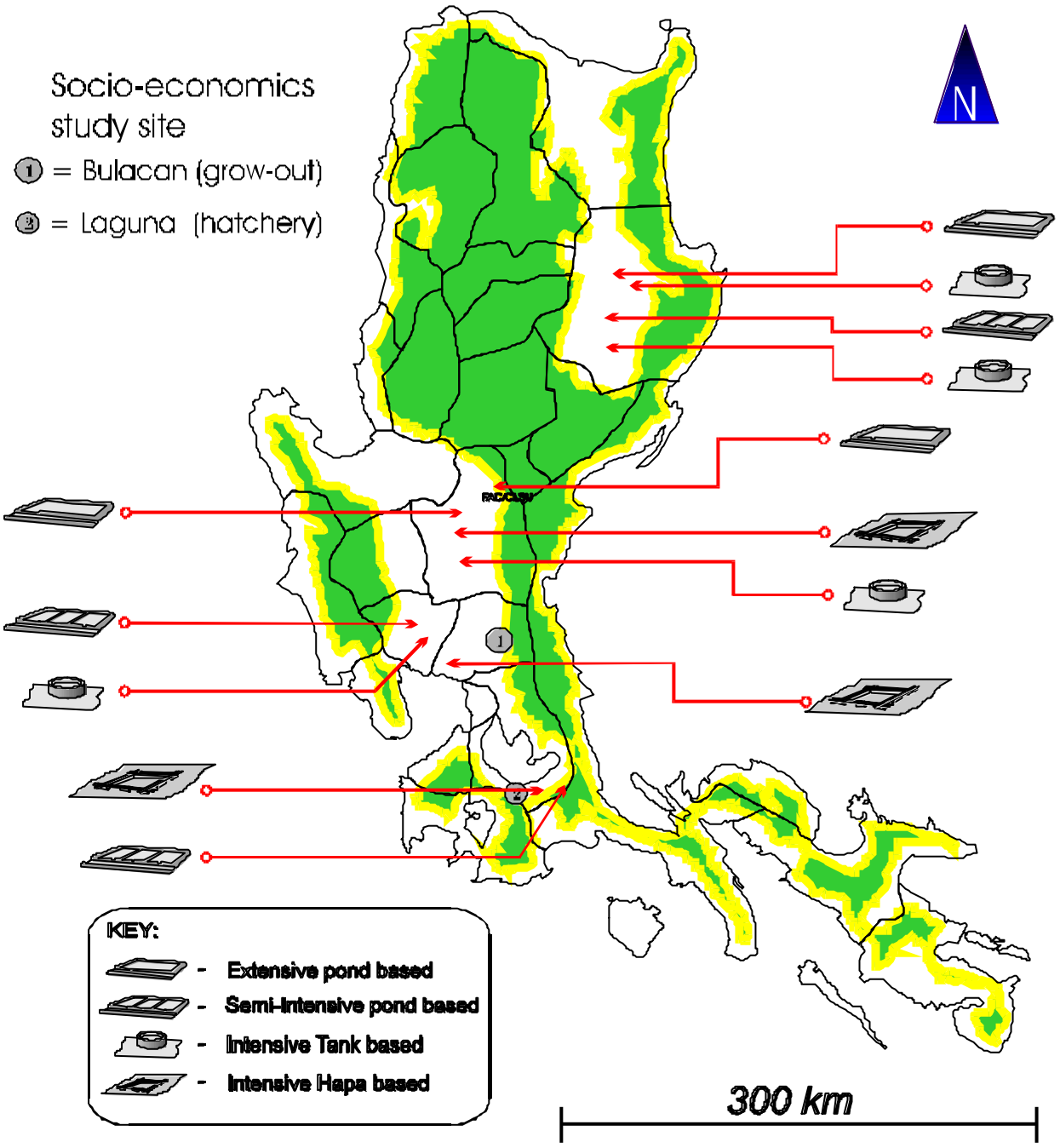
<u>According to the weight of females</u>: Fingerling production is expressed per kilogram of female weight. This measure was used to account for possible differences in the size of females between GMT producing and control broodstock.

According to surface area of the production facility: This measure was designed to give indication of the comparative production at different levels of intensity. Surface area was based on the total area of ponds and tanks used for fry production and also for the nursing of fingerlings. In the case of hapa based production the area was based only on the area of the breeding and nursing hapas and not on the area of the pond in which they were installed.

The principal hypotheses prior to conducting this trial included the following:

- A number of trials would fail to give useful results due to poor management and man made or natural disasters.
- Production of fingerlings per unit broodfish would increase with the level of intensity of the hatchery system, being highest in the hapa based hatchery and lowest in the extensive pond based.
- There would be no significant difference in fry production parameters between GMT producing and control broodstock.
- There would be no significant difference in the survival and growth of the GMT producing and control broodstock.
- GMT production would produce higher returns if higher prices were charged for the GMT.
- Sex ratios of GMT would be more variable in the more extensive pond based hatcheries due to the greater difficulty in preventing broodstock contamination.

Figure 4 Map showing the locations and system type of co-operator hatcheries for the on-farm trials of GMT producing broodstock.



Socio-economics of tilapia culture in the Philippines

This study took the form of a Ph.D. research study and thesis carried out by Ruben Sevilleja , director of FAC, CLSU. He conducted a case study of two aquaculture communities to collect and collate baseline data on the socio-economic issues pertinent in Philippine tilapia culture.

The study investigated and analysed the effects and consequences of tilapia technology adoption among farmers, leading to a better understanding of the circumstances and

motivations that shape farm operators' decision-making processes. Specifically, this research pursued the following objectives:

- to study tilapia farmers, their farms and their farming practices in one community of tilapia growers and one community of seed producers;
- to determine how farmers utilize their available resources and identify the factors which affect their decisions on resource use;
- to compare the productivity and efficiency of tilapia farming according to size of farm and according to tenurial arrangement;
- to identify the factors which influence farmers to adopt tilapia farming; and
- to formulate some policy recommendations for the promotion of tilapia technology utilization in particular, and for the development of the freshwater aquaculture industry in general.

The methodology adopted for this research was to conduct two case studies. After careful consideration of possible locations, two villages were selected as study sites: Kabaritan, Sto. Domingo in the municipality of Bay, province of Laguna (Southern Luzon), where tilapia hatchery operation is the main culture system practised by farmers; and Partida in the municipality of San Miguel in the province of Bulacan (Central Luzon) where the technology of tilapia grow-out is widely adopted (see Figure 4).

Field investigations were made on the livelihood, social relations and economic behaviour of the different groups of farmers involved in tilapia production; the functioning and make-up of the agrarian structure; and the operation of units of tilapia production. An informal interviewing policy was adopted. After spending some time among the community the research identified a number of respondents, being those farmers involved in some way in aquaculture production. Working to a questionnaire and guidelines (see Appendix 3) all farms were visited and interviewed on several occasions over a period of 8-10 months in 1995.

The results were analysed and discussed (at some considerable length in Dr. Sevilleja's thesis itself) in the context of the adoption of tilapia farming and culture technologies, the allocation and use of the main factors of tilapia production, and production efficiency and indicators of financial feasibility. This report deals only with aspects of this research most relevant to the dissemination of genetic technologies in aquaculture.

Evaluation of technology transfer to Thailand

This study is a continuation of research that began under R. 5068A to investigate the feasibility of different methods of transferring the YY male technology to Thailand. Thailand, like the

Philippines, has a vibrant tilapia culture industry although the culture systems and practices differ substantially from those in the Philippines. There are two components to the study, investigating different options for breeding programmes, and comparing the growth rates of transferred fish, local Thai strains and their crossbreeds. We are evaluating four methods of breeding for technology transfer.

Direct transfer

This involved, under R 6058A, the direct transfer of broodstock fish from the Philippines to Thailand. This enabled the production of YY male and normal female broodstock for the production of Egypt-Swansea GMT as used in the Philippines. These broodstock were transferred to both AIT and NAGRI. However, most of this work has been done at NAGRI where production of YY males and normal females has become routine and large numbers (many thousands) of YY males and over one thousand YY females are maintained. The main role for these fish in this project was in their culture performance evaluation compared to alternative strains and strain combinations.

Indirect transfer by crossbreeding

This method of technology transfer simply involves the crossing of YY males of the Egypt-Swansea strain with females of the local Thai strain(s) to produce crossbred GMT. Results from work at AIT under R. 5068A indicated that sex ratios of this cross were somewhat variable and low (ranging from 84-100% male). At AIT attempts were being made to select a female line of the Egypt-AIT strain for its combining ability with YY males of the Egypt-Swansea strain. This was initiated by progeny testing seven Egypt-AIT females with Egypt-Swansea YY males. Three Egypt-AIT females were selected based on the sex ratios produced in these progeny tests (>98% male). These three selected females were crossed with sex reversed XX $\Delta\male$ to produce the next generation of females to be used for producing crossbred GMT. These females were then progeny tested to determine any response to selection. By the end of the project a total of 21 selected and 20 normal control females were progeny tested with YY males.

Indirect transfer by crossbreeding and selection

The objective of this form of technology transfer was to introduce YY males from the Egypt-Swansea strain and to use these to 'fast track' a breeding programme for YY male production in the local strain. Under R. 5068A, hybrid XY progeny were produced by crossing the YY males with females of the Egypt-AIT strain. These were then feminised and progeny tested to identify hybrid $\Delta\female$ (XY). The concept was that these fish could then be back-crossed to the local Egypt-AIT strain at each generation of the breeding programme for mass production of YY males, thereby maximizing the genetic contribution of the local strain. It was thought that this approach would enable the earlier production of YY males in the local strain.

Application in pure Thai strains

In this approach it was intended to develop YY males in the local Egypt-AIT strain from scratch, applying the breeding programme approach developed in the Philippines. Significant progress was made under R. 5068A and at the start of this project, XY Δ♀♀ were available in the strain. Whilst proceeding with the breeding programme, it was decided to take a closer look at the sex determining mechanisms in this strain through the analysis of sex ratio variation in crosses of normal fish. These results could then be compared with those obtained in the past for the Egypt-Swansea strain. The baseline information would be of value in the interpretation of sex ratios arising from manipulated fish.

We adopted two approaches to gathering this baseline data. The first was to collect a series of data from single pair matings of normal fish. Over an 18 month period 95 family sex ratios were obtained in single pair matings of untagged fish selected from AIT's population of *O. niloticus*.

A second study was designed to determine maternal and paternal effects on sex ratio by attempting a 5 (♂) x 7 (♀) di-allele type cross. A total of 26 of the possible 35 cells in the matrix of crosses were completed by the end of the experiment, sex ratios with blank cells resulting either from mortality of one of the male or female parents, loss of a progeny group or simply the failure of a single female to spawn. The matings were done in single pairings, with fish spawned in hapas.

Males from crosses of XY ♂ x XY Δ♀ were progeny tested in the first year of the project to identify YY males. A number of YY males were identified in the AIT strain (based on the high proportion of male in their progeny). Once identified these YY males were then progeny tested to a number of females to determine the consistency of sex ratio. In the case of one male it was possible to produce 12 families which was a sufficient number to give a reliable indicator of sex ratio variability. Successful feminization was obtained following DES treatment of progeny from several crosses of XY♂ x XYΔ♀ in the Egypt-AIT. These treated fish were raised to sexual maturity and were available at the end of the project to be progeny tested using with XX Δ♂ in the hope of identifying YYΔ♀♀.

Growth comparisons

A number of important growth trials have been completed during this project. The emphasis of these trials is to determine the relative growth performance of GMT (pure bred or crossbred) compared to that of presently available alternatives, in culture environments that are representative of common culture environments in the countries. Most of these trials were conducted in Thailand but a number of trials were also carried out in the Philippines.

This section lists the basic details for each trial.

Lake Sebu, Mindanao - Replicated (x3) cage trial

Strains compared:

Local strain	A strain of *O. niloticus* that has been cultured in the lake for the past ten years. Source unknown
Selected GIFT	Fish from the second generation of selection in ICLARM's GIFT Project which utilised combined selection.
Selected IDRC	Fish from the eighth generation of within family selection for growth in a project based at CLSU and funded by IDRC.
Egypt-Swansea GMT	GMT obtained from the Breeding Center at FAC\CLSU

Objective: To compare the relative growth performance of GMT (in terms of growth, survival , food conversion ratio, and yield) with that of mixed sex versions of alternative genetically improved tilapia. in a commercial cage culture system. A simple cost and return analysis was also performed in this, a commercial system.

Environment: High stocking density (30 fish per m^2) in 4 m^2 floating cages (three replicates per strain), with feeding of commercial feeds and five, reducing to three % of biomass per day..

Duration: Planned for 120 days but terminated after 84 days

Additional information: The study was conducted by Mr. Zosipat Beniga, a masters student from CLSU. Fifteen percent of the population were sampled (weighed and measured) every three weeks with a total count to determine survival. Problems were encountered with a mass fish kill in the lake after 94 days of culture. Data collected at the last sampling on day 84 was analysed.

FAC, Philippines - Replicated (x3) pond trials under various fertilization regimes

Strains compared:

Sex reversed GIFT	Fish from the second generation of selection in ICLARM's GIFT Project which utilised combined selection. Fish were first hormone treated to induce masculinization by oral application or 17 ∝-methyltestosterone (MT)
Egypt-Swansea GMT	GMT obtained from the Breeding Center at FAC\CLSU

Objective: A 2 x 2 factorial (genotype x nutrient input) experiment designed to test the relative growth performance of all male fish (SRT) in a strain selected for growth with that of

the GMT under two different management regimes, one based on fertilisation only and the other sequentially combining fertilization and full feeding.

Environment: Earthen ponds (500m²) stocked at three fish per m². The SRT GIFT and GMT were each reared under two pond fertilization regimes; i) Inorganic fertilizer at 28 kg N and 5.6 kg P /ha/wk fish and, ii) inorganic fertilizer as in (i) for 2.5 months and complete feeding at 3% of body weight per day for the remaining 1½ months of the culture period.

Duration: Four months.

Additional information: The study was conducted by Mr. Eduardo Lopez under the Pond Dynamics Aquaculture Collaborative Support Programme (PD/A CRSP) with the principal objective of evaluating the management regimes, the strain comparison being a secondary objective. There was a problem with the experimental design in that the initial age and size of the fish were not well matched at the beginning of the experiment, the GMT having an initial weight of approximately 6g compared to 18g for the SRT GIFT. The researchers also failed to determine the sex ratio of the fish at harvest. A minimum of 25 fish were sampled monthly and all fish were harvested.

AIT, Thailand - Replicated (x3) cage-in-pond trial

Strains compared:

Egypt-AIT - SRT	The local strain sex reversed using AIT's standard sex reversal protocol of oral administration of MT.
E-S x E-A - SRT	Hybrid between the introduced (♀) and local (♂) strains, sex reversed to male.
E-A x - E-S GMT	Hybrid between a female of the local strain and the YY male of the introduced strain.
E-S x (E-A x E-S hybrid) - GMT	Hybrid between a female of the local strain and an $_{F1}$ hybrid YY male identified by progeny testing from the indirect transfer breeding programme (see page 30).
Egypt-Swansea - GMT	Pure strain GMT produced following direct transfer of the technology (see page 30)

Objective: To compare the growth performance of the various genotypes of all male tilapia resulting from the breeding work investigating the different methods of technology transfer at AIT.

Environment: Four m³ cages stocked with 50 fish per replicate per treatment. All cages stocked in a single 500m² earthen pond. Full feeding with commercial catfish pellets.

Culture period: 188 days

Additional information: This trial was actually initiated under R. 5068A but was not complete by the end of that project. The trial followed a similar design to an experiment that was completed under R. 5068A. This first trial indicated that the Egypt-AIT and hybrid SRT were superior in growth to Egypt-Swansea SRT and the pure Egypt-Swansea or hybrid GMT (Mair, 1995). The hybrid GMT in this first experiment had a low sex ratio of only 82 %♂ which may have affected its growth hence the need to repeat the experiment (with the addition of a backcross GMT).

AIT, Thailand - Replicated (x3) cage-in-pond trial

Strains compared:

Egypt-AIT - SRT	The local strain sex reversed using AIT's standard sex reversal protocol of oral administration of MT.
Egypt-AIT - GMT	A GMT of the local strain produced by crossing identified YY males developed under the breeding programmes at AIT with randomly selected females of the same strain.
E-A x E-S - SRT	Hybrid between the local (♀) and introduced(♂) strains, sex reversed to male.
E-A x E-S - GMT	Hybrid between a female of the local strain and the YY male of the introduced strain.
E-A x (E-A x E-S hybrid) - GMT	Hybrid between a female of the local strain and an F_1 hybrid YY male identified by progeny testing from the indirect transfer breeding programme (see page 30).
Egypt-Swansea - GMT	Pure strain GMT produced following direct transfer of the technology (see page 30)

Objective: To compare the growth performance of the various genotypes of all male tilapia resulting from the breeding work investigating the different methods of technology transfer at AIT.

Environment: Four m^3 cages stocked with 20 fish per replicate per treatment. All cages stocked in a single 500m^2 earthen pond. Full feeding with commercial catfish pellets.

Culture period: 166 days

Additional information: This was the third cage based trial conducted at AIT and was set up to try to resolve some of the ambiguities resulting from the first two trials. This trial used a lower stocking density in an attempt to produce faster growth rates that may yield clearer differences between the strains.

AIT, Thailand - Replicated (x3) pond trial at AIT

Strains compared:

Egypt-AIT - SRT	The local strain sex reversed using AIT's standard sex reversal protocol of oral administration of MT.
Egypt-AIT - GMT	A GMT of the local strain produced by crossing identified YY males developed under the breeding programmes at AIT with randomly selected females of the same strain.
E-A x E-S - GMT	Hybrid between a female of the local strain and the YY male of the introduced strain.
Egypt-Swansea - GMT	Pure strain GMT produced following direct transfer of the technology (see page 30)

Objective: To compare the growth performance in ponds of the most promising genotypes of all male tilapia resulting from the breeding work investigating the different methods of technology transfer at AIT.

Environment: Fish were stocked at a low density of 1 per $2m^2$ in $200m^2$ earthen ponds. Ponds were managed extensively, fertilized with a combination of organic and inorganic fertilizer.

Culture period: 124 days

Additional information: This was the first pond based trial conducted at AIT. Although the results from the cage based trials were informative it was considered that the cage-in-pond environment was unrepresentative of any commercial culture systems. There was therefore a worry that, given that significant genotype interactions can occur in tilapia, any comparative growth differences from these cage environments may not apply to commercial systems. The pond environments in which fish were tested in this experiment were representative of a common commercial culture system for tilapia in Thailand.

DOF Station, Surin Province, Thailand - Replicated extensive (x3) pond trial

Strains compared:

Egypt-AIT - MST	The local strain. In this experiment the strain from AIT was used to enable direct comparisons with the results of studies conducted in cages at AIT.
Egypt-AIT - SRT	The local strain sex reversed using standard sex reversal protocol of oral administration of MT.
Egypt-Swansea - GMT	Pure strain GMT produced following direct transfer of the technology (see page 30)

Objective: To compare the growth performance of the introduced GMT with the alternative fish presently available to local farmers (in this case either MST or SRT of the strain from AIT) to determine the potential benefits of direct transfer.

Environment: Fish were stocked at a low density of 2 per m² in 1,000m² earthen ponds. Ponds were managed extensively, fertilized with a combination of organic and inorganic fertilizer.

Culture period: 6 months

Additional information: This trial, conducted by NAGRI, was set up as it was necessary to make field based study, in ponds, to back up the data coming from the cage based experiments at AIT.

Nakonphanom DOF station, NAGRI, Thailand - Replicated (x2) cage-in-pond trial

Strains compared:

Chitralada - MST	The local Chitralada strain produced from broodstock collected directly from the founder stock at the royal palace.
Egypt-Swansea - MST	Mixed sex tilapia produced in crosses of normal males and females in the Egypt-Swansea strain introduced from the Philippines.
Chit x E-S - MST	A mixed sex hybrid between the local (♀) and introduced(♂) strains.
E-S x Chit - MST	The reciprocal mixed sex hybrid between the introduced (♀) and local (♂) strains
Chit x E-S - GMT	Hybrid between a female of the local strain and the YY male of the introduced strain.
Egypt-Swansea - GMT	Pure strain GMT produced following direct transfer of the technology (see page 30)

Objective: To compare the relative growth performance of the most commonly cultured strain of *O. niloticus* in Thailand (known as the Chitralada strain) and the introduced Egypt-Swansea strain. The relative growth of the MST will be compared with that of the purebred and hybrid GMT. This experiment should enable elucidation of strain and sex effects on growth.

Environment: Moderate stocking density (30 fish per 4m² cage), weekly pond fertilization, full-feeding of commercial pelleted diet.

Culture duration: 5 months

Additional information: This comparison, carried out by NAGRI, used the Chitralada strain in place of the Egypt-AIT strain. The strain is named after the royal palace in which stocks have been held since the fish was gifted to the King of Thailand by the Emperor of Japan in 1965. The fish has been widely dispersed, by DOF stations, to farmers around the country since

1967 and is the now the strain grown by the large majority of tilapia farmers in the country. The Egypt-AIT strain is derived from the Chitralada stock. We wanted to investigate this stock as an alternative to the Egypt-AIT strain as we thought that the latter may have become locally adapted to conditions at AIT.

Surin and Pitsanulok DOF Stations, Thailand - Replicated (x2) pond trial at two stations

Strains compared:

Chitralada selected - SRT	This is a strain that has been subjected to 3 generations of within family selection for growth and is said to out-perform the presently cultured stocks of the strain by approximately 17% (Pongthana, pers. comm.)
GIFT selected - SRT	The second generation of the GIFT selected strain (ICLARM) which was introduced to Thailand in 1994.
Egypt-Swansea - GMT	Pure strain GMT produced following direct transfer of the technology (see page 30)

Objective: To compare the growth performance of the introduced GMT with monosex (produced by sex reversal) progeny of the presently available alternative genetically improved strains in Thailand.

Environment: Fish were stocked in very similar, extensive culture environments at both stations. At Surin Fisheries College in Northeast Thailand, the fingerlings were stocked at a low density of 1 per m² in 330m² earthen ponds. Ponds at the Pitsanulok Genetics Center (of NAGRI\DOF) were larger at 500m² but were stocked at the same low density. In both trials ponds were fertilized (4 kg N/ha/da and 2 kg P/ha/da) and 30 fish per pond replicate were sampled monthly.

Culture period: 5 months

Additional information: This trial, conducted by NAGRI, was set up as it was necessary to make field based study, in ponds, to back up the data coming from the cage based experiments at AIT.

Investigations into Salinity Tolerance

The long term objective of these studies was to identify fast growing saline tolerant tilapia. However, given the time and facilities available it was planned only to conduct preliminary trials. Nevertheless, progress in these trials has been poor for a number of reasons. We decided early on that this research should be conducted in real brackishwater environments rather than trying to simulate these environments in aquaria or tanks. The brackishwater environment is a complex environment in which salinity and other water quality variables

change seasonally and can even change with the tides. Thus, trials done in constant salinity environments on-station might not present an accurate picture of how strains would respond in the field. With the project being based at an inland research station, it was not feasible for us to carry out these trials directly. We established a collaborative link with Miss Nelia Estabillo (an alumni of FAC, CLSU who had conducted research on the YY male technology while taking her masters degree) at Pangasinan State University (PSU) which is located in an area where brackishwater estuaries and fishponds are common. The first stage of the research was to conduct an on-farm trial comparing the growth of all-male (GMT) and all-female (GFT) populations in a brackishwater environment.

The objective of this study was to determine if there were any differences between the sexes in the tolerance to higher salinity as measured by growth and survival in a brackishwater environment. The experiment, conducted in farmers cages in a river in Binmaley, Pangasinan was carried out over two phases.

In the first phase, fry (mean weight of 0.001g) were transferred from FAC\CLSU and stocked in 1 m^3 fine mesh cages in the river at a density of 500 per m^3 with three replicates each for GMT and GFT. Fry were introduced to the brackishwater, which at that time had a salinity of less than 5ppt, gradually with water exchange over a period of 24hrs.

Fry were fed with powdered form feed, consisting of 75% rice bran and 25% fish meal, three times a day at 10% of fish biomass per day.

Fish sampling by individual weighing and measuring of 100 individuals per cage was carried out every three weeks. Monitoring of water quality parameters i.e., dissolved oxygen, salinity, temperature and pH was carried out three times a day.

After the four month period of Phase 1, all the fingerlings from the replicates of each genotype were pooled and redistributed to cages at two densities (100 and 200 m^3) with three replicates for each density-genotype combination. The fish were grown for a further four months in the cages, with monthly sampling, prior to total harvest at the end of the experiment. One replicate of the GFT held at 200 fish per m^2 was lost after the first month of culture due to escape. During the trial all cages were fed at an appropriate level with commercial tilapia feeds.

This experiment took a long time to complete and further delays were encountered in obtaining the data for the experiment. Furthermore, Miss Estabillo, the principal collaborator, expressed her intention to take up Ph.D. studies which would consume more of her time. Thus, it was considered unwise to attempt further work of this nature and the collaboration was discontinued. We made attempts to identify suitable alternatives and had promising discussions with Dr. Carlos Baylon at the University of the Philippines, Visayas (UPV). However, the long travel time and the limited budget available for this collaboration prevented

it from being pursued. With the failure to identify a suitable collaborator no further progress was made with this part of the project.

Additional Complementary Research Activities

The majority of the aforementioned research constituted the core activities of the project that were anticipated at the time that the proposal was submitted and were directly targeted at producing the required outputs of the project. However, as might be expected, further researchable constraints and opportunities came to light during the progress of the project and we were able to conduct a number of important studies complementary to the core research. The previously described studies on gathering baseline data on hatchery management practices in the Philippines and the work on intra strain crosses of the Egypt-AIT strain both constituted additional complementary research. A further study investigated methods of tagging and marking in tilapia, in response to an identified constraint.

Fish marking and tagging

During the early dissemination of the products of the YY male technology it became clear that a reliable method of marking the YY males would greatly facilitate this process. If the researchers could be provided with a method of identifying the fish it would reduce the possibility of on-station contamination and would enable us to perform troubleshooting diagnosis in hatcheries which were experiencing problems. It would be preferable if the method of marking could also be detected by the farmer as this would permit them to have better control over their broodfish. During the earliest part of the dispersal programme for YY males to accredited hatcheries we adopted the policy of clipping the right or left pelvic fin of the YY males. This worked reasonably well but some confusion did arise when females were also fin clipped and in some hatcheries where some females were detected among the YY males, we could not tell these apart from the normal XX females. Also, as fin clipping is easy to do it was thought not unlikely that unscrupulous people would swap the valuable YY males with freshly clipped normal males (we have already identified one incident where this may have occurred). Furthermore, it seemed likely that future growth trials would compare only monosex fish (for example pure bred and crossbred GMT), comparisons which could be made under communal stocking. Such trials would require suitable batch marking techniques to be applied. Thus, it was decided to conduct two experiments evaluating individual and batch marking techniques including, where possible, some newer methods not previously applied in tilapia.

The first experiment investigated the utility of five different methods of individual marking, in two size groups of fish (10-20g and 25-35g at marking) using the following techniques:

PIT tag Spaghetti tag

Binary coded tag Fingerling Floy tag

Elastomer dye marking site combinations

A total of one hundred fifty tagged/marked individuals were stocked for each type of tag/mark. This experiment has three replications with 50 fish tagged with each tag type which were then communally reared in cages.

Total fish sampling was carried out once a month. Fish were weighed after which the amount of supplemental feeds given was adjusted at 5% of total weight per cage per day, given twice daily. This study was carried out for 180 days.

At harvest, the total number of surviving fish was counted and divided up into those still bearing identifying tags/marks and unmarked fish. Overall survival of fish was calculated based on the number of surviving fish. On the basis of the assumption that there was no differential mortality between the fish tagged using the different methods, it was possible to estimate tag retention, by correcting for estimated mortality as follows:

$$\frac{\text{no. of fish recovered with tag/mark} \times 100}{\text{initial no. with tag/mark}} \times \frac{\text{total number of fish initially stocked}}{\text{total number of fish harvested}}$$

Data collected in this experiment was analysed using Duncan's Multiple Range Test

The second experiment investigated the utility of six methods of batch marking:

Fin clipping Premaxilla clipping

Alcian blue dye marking Fluorescent elastomer dye marking

Binary coded tags Colored fingerling Floy tags.

A total of one hundred eighty tagged/marked fingerlings with a size range of 5.0-7.0 g were stocked for each type of tag/mark. This experiment had three replications (with 60 fish per replicate) for each tag type and the fish were communally reared in cages.

Outputs

Performance testing of GMT and YY males

Completion of on-farm trials of GMT

The failure of three of the four remaining on-farm trials brought the total failure rate of these trials to 14 out of 33 (43%) initiated.

Table 2 Failure rate of on-farm growth trials of GMT (conducted primarily under R. 5068A but completed under this project)

System type	Stocked	Completed	Failed	Failure rate
Ponds	18	8	10	55.5%
Cages	9	6	3	33.3%
Tanks\others	6	5	1	16.7%

The highest failure rate was in the pond based trials due to their susceptibility to flooding during typhoons. The major reasons for the failures were typhoon related losses (64% of cases), farmer negligence (29%) and accidents (7%). Whilst not all of these failures resulted in loss of fish (mixing of fish was sufficient to invalidate the trial) these high failure rates emphasised the difficulties in conducting on-farm research of this nature and, from the farmers perspective, highlighted the risks inherent in aquaculture operations in this climatic zone.

One remaining trial, with the GMT compared to MST and SRT under intensive conditions in concrete tanks with full feeding, was successfully completed early on in the project. The results from this trial were in accordance with those obtained in previous trials and are summarised in (Table 3), confirming the overall superior performance of GMT compared to existing farmer's fish in the Philippines.

The increase in harvest weight wasere slightly lower than the average across all trials bringing about relatively smaller increases in harvested yield. However, size uniformity and food conversion efficiencies in this controlled environment were considerably improved over the controls which included SRT with sex ratio close to that of the GMT. No analysis was applied to this data due to the absence of replication in the on-farm trials. However, this data was included in the overall summary of the on-farm trials analysed and presented in the final report of R. 6058A (Mair, 1995).

Table 3 Comparative harvest characteristics from the performance testing of GMT, compared to farmer's MST and SRT, grown under intensive conditions in concrete tanks.

Parameter	GMT	SRT control	MST control
Mean harvest weight (g)	114.9	108.2 (+6.2)	104.4 (+10.1)
% survival	82.0	74.0 (+9.2)	76.0 (+7.9)
Sex ratio (% male)	97.5	94.6 (+3.1)	60.5 (+61.1)
CV of weight	22.7	28.9 (-21.4)	32.0 (-29.1)
Food Conversion Ratio (FCR)	1.64	1.93 (-15.0)	1.95 (-15.9)
Yield ($g.m^2.day$)	4.2	3.6 (+16.6)	3.5 (+20.0)
Net return ($P.m^2.day$)	4.92	2.08 (+136.5)	1.96 (+151.0)

Values in parentheses represent differences with GMT as a percentage of the control

On-station evaluation of YY males

These experiments were designed to evaluate the reproductive capacity of the GMT producing broodstock compared to normal fish.

Sperm count and fertilising abilities of YY males

Table 4 presents the results from the study on sperm count in XY and YY males. The sperm count of XY males was consistently higher than that of YY males. Although variation between males was high this difference was shown to be significant ($P < 0.05$).

Table 4. Sperm count ($sperm.ml^{-1} \times 10^{-9}$) of the heterogametic (XY) and homogametic (YY) males of Nile tilapia (means from 25 males of each genotype)

Male genotype	Mean sperm count (±SD)
XY	2.09 (±0.36)
YY	1.77 (±0.40)

Table 5 summarises the results from the evaluation of fertility rates in eggs stripped from common females and fertilised with sperm from XY or YY males. These results indicate that hatching rates were lower in eggs fertilised with sperm from YY males although this difference was not significant.

Table 5 Fertility rate (number of hatchlings as a % of the number of eggs fertilised) of the heterogametic (XY) and novel homogametic (YY) males of Nile tilapia.

Male genotype	Mean % hatching rate (± SD)
XY	71.16 (±11.21)
YY	60.44 (±10.73)

The subjectivity of the assignment of degrees of motility of a particular sperm sample rendered this measure rather ineffectual and the results are not presented here. It would be necessary to use video techniques to quantify sperm motility in order to make valuable comparisons between the sperm samples.

This work was conducted as a BS thesis study and there were some concerns over the validity of the experimental design and we do not place too much emphasis on these results other than as a general guide. With consideration to the limitations of the study the results do seem to indicate a diminished reproductive capacity in YY males compared to XY genotypes. This may be due to negative effects of the absence of an 'X chromosome' or the homozygosity at or around the male determining region of the 'Y chromosome'. However, it is also possible ~~to~~ that inbreeding depression may be the major influencing factor. It is assumed that the YY producing line is more inbred than that used to produce normal XY males due to the greater severity of genetic bottlenecks in the breeding programme for production of YY males.

The results of this study emphasised the importance of the major study, reported below, on the application of YY males on farm. It is clearly important to determine any potential negative factors related to the use of YY males as broodfish, particularly in relation to effects on fry production, that might impact upon the uptake of the technology by hatcheries.

Comparisons of fry production in XY and YY males

Although the on-station trials did not run for a twelve month period as first anticipated, they did provide sufficient data for valid comparison of GMT and MST fingerling production within the same Egypt-Swansea strain.

Table 6 and Table 7 show the comparative data on initial weight and standard length and the gain in weight and length over the period of the trial.

Table 6 illustrates that the initial size of the males was greater than that of the females in both sets of broodstock. The same data analysed for differences between GMT producing and control broodstock (Table 7) revealed that there were no differences in initial size and only the length was statistically different in the males, being greater in the normal XY males despite the latter weighing slightly less. Strangely there were highly significant differences in the weight gain of the females with those stocked with the YY males gaining significantly more weight than the control females. This could be indicative of a lower spawning frequency in these females or possibly reduced interaction between males and females. The initial weight and weight gain for the two sets of broodstock are illustrated in Figure 5.

Table 8 shows the mean monthly production data for GMT and control fingerlings in the on-station hapa based production trials. On average the production in controls was higher than

for GMT although these differences were not significant. GMT production per unit area was slightly higher due to the smaller number of nursing hapas required to nurse the fingerlings but this change in ranking is considered as an artefact of the experimental design.

Table 6 Comparison of mean initial and gain in weight and standard length (s.d.) between the males and females within the GMT producing and control broodstock packages

	YY packages			Control		
	YY	XX	Mean	XY	XX	Mean
			INITIAL			
SL (cm)	13.1**	9.8	11.4	12.6**	9.5	11.1
	(0.202)	(0.202)	(0.273)	(0.208)	(0.135)	(0.257)
Weight (g)	67.7**	36.2	51.9	70.1**	32.8	51.5
	(3.271)	(1.825)	(2.918)	(2.863)	(1.093)	(3.069)
			GAIN			
SL gain (cm)	3.7	3.8	3.8	5.6**	1.7	3.3
	(0.290)	(0.451)	(0.330)	(0.339)	(0.323)	(0.453)
Weight gain (g)	80.3	69.7	72.7	79.4**	35	52.8
	(9.975)	(6.099)	(5.193)	(5.818)	(7.772)	(6.777)

**significantly greater - $P < 0.01$

Table 7 Comparison of mean initial and gain in standard length and weight (s.d.) of the males and females between the GMT producing and control broodstock packages.

	INITIAL				GAIN			
	Male		Female		Male		Female	
	YY	XY	XX	XX	YY	XY	XX	XX
SL (cm)	13.1	12.6ns	9.8	9.5ns	3.7	5.6*	3.8**	1.7
	(0.202)	(0.208)	(0.202)	(0.135)	(0.290)	(0.451)	(0.451)	(0.323)
Weight (g)	67.7	70.1ns	36.2	32.8ns	80.3	79.4	69.7**	35
	(3.271)	(2.863)	(1.825)	(1.093)	(9.975)	(5.818)	(6.099)	(7.772)

*significantly greater - $P < 0.05$, ** significantly greater - $P < 0.01$

Figure 5 Histogram showing comparative initial weight and weight gain for YY male and control broodstock packages.

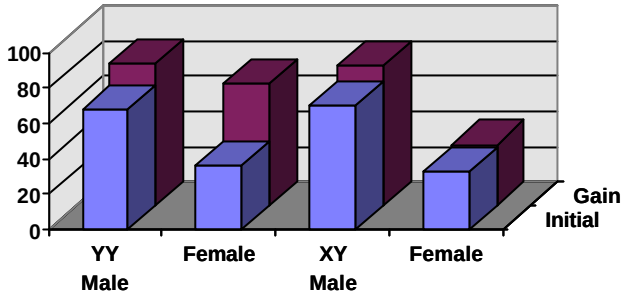


Table 8 Comparative average monthly production of fingerlings from on-station comparisons of GMT and mixed sex control producing broodstock.

Genotype	Production		
	Fingerling/package/month	Fingerling/kg of female/month	Fingerling/m²/month
YY packages	235 ± 40.784	1053 ± 259.090	354 ± 63.006
Control	308 ± 65.521	1438 ± 430.595	342 ± 48.869

The overall conclusion from this on-station study comparing fingerling production of XY and YY males in the same strain, is that there are no major differences that would be likely to significant effects on quality or quantity of fingerling production using YY males. It was important nevertheless to verify these findings with on-farm research.

On-farm evaluation of YY males

Rearing of broodstock up to sexual maturity

Prior to initiation of the trials of the fish broodstock, they were delivered to the farm as large fingerlings (0.5 to 1g). YY males were separated from the XX females to avoid contamination from the XX males and on-grown in similar facilities to those which would .be used to spawn the fish. Table 9 shows the average rearing time taken by the hatcheries to rear the fish up to the point that they fish could be stocked for breeding which averaged 4.15 months. In reality this period could be shortened as some fish were not stocked as soon as they attained sexual maturity due to temporary shortages of resources on the farm. Most fish were sexually mature within 3 months. In the existing procedure for supplying broodstock to commercial accredited hatcheries (see page 90), we estimated a period of only three months before the fish could be stocked for breeding, this period does appears to be sufficient.

Furthermore, when fish are provided to hatcheries an allowance of 15% is given on the number of males and 20% on the number of females (i.e. if 1,000 packages are requested, a

total of 1,150 males and 3,600 females would be sent to the farm as fingerlings). The additional 5% of females is to take account of the anticipated 5% of males that would be expected in the progeny of XX x XX crosses. Even with these allowances taken into account, mean survival of males during rearing was only 67.4% and females 83.0%. The mean proportion of 'rare males' removed from the female population was 3.46%, less than the anticipated 5%). These findings indicate that male survival is lower than that of females and also that overall these mortality allowances given to accredited hatcheries are insufficient. As the control fish came from on-farm sources, the survival during rearing could not be compared with that the GMT producing broodstock.

Growth and survival of broodstock during the period of fry production

Appendix 5 and Appendix 6 show the initial and final weights and lengths of the broodstock used in these trials. There were several farms in which there were significant differences between the size of the YY male packages and the control broodstock at the beginning of the trial (when the broodstock were first paired). There was however no apparent correlation between the initial and final size differences, indicating that these initial differences are unlikely to have had a major effect on either the growth or productivity of the fish. Table 10 shows the mean weight gains for the GMT producing and control broodstock, summarised for the four different production systems. In all systems the weight change in the GMT producing broodstock is significantly different to the control. Weight gain was lower in the extensive and in the two intensive systems but higher in the semi-intensive pond based system. No clear consistent trend was apparent. It is unlikely that these size and growth difference would have had a major impact upon fry production. An allowance of an additional 20% was added to the number of females and 15% for the males to cover mortality during transport and during the rearing phase and in the case of the females, to anticipate the presence of approximately 5% spontaneously sex reversed XX males which would need to be discarded). The percentage survival is based on the number of packages ordered (i.e. does not allow for the additional fish added to cover for mortality) hence the possibility to have survival apparently > 100% if the mortality allowance was surplus.

The survival of the broodstock was monitored for each cycle of production, counting the number or male and female broodstock stocked and then harvested. Table 11 shows the survival of male and female broodstock over the whole period of the experiment, for each farm in each culture system. This data could not be tested statistically but overall there appeared to be a consistently higher survival of GMT producing broodstock, compared to the controls, especially in the case of females, which overall had a 33% higher survival than control females (see Figure 6).

Table 9 Showing mean rearing times and survival for males and females in GMT producing packages during the rearing period from fingerling to broodstock.

System/Farm	Number of Packages	Rearing time (months)	Survival (%) ♂	♀
EPB				
1	1500	4	96	83
2	1200	3	73	N\A
3	300	5	50	76
SIPB				
4	400	3	100	114
5	400	4	31	78
6	1500	3	73	87
ITB				
7	400	3	30	41
8	1000	6	18	56
9	300	6	75	80
10	280	3	86	95
IHB				
11	276	5	82	91
12I	276	3	62	95
13	500	6	100	100
Mean		4.15	67.4	83.0

Table 10 Comparison of mean gain in weight (± s.e.) of breeders between genotype per production system.

System	Broodstock		Level of Significance
	YY broodstock	Control	
EPB	41.2 ± 0.613	44.1 ± 0.996	P<0.001
SIPB	51.0 ± 1.889	41.5 ± 0.943	P<0.001
ITB	79.0 ± 1.565	90.0 ± 2.370	P<0.001
IHB	57.8 ± 1.036	66.0 ± 1.399	P<0.001

Comparative fingerling production

Fingerling production, which is based on estimates of the number of fingerlings produced and reared up to the age and size at which they can be dispersed, in the four hatchery systems in the three farms is summarised in Table 12. The numbers of fingerlings are expressed in the relevant measures, according to the number of packages, the weight of female broodstock and according to the water area of the hatchery system.

Data were analysed by student's *t*test, treating the production cycles in each farm as replicates. There were no differences in the ranking of GMT and control fingerling production for the different measures by which it was assessed although there were some minor changes in the magnitude and significance of the differences.

Significant differences were observed in the fingerling production of controls and GMT producing broodstock (per package) in several of the farms. In extensive pond based systems GMT production was significantly higher in two of the three farms. This also applied one of the semi-intensive pond based hatcheries and two of the four intensive tank based hatcheries. Control production was only significantly higher than that of GMT in one of the three intensive hapa based hatcheries.

Whilst there were significant differences in the GMT and control fingerling production between hatcheries there was no consistent trend to these differences. It is our conclusion therefore, that over all the trial, fingerling production did not differ significantly between the GMT producing and the control broodstock.

In the dissemination programme in which GMT producing broodstock are supplied to accredited hatcheries (see page 90), we developed a series of assumptions on which to base our estimates of the number of fingerlings that would be produced by the accredited hatcheries. The royalty fees paid by these hatcheries was based on these estimates. At the commencement of the dissemination programme, fingerling production estimates were based on 500 fingerlings per package month. Following an evaluation of the dissemination procedures after some months, it seemed likely that this figure was an over-estimate and a revised estimate of 250 fingerlings per package per month was proposed. Figure 7 shows the comparative production of GMT and control fingerlings and illustrates the lack of a clear trend in the differences between the two.

Table 11 Percentage survival of GMT producing and control broodstock for the duration of the fry production trials (from commencement of the first production cycle up to the end of the last cycle).

System/ Farm	Survival (%) YY Broodstock		Survival (%) Control		% difference YY/Control	
	Male	Female	Male	Female	Male	Female
EPB						
1	72	78	23	24	213	225
2	19	35	20	35	-5	0
3	37	28	35	31	6	-10
Mean	**43**	**47**	**26**	**30**	**65**	**56**
SIPB						
1	25	63	8	24	212	162
2	19	68	19	66	0	3
3	n\a	n\a	n\a	n\a	n\a	n\a
Mean	**22**	**66**	**14**	**45**	**57**	**47**
ITB						
1	73	79	69	69	-8	14
2	98	97	95	100	3	-3
3	93	86	93	82	0	5
4	93	79	72	36	29	119
Mean	**89**	**85**	**82**	**72**	**6**	**34**
IHB						
1	40	30	59	24	-32	25
2	97	98	97	77	-1	27
3	74	47	93	34	-20	38
Mean	**70**	**58**	**83**	**45**	**-16**	**29**
Overall mean	**56**	**64**	**51**	**48**	**10**	**33**

Figure 6 Comparative survival of GMT producing and control broodstock over the duration of the production trials (GMT producing broodstock survival as percentage of survival of controls).

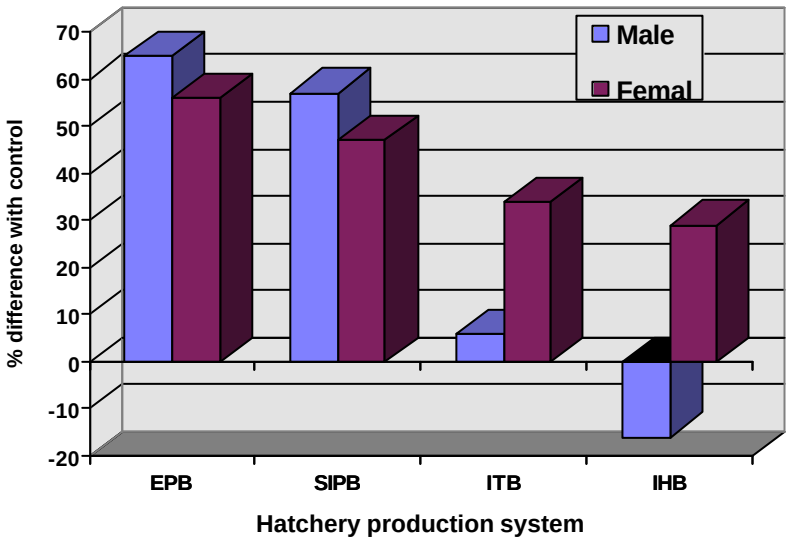


Table 12 Average monthly production of fingerlings (expressed according to production per package, per kg of female weight and per unit area) for GMT producing and control broodstock.

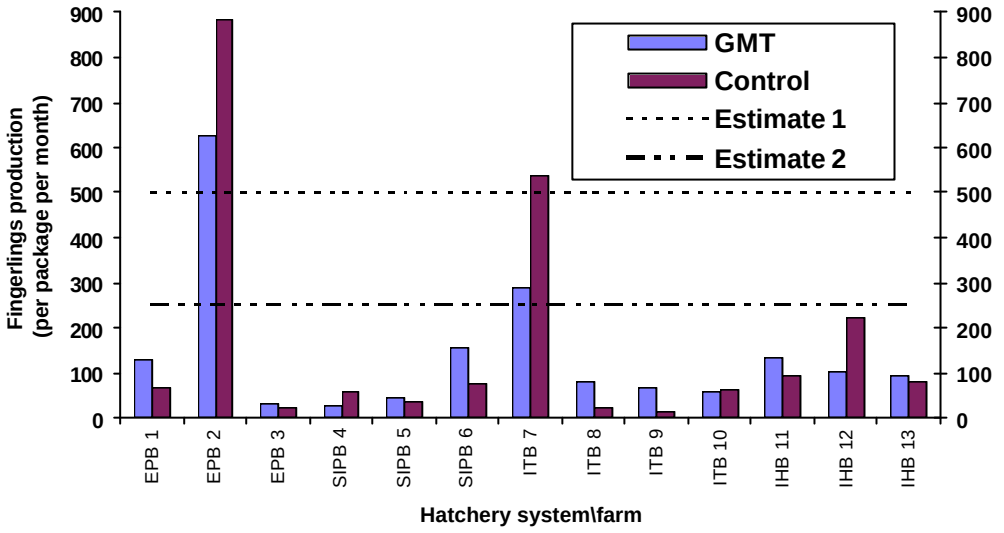
	Average monthly fry production					
	per package (1♂:3♀)		per kg of female		per unit area (m^2)	
System/Farm	GMT	Control	GMT	Control	GMT	Control
EPB 1						
1	131**	68	585**	226	31**	18
2	628	886	1701	2005	44	60
3	33**	21	166**	158	7**	5
mean						
SIHB						
4	28	59	264	440	8	17
5	45	36	335	273	16	12
6	156*	74	565	545	95*	69
mean						
ITB						
7	289**	536	889**	1119	99	117
8	81	24	308	110	261	104
9	67*	16	307	125	45	18
10	58	63	229	229	40	46
mean						
IHB						
11	132	92	1141	663	130	95
12	103	221**	446	824**	103**	195
13	95	82	301	174	91	78
mean						
overall mean						

* - significantly greater at P<0.05, ** significantly greater P<0.01, *** significantly greater at P<0.001.

The graph also indicates some rather surprising findings. It had been predicted that the more intensive hatcheries (tank and especially hapa based) would produce more fingerlings per package than the lower intensity systems. However, this was clearly not the case, there being no apparent relationship between intensity of the operation and fingerling production and in fact the highest production of any farm came from the extensive pond based system. Furthermore, it is clear that only two hatcheries exceeded or came close to either of the estimated fingerling production targets. It is interesting to note that these two hatcheries, BFAR (EPB 2) and Jonathan Reyes (ITB 7) represented probably the two most experienced hatcheries in the trials. None of the less experienced hatcheries (less than two years of experience in hatchery production) produced high numbers of fingerlings but there were also two experienced hatcheries which had relatively low production (6 and 7). Figure 8, showing fingerling production per unit area, does illustrate that the more intensive hatchery systems generally produced a higher productivity per unit area due principally to the higher stocking densities of broodstock and of fingerlings.

In addition to inexperience in hatchery production, a very major factor in the poor production rates achieved in most of the hatcheries, was the small size and young age of the broodstock. It is considered that tilapia broodstock have a productive life of 18 to 24 months. During this time the productivity per broodfish increases steadily, peaking at 14-18 months after which time spawning frequency in the females begins to decline. The production recorded in these trials represented the first 4-6 months of production and it is very likely that substantial increases would have been realised in the succeeding months.

Figure 7 Histogram showing actual and estimated production of fingerlings (per package per month) from GMT producing and control broodstock in the hatchery trials of the GMT producing broodstock.

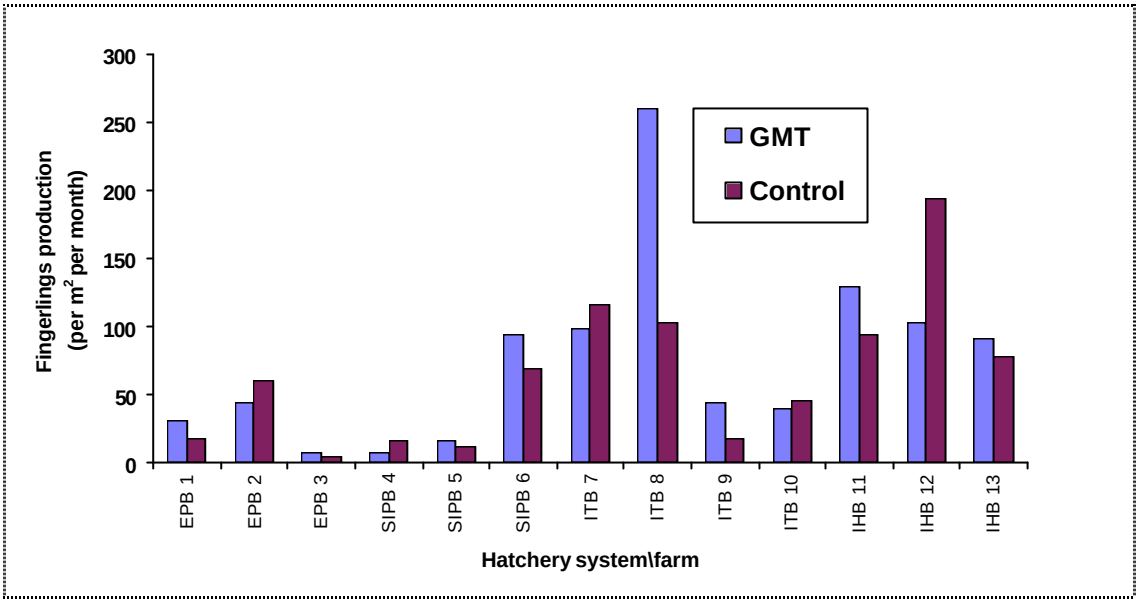


In informal canvassing of opinion, none of the hatchery managers or owners expressed dissatisfaction or surprise at their productivity. Furthermore none observed, either from a technical or subjective viewpoint, any major advantages or disadvantages in the production of GMT or control fingerlings, except of course for the fact that the former fingerlings were putatively all-male. All hatcheries producing SRT as control appreciated the benefits of producing GMT which simplified their production process. However, there were no clear trends in the relative production of GMT and SRT in the hatcheries producing the latter as controls (6,7,8,11,12 and 13).

Sex ratio

It was originally intended to collect sex ratio data from every production cycle in each farm. However, farmers were unwilling to sacrifice fingerlings too regularly and on occasions, due to high demand, all fingerlings had been sold before the farm was visited to collect samples. Nevertheless, some sex ratio data was collected from the majority of the farms as shown in Figure 9.

Figure 8 Histogram showing monthly production of fingerlings (per unit area) from GMT producing and control broodstock in the hatchery trials of the GMT producing broodstock.



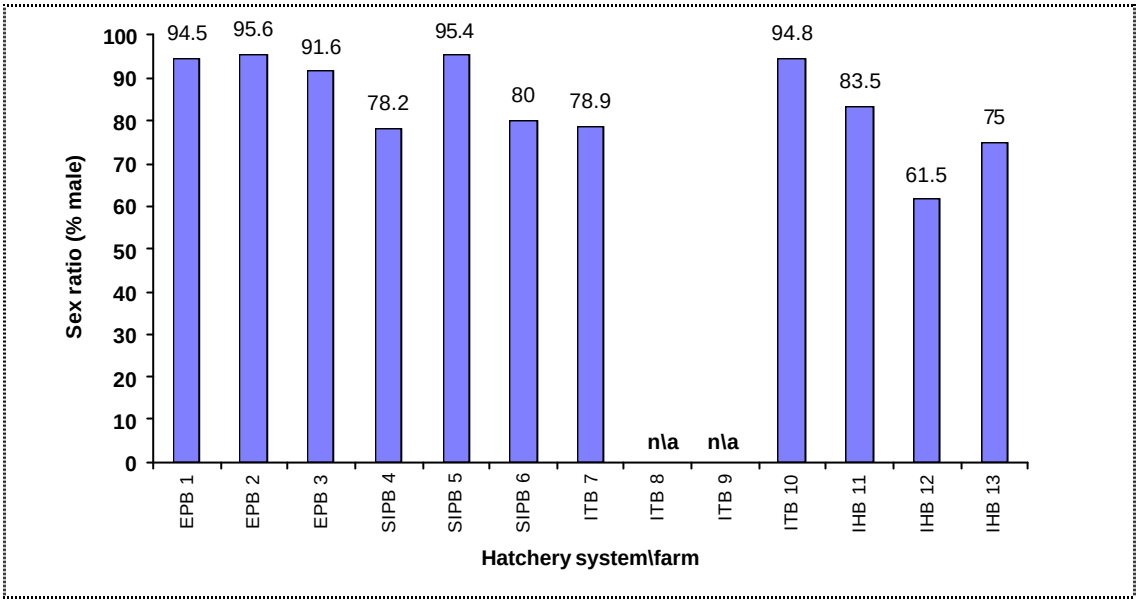
Overall, sex ratios averaged 84.5% males across all the farms from which data was collected, a low sex ratio considering that YY males are known to produce a mean of 95% male progeny based on several years of on-station experimentation. As can be seen from Figure 9, there was one hapa based farm which produced a sex ratio of only 61.5% male. Such a low ratio had not previously been seen, even in a single pair mating involving YY males and it is highly unlikely that this ratio came from the fingerling production of YY males. Experimental error involving a mix up of the fish is the only logical explanation for this ratio. Five other farms, two semi-intensive pond based, one tank based and one hapa based produced sex ratios lower than 85% male. This is again lower than expectations and is likely to be indicative of low levels of contamination of the broodstock or of the fingerlings during nursing. The effect of, as yet undetermined environmental factors, on sex differentiation should also not be discounted. It had been predicted that broodstock contamination would be more likely in the more extensive pond based hatcheries. Contrary to expectations, sex ratios were highest in the extensive pond based systems, indicating that the technology can be effectively applied in such systems. Whilst further investigations into the causes for lower than expected sex ratios are required, it is predicted that all the raised sex ratios were sufficiently high (with the exception of hatchery 12) to result in significant increases in production during grow out compared to mixed sex progeny.

Cost and return analyses

During the course of the trials, attempts were made to record all costs associated with the production of the fingerlings including depreciation costs of equipment, labour, food and

fertilizer etc. The vast majority of costs did not differ between the production of GMT and control fingerlings. In the case of those hatcheries

Figure 9 Mean sex ratios of GMT collected at each of the co-operator hatcheries.



producing SRT (7, 8, 11, 12, 13) fingerling production costs were higher due to increased costs of facilities, labour, hormones and alcohol (see details in the Farm Fact Files - Appendix 2). There was an additional cost associated with the production of GMT. Under the accreditation procedures (see page 90) developed for the dissemination programme, the cost to hatcheries of GMT production were in one of two forms i) the purchase of the broodfish at P300 per package or ii) the payment of royalties of P17.5 per 1,000 fingerlings, with fingerling production estimated at 250 per package per month.

Table 14 shows the cost of fingerling production under the two schemes and a hypothetical cost if the hatchery paid a royalty based on the actual production rather than on an estimate. It can be seen from the same table that nine of the 13 hatcheries sold the GMT at the same price as the control fish. In the case of hatcheries producing SRT it was logical to value the GMT and SRT, as all male fish, at the same price. In the case of those selling mixed sex tilapia, those that chose to maintain the same price did so either out of government policy (in the case of BFAR) or because they were young, non established hatcheries, or they were located in an areas where GMT was not yet commonly known. All hatcheries except BFAR expressed their intention, in the long term, to sell GMT at a 15 to 20% higher price than MST. This should more than cover the royalty paid on the fingerlings which is equivalent to less than 10% of the cost.

Scheme one costs are clearly very high and would have been very unprofitable at the low rates of production achieved by most of the hatcheries. Scheme 2 costs are also high, proportionally more so due to the low productivity of fingerlings.

Table 13 Comparison of total GMT production over the experimental period and the estimated production based on Scheme 2 of commercial accreditation under the dissemination programme for the YY male technology. The estimate is based on the assumption of a production of 250 fingerlings per package per month from the actual number of breeders used during the initial pairing.

System\Farm	Actual production	Estimated production	% difference actual/estimated
EPB			
1	206,040	703,750	-70.72
2	1,332.647	1,032,500	29.07
3	9,800	457,500	-97.86
mean			**-46.50**
SIPB			
4	48,115	951,500	-94.94
5	47,387	471,000	-89.94
6	101,000	2,025,000	-95.01
mean			**-93.29**
ITB			
7	357,700	492,000	-29.43
8	10,000	45,000	-66.67
9	12,430	358,500	-96.53
10	19,730	150,000	-86.85
mean			**-69.89**
IHB			
11	65,112	945,000	-93.10
12	68,600	585,750	-88.29
13	269,252	875,000	-69.23
mean			**-83.54**
Overall mean	187,097	699,423	
Overall total	2,432,272	9,092,500	**73.2%**

The inputs, sales and net returns for the 13 hatcheries over the period of the trial are shown in Appendix 7 . Under Scheme 1, only one of the hatcheries would have made a higher return producing GMT. Under Scheme 2 this rose to four out of the twelve, and if royalties could be charged based on actual production rather than estimates, nine of the twelve hatcheries would have made a greater profit producing GMT.

It is worth noting that four of the twelve hatcheries actually made a loss on their own fry production. At a time when tilapia hatchery production is popular with aquaculturists and entrepreneurs alike due to its high profitability, this is a strong indicator of poor production in these hatcheries, largely due to inexperience. With even modest increases in fry production, to bring production closer to the revised estimates set by the dissemination programme, GMT

would clearly become a more profitable activity than the production of the farmer's present fish, especially if combined with an increase in the selling price of GMT.

Table 14 Costs of production of control and GMT fingerlings in the hatchery trials. Cost of GMT are based on three different options. Scheme 1 - outright purchase of the broodfish; Scheme 2 - payment of royalties based on an estimated production of 250 fingerlings per package per month; Actual - payment of royalties based on actual production of fingerlings. Prices in Philippine Pesos (OER £1 = P40)

System /Farm	Productions cost per fingerlings				Selling Price	
	Control	[1]Scheme 1	Scheme 2	Actual	Control	GMT
EPB						
1	0.44	0.39	0.23	0.18	0.25	0.30
2	0.04	0.12	0.07	0.07	0.30	0.30
3	0.79	2.57	1.19	0.80	0.50	0.55
Mean	0.42	1.03ns	0.50ns	0.35ns	0.35	0.38
SIPB						
4	0.14	1.58	0.60	0.27	0.25	0.30
5	0.22	0.49	0.19	0.16	0.18	0.25
6	0.21	1.39	0.41	0.07	0.40	0.40
Mean	0.19	1.15*	0.40ns	0.17ns	0.28	0.32
ITB						
7	0.02	0.11	0.04	0.04	0.40	0.40
8	1.53	0.73	0.36	0.20	0.40	0.40
9	0.57	2.40	0.64	0.18	0.35	0.35
10	0.17	0.61	0.24	0.13	0.35	0.35
Mean	0.57	0.96ns	0.32ns	0.14ns	0.37	0.37
IHB						
11	0.21	1.06	0.35	0.11	0.40	0.40
12	0.04	0.48	0.17	0.09	0.30	0.30
13	0.11	0.31	0.15	0.11	0.40	0.40
Mean	0.12	0.62ns	0.22ns	0.10ns	0.37	0.37

= significant at $P<0.05$; ns = not significant

1- Scheme one costs are estimated based on the depreciation costs of the broodfish over the period of the trial as a proportion of the total useful life of the broodstock (18 months).

Summary

At the outset we established a number of hypotheses for these on farm trials which can now be reexamined:

- A number of trials would fail to give useful results due to poor management and man made or natural disasters - ***rejected****, all trials produce valid data although one had only a single production cycle.*
- Production of fingerlings per unit broodfish would increase with the level of intensity of the hatchery system, being highest in the hapa based hatchery and lowest in the intensive tank

based - ***rejected***, *there appeared to be no relationship between fingerling production efficiency and intensity of the production system.*

- There would be no significant difference in fry production parameters between GMT producing and control broodstock. - ***accepted***, *whilst some differences were significant there was no consistent trend.*
- There would be no significant difference in the survival and growth of the GMT producing and control broodstock - ***accepted***, *there were no consistent differences observed*.
- GMT production would produce higher returns if higher prices were charged for the GMT - ***Further investigation is required***. *Based on present accreditation schemes not all farmers charging higher prices, made higher returns, mainly due to low fingerling production*
- Sex ratios of GMT would be more variable in the more extensive pond based hatcheries due to the greater difficulty in preventing broodstock contamination - ***rejected***, *sex ratios were variable but they were actually lower in intensive hapa based systems, and highest in extensive pond based!*

These on-farm trials were very successful and achieved their objective, providing very valuable feedback on technical and economic aspects of the production of GMT fingerlings in commercial hatcheries. The most important finding was that there were no major differences in the performance of GMT producing and control broodstock and therefore the principal limitations (if they exist) to the uptake of the technology in hatcheries will be economic and social. The results have provided valuable information which will be used to optimise dissemination programmes.

Socio-economics of tilapia culture in the Philippines

As previously stated this study took on two approaches, a case study of two aquaculture communities to collect and collate baseline data on the socio-economic issues pertaining to Philippine tilapia culture, and a survey of common hatchery management practices.

Some of the important issues pertaining to the application of the YY male technology were identified in the surveys carried out in the two aquaculture communities. This section of the report highlights some of the major findings. A fuller analysis of the findings can be found in Appendix 8.

More than 100 hatcheries were identified in the Laguna area and 66 tilapia growers in Bulacan. More than 50 of the hatchery owners\managers were interviewed along with 37 of the tilapia growers. At the time of the survey, none of the hatcheries or growers had adopted the YY male technology principally due to the lack of availability of GMT fingerlings and YY

male broodstock. Table 15 shows some of the key social and demographic characteristics of these two groups of farmers. The only major difference between the two groups of farmers were that the hatchery owners in Laguna had taken up aquaculture much earlier than the growers in Bulacan. Otherwise the general character of the tilapia farmers was that of a middle aged, partially educated male, with a medium to large sized family.

Table 15 Selected socio-demographic characteristics of farmers by production system in Laguna and Bulacan, Philippines, 1994 - 1995.

Item	*Production System*	
	Hatchery (Laguna)	Grow-out (Bulacan)
Total tilapia farmers	116	66
No. of case farmers	50	37
% to total farmers	43.1	56.1
Age (years)	43.5	50.8
Sex (no. & proportion of males)	45 (90%)	35 (95%)
No. of household members	5.4	4.3
Years in school	7.4	8.5
Years experience in tilapia farming	11.1	2.9

A very major difference between the two groups of farmers, as shown in Table 16, is that for the hatchery owners in Laguna tilapia fingerling production was the primary source of income whereas tilapia grow-out was clearly a secondary activity for the farmers in Bulacan. However, it was apparent that aquaculture was more profitable that the other forms of agriculture adopted by the farmers, with hatchery production being more profitable than grow-out. Most farmers were optimistic about their involvement in the industry with 60-70% planning to continue with their operations and a further 20-25% were planning to expand.

This group of hatcheries in Sto. Domingo in Laguna are quite unique in the Philippines, established in the 1980s to provide fingerlings for the flourishing cage and pen culture in the nearby shallow lagoon of Laguna de Bay. Most are very small scale hatcheries, averaging less that 1,000 m² each, with some being considerably smaller. One relevant factor here would be the relative risks involved in trying out new technologies which would be much greater for the hatcheries.

Table 16 Composition of income among hatchery and grow-out farmers.

Income Source	(% of total income)	
	Hatchery (Laguna)	Grow-out (Bulacan)
Rice production	2.9	61.0
Tilapia farming	82.1	14.1
Animal Production	0.9	9.5
Crop production	-	4.9
Business and trade	6.1	4.4
Salaried job	3.6	4.2
Non-farm labour	-	1.1

Tilapia production - related sources	3.9	-
Others	0.5	0.8

When asked what were the principal problems in their respective tilapia culture activities the most major problem cited was that of obtaining suitable fish stocks (Table 17). This was perhaps a surprising finding as tilapia is a ubiquitous fish in the Philippines. In the case of the tilapia growers in Bulacan, there are few hatcheries in the area so they would need to source fingerlings from distant areas. In the case of Laguna, the problem probably referred to obtaining broodstock sized fish, which are difficult to obtain live due to the difficulty of transportation. Water resources were also a major factor for the pond growers due to irregular supply of irrigation water. Availability of capital and land area for expansion were also cited as problems but surprisingly security of tenure was not cited as a problem.

Table 17 Problems faced by tilapia hatchery and grow-out farmers.

	(% of responses)	
Problems	Hatchery (Laguna)	Grow-out (Bulacan)
Obtaining fish stock	76	57
Poor growth of fish	12	40
Mortality of fry / fingerlings	70	19
Water shortage	-	78
Lack of capital	24	65
Small land area	66	-
High cost of feeds	16	41
Limited expertise	10	95
Lack of technical assistance	8	65
Proliferation of producers	64	14
Poaching	4	62

The farmers did not make reference to the quality of the fish and non of the grow-out operators specifically cited reproduction in their fish ponds as a problem. Furthermore most of the farmers were either not aware of only peripherally aware of the existence of the YY male technology and GMT. Approximately 60% of the hatcheries had heard of the technology but knew little of the detail or potential benefits. Only 5% of tilapia growers in Bulacan had heard anything of the technology. Once the technology was explained to the farmers most (more than 90% of hatcheries and 60% of growers) were willing to use the products of the technology provided that is was affordable.

There were some reservations expressed identifying some constraining factors to the uptake of the technology. These included a possible limited market for all-male tilapia (clients of hatcheries do not specifically request all-male fingerlings), a lack of information about the technology and a lack of capital. Most tilapia hatcheries in Sto. Domingo produce their own replacement broodstock and would have to be persuaded of the benefits of YY males before making a switch to them, especially if this involved significant capital outlay.

Few of the tilapia growers actually sought monosex to grow in their ponds and some did not consider that the sex or the 'genetic quality' of the fish had much influence on growth and could not even distinguish the male and female tilapia. Some growers also perceived the benefit to them of selling fingerlings caught during the harvest of the stocked fish, a benefit that would be lost in growing GMT. Clearly lack of information, through the weakness of government and private extension agencies, is a major factor in the introduction and uptake of new technologies such as the GMT and one that should be addressed in further research and extension of the technology.

One interesting finding was the inverse relationship between farm size and productivity in the hatcheries in Laguna indicating that the small farms used labour and space resources more efficiently. This inverse relationship did not appear to apply for grow-out operations. This provides some optimism that small scale hatcheries can be competitive with larger farms in adopting the YY male technology, the properties of which appear more or less scale neutral.

Evaluation of technology transfer to Thailand

This study is a continuation of research begun under R. 5068A to investigate the feasibility of different methods of transferring the YY male technology to Thailand. We continued the evaluation of four methods of breeding for technology transfer.

Direct transfer

This involved the direct transfer of broodstock fish from the Philippines to Thailand. The original transfer was of YY males, normal females and some estrogen treated females from XY x YY crosses. These were progeny tested under R 5068A and some YY male successfully identified. This enabled the production of YY male and normal female broodstock for the production of Egypt-Swansea GMT as used in the Philippines. Most of this work with this strain is being done at NAGRI where production of YY males and normal females has become routine and large numbers (many thousands) of YY males and over one thousand YY females are maintained. Some fish have been lost in storms and in flooding and it has proved necessary, from time to time, to move the fish around between Dept. of Fisheries stations. The YY males are routinely crossed with females of the Egypt-Swansea strain or with Egypt-Stirling females. Sex ratios of the pure bred GMT are reported to be consistently high, ranging from 95-100% male. It can be considered that, using this simplest form of technology transfer, that the technology has indeed been successfully transferred to Thailand, where GMT and GMT broodstock can be and are being mass produced. The main use of these fish in research has been as controls in growth evaluation experiments (see page 46).

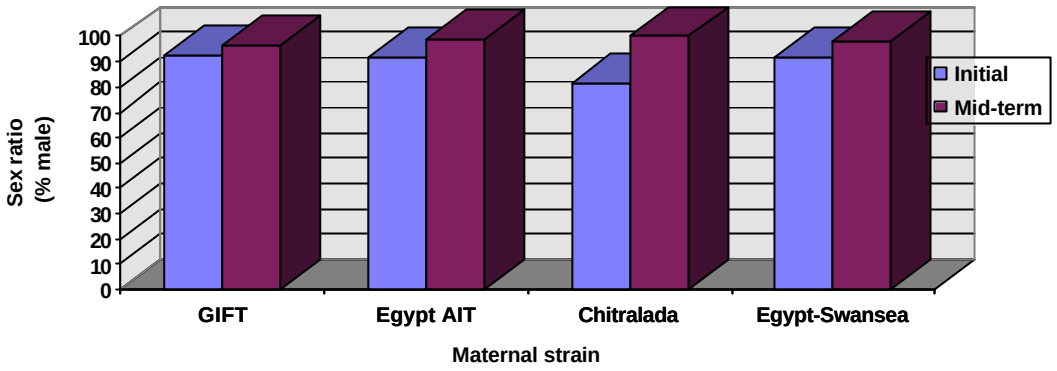
Indirect transfer by crossbreeding

This method of technology transfer simply involves the crossing of YY males of the Egypt-Swansea strain with females of the local Thai strain to produce crossbred GMT. An informal selection programme was initiated to develop a female line in the Egypt-AIT strain. A total of 21 females arising from crosses of the selected females, were progeny tested against YY males of the Egypt-Swansea strain. Sex ratios from the progeny testing of selected females ranged from 89.9 - 100% male with a mean of 99.3% which was not significantly different to the sex ratios obtained from the non-selected females which ranged from 83.3 - 100% male with a mean of 98.3%. This data should be regarded only as a very preliminary indication of the lack of response to selection and a lot more data are required to make firm conclusions on this. Overall the sex ratios in the progeny testing of this second generation of females were much higher than in the first generation (88% male and 92% male in two separate studies). There is a need to identify other possible sources of this variation in sex ratio.

A response to selection would be indicative of a polygenic or multi-factorial mechanism of sex determination in this strain.

Some further data on crossbred sex ratios was collected as part of a growth performance trial being conducted in Trad Province in Thailand. This study produced crossbred GMT by crossing the YY male of the Egypt-Swansea strain with females of ICLARM's GIFT strain, Egypt-AIT, and the widely cultured Chitralada strain. Sex ratios were obtained at the beginning of the trail using the reliable gonad squash technique and then again at the mid term point, by external morphology. The summary of the results are shown in Figure 10 illustrating that none of the sex ratios in the crossbred differ significantly from that of the control within strain GMT. The mid-term sex ratios are higher than the initial sex ratios reflecting either a differential mortality of the small proportion of females in the populations, or a greater error in mis-identifying females using external morphology.

Figure 10 Sex ratios of three crossbred GMT compared with the pure bred Egypt-Swansea GMT. Values represent mean sex ratios (% male) from three replicates of fish prior to (initial) and three months after (mid-term) stocking in ponds for growth comparisons



Indirect transfer by crossbreeding and selection

Following on from the progress made under R. 5068A the breeding programme was continued enabling identification of sex reversed XY females (Δ♀♀) in the Egypt-AIT strain and thereafter YY males and sex reversed YY females in the hybrid progeny derived from them. A total of 13 crossbred YY males were identified together with six YY females. In back crosses to XX genotypes in the Egypt-AIT strain, the mean sex ratio from the males was just below 90% male but from the females this was higher at 98% males (see Table 18).

Table 18 Number of manipulated genotypes available at the end of 1996

Genotype (number identified)	Strain contribution	Progeny tested with?	Mean sex ratio(%♂) in progeny tests
XY Δ♀ (4)	Egypt-AIT	XX EA or ES	47.8
YY ♂ (13)	Crossbred (XY E-A x YY E-S)	XX ♀ Egypt-AIT	89.6
YY Δ♀ (6)	Crossbred (XY E-A x YY E-S)	XX Δ♂ Egypt-AIT	98.1

E-A = Egypt-AIT strain, E-S = Egypt-Swansea

The crossbred YY Δ♀♀ can now be crossed to YY male of the Egypt-AIT strain (see below) to generate YY males carrying 75% of the Egypt-AIT genome. This approach was intended to accelerate the process of transferring the technology to a local strain. However, early on in the project it was clear that YY males would be produced in the breeding programme (see below) in the pure Egypt-AIT strain. As this approach appeared to have little advantage in terms of speed and ease of transfer, or in sex ratios produced, it was decided to abandon further research on this form of technology transfer.

Application in pure Thai strains

This approach made major advances in the development of YY males in the local Egypt-AIT strain from scratch, applying the breeding programme approach developed in the Philippines. Whilst continuing with the breeding programme to develop YY males we also collected some baseline data on sex ratios in normal crosses within the strain in an attempt to further elucidate the mechanism of sex determination.

Sex ratios in normal crosses

Table 19 presents the summary of 95 single pair matings performed to investigate the distribution of family sex ratio in this strain.

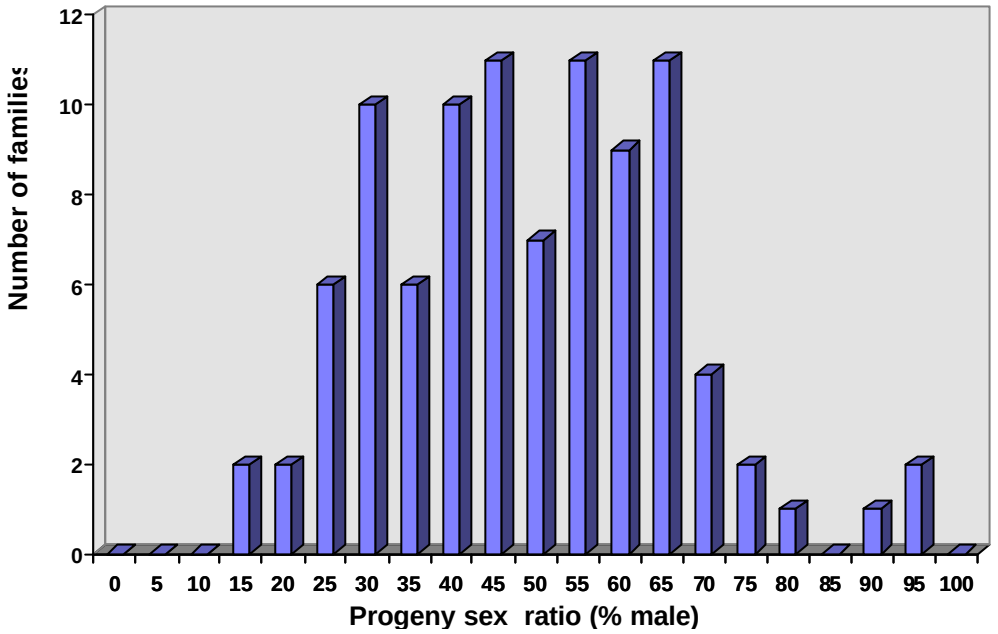
Overall the proportion of males was dose to 50% as was the mean sex ratio of the 95 families. The large and significantly heterogeneous distribution of sex ratios, with more than half of the ratios being significantly different from 1:1, is indicative of polyfactorial sex determination in this strain. This is borne out by shape of the frequency distribution of sex ratio (Figure 11) which, whilst showing an underlying normal-type distribution around 50% males, is very wide and somewhat skewed. This frequency distribution is much wider than that observed by Mair *et al.* 1991a for the Egypt-Swansea strain of the same species. This may reflect a more polygenic system of sex determination with greater genetic variance of the character. The observation of three family sex ratios in excess of 90% male sounds a note of caution to be considered when progeny testing to identify YY males.

Table 19 A summary of progeny sex ratios of normal broodstock of the Egypt-AIT strain of *O. niloticus*.

Number of families produced and sexed:	95
Total number of progeny sexed	7,822
Average sample size per family	82.3
Total number of males in the progeny (overall % male)	3,951 (50.5%)
Total number of females in the progeny (overall % female)	3,871 (49.5%)
Range of family sex ratios observed	15.5 - 100% ♂
Mean family sex ratio	50.97% ♂
Number of families with sex ratio skewed to male ($P < 0.05$)	27 (28.4%)
Number of families with sex ratio skewed to female ($P < 0.05$)	24 (25.3%)
Pooled χ^2	$1.278_{[1]}$
Total χ^2	$883.673_{[95]}$
Heterogeneity χ^2	$882.395_{[94]}$ ***

*** significant ($P < 0.001$) Values in parentheses represent modified degrees of freedom.

Figure 11 Sex ratio frequency distribution of progeny derived from 95 single pair matings of normal broodstock of the Egypt-AIT strain of *O. niloticus*. (classes intervals of 5 percentage points have been used have with the X-axis showing the lower limit of each class).



A second experiment attempted to investigate specific maternal and paternal effects on sex ratio in a seven (female) by five (male) di-allele type cross. Table 20 shows that of the 35 possible, only 26 crosses were completed. Sex ratios varied from 27.1 to 97.9% male, overall highly heterogeneous. When analysed across individual males or females, three males and four females produced significantly heterogeneous sex ratios. Test for independence between the two classification criteria (male and female parent) produced a highly significant χ^2 ($P<0.001$) rejecting the hypothesis of independence indicating that the sex of the progeny groups was dependant upon male and female parents, i.e. that there were significant maternal and paternal effects on sex ratio.

Table 20 Sex ratios (with % male shown in parentheses) of a di-allele type cross of five males and seven females in the Egypt-AIT strain of *O. niloticus* .

Females (tag no.)	Male Parents (tag no.)					χ^2	P
	M054	M569	M872	M890	M311		
FM 029	29 : 17 (63.0)	93 : 25 (78.8)			8 : 5 (61.5)	4.088	ns
FM 537	51 : 49 (51.0)	68 : 32 (68.0)	43 : 57 (43.0)	27 : 60 (31.0)		27.268	<0.01
FM 283	7 : 1 (87.5)	61 : 19 (76.3)	13 : 35 (27.1)	41 : 59 (41.0)		39.602	<0.01
FM 047	26 : 8 (76.5)	70 : 30 (70.0)	25 : 26 (49.0)	51 : 49 (51.0)	46 : 32 (59.0)	13 : 42	<0.01
FM 367	22 : 28 (44.0)		48 : 50 (48.9)			0.328	ns
FM 286	47 : 1 (97.9)	12 : 11 (52.2)	42 : 58 (42.0)	19 : 37 (33.9)	46 : 50 (47.9)	51.727	<0.01
FM 313		51 : 38 (57.3)	2 : 1 (66.7)	51 ; 49 (51.0)		0.902	ns
χ^2	40.730	13.706	7.705	12.049	2.466		
P	<0.01	<0.05	ns	<0.05	ns		

χ^2 test was for heterogeneity applied to sex ratios from females (rows) and males (columns); ns = not significant.

Development of YY males

Four YY males (producing sex ratios ranging from 85.9 - 98.6% male) were identified following the progeny testing of 17 males produced in XYΔ♀ x XY♂ crosses (Table 21).

Two of these YY males, with tag numbers 829 and 840, were used in repeat matings with females of the same strain to determine the repeatability of sex ratios produced by them. These two males had been identified as YY males by the sex ratios of 85.9 and 98.6% male respectively produced in their original progeny tests. Table 22 shows the sex ratios produced in 20 of these repeat matings. The results were somewhat surprising. Most sex ratios were highly significantly different from 1:1 but two families produced by male #829 had sex ratios close to 1:1 and one that was significantly skewed to female. In the case of male #829 there were even some significant differences between full sibling families. The mean family sex ratio produced by YY male #829 was only 74.5 male compared to 93.8% male with #840. Whilst more progeny testing is required to confirm this, the male which produced the highest progeny sex ratio in the original cross also produced the highest sex ratios in the repeat mating.

These results provide further evidence for the greater lability~~lability~~ of sex ratio in the Egypt-AIT strain compared to the Egypt-Swansea strain. It is likely that the proportion or effect of autosomal sex modifying genes in the Egypt-AIT strain is greater, possibly as a result of higher overall levels of genetic variation in this strain. The strain has passed through fewer genetic

bottlenecks than the Egypt-Swansea strain and is customarily maintained at higher effective population sizes at AIT than has been possible with the latter strain at CLSU. Introgression with other species could also account for many of the deviations from the expected sex ratios but this does not seem likely to have occurred at AIT.

Table 21 Sex ratios from progeny testing of males, resulting from crosses of sex reversed females (XY) with normal males (XY), for identification of YY-males in the Egypt-AIT strain of *O. niloticus*.

Males (XY/YY) (tag no.)	No. progeny sexed	Sex ratios (♂ : ♀)	% male	$\chi^2_{[1]}$ (1:1)
124	100	55 : 45	55.0	1.00
046	100	56 : 44	56.0	1.44
103	41	9 : 32	22.0	12.90
829	85	73 : 12	85.9	43.77 ***
822	19	14 : 5	73.7	4.26
800	6	1 : 6	16.7	2.67
339	45	3 : 42	6.7	33.80
840	73	72 : 1	98.6	69.05 ***
110	133	50 : 83	37.6	8.19
821	100	92 : 8	92.0	70.56 ***
107	100	44 : 56	44.0	1.44
863	93	56 : 37	60.2	3.88
288	25	14 : 11	56.0	0.36
859	100	58 : 42	58.0	2.56
880	81	48 : 33	59.3	2.78
315	100	55 : 45	55.0	1.00
337	80	77 : 3	96.3	68.45 ***

*** Significantly different from 1:1 ($P < 0.001$); only those sex ratios skewed to male were tested

Given the results and the aforementioned assumptions, it appears likely that sex ratios produced by the YY males in the Egypt-AIT strain could be improved both by the selection of YY males and females, based on progeny sex ratio.

A total of seven DES treated fish from crosses of XY♂ x XYΔ♀ in the Egypt-AIT strain have been raised to sexual maturity and are available to be progeny tested with XX Δ♂ in the hope of identifying YYΔ♀♀. This progeny testing did not yield any data by the end of the project.

Growth comparisons

A number of important growth trials have been completed during this year.

Full details have either been supplied quarterly reports or will appear in the Final Report for the project but the following summarises the results from the different trials:

Table 22 Sex ratios in progeny from crosses of normal females with two YY males in the Egypt-AIT strain of *O. niloticus* including a number of repeat matings (boxed).

Crosses (♀ x ♂)	No. progeny sexed	Sex ratios (♂ : ♀)	% male	χ^2 [1] (1:1)
007 x 829	65	63 : 2	96.9	57.2 ***
007 x 829	100	66 : 34	66.0	10.2 **
041 x 829	65	63 : 2	96.9	57.2 ***
041 x 829	57	47 : 10	82.5	24.0***
090 x 829	37	35 : 2	95.0	29.4 ***
090 x 829	100	69 : 31	69.0	14.4 **
090 x 829	100	82 : 18	82.0	40.9 ***
090 x 829	73	51 : 22	69.9	11.5 **
047 x 829	35	17 : 18	48.6	0.028
524 x 829	20	11 : 9	55.0	0.100
006 x 829	32	28 : 4	87.5	18.0 ***
103 x 829	100	36 : 64	36.0	7.84 **
295 x 829	71	58 : 13	81.7	28.5 ***
804 x 829	98	75 : 23	76.5	27.6 ***
047 x 840	100	94 : 6	94.0	77.4 ***
524 x 840	100	76 : 24	76.0	27.0 ***
362 x 840	82	82 : 0	100.0	82.0 ***
362 x 840	100	99 : 1	99.0	96.0 ***
362 x 840	100	100 : 0	100.0	***

** significantly different from 1:1 ($P < 0.01$); *** significantly different from 1:1 ($P < 0.001$)

FAC, Philippines - Replicate (x3) cage trial in L. Sebu, Mindanao

Despite being curtailed by a fish kill after less than 100 days, the experiment yielded some useful results and provided an good comparison of the different options available in genetically improved fish in the Philippines. There were no significant differences in initial weight and length between the four treatments so valid comparisons of final weight and length could be made (Table 23).

Table 23 Initial and harvest characteristics of four strains of *O. niloticus* grown in commercial cages in Lake Sebu, Mindanao.

Strain	Initial		Final		Survival (%)	Condition factor (g/cm)	Food Conversion Ratio	Yield (kg)
	Wt.(g)	SL(cm)	Wt.(g)	SL(cm)				
Local	3.5[a] (0.4)	4.5[a] (0.2)	58.1[c] (1.6)	11.4[c] (0.5)	86.6[bc] (1.4)	0.0393[b]	1.2[a] (0.2)	6.0[b] (0.6)
IDRC	3.2[a] (0.9)	4.2[a] (0.5)	82.0[a] (1.8)	12.5[a] (0.4)	85.3[c] (2.7)	0.0417[a]	1.1[a] (0.5)	8.4[a] (0.9)
GIFT	3.3[a] (0.6)	4.5[a] (0.5)	67.3[b] (2.2)	11.8[bc] (0.5)	94.2[ab] (1.2)	0.0413[a]	1.1[a] (0.3)	7.6[a] (0.7)
GMT	3.4[a]	4.6[a]	69.3[b]	12.0[b]	96.6[a]	0.0407[ab]	1.0[a]	8.0[a]

	(0.6)	(0.3)	(1.8)	(0.4)	(1.1)	(0.2)	(0.6)

different superscripted letters within a column denote significant differences (P <0.05)

Table 23 shows the initial weight and length and the harvest characteristics of the four strains. The IDRC selected strain had the highest weight and standard length at harvest being significantly larger than the other strains. The local strain was significantly smaller than the genetically improved strains, had significantly poorer condition factor (weight in proportion to length) and a significantly lower overall yield than the three improved strains. GMT had significantly higher survival than the other three strains. Due to a combination of good growth, high survival and low food conversion ratios, the GMT produced the highest net return of all the strains (see Table 24).

Table 24 Costs and returns of growing local and three genetically improved strains in cages in Lake Sebu, Mindanao.

Strain	Total production (kg)	Total sales (P)	Production costs (P)	Net return (P)	Profitability Index	
					Cost (P/kg fish)	Return (%)
Local	300	7,500	6,888	612	22.96	8.8
IDRC	420	10,500	8,149	2,306	19.50	28.1
GIFT	380	9,500	7,630	1,870	20.05	24.5
GMT	400	10,000	7,400	2,600	18.50	35.1

This experiment was the first to compare the culture performance of different genetically improved tilapia. The result demonstrated that all were considerably superior to the unimproved local strain. The fact that GMT produced the highest net return in an environment where unwanted reproduction is not considered a major problem, due the absence of competition from recruits, is an indication of the potential of the YY male technology. The result demonstrates that the products of the YY male technology can produce similar or greater benefits than more traditional selection programmes which required greater resources (as in the development of the GIFT strain) and\or a significantly longer time to achieve gains (as in the 13 generations of selection applied in the IDRC programme).

FAC, Philippines - Replicated (x3) pond trials under various fertilization regimes

The data for this trial is only available in terms of daily weight gains and specific growth rates as summarised for the three replicates under the two fertilisation regimes in Table 25.

The results from this study are qualified by the problem with the experimental design in that the initial age and size of the fish were not well matched for the two types of improved fish and the failure to determine the sex ratio of the fish at harvest.

Table 25 Mean growth rates (absolute and relative) of sex reversed GIFT and GMT in ponds under two different fertilisation regimes over 126 days

	Egypt-Swansea GMT		SRT GIFT	
Pond input	Daily weight gain ($g.day^{-1}$)	Specific growth rate (% per day)	Daily weight gain ($g.day^{-1}$)	Specific growth rate (% per day)
Fertilizer only	0.54	1.93	0.65	1.34
Fertilizer then feed	0.96	2.30	1.38	1.97
Strain means	0.75	2.11	1.02	1.65

After 125 days of culture ANOVA on harvested fish weight showed significant effects of both pond inputs and strain with the former explaining 79% of variation in mean fish weight and the latter 27.5%. The pooled mean weight of the SRT GIFT was higher at 144.5 than the GMT at 101.0g. GMT had significantly higher ($P<0.01$) specific growth rates but lower daily weight gain due to smaller initial weight. Due to the flaws in this experimental design, the results of this study are ambiguous and inconclusive.

AIT, Thailand - Replicate (x3) cage-in-pond trial at AIT

This experiment compared various options of monosex male fish available following several years of work on technology transfer of the YY male technology at AIT. Table 25 summarises the results of this experiment alongside two previous trials completed under R 5068A and these results are illustrated in Figure 12.

The relative growth performance of the strains in cages is somewhat variable and confounded by variation in sex ratio and survival which impact considerably upon growth. A number of trends can be seen. It is apparent that the Egypt-AIT strain of *O. niloticus* showed better growth performance than that of other treatments. Mean harvest body weight of EA, SRT in two of the three cage trials were among the highest, the poorer performance in experiment 3 probably being due to its lower proportion of males (66.9% male). The Egypt-Swansea strain *O. niloticus* presented poorer growth performance than that of the Egypt-AIT in most comparisons (see Table 26). This may be a result of adaptation of the Egypt-AIT strain to the cage culture conditions and pond environment at AIT. This strain has been maintained and bred over at least eight generations in hapas in ponds, an environment not dissimilar to the cage environment used in the trials. The Egypt-Swansea strain was only recently introduced to Thailand and has evolved predominantly in closed recirculating systems and ponds and therefore may not be well adapted to cage conditions.

Studies under previous ODA funded projects in the Philippines indicated that, contrary to expectations, GMT performed better than androgen treated sex reversed fish both within and between strains. This was attributed to lower recruitment and slower growth in sex reversed

female genotypes. In these trials, there were no significant differences in mean harvest body weight or yield between SRT and GMT of the same strain although the Egypt-AIT GMT had improved harvest characteristics over SRT in the same strain in experiment 3, the only one in which they were compared.

Figure 12 Histograms showing sex ratio, survival, harvest weight and yield from three cage based experiments comparing the culture performance of various monosex male populations available at AIT.

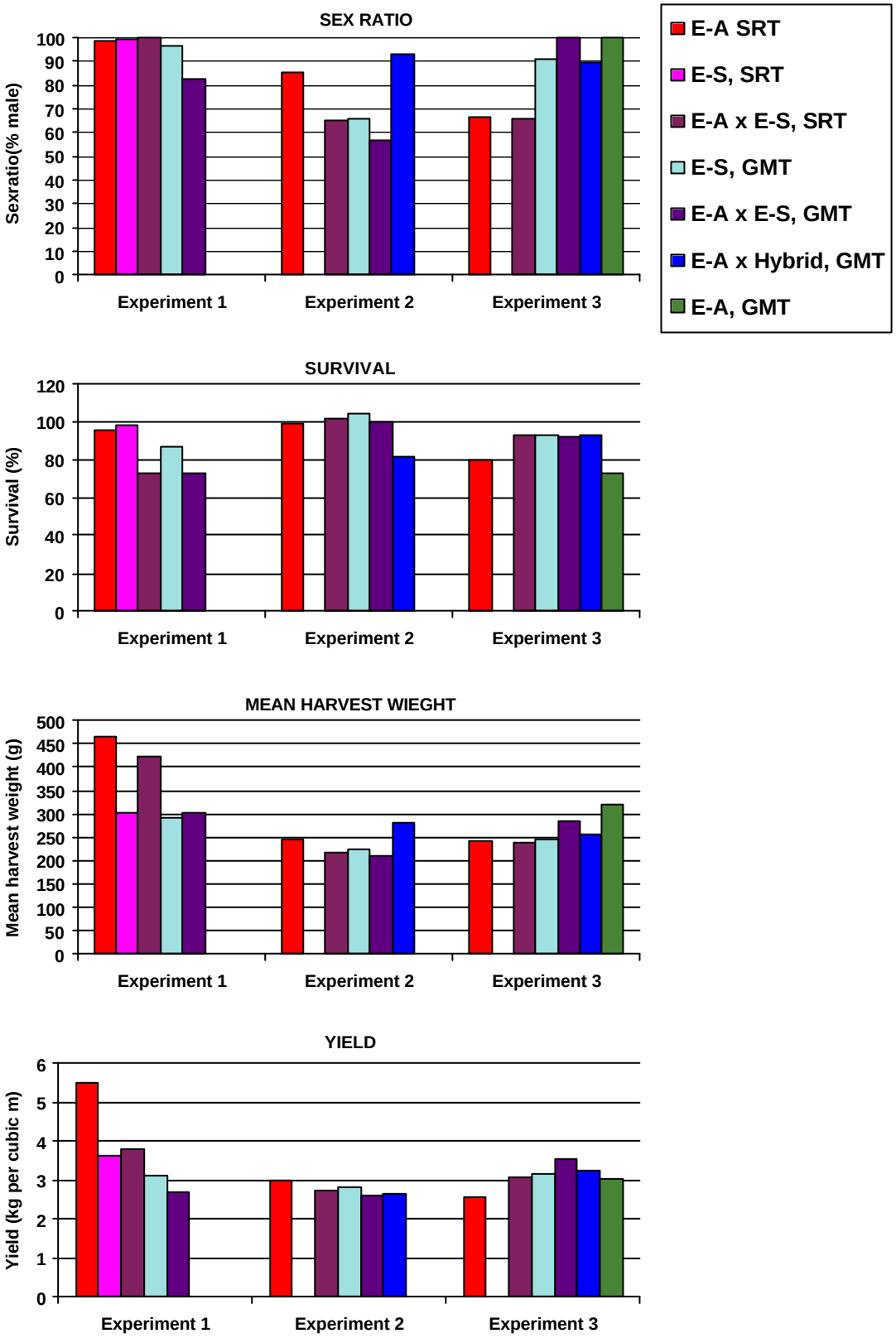


Table 26 Summary results of three growth performance trials of monosex *O. niloticus* in cages in fertilised pond with supplementary feeding.

Trial	Treatments	Sex ratio (% ♂)	Survival (%)	Mean Harvest Weight ♂ & ♀	Mean Harvest Weight ♂	Mean Harvest Weight ♀	CV of Weight	Daily weight gain ($g.day^{-1}$)	Yield ($kg.m^{-3}$)
1	E-A, SRT	98.6^{a}	95.3^{ab}	466.3^{a}	N/A	N/A	25.5^{a}	2.46^{a}	5.50^{a}
	E-S, SRT	99.3^{a}	98.0^{a}	300.9^{b}	N/A	N/A	24.1^{a}	1.52^{b}	3.64^{b}
	E-S, GMT	96.2^{a}	87.3^{ab}	289.3^{b}	N/A	N/A	21.0^{a}	1.46^{b}	3.09^{b}
	E-A x E-S, SRT	100.0^{a}	73.3^{b}	423.6^{a}	N/A	N/A	19.8^{a}	2.14^{a}	3.80^{b}
	E-A x E-S, GMT	82.6^{b}	72.7^{b}	300.8^{b}	N/A	N/A	23.4^{a}	1.51^{b}	2.70^{b}
2	E-A, SRT	85.3^{a}	99.3^{a}	244.5^{ab}	252.0^{a}	182.9^{a}	27.7^{a}	1.28^{ab}	3.00^{a}
	E-S, GMT	65.9^{b}	104.0^{a}	223.1^{ab}	243.2^{a}	185.3^{a}	23.8^{a}	1.16^{ab}	2.81^{a}
	E-A x E-S, SRT	64.8^{b}	102.0^{a}	218.0^{b}	239.0^{a}	177.2^{a}	25.0^{a}	1.14^{b}	2.73^{a}
	E-A x E-S, GMT	56.7^{b}	100.0^{a}	211.4^{b}	238.9^{a}	175.6^{a}	23.3^{a}	1.11^{b}	2.60^{a}
	E-A x Hybrid, GMT	93.0^{a}	82.0^{a}	282.0^{a}	286.9^{a}	210.3^{a}	17.4^{a}	1.48^{a}	2.66^{a}
3	E-A, SRT	66.9^{b}	80.0^{a}	241.0^{b}	256.5^{ab}	198.5^{a}	22.8^{a}	1.42^{b}	2.58^{a}
	E-A, GMT	100.0^{a}	73.3^{a}	317.8^{a}	317.8^{a}	N/A	20.6^{a}	1.89^{a}	3.02^{a}
	E-S, GMT	90.7^{a}	93.3^{a}	246.9^{b}	252.7^{b}	168.9^{a}	20.2^{a}	1.46^{ab}	3.18^{a}
	E-A x E-S, SRT	65.4^{b}	93.3^{a}	240.2^{b}	257.5^{ab}	201.6^{a}	17.4^{a}	1.42^{b}	3.07^{a}
	E-A x E-S, GMT	100.0^{a}	91.7^{a}	284.0^{ab}	284.0^{ab}	N/A	20.8^{a}	1.69^{ab}	3.56^{a}
	E-A x Hybrid, GMT	89.5^{a}	93.3^{a}	255.8^{ab}	266.0^{ab}	147.9^{a}	21.9^{a}	1.51^{ab}	3.26^{a}

Within trial values superscripted with different letters are significantly different ($P<0.05$); N/A: not applicable or not available.

AIT, Thailand - Replicated (x3) pond trial at AIT

This replicated pond experiment was conducted partially to test the hypothesis that the local strain was pre adapted to culture in cages in ponds. The trial compared the performance of Egypt-AIT SRT with GMT in the two strains and the hybrid between them. Table 27 summarises the harvest characteristics of the four monosex populations. It is evident that there is very considerable between replicate variance in weight, survival and yield, the majority of which can be explained by variable water quality, especially chlorophyll-a counts.

Table 27 Growth performance of monosex male *O. niloticus* in fertilised ponds without feeding. Values represent those from individual replicate ponds

Treatments	Sex ratio (% male)	Survival (%)	Mean Weight (g)	CV of weight (%)	Yields (kg/pond)
E-A, SRT	98.4	62.0	183.7	15.5	10.9
	98.4	63.0	106.5	15.9	6.2
	95.5	67.0	137.7	19.1	9.1
Mean	**97.4**[a]	**64.0**[a]	**142.6**[a]	**16.8**[ab]	**8.7**[a]
E-A, GMT	98.4	62.0	118.4	30.7	6.8
	90.5	84.0	125.3	33.4	10.5
	95.0	40.0	512.3	16.4	20.2
Mean	**94.6**[ab]	**62.0**[a]	**252.0**[a]	**26.8**[a]	**12.5**[a]
E-S, GMT	96.0	75.0	119.5	17.6	8.0
	72.5	80.0	239.1	19.8	18.1
	84.6	52.0	112.8	37.1	6.7
Mean	**84.4**[b]	**69.0**[a]	**157.1**[a]	**24.8**[ab]	**10.9**[a]
E-A x E-S,GMT	93.8	80.0	357.1	22.1	21.1
	96.3	80.0	236.7	11.4	18.6
	86.1	86.0	119.5	11.7	9.7
Mean	**92.1**[ab]	**82.0**[a]	**237.7**[a]	**15.1**[b]	**16.5**[a]

Means within a column superscripted with different letters are significantly different between treatments ($P<0.05$)

Factorial analysis showed significant genotype (strain) and environment (pond) interaction. However, growth performance in terms of final body weights and yields were not significantly different between the populations.

Previous reports on the growth performance of GMT have indicated that females in GMT populations are late maturing, limiting or negating recruitment. In this pond based study recruits were generated from females in both SRT and GMT populations in some replicates of all the strains, this being most significant in replicate 2 of the Egypt-AIT SRT and replicates 2 and 3 of the Egypt-AIT GMT. This recruitment did not seem to impact greatly on yields. Recruitment in the Egypt-Swansea GMT which had a high proportion of female, was negligible, confirming that these females may be later maturing and largely unreproductive.

The inter-strain hybrid GMT (E-A x E-S, GMT and E-A x hybrid, GMT) presented good growth performance in both culture systems, cage-in-pond and in a fertilised earthen ponds, however variable sex ratio of the inter-strain hybrid GMT may be a constraint to the application of the inter-strain crosses using YY-males in tilapia aquaculture.

The results from this pond trial were inconclusive due to the high between replicate variance but there were indications that the GMT in the Egypt-AIT strain and in the inter strain crosses have potential to increase yields from pond culture. If sex ratios of these different monosex populations can be made more consistant, possibly through the selection of the females, it would be more appropriate to conduct such comparisons in communal stocking as it would be logistically difficult to eliminate the effect of replicate variance in the intepretation of results from the kind of experimental design used in this study.

NAGRI, Thailand - Replicate (x3) pond trial at Surin DOF station

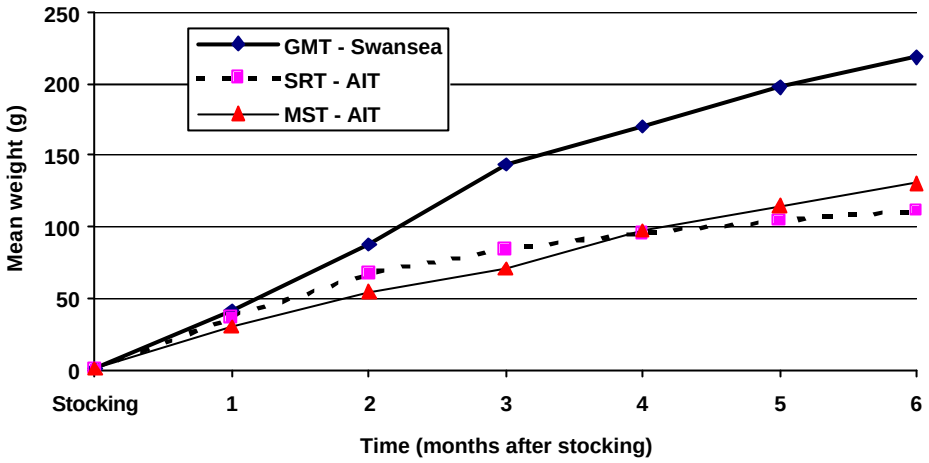
Results, including statistical analysis, are shown in Table 28. Sex ratios of both SRT and GMT were 100% male making the comparison a very valid one in terms of a comparison of strains and of the methods of producing monosex fish. The result demonstrated a large and significant ($P < 0.001$) improvement in yield of the Egypt-Swansea GMT over the MST and SRT of the Egypt-AIT strain with the growth curve (Figure 13) illustrating that the growth of GMT began to diverge early on in the trial. This result is somewhat surprising as previous trials in cages at AIT had indicated that the growth of the Egypt-AIT strain was superior to that of the Egypt-Swansea strain. This result lends support to the hypothesis that the Egypt-AIT strain is has been selected to be locally adapted to conditions at AIT. The production performance of Egypt-Swansea GMT appears very promising in this environment.

Table 28 Final weight, survival, yield and sex ratio of GMT (Egypt-Swansea) compared to SRT and MST (Egypt-AIT) in replicated ponds at a DOF station in Surin province, Thailand, after six months of grow-out.

Treatment	Strain	Sex ratio % ♂	Mean harvest wt (g).	Mean survival (%)	Mean yield (kg)
MST	Egypt-AIT	78.37	130.25[b]	44.53[a]	19.26[b]
SRT	Egypt-AIT	100	111.07[b]	50.02[a]	20.10[b]
GMT	Egypt-Swansea	100	218.79[a]	77.23[a]	54.30[a]

ANOVA for weight ($F=323.50$; $P < 0.001$), GMT significantly higher than MST & SRT; ANOVA for survival ($F = 1.78$; not significant); ANOVA for yield ($F = 15.73$; $P < 0.01$), GMT significantly higher than MST & SRT.

Figure 13 Growth curves (mean of 3 replicates) in the pond based comparison of GMT (Egypt-Swansea) with MST and SRT of the Egypt-AIT strain.



NAGRI, Thailand - Replicate (x2) cage-in-pond trial at Nakhonpanom DOF station

The results from this study are shown in Table 29. Some significant differences were observed despite there being only two replicates. The Egypt-Swansea GMT produced significantly higher weight, survival and yield than the other strains and strain combinations. The remaining results were somewhat unpredictable with the pure Chitralada strain MST and the crossbred GMT producing the next highest mean weights and yields respectively.

Table 29 Results from the growth comparison, in cages over a five month period, of purebred and crossbred Nile tilapia at Nakhonpanom (mean of two replicates with 30 fish per cage)

♀	♂	cross type	mean harvest weight (g)	mean survival (%)	mean yield (kg)
E-S	YY E-S	purebred E-S GMT	166.47[a]	91.43[a]	4.57[a]
Ch	YY E-S	crossbred GMT	121.94[b]	60.00[b]	2.19[b,c]
Ch	Ch	purebred Ch MST	153.84[a]	65.71[a,b]	3.03[b]
E-S	E-S	purebred E-S MST	87.89[c]	67.14[a,b]	1.77[c,d]
Ch	E-S	crossbred MST	116.46[b,c]	52.86[b]	1.75[c,d]
E-S	Ch	crossbred MST	88.38[c]	37.14[b]	0.98[d]

E-S = Egypt-Swansea strain; Ch = Chitralada; ANOVA for weight ($F=15.83$; $P < 0.01$); ANOVA for survival ($F=4.51$; $P < 0.05$); ANOVA for yield ($F=26.36$; $P < 0.001$)

The culture performance of the GMT appears promising in this environment even though MST of the Egypt-Swansea strain does not rank well for growth. This may be associated with the 100% male sex ratio of the GMT. When the relative performance of the strains in this trial are compared with those of the cage trials at AIT, it would seem probable that the growth performance of the Egypt-Swansea superior to that of the Chitralada strain.

NAGRI, Thailand - Replicate (x2) pond trial at Surin DOF station (experiment repeated at Pitsanulok DOF station)

These two trials compared the monosex growth performance of the selected local Chitralada strain and the introduced selected GIFT - SRT with that of the introduced GMT in the Egypt-Swansea strain in extensively managed ponds. Egypt-Swansea GMT had the highest weight in first trial but lowest in second. Survival of the GIFT ranked highest in both trials, with the local Chitralada strain having the lowest survival. The SRT GIFT produced marginally higher yield in both trials but none of the differences were significant. GMT had 100% male sex ratios in both trials compared to mean sex ratios of 65 to 86% male in the sex reversed fish, further illustrating the limitations of the latter technique in consistently producing all male populations.

Table 30 Summary of harvest characteristics (s.d.) of replicated pond trials at Surin and Pitsanulok comparing the culture performance of SRT of Chitralada and GIFT strains with that of the Egypt-Swansea GMT.

Strain	Survival (%)	% male	Mean weight (kg)	Yield (kg)
Surin				
Chitralada SRT	38.33 (21.64)	72.91 (6.35)	159.37 (81.20)	17.26 (11.79)
GIFT SRT	53.33 (15.0)	86.29 (0.33)	143.87 (1.90)	25.51 (6.20)
Egypt-Swansea GMT	43.33 (7.29)	100.0 (0.0)	157.99 (45.50)	22.04 (1.32)
Pitsanulok				
Chitralada SRT	40.20 (2.26)	97.13 (4.06)	392.58 (22.13)	78.82 (0.04)
GIFT SRT	64.20 (12.45)	67.64 (10.90)	266.12 (15.27)	85.09 (11.70)
Egypt-Swansea GMT	52.40 (5.66)	100.0 (0.0)	261.94 (12.73)	68.20 (3.72)

Summary of growth trials

When viewed collectively the growth trials conducted in Thailand did not yield an entirely consistent picture. The following list summarises some of the principal findings from these growth trials.

- There is no one strain or strain combination that can be universally recommended for all environments.
- There is some indication of local adaptation of strains, specifically the relative performance of the Egypt-AIT in cages at AIT.

- The Egypt-Swansea strain, in its mixed sex form, rarely out grows other strains in Thai environments but its survival is good.
- There appears to be advantage to culturing a crossbred GMT which usually produces growth rates on a par with or exceeding that of the pure strains. Sex ratios or crossbred GMT seem to be acceptable in all cases.
- Of the selected fish the selected Chitralada does not seem to be a good candidate. The GIFT does not have exceptional growth but usually has high survival.
- GMT outperforms SRT in most cases even if SRT has a higher sex ratio.
- It may, in due course, be recommended that we use YY males with any available pure strain *O. niloticus*.

Investigations into Salinity Tolerance

During the early stage of fry nursing, the salinity varied from 0 to 12 ppt. Table 31 shows that there were no significant differences in size of the fish after this period of nursing. Unfortunately, survival data is not available.

Table 31 Mean weight (g), standard length (cm) and standard errors of GFT and GMT cultured in net cages at 500 fry per m^3. Based on the third month of Phase 1, harvest data, including survival, is not available

Genotype	Weight (g)	Standard Length (cm)
GFT	1.9 ± 0.051	4.6 ± 0.045
GMT	2.0 ± 0.075	4.7 ± 0.046

Salinities recorded for the three month duration of the Phase 2 culture period ranged from 13ppt to 27ppt, with a gradual increased in salinity up to April 1996. During the third month of culture 20 to 60% of the fish stocked in each replication were infected with a range of diseases. This coincided with, and was presumably caused by, the highest levels of salinity although temperature was also quite low at this time (24°C). The outbreak of disease resulting in high mortality ranging from 85 to 97%. This high mortality which varied between treatments and replicates largely invalidated the comparisons of weight and length which did indicate that GMT had significantly higher weight.

Clearly this strain of *O. niloticus* is not suited to culture in this kind of brackishwater environment with salinities as high as 27ppt.

Table 32 Mean weight, standard length, standard errors and percentage survival of genetically female tilapia (GFT) and genetically male tilapia (GMT) in cages at two stocking densities.

Genotype	Weight (g)		Standard Length (cm)		Survival
	100	200	100	200	(%)
Initial					
GFT	3.5 ± 0.191	5.1 ± 0.327	5.9 ± 0.103	6.6 ± 0.132	14.7
GMT	4.3 ± 0.271	4.3 ± 0.235	6.3 ± 0.133	6.5 ± 0.109	9.7
Harvest					
GFT	35.9 ± 1.590	45.7 ± 3.019[a]	12.2 ± 0.183	13.1 ± 0.292[b]	6.7
GMT	50.5 ± 3.008***	50.1 ± 5.553	13.6 ± 0.287***	13.5 ± 5.553	2.7

Numbers with asterisks within column are comparison between GFT and GMT at the same stocking density. Note: *** P<0.0001; Numbers within row with superscript letters are comparison of weights or standard length between the two stocking densities within GFT or GMT. Note: a = P<004; b = P<0.02.

Additional Complementary Research Activities

Fish marking and tagging

Experiment 1 - Individual marking

Analysis showed that there were no significant differences in the mean initial weight of fish stocked for the five different types of tags/mark in size group 1 (6.4 - 38.3 g). However, in size group 2 (15.7 - 44.9 g), fish stocked for spaghetti and binary coded-wire tag had had significantly higher (P<0.05) mean weight than the fish marked with the other three types of tagging, presumably due to non random selection of fish prior to tagging. Since the mean weight and standard length of the fish at harvest was not influenced by their initial weight and standard length, corrections for these minor differences in initial weight were not made.

Comparisons among treatment means of weight (g), standard length (cm) and estimated percentage tag retention at the end of the 180 day grow-out period are presented in Table 33. It is interesting to note that the fish marked or tagged using internal tag/marks that do not create or cause large or persistent wounds on the body of the fish, such as PIT tags, binary-coded wire tag and fluorescent elastomer marks have significantly faster growth than the fish that were tagged with either spaghetti or fingerling floy tag. This result was true for both size groups of fish evaluated in this experiment.

In terms of estimated tag retention however, the spaghetti tag had significantly better retention (estimated to be 100% in both size groups) than the alternative types of tag evaluated other than the binary-coded wire tag. However, there were apparent disadvantages in using spaghetti tags compared to binary-coded wire tags. These limitations are that the spaghetti tag can only be applied to larger fish, they are expensive, they wear out or get soiled and become unreadable in a relative short time, infections can result after application, the tag can get snagged in nets or even vegetation. For the above reasons, the binary-coded wire tags seem more appropriate although they have the major disadvantage of the high cost of the equipment and the fact that the fish would most likely have to be sacrificed in order to read the tag number (but not to detect its presence).

Table 33. Mean weight (g), standard length (cm), standard error and estimated percentage tag retention of tilapia tagged or marked using individual tagging or marking techniques after 180 days of grow out in cages.

	Size range for group					
	6.4 - 38.3 g			15.7 - 44.9 g		
Tag Type (Treatment)	Wt (g)	SL (cm)	Estimated % retention	Wt (g)	SL (cm)	Estimated % retention
Pit tag	57.8^{a}±1.77	11.3^{a}±0.13	63.4	74.2^{a}±1.54	12.6^{a}±0.09	57.6
Spaghetti tag	42.7^{b}±1.51	10.3^{b}±0.12	100	63.5^{b}±1.40	11.9^{b}±0.08	100
Binary-coded	59.5^{a}±1.37	11.4^{a}±0.10	95.5	75.0^{a}±1.12	12.6^{a}±0.06	90.9
Floy tag	51.9^{b}±7.3	10.6^{b}±0.54	6.2	59.8^{b}±4.05	11.5^{b}±0.34	5.3
Elastomer	60.4^{a}±1.67	11.5^{a}±0.11	58.0	72.9^{a}±2.12	12.5^{a}±0.13	56.8

Experiment 2 - Batch marking

Comparison among means using showed that mean initial weights of fish varied significantly between the fish marked with the different techniques. However, this was not thought to have had a substantial effect on harvest weight.

The results in terms of harvest weight and percentage tag retention following 180 days of grow-out in cages are summarised in Table 34. These show that fish marked with Alcian blue dye and fluorescent elastomer marks were significantly faster growing than the fish having different kinds of tag/mark. However, in terms of tag retention, pectoral fin clipping was significantly better than the remaining five methods. It can be noted that tag retention in binary-coded wire tag was significantly lower for this experiment than observed in experiment 1. This was a somewhat surprising result and may have been due to poor application technique on smaller fish.

Table 34 Mean body weight (g), standard length (cm), (± standard error) and estimated percentage tag retention of batch marked fish. Comparisons among treatment means were carried out using a range test

Tag Type (Treatment)	Bdwt (g)	SL (cm)	Estimated % tag retention
Alcian Blue	38.4^{a}±1.29	9.9^{a}±0.10	58.8^{c}±4.55
Pectoral fin clipping	27.3^{c}±0.92	8.7^{c}±1.18	98.6^{a}±1.37

Premaxillae clipping	16.6^{d}±0.79	7.6^{d}±1.28	79.9^{b}±4.05
Elastomer marks	38.0^{a}±1.66	9.7^{a}±1.36	66.8^{c}±9.28
Binary-coded wire tag	32.4^{b}±2.56	9.3^{b}±1.16	21.8^{d}±6.66
Floy Tag	24.1^{c}±1.74	8.4^{c}±1.41	31.9^{d}±2.17

Means within a column sharing the same superscript letters are not significantly different (P>0.05)

It can be concluded from these experiments that tagging or marking techniques which can carry the risk of infections due to the wounds created by the tagging/marking technique can result in growth inhibition. However, tag retention may still be high such as with the spaghetti tag.

Pectoral fin clipping if carried out properly can be cheap and simple technique for batch marking. This method does however carry some potential disadvantages such as stress during handling and clipping the fins and the limited number of fin clipping combinations.

It can be concluded from these experiments that tagging or marking techniques which carry the risk infections due to the wounds created by the tagging/marking technique can result in growth inhibition. However, tag retention may still be high such as with the Spaghetti tag.

Pectoral fin clipping if carried out properly can be cheap and simple technique for batch marking. This method does however carry some potential disadvantages such as stress during handling and clipping the fins and the limited number of fin clipping combinations.

Based on these results the initial decision was taken to fin-clip YY males and normal females transferred to hatcheries. Left and right pectoral fins are clipped for males and females respectively.

Based on literature and the findings of other researcher, binary coded tags have the potential to be effective in tilapia. Late in the project we were successful in procuring a hand held tag applicator, which was much more efficient than the syringe used in the initial trials. This was used to mark fish in a new location, the cheek just below the eye. At the time of writing retention of this tag is >96%. A decision has been taken to use this method to mark YY males that are kept on station or distributed to hatcheries. Whilst the presence of the tag can only be detected using an expensive, purpose made, metal detector, this will not provide a method for farmers to identify the fish but will enable the researchers to check the broodfish for contamination.

Publications and presentations

A number of important publications were accepted for publication or came to press during the project. With this being an adaptive project, conducting principally applied research, the major emphasis on outputs related the dissemination of the products of the research to the farmer. A major written output of the project however, was a technical manual targeted at hatchery managers using or applying to use YY male broodstock for the commercial

production of GMT (see the list below for titles of the book and the chapters therein). Further dissemination and extension related outputs include a 22 minute video, entitled "No sex please, we're growing" or "The YY Male Technology - Breeding better fish for the enhancement of aquaculture production". This video, paid for out of a dissemination fund resulting from the income generating project attached to this research project, has been distributed to NARS institutes throughout the Philippines and will be used for demonstrating the technology to NGOs, PO and private farmers. Also, in aid of information dissemination, a brochure describing the YY male technology and GMT was printed for general dispersal to any and all interested parties.

The following lists the papers that were published or came to press during the course of the project~~proejct~~:

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

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

Beardmore, J.A. (1995) Sex and the single tilapia. Aquaculture News 21: p25**

Capili, J.B. (1997) Dissemination strategies for the YY male technology and its products. . *In*: Mair, G.C. and Abella, T.A. (eds.) Technoguide on the Production of Genetically Male Tilapia (GMT). Freshwater Aquaculture Center, Central Luzon State University, Philippines: 58-66. *

Mair, G.C. (1997) The problem of early sexual maturity in tilapia culture. *In*: Mair, G.C. and Abella, T.A. (eds.) Technoguide on the Production of Genetically Male Tilapia (GMT). Freshwater Aquaculture Center, Central Luzon State University, Philippines: 6-13. *

Mair, G.C. and Abella, T.A. (eds.) (1997) Technoguide on the Production of Genetically Male Tilapia (GMT). Freshwater Aquaculture Center, Central Luzon State University, Philippines. 67p *

Mair, G.C., Dahilig, L.R., Morales, E.J., Beardmore, J.A. and Skibinski, D.O.F. (1997) Application of genetic techniques for the production of monosex male tilapia in aquaculture: Early experiences from the Philippines. Proceedings of the Fourth

Central America Symposium on Aquaculture, Tegucigalpa, Honduras, April 22-24, 1997. 225-227. **

Morales, E.J. (1997) Intensive tilapia hatchery production systems. . *In*: Mair, G.C. and Abella, T.A. (eds.) Technoguide on the Production of Genetically Male Tilapia (GMT). Freshwater Aquaculture Center, Central Luzon State University, Philippines: 39-51. *

Pascual, L.P. and Mair, G.C. (1997) Culture performance of genetically male tilapia (GMT). *In*: Mair, G.C. and Abella, T.A. (eds.) Technoguide on the Production of Genetically Male Tilapia (GMT). Freshwater Aquaculture Center, Central Luzon State University, Philippines: 27-38. *

Roderick, E.E., Garcia-Abiado, M.A.R., and Mair, G.C. (in press) Fish tagging and marking methods for genetic programmes in aquaculture. Proceedings of the Second AADCP International Workshop on Genetics in Aquaculture and Fisheries Management, Phuket, Thailand, Nov. 7-11, 1994. **

Sevilleja, R.C. (1996) Freshwater Aquaculture Development in the Philippines: The Case of Tilapia. Ph.D. thesis, University of Wales. 345p

Tuan, P.A., Abucay, J.S., Little, D.C. and Mair, G.C. (in press) Preliminary investigation in to the feasibility of transfer of the YY-male technology to the Thai Chitralada strain of Oreochromis niloticus L. Proceedings of the Second AADCP International Workshop on Genetics in Aquaculture and Fisheries Management, Phuket, Thailand, Nov. 7-11, 1994. **

Tuan, P.A., Little, D.C. and Mair, G.C. (in press) Genotypic effects on comparative growth performance of all-male tilapia *Oreochromis niloticus* (L.). Aquaculture. **

Vera Cruz, E.M. (1997) Biology and reproduction of tilapia in the Philippines. *In*: Mair, G.C. and Abella, T.A. (eds.) Technoguide on the Production *of* Genetically Male Tilapia (GMT). Freshwater Aquaculture Center, Central Luzon State University, Philippines: 1-5. *

* - see accompanying documentation

** - see copies in Appendix 9

In addition to these formal publications there has been considerable press coverage of the technology including articles in national newpapers and several features on agricultural documentary~~documentar~~ programmes on television (see Appendix 10).

Meetings held

Network planning with State Colleges and Universities

A two day workshop for fisheries scientists from State Colleges and Universities (SCUs) was held in early October 1995. Details of the Project's progress and objectives were presented on the first day. The second day was taken up with discussions of the prospects for a National Network of hatcheries producing and disseminating GMT. The majority of delegates (21 people representing 14 SCUs) were enthusiastic to proceed with this network with several committing to fund the establishment of their own hatcheries. Others will clearly need to source financial support to develop or upgrade their hatchery facilities.

Consultation Workshop with Philippine NGOs

Following dialogue on internet fora, much of it misinformed, concerning environmental and socio-economic impacts of the introduction of genetic technologies, the project sponsored a meeting between scientists and representatives of NGOs. This meeting, held at CLSU, was attended by representatives from five Philippine NGOs concerned with aquaculture and fisheries and two People's Organisations (POs). The meeting consisted of presentations on the YY male and transgenic technologies and was followed by discussions of important socio-economic and environmental issues. There was a fairly broad consensus that the YY male technology represents little or no environmental risk, and as a replacement for sex reversal, may have real benefits. Discussion helped in identifying the important social and economic issues.

Several of the NGOs expressed an interest in collaboration, especially in relation to information dissemination (a fair criticism levelled at the scientist was that they seldom did this effectively) and assistance to small farmers. Some of these contacts were followed up. Meetings with a fisherfolk's NGO, Tambuyog has resulted in plans being formulated to collaborate on the production of information material and to investigate the potential for GMT culture as an alternative livelihood resource for a coastal project area in Bicol.

A linkage has also been established with a PO, the Integrated Multi-Purpose Co-operative Incorporated (IMPCI). It is hoped that the project can work with the co-operative to assist their fish farming members (10 in all) to grow GMT and later to help them establish their own GMT hatchery. IMPCI is a member of a larger organisation and if collaborative efforts were successful, could provide a link to a wider state and even nation-wide network of people's organisations (POs).

In-house project review

Attended by 34 staff of the project, CLSU's FGBP and FAC faculty and staff. The workshop was held in two parts. The first held at FAC, CLSU on January 19 and 21, 1997 reviewed the progress of research and dissemination activities up to the present and identified

key constraints to the application of the technology. Stage two held in Boracay Island, Panay from January 22-24, 1997 consisted of four working group sessions. These discussed i) research constraints to the further development and improvement of the technology, ii) constraints to maximising GMT fry production for dispersal to farmers, iii) the structure and function of Phil-Fishgen, the income generating project, and iv) social and economic issues related to the dissemination of GMT.

The meeting provided an invaluable opportunity to review the progress of recent research on the YY male technology. The workshop produced a series of priorities for future research which were used in the design of proposals to conduct research following on from that presented in this report.

Contribution of outputs

Summary of outputs

The anticipated outputs for this project and the progress made towards these outputs are summarised in the list below:

- *Programme and necessary broodfish for mass production and appropriate dissemination of the YY male technology developed and initiated.*

  Achieved - Based on the results of this project (and with regard to results obtained under previous projects) a dissemination programme was designed and initiated (see below under "uptake of the research products"). The programme was initiated earlier than expected and has been exceeded expectations to the extent that the technology is already likely to be having an impact upon the industry.

- *Feasible and effective methodology for technology transfer of YY male technology developed.*

  Achieved - The research at AIT demonstrated that indirect transfer and application of the technology anew can also be used to transfer the technology in addition to the simplest form of direct transfer. The growth trials indicated that the crossbred (local x introduced YY) and the local (Egypt-AIT strain) GMT exhibit superior growth performance to the introduced GMT in most environments and are likely to become the preferred GMT in Thailand.

- *YY male technology transferred to Thailand*

  Achieved - The technology was effectively transferred to Thailand. Under the method of direct transfer NAGRI has even commenced dissemination of the Egypt-Swansea on a medium to large scale and the Thai Department of Fisheries has made a policy decision to disseminate the products of the technology throughout the country.

- *Case studies on socio-economic status of tilapia farmers produced and likely long term impacts of the introduction of YY male technology determined.*

  Partially achieved - Two case studies were completed and the principal factors related to the adoption of technologies were identified. However, without setting up farm trials it proved difficult to accurately predict the likely long term impacts of the introduction of the YY male technology.

- *Brackishwater tolerant strain of O. niloticus identified*

  Not achieved - with the benefit of hindsight this activity did not fit well with the principal activities. Only one field trial was completed which did not yield much useful data. This topic should be researched in a single project dedicated to this objective.

A further output of the project was the identification of appropriate tagging methods for use in identifying YY males used in the dissemination programme.

Uptake of the research products

The YY male technology, through the success of this and preceding project, is beginning to have a major impact through the dissemination of the products of the technology, namely GMT producing broodstock (YY males and normal females) for hatcheries and the GMT themselves for tilapia growers.

Phil-Fishgen, a mechanism for the dissemination of the products of the YY male technology in the Philippines

The project has supported very significant advances in the promotion and dissemination of the products of past and present research and development on the YY male technology. Early in 1995 CLSU, with the assistance and support of UWS and the project, established Phil-Fishgen. Phil-Fishgen is an income generating project of CLSU, initiate with approval from its Board of Regents. The relationship of Phil-Fishgen with the other organisations involved in this project is summarised in Figure 3.

Rationale and objectives

It is important that the improved yields that have been demonstrated in controlled growth trials be translated into sustainable increases in production on-farm, improved availability of a much needed and cheap source of protein, and improved livelihood for resource poor, rural fish farmers. In order for these objectives to be attained Phil-Fishgen was initiated to establish a national network of producers as mechanism for further research and development of the technology, and the distribution of YY male broodstock and, consequently, production of genetically male tilapia in all the tilapia growing regions of the country. Specific objectives of the program include:

- Support and conduct of research on the improvement of the YY male technology.
- Establishment of a national network for the dissemination of genetically male tilapia.
- Transfer of the technology to the tilapia culture industry through training and production of extension materials such as brochures, technoguides, posters, manuals, etc.
- Establishment of mechanisms for the financial sustainability of the aforementioned operations.

Structure

As a direct result of the success of this and previous ODA FGP projects under the collaboration, CLSU established its Fish Genetics and Biotechnology Programme (FGBP) in 1995. The local scientific and support staff trained under this and previous project were

institutionalised under this project with the majority the their salaries paid by CLSU. The research projects and Phil-Fishgen were major activities under the FGBP.

The network for the dissemination of the products of the YY male technology has a three tier structure, the first tier involves the continued research, testing and development of the technology at FAC\CLSU, designated at the Breeding Center. The research done under this project and under R 6058 formed part of the activities of this Breeding Center. Products of the research and development (i.e. YY male and normal female broodstock) will be mass produced here (at a ratio of 1 YY male to 3 normal females) for distribution to GMT fry producers. In addition, the Breeding Center will also produce GMT from YY male broodstock, for distribution directly to farmers. It was proposed that production capacity of GMT at FAC should be, in the first instance, approximately 10 million GMT *per annum*.

The second tier of the network are GMT producers who obtain their broodstock from the Breeding Center. It was proposed that the primary GMT producers are private sector commercial hatcheries, State Colleges and Universities (SCUs), and peoples organisations (POs), distributed throughout the Philippines. In areas where tilapia production is concentrated, additional producers will be identified in Local Government Units (LGUs). All these producers will sell fry direct to the third tier, the farmers, with co-ordinated extension activities to make farmers aware of the availability of GMT. The structure of the network is described in Figure 14.

Due to administrative protocols required to establish fingerling production in SCUs and LGUs, the private sector hatchery operators have been the first beneficiaries of the technology and have been producing GMT for dispersal to farmers.

Phil-Fishgen is managed and run by a management committee made up of representatives of FAC, CLSU, UWS, The research project and ODA Fish Genetics Programme.

GMT production at the Breeding Center

Fingerling production is done in two demonstration tilapia hatchery facilities. One is a semi-intensive pond based system on a new one hectare site with 32 x 200m² ponds constructed by Phil-Fishgen. The second system is a more innovative and intensive hapa based system utilising artificial incubation of eggs. The breeding is and nursing of fingerlings is done hapas installed in ponds which are rented by Phil-Fishgen from CLSU, and the eggs are incubated in a hatchery constructed jointly by this project and Phil-Fishgen. Both facilities are also used for training of farmers and students alike.

Figure 14 Structure of the dissemination network for GMT and GMT producing broodstock under Phil-Fishgen.

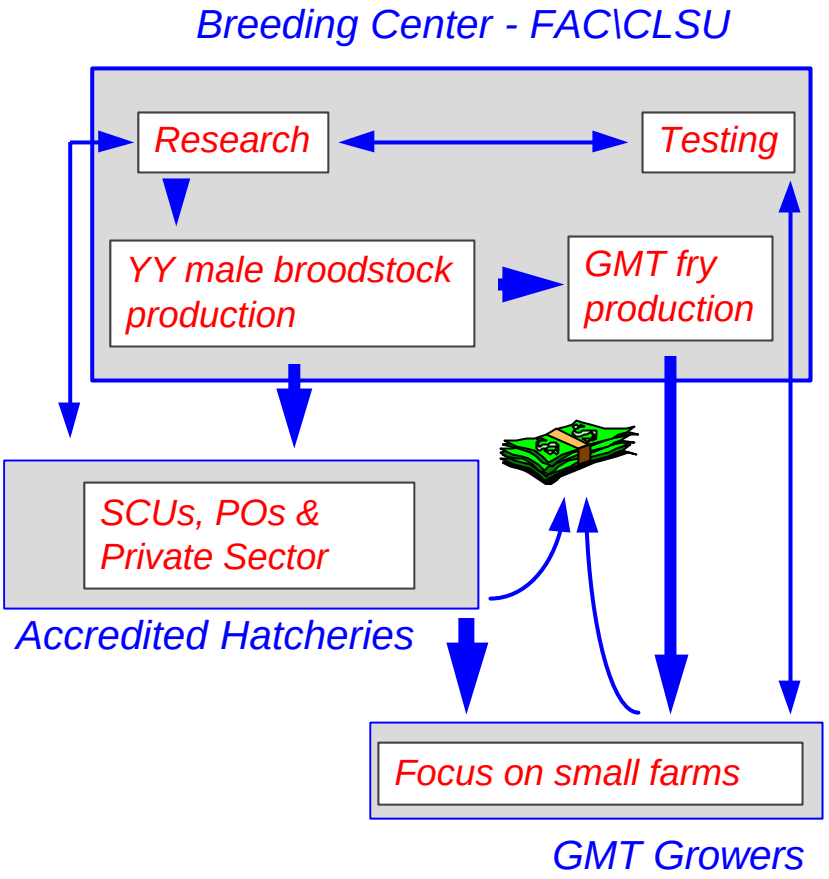


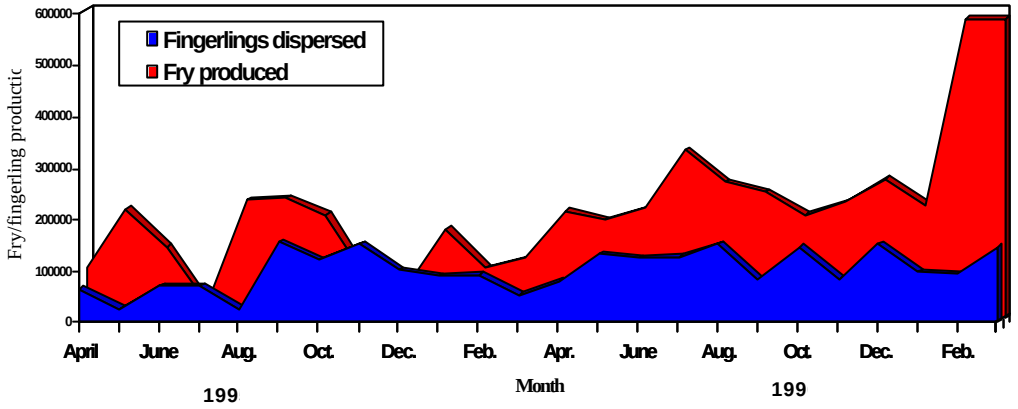
Figure 15 shows that the production of GMT fingerlings has varied considerably throughout the two year period, from April 1995 when it was initiated. The last two months of the project saw a rapid rise in production as new broodstock and new facilities were added to increase hatchery capacity.

This fry production is almost entirely from the pond based hatchery. Hapa based hatchery production has been very low as we suffered the effects of shortage of nets and various teething problems associated with the hatchery. For the duration of the project (two years of production) a total of 5.2 million fry were produced, of which 2.5 million were dispersed as fingerlings. This is still a long way short of the targeted 10-12 million per annum but the trend during the last year of the project has been one of increasing production. With the large increase in production associated with the implementation of further improvements to the pond based hatchery management in February 1997 we are now optimistic of attaining the target for pond based production within 12 months from the end of this project.

With over half a million fry being produced from 2,300 packages, this is equivalent to a monthly production of 225 fry per package per month. This compares favourably with most of the commercial producers but it is clear that this figure could be and will become

substantially higher. With reference to the on-farm trials (see page 21), our own on-station production was considerably greater than most of the commercial hatcheries and reflects the lack of experience for many of the new hatcheries who joined in the experiment as co-operators. The average sex ratios for GMT produced on station ranged from 91.5 to 95% male.

Figure 15 Chart showing monthly fry production and fingerling dispersal of GMT from the Breeding Center at FAC, CLSU.



Dispersal of GMT fingerlings

Fingerling dispersal, has for the most part mirrored the fluctuation in fry production, with a one month time lag, the period required to nurse the fry. The survival rate during nursing has ranged from 65-70%. Figure 16 illustrates that GMT dispersal has been concentrated in the Provinces of Central Luzon with nearly 50% of GMT being sold to farmers in Nueva Ecija with the bulk of the remaining fry going to the neighbouring provinces of Tarlac (10.7%), Pampanga (10.7%), Bulacan (8.5%) and slightly more distantly to Isabela (6.9%). However, the GMT have been dispersed widely, although not in great numbers. It has now been cultured in 20 Provinces on over 170 farms.

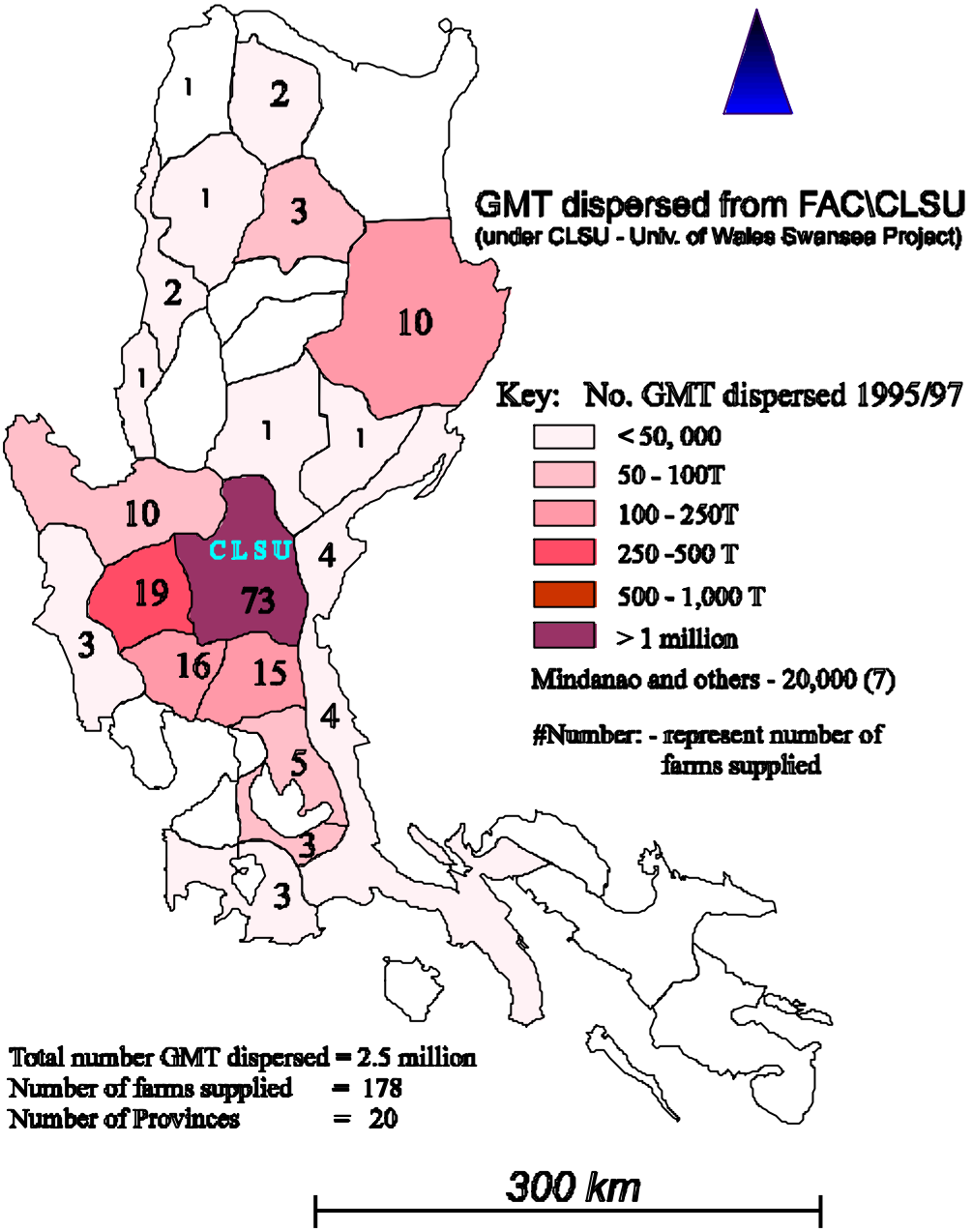
Broodstock Production at the Breeding Center

YY males were mass produced in YY male by YY female crosses at the Breeding Center. These YY male lines are perpetuated by occasional feminization of YY male to female to create sufficient YY females. All-female lines are similarly perpetuated by occasional masculinization of XX female to male. As part of the research under R. 6058, these YY males, YY females and all-female lines were selected for their combining ability for growth

and sex ratio in GMT. Initially all broodstock were marked by fin clipping at the juvenile stage and distributed to fry producers as post fingerlings (5-10g). Following some problems with contamination and/or loss of YY male broodstock by hatcheries this policy was modified so that only females were dispersed as post-fry and the YY males were grown up to sexual maturation prior to stocking in hatcheries.

At the end of the project, based on the results from our tagging work, it was decided that all YY males will be marked with a binary coded wire tag.

Figure 16 Map showing dispersal of GMT from Phil-Fishgen's hatchery at FAC-CLSU from the time that production began in April 1995 up to March 1997.



The breeding center maintains the capacity to produce GMT producing broodstock at 5,000 to 10,000 packages per month although this capacity was not met due to phased accreditation.

Accreditation and monitoring

A system of accreditation for GMT fry producers has been developed and initiated in the form of an annually renewable certification and licensing system. Interested parties are invited to apply for accreditation and the application is then considered following a farm visit. There are two forms of accreditation related to how the hatchery pays for the technology. Broodstock can either be purchased under Scheme 1 or a royalty is paid on estimated fingerling production. Details of these schemes are shown in Appendix 11.

If accreditation is granted, the requisite number of fish are set aside and then delivered to the hatchery. The hatchery initially receives a preliminary six month licence followed by annually renewable full licences. Hatcheries also receive booklets of certificates which are given to the GMT buyers, certifying the fish as GMT. Accredited hatcheries are also required to sign a memorandum of agreement governing the conditions of accreditation (see Appendix 11 for samples of these documents).

Support services are given to accredited hatcheries in the form of training for the hatchery managers and regular farm visits are made to check on production and to collect samples for sex ratio analysis. Fry should have a sex ratio close to 95% male and sex ratios significantly lower than this would indicate a problem such as contamination of broodfish. If a hatchery is consistently unable to produce the required quality of GMT then accreditation can be withdrawn.

A total of 17 private sector hatcheries were accredited in two phases in 1996. After these two phases of accreditation the process was postponed for evaluation of procedures. Accreditation is expected to recommence in July 1997 and more than 30 applications were being held on file at the end of the project. The location of the accredited hatcheries can be seen in Figure 17.

A total of 19,500 YY male packages have been dispersed to these hatcheries and we estimate these are capable of producing over three million GMT fingerlings per month.

Training

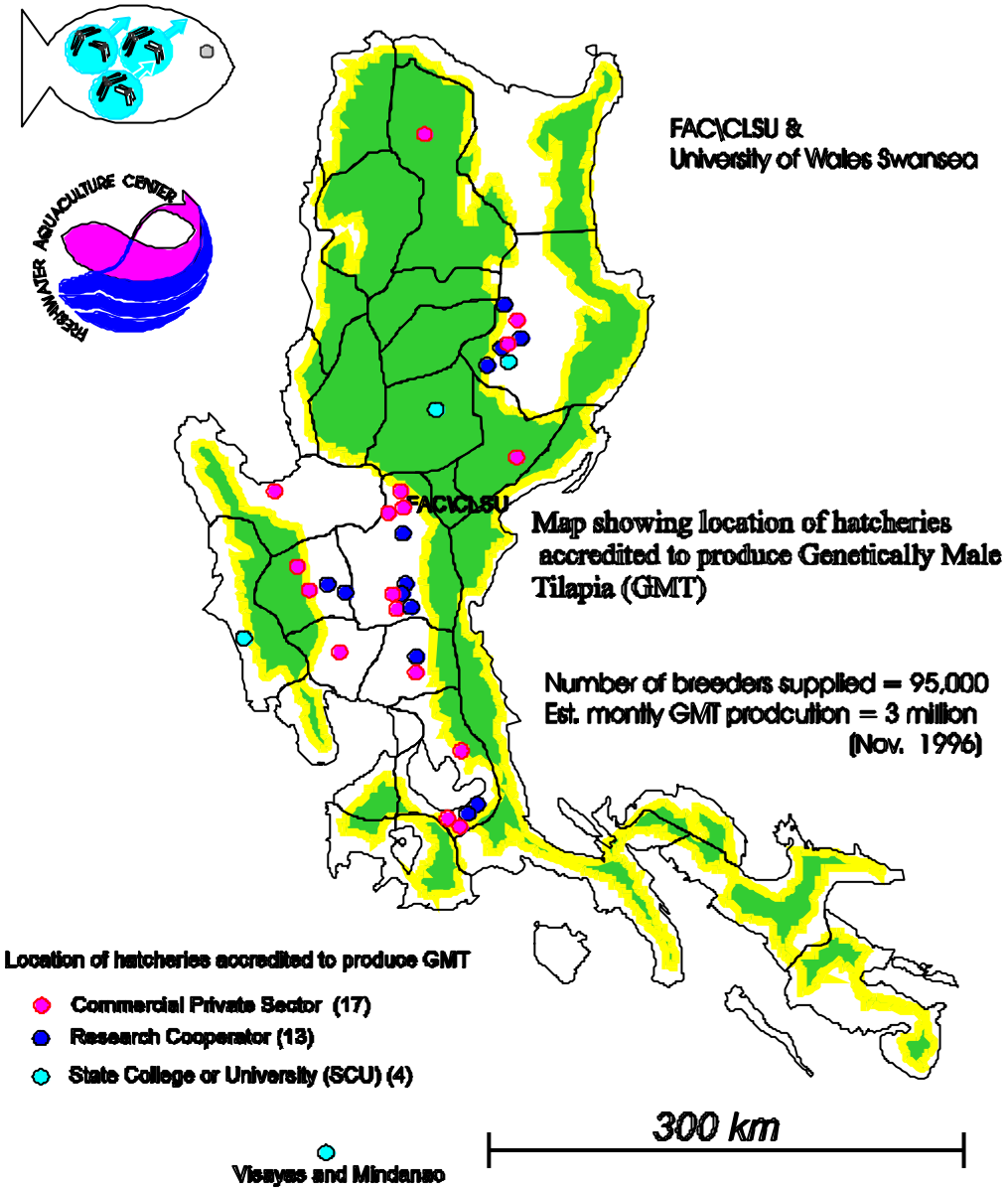
Training in hatchery and broodstock maintenance techniques is considered as a very important component of this network. To date one training has been held for the accredited hatchery managers. The training, sponsored by a commercial fish feed company, was held over two days in April 1996 and was attended by over 30 participants. The trainees received a full

orientation on the YY male technology and its application. The lecture notes used for this training were later developed into the technoguide (see publications - page 79).

Sustainability

Although funding is made available by CLSU through the institutionalisation of the majority of the staff trained under this and previous ODA funded projects, there is still a requirement for funds to support its activities together with those of the dissemination network. It is the long term objective that the activities of Breeding Center should be financially self sustaining including the funding of research. It is considered that ultimately all the activities of the network should be financially self-sustaining, being independent of external sources of funding. It is hoped that this scenario could be attained by the end of the century.

Figure 17 Map of Luzon island showing the location of accredited hatcheries including private sector, hatchery co-operators and SCUs.



Phil-Fishgen has four possible sources of income to the Breeding Center in addition to core support that it may receive through CLSU; (i) the revenue from direct sale of GMT to farmers:(ii) sale of broodstock to fry producers and/or, (iii) collection of royalties on fry sold by the fry producers. Phil-Fishgen has been in operation for two calendar years. In 1995 it generated a net income equivalent to approximately £18,000 but in 1996 this fell to only £4,700 due to fewer accreditations.

The value of the technology

Due to the variability in production rates and profitability for the different hatcheries it is difficult at present to determine accurately the economic impact of the introduction of the YY male technology and its products. It is however possible to estimate the potential value of the industry that will be impacted by the technology.

We estimated that at the end of the project, GMT fingerling production in the Philippines (combining that from FAC and the accredited hatcheries) represents approximately three to five percent of the total demand for fingerlings. Based on a present estimate of 40 million GMT fingerlings produced per year, we can estimate the total value of GMT *fingerlings sales* to be 12.2 million Pesos or £300,000 per annum. Given assumptions of survival and productivity during grow-out, this equates to a gross income from the *grow-out* of GMT of 200 million pesos or five million pounds.

It is clear therefore, that even if GMT gave only modest increases in profitability (and previous projects estimated substantial increases) it is likely to have a very major impact on the value of the tilapia culture industry of the Philippines. This is likely to extend to increased profitability to hatcheries, growers and consumers.

Relationship to other national programmes

BFAR's National Freshwater Fisheries Training and Research Center (NFFTRC) and FAC, CLSU co-ordinate the activities in the Philippine National Tilapia Breeding Programme (PNTBP) at CLSU. The PNTBP has been created with the principal objective of continuing the selective breeding programme and to effectively disseminate the genetic gains of the Genetic Improvement of Farmed Tilapia (GIFT) project, being executed by the International Center for Living Aquatic Resources Management (ICLARM). This activity has been subsumed by the newly formed GIFT Foundation International. As a result the activities in the dissemination through Phil-Fishgen and under the auspices of FAC's Fish Genetics and Biotechnology Programme (FGBP) are set to become a major activity of the PNTBP.

Summary

Currently in the Philippines, there exists an acute shortfall in the availability of quality tilapia fry for grow-out. The hatcheries that exist are operating largely inefficient hatchery technologies, using poor quality broodstock. In addition, these hatcheries are concentrated in certain

regions of the country, and no mechanisms exist for fry trading or distribution. It is to be hoped that the establishment of a network for the dissemination of genetically male tilapia, utilising the proposed network structure and incorporating improved hatchery technologies, will greatly alleviate these problems. It is hoped thereby providing support for improved livelihood of rural fish farmers through the ready availability of improved tilapia fry.

Dissemination of the YY male technology in Thailand

The Thai Department of Fisheries, through NAGRI, has released information material to farmers and have had a number of press articles on their work on the YY male technology. The Thai department of fisheries commenced distribution of GMT and YY male broodstock to farmers early in 1996. A programme for dispersal of YY male and normal female broodstock to regional DOF stations has developed slowly due to problems with loss of important broodfish in widespread flooding in 1996. However, NAGRI was able to conduct a training course on the YY male technology and its products, for the DOF station managers, in mid 1996. At the end of the project GMT are being produced at NAGRI's own research station and in two provincial stations, the latter two having received 300 YY males packages. To date there has been no dispersal of YY males to private sector hatcheries. It is planned, within the next year, to disperse GMT producing broodstock to approximately half (60-70) of the Government fisheries stations , possibly requiring up to one million YY male packages. The GMT, which sell at four times the normal price of fingerlings (B0.20), are nevertheless in great demand. It is estimated that NAGRI and the two fisheries stations are presently dispersing one to two million GMT fingerlings into the industry in Thailand. No information is presently available relating to the location and economic status of the present beneficiaries in Thailand.

Implications and Priorities

As previously stated a project workshop was held in the Philippines in January to review progress of the research and to develop recommendations for future research and dissemination (exclusively those pertaining to the Philippines). Some important recommendations arising from this project are summarised below:

Research and Development in the Philippines

This and previous projects funded by the ODA Fish Genetics Programme have placed emphasis on the more technical and biological development and evaluation of the technology. The results have supported the conclusion that the technology can be applied to enhance yields and profitability in tilapia culture. A programme for the dissemination of the products of this technology (YY male broodstock packages and genetically male tilapia themselves) has been established without external funding support.

The outputs of this project have contributed directly to a number of improvements in the programme including the following recommendations:

- Provide YY males to the hatcheries at a larger size, preferably after sexing by external morphology to minimize mortality and contamination.
- Use a better marking technique for the YY males. This has actually already been acted on with the purchase of a binary coded metal tag applicator which will be used to mark YY males.
- To reevaluate the charging of royalties to hatcheries based on estimated production of GMT. It appears initial estimates were high and have reduced the potential for improved profitability in the production of GMT fingerlings.
- To develop more information material, in local dialects, to better inform the farmers about the technology.

These recommendations relate to fine tuning of the dissemination programme. However, a major recommendation for further research has been identified based on clear limitations in the present structure of the dissemination programme.

To date the dissemination process has been a passive one. The beneficiaries of the technology have tended to be those that have become aware of it through various national media and word of mouth, are mobile, fairly well educated, and have some capital to invest in new technologies. This passive process has effectively excluded small hatcheries which either do not know of the technology or are unable to obtain either the information or the improved fish themselves, for a variety of reasons associated with their lack of resources. At present we do not know which sector of the tilapia grow-out industry is benefiting from the technology and what impact it is having upon them.

It is clear that a greater understanding of the structure and functions of the tilapia culture industry in the Philippines is required in order to target this technology at appropriate key beneficiaries and stakeholders.

Existing surveys and assumptions on the nature an structure of the industry have not accounted for the possible existence of significant numbers of truly small-scale farmers (defined as those that generally have less than 1,000 m² of water area available for aquaculture, have limited or no capital, use much of their fish production for home consumption and have insecure tenure over land or water used for their production). Furthermore little is known of the nature and strengths of the links between the different sectors of the industry.

Having established the potential and demand for the YY male technology and its products there is now a clear need to examine the social and economic aspects related to the uptake of this technology. Research is required to improve the uptake of the products of technologies

developed to improve tilapia used in aquaculture with emphasis on small-scale farmers, ultimately to sustainably enhance aquaculture production.

In this regard a project has been proposed to the ODA - FGP to research these issues. The project will have the general objective of evaluating the applicability and potential impacts of the YY male technology and other competing technologies to each sector of the tilapia culture industry in the Philippines, with emphasis in Luzon island. An inadequate understanding of the structure and functioning of the various sectors of the industry (especially the hatchery sector) is a major constraint to the design of appropriate and effective dissemination strategies for technologies designed to improve aquaculture production of tilapia.

Small-scale aquaculture in the Philippines is poorly defined and understood and one of the first tasks of the project will be to characterise this sector of the industry and determine the social, economic and institutional constraints to the adoption of these technologies by this sector. A further major thrusts of the project will be a comprehensive social and economic evaluation of the impact of the introduction of YY males in hatcheries and GMT in tilapia farms.

Whilst the aforementioned proposal has been submitted to ODA for funding it is also intended that some support for research of this nature be generated by the dissemination programme itself and a medium term objective is to make all research activities predominantly self-sustaining by the year 2000.

Research and Development in Thailand

Based on the research progress in Thailand a number of research needs were identified. This project has gathered considerable data on the relative growth performance of different strains and strain combinations. Whilst this has provided some indicators of relative performance some of the results have been ambiguous and relative ranking differs between environments. The environments in which these comparisons have been made have not been truly representative of common culture systems in Thailand and there is now clearly a need to move on to conducting on-farm trials of the most promising genotypes.

The nature and structure of the tilapia culture industry in Thailand and other parts of Southeast Asia differ considerably to that in the Philippines, with the latter being dominated by non-integrated monoculture. It is thus recommended that, alongside the on-going dissemination of the technology by the Thai DOF, we investigate and evaluate the potential of uptake of the technology in the private sector. This can be achieved through a repetition of some of the hatchery trials conducted in this project, in common hatchery systems in Thailand and the Philippines.

A proposal has been submitted, again to the FGBP, in which further technical improvements in the technology in the Philippines be combined with on-farm evaluations of the technology in Thailand and Vietnam.

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Glossary of Acronyms

AASP	Agricultural and Aquatic Systems Program (at AIT)
AIT	Asian Institute of Technology
BFAR	Philippine Bureau of Fisheries and Aquatic Resources
CDS	Centre for Development Studies (UWS)
CLSU	Central Luzon State University
DES	Diethylstilboestrol - oestrogen hormone
EPB	Extensive pond based (hatchery system)
FAC	Freshwater Aquaculture Center (of CLSU)
FGBP	Fish Genetics and Biotechnology Programme (of FAC, CLSU)
GIFT	ICLARM's Genetic Improvement of Farmed Tilapia (Project)
GFT	Genetically Female Tilapia (derived from crosses of $\Delta\male$ XX)
GMT	Genetically Male Tilapia (derived from YY males)
GMIT	Genetic Manipulations for Improved Tilapia (Project)
ICLARM	International Center for Living Aquatic Resources Management
IDRC	International Development Research Council (of Canada)
IHB	Intensive hapa based (hatchery system)
ITB	Intensive tank based (hatchery system)
MST	Mixed-Sex Tilapia (from crossing of normal broodstock)
MT	17-α methyltestosterone - androgenic hormone
NAGRI	National Aquaculture Genetics Research Institute (Thailand)
NARS	National Aquatic Resources Systems
NFFTRC	National Freshwater Fisheries Training and Research Center (of BFAR)
NGO	Non-governmental organisation
ODA	Overseas Development Administration
PNTBP	Philippine National Tilapia Breeding Programme
PSU	Pangasinan State University
PO	People's Organisation (farmer groups, co-operatives etc.)
PD/A CRSP	Pond Dynamics Aquaculture Collaborative Support Programme supported by USAID.
SIPB	Semi-intensive pond based (hatchery system)
SRT	Sex Reversed Tilapia (treated with MT)
TAD	Technology Adaptation and Development (Project)
TCTP	Technical Co-operation Training Programme (British Council, Manila)
UWS	University of Wales Swansea

Appendices

Appendix 1 Logical Framework for the project as submitted to the ODA Fish Genetics Programme

Appendix 2 Farm fact files for the hatcheries co-operating in the on-farm trials of GMT producing broodstock.

Appendix 3 Guide for social and economic data gathering.

Appendix 4 Protocol for progeny testing to identify manipulated genotypes

Appendix 5 Mean body weight (g) at the time of stocking of breeders and at the end of the experimental period for each hatchery according to production system.

Appendix 6 Mean standard length (cm) at stocking of breeders and gain in standard length at the last day of fingerlings collection under four production system in the on farm trials of YY male broodstock.

Appendix 7 Inputs, sales and net return. Table showing the comparison between the average cost of production per set of breeder and the average income/loss per set of breeder for the whole period of the experiment. Average production cost and income/loss for the GMT were computed in based on the initial number of broodstock during initial pairing under scheme 1 and 2 and based on the actual production

Appendix 8 Freshwater aquaculture development in the Philippines: the case of tilapia

Appendix 9 Copies of publications produced under the project

Appendix 10 A sample of media articles relating to the YY male technology in the Philippines.

Appendix 11 Details and supporting papers for the accreditation of private sector hatcheries under Phil-Fishgen's dissemination programme for the products of the YY male technology.

Appendix 1 Logical Framework for the project as submitted to the ODA Fish Genetics Programme

Project Title: Genetic Manipulations for Improved Tilapia Technology Adaptation and Development II **Period of ODA funding:**
Brief Description of Project: Collaboration between UWS , CLSU (Philippines), AIT and NAGRI (Thailand) From April '94 to March '97
to test and verify the YY-male technology . **Total ODA funding:** £150,080
His Code No.: R 6070A **Date Framework Prepared/Revised**
File Reference: TAD2.fmk November 21, 1995

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
GOAL: Sustainable aquaculture production enhanced by genetic technology	Widespread increases in tilapia yields attributable to introductions of YY male technology and/or saline tolerant *O. niloticus* Tilapia production expands at a faster rate than the demand caused by increasing population Tilapia culture represents increasing proportion of total aquaculture production Evidence of improved livelihood of farmers adopting project technologies	Statistics obtained from government and development agencies	
PURPOSE: Increase in tilapia production through adoption of YY male technology and the culture of *O. niloticus* in brackishwater.	YY male technology successfully adopted in at least 5 less developed countries by 2000. 40% increase in yields in on-farm trials compared to tilapia presently grown by project end Sustainable production of *O. niloticus* in brackishwater including abandoned shrimp ponds	Culture performance and socio-economic records and reports obtained by: (i) FAC\CLSU-UWS for on-station and on-farm trials (ii) Government statistics (iii) Non-governmental organisations monitoring farm production (iii) Commercial organisations testing and utilising the technology	Sufficient interest in disseminating technology by state, NGO and private sector stakeholders (especially small farmers) in tilapia growing counties exists. National extension programmes are able to adopt and disseminated technologies and their products Examples of technology application in the Philippines and Thailand are repeated in other countries

OUTPUTS: 1. Programme and necessary broodfish for mass production and appropriate dissemination of the YY male technology developed and initiated 2.1 Feasible and effective methodology for technology transfer of YY male technology developed 2.2 YY male technology transferred to Thailand 3 Case studies on socio-economic status of tilapia farmers produced and likely long term impacts of the introduction of YY male technology determined 4 Brackishwater tolerant strain of *O. niloticus* identified	By project end: 1.1 At least 200 Filipino farmers growing GMT 1.2 At least 25 Philippine hatcheries producing GMT 1.3 Income generating project in place and research and dissemination activities targeted to be self-sustaining by March 1998. 1.4 Brochures and training manuals for YY male technology produced and 3 training courses for hatchery managers completed 1.5 Trials of YY male broodstock on at least 10 farms completed 2. GMT producing broodstock available for dispersal in Thailand and at least 5 hatcheries producing GMT. One publication on technology transfer 3. Case studies completed and analysed and published as Ph.D. thesis 4. Strain of *O. niloticus* that survives in Philippine brackishwater pond environments and produces higher yields than existing cultured tilapia	Publications in refereed journals and conference proceedings, lectures, seminars and popular articles. Training materials for tilapia seed producers and growers	YY male technology and its products are competitive with alternative improvement technologies Environmental and socio-economic impacts of YY male technology are neutral or positive Environmental and socio-economic impacts of culture of *O. niloticus* in brackishwater are neutral or positive Adoption of improved technologies\strains is not adversely affected by political interests

ACTIVITIES: 1.1 Evaluation of productivity and economic viability of the application of the YY male technology on-station and in private hatcheries 1.2 Mass production of GMT producing broodstock developed under R 6058 1.3 Design and initiation of mechanism for sustainable research, development and dissemination of the YY male technology and its products 2. Compare three alternative mechanisms for transfer of YY male technology to Thailand 3.1 Conduct case studies of farmers involved in tilapia culture in two Philippine communities representing hatchery and grow-out operators 3.2 Evaluate response of farmers to GMT 4.1 Aquarium based comparisons of salinity tolerance of strains of *O. niloticus* held at CLSU 4.2 Evaluation of culture performance of promising strains of *O. niloticus* in brackishwater conditions	Inputs/resources: ODA Project Budget (£) Staff costs: 99,817 Capital costs: 17,530 Running costs 33,028 Total (3 years) **150,080** Facilities and local counterparts from CLSU, AIT and NAGRI. Additional financial input for institutionalization programme from CLSU and for dissemination of YY male technology from NAGRI	Quarterly, annual and final reports to ODA Internal discussion groups and written documents	1.1 Willing and qualified farmer cooperators can be identified 1.2 Development of improved broodstock under R 6058 is successful 2. At least one technology transfer mechanism is feasible and appropriate 3.1 Suitable sites for case studies are identified and farmers are cooperative 4.1 Appropriate practical methods for quantifying salinity tolerance can be identified 4.2 Genetic variation for salinity tolerance exists between strains General Institutionalization programme at CLSU is successfully initiated and maintained. Key trained scientific and extension personnel stay with the project No natural or anthropogenic disasters hinder, delay or prevent research

Appendix 2 Farm fact files for the hatcheries co-operating in the on-farm trials of GMT producing broodstock.

Appendix 3 Guide for social and economic data gathering.

Appendix 4 Protocol for progeny testing to identify manipulated genotypes

Progeny testing is carried out to identify manipulated genotypes, the principal being that we can determine the genotype of the parental fish by the sex ratio of its progeny.

The main manipulated genotypes of interest in the Nile tilapia *O. niloticus* are as follows:

Origin (♀x♂)	Treatment	Name	Symbol	Description
XX x XY or XX x YY	Diethylstilboestrol at 1,000 mg kg^{-1} for 21 days	Sex reversed XY females	XY Δ♀	Genetically male, phenotypically and functionally female
XX x XY or XX x XX	Methyltestosterone at 40 mg kg^{-1} for 25 days	Sex reversed XX males	XX Δ♂	Genetically female, phenotypically and functionally male
XY x YY or YY x YY	None	YY males	YY ♂	Novel YY males or "supermales"
XY x YY or YY x YY	Diethylstilboestrol at 1,000 mg kg^{-1} for 21 days	YY females	YY Δ♀	Genetically male, phenotypically and functionally female

The Δ prefix denotes functional sex reversal

In progeny testing the fish are spawned in single pair matings, usually in 1m² hapas. The progeny are then collected and reared in hapas for a period of approximately 3 months or until then attain a mean weight of 3-5g. They are then sacrificed (all or sub-sample depending on whether the progeny are required for breeding) and the sex determined using a gonad squash technique. The following crosses are made to identify the aforementioned genotypes:

Genotype being tested for.	Crossed with	Expected sex ratio
XY Δ♀	XY ♂ or XX Δ♂[1]	3:1 or 1:1
XX Δ♂	XX ♀	0:1
YY ♂	XX ♀	1:0
YY Δ♀	XY ♂ or XX Δ♂[2]	1:0

Sex ratios are tested against the expectations using χ^2 contingency tests. For example if the progeny test is to distinguish XY Δ♀ from normal XX ♀ by crossing them to an XX Δ♂ then the sex ratio will be tested against an expectation of 1:1.

A stringent statistical criterion was adopted for the designation of parental genotypes when progeny testing for 'YY' males or females, hypothesised to produce all- or nearly all-male progeny. A 5% level of probability was not deemed sufficiently stringent to permit the confident designation of genotypes. Thus potential YY genotypes producing sex ratios, in crosses with XX genotypes, that were not significantly different from 1:1 or only significant at the 5% level ($P > 0.01$) were designated as XY. Only those producing male-skewed sex ratios different from 1:1 at a probability level of 0.1% ($P < 0.001$) were designated YY. This procedure also helped to minimise the chance of making a type I error in the identification of 'YY' males. No genotype was designated for parents of families falling between these two criteria ($0.001 > P < 0.01$).

[1] XX Δ♂ are preferred over XY ♂, it is statistically easier to distinguish 1:1 ratios from 0:1 than 3:1from 1:1

[2] XX Δ♂ are preferred over XY ♂, it is statistically easier to distinguish 1:0 ratios from 1:1 than from 3:1

Appendix 5 Mean body weight (g) at the time of stocking of breeders and gain (GGM1) and at the end of the experimental period for each hatchery according to production system.

System	Farm	Initial Body weight (g)						Gain Body weight (g)					
		YY packages			Control			YY packages			Control		
		♂male	♀	Mean Ave. of Geno 1	♂male	♀	Mean Ave. of Geno 2	♂male	♀	Mean Ave. of Geno 1	♂male	♀	Mean Ave. of Geno 2
EPB1	1	57.7ns	55.9	56.5	79.9ns	76.6	77.7**	182.8**	94.7	124.1	184.1**	120.3	141.6**
		(8.9)	(7.9)	(8.3)	(16.2)	(14.9)	(15.4)	(29.8)	(26.7)	(50.0)	(65.3)	(28.9)	(53.5)
	2	31.7	22.3	25.7**	31.7**	22.3	22.4	740.0**	369.4	462.1	853.8**	685.5	727.6**
		(9.3)	(5.4)	(8.4)	(9.3)	(5.4)	(10.2)	(246.7)	(167.9)	(248.8)	(180.3)	(285.8)	(272.7)
	3	25.1**	20.8	40.5**	63.2**	29.9	26.7	49.0	67.7*	61.0	60.7ns	63.9	62.9ns
		(11.8)	(8.8)	(17.1)	(8.1)	(6.5)	(6.9)	(28.9)	(21.9)	(25.0)	(30.0)	(22.4)	(25.4)
							±						
SIPB2	4	29.6	25.3	27.0	37.7**	22.1	30.1**	90.8**	35.5	53.9	125.1**	49.9	74.9**
		(6.5)	(6.7)	(10.5)	(8.5)	(7.2)	(10.7)	(12.8)	(13.5)	(29.4)	(31.7)	(18.8)	(42.8)
	5	37.4**	26.8	23.7	23.7ns	23.8	32.1**	56.4**	37.1	41.0**	41.9**	24.6	28.0
		(9.4)	(9.6)	(8.1)	(7.8)	(8.4)	(10.8)	(10.5)	(17.5)	(18.1)	(20.3)	(11.4)	(15.3)
	6	39.6**	24.5	121.2**	110.9	126.5*	72.1	143.3**	107.9	119.7**	4.27	20.4*	15.0
		(7.5)	(7.0)	(28.6)	(32.2)	(25.1)	(18.3)	(64.4)	(28.3)	(46.6)	(21.4)	(29.6)	(28.1)
ITB3	7	82.5**	66.9	105.2	233.9**	108.9	150.0**	150.9**	36.4	98.659	116.3**	69.3	105.2
		(12.0)	(18.4)	(26.4)	(48.5)	(24.9)	(68.2)	(49.5)	(44.3)		(65.4)	(54.6)	
	8	131.5**	90.8	104.4**	116.1**	73.6	87.8	n\a	n\a	n\a	n\a	n\a	48.9
		(21.2)	(16.8)	(26.6)	(26.3)	(21.8)	(30.8)						
	9	26.4	27.5ns	27.0	40.5*	35.8	37.7**	80.1**	46.5	54.3	75.7**	39.3	49ns
		(14.0)	(9.2)	(11.6)	(6.9)	(9.0)	(8.5)	(18.8)	(12.2)	(19.9)	(14.5)	(15.2)	(22.1)
	10	55.9ns	53.6	54.3	67.8ns	67.2	67.4**	45.6ns	39.9	75.7**	100.4**	60.6	39.3
		(9.4)	(9.6)	(9.6)	(12.0)	(11.4)	(11.8)	(19.6)	(19.9)	(14.5)	(30.5)	(25.8)	(15.2)
IHB4	11	26.5*	22.5	24.5**	21.6*	18.3	20.0	157.4**	69.2	98.6	122.5**	96.7	105.1ns
		(8.2)	(7.0)	(8.3)	(7.6)	(5.7)	(6.9)	(49.5)	(16.4)	(52.2)	(41.0)	(30.4)	(36.6)
	12	86.7*	67.3	72.2ns	78.0*	67	69.8	40.9**	24.2	29.8	72.7**	37.0	48.9**
		(13.5)	(10.4)	(13.0)	(23.8)	(17.7)	(19.9)	(25.2)	(19.1)	(22.6)	(21.4)	(22.0)	(28.0)
	13	66.4**	55.9	60.2	71.4	88.9**	83.1**	86.7*	71.2	76.4	100.4ns	95.6	97.3

	(17.9)	(15.0)	(17.1)	(25.6)	(27.4)	(27.0)	(43.0)	(30.1)		(52.5)	(57.7)

- significant at P0.05,

** - significant at P0.001,

- ns - not significant; n\a = not available

Appendix 6 Mean standard length (cm) at stocking of breeders and gain in standard length at the last day of fingerlings collection under four production system in the on farm trials of YY male broodstock.

		Standard length (g) at stocking						Gain Standard length (cm) at harvest					
		YY packages			Control			YY packages			Control		
System	Farm	♂	♀	Mean	♂	♀	Mean	♂	♀	Mean	♂	♀	Mean
EPB	1	11.3	11.5*	11.4	12.6**	12.3	12.4**	6.7**	3.9	4.8ns	5.5**	4.6	4.9
		(0.7)	(0.6)	(0.6)	(0.8)	(0.9)	(0.9)	(0.7)	(1.1)	(1.6)	(1.5)	(0.9)	(1.2)
	2	12.1**	10.1	11.3**	11.1**	10.2	10.5	11.5**	10.1	10.5	14.0**	10.7	11.5**
		(1.2)	(0.8)	(1.2)	(1.7)	(1.5)	(1.6)	(1.1)	(1.7)	(1.7)	(1.8)	(1.7)	(2.2)
	3	11.8**	9.1	10.0**	9.2**	7.9	8.3	2.6	4.7**	3.9	4.2	5.5*	5.0**
		(0.5)	(0.7)	(1.4)	(0.7)	(0.7)	(0.9)	(1.4)	(1.1)	(1.6)	(1.2)	(1.2)	(1.3)
SIPB	4	12.7**	10.7	11.3	13**	11.3	11.8**	2.4**	1.3	1.7	3.4**	1.7	2.3**
		(0.9)	(1.1)	(1.4)	(1.1)	(1.2)	(1.4)	(0.5)	(0.9)	(0.9)	(1.1)	(1.1)	(1.4)
	5	10.6ns	10.6	10.6	13.0**	11.1	12.0**	2.6**	1.4	1.6**	0.9*	0.4	0.5
		(1.1)	(1.1)	(1.1)	(0.8)	(1.1)	(1.3)	(0.6)	(1.0)	(1.0)	(1.2)	(0.9)	(1.0)
	6	14.6ns	15.0	14.9**	12.8**	12.0	12.2	2.6**	0.8	1.4*	0.4	1.2**	0.9
		(1.3)	(1.1)	(1.2)	(0.7)	(1.0)	(1.0)	(1.8)	(1.4)	(1.4)	(1.2)	(1.4)	(1.4)
ITB	7	12.3*	12.8	12.6	15.9**	12.4	13.5**	6.1**	3.0	4.0	5.2**	4.0	4.4ns
		(0.8)	(1.0)	(1.0)	(1.2)	(0.8)	(1.9)	(1.1)	(1.7)	(2.1)	(1.4)	(1.8)	(1.8)
	8	14.7**	13.4	13.8**	14.3**	12.1	12.8	n\a	n\a	n\a	n\a	n\a	n\a
		(0.7)	(0.7)	(0.9)	(1.0)	(1.1)	(1.5)						
	9	11.2	11.4ns	11.3	13.0*	12.5	12.7**	3.3**	0.8	2.2**	3.3**	1.9	1.0
		(1.9)	(1.1)	(1.6)	(0.9)	(0.9)	(0.9)	(1.0)	(1.1)	(1.0)	(0.9)	(0.7)	(1.0)
	10	9.9ns	9.9	9.9	10.8ns	10.7	10.7**	4.7**	3.9	4.1	4.7**	3.9	5.5**
		(0.5)	(0.6)	(0.6)	(0.8)	(0.6)	(0.7)	(0.7)	(0.8)	(1.4)	(1.7)	(1.1)	(1.4)
IHB	11	11.4*	10.1	11.0**	9.5**	11.0	10.1	6.3**	5.1	3.9	5.9**	2.9	5.5**
		(1.2)	(1.2)	(8.3)	(0.9)	(1.1)	(1.2)	(0.8)	(1.5)	(1.8)	(1.6)	(0.8)	(1.5)
	12	13.7**	12.4	12.7**	13.0*	12.1	12.3	5.5ns	5.4	5.5**	2.3**	1.7	1.8**
		(0.7)	(0.7)	(0.9)	(1.1)	(1.1)	(1.2)	(1.7)	(1.3)	(1.5)	(0.7)	(0.9)	(0.9)
	13	12.1**	11.7	11.9	12.4	13.1**	12.9**	4.3ns	3.9	4.0	4.2**	4.2	4.2ns
		(1.2)	(1.0)	(1.1)	(1.3)	(1.4)	(1.4)	(1.3)	(1.3)	(1.3)	(1.6)	(1.8)	(1.7)

significantly greater ($P < 0.05$).

? ** - significantly greater (P<0.01), *** - signficantly greater (P<0.001),

ns - not significant; n\a = not available **in bodyweight at the last day of fingerlings collection under four production system.**

Appendix 7 Inputs, sales and net return. Table showing the comparison between the average cost of production per set of breeder and the average income/loss per set of breeder for the whole period of the experiment. Average production cost and income/loss for the GMT were computed in based on the initial number of broodstock during initial pairing under scheme 1 and 2 and based on the actual production

System/ farm	Input				Sales		Income/Loss						
	Control	GMT Scheme 1	GMT Scheme 2	Actual	Control	GMT	Control	% Diff Gmt/cont	Scheme1	% Diff GMT/cont	GMT Scheme2	% Diff GMT/cont	Actual
EPB													
1	96.08	144.44	82.99	67.51	50.38	94.69	-45.70	-8.88	-49.76	N/A	11.7	N/A	27.17
2	152.98	386.31	214.23	207.37	587.86	517.99	434.88	-69.72	131.68	-30.15	303.76	-28.57	310.62
3	37.98	137.55	63.80	38.49	24.04	29.45	-13.94	675.47	-108.10	146.41	-34.35	-35.22	-9.03
Mean	95.68	222.77^{ns}	120.34^{ns}	104.46^{ns}	.		125.08^{ns}.-		8.737		93.70		109.60
SIPB													
4	43.41	219.27	82.78	37.04	77.90	41.76	34.50	N/A	-177.62	N/A	-41.02	-86.35	4.71
5	24.75	121.72	47.97	24.37	20.18	61.61	4.57	N/A	-60.11	198.47	13.64	715.10	37.25
6	16.72	156.23	45.61	8.20	37.78	44.89	21.06	N/A	-111.34	N/A	-0.72	74.22	36.69
Mean	28.29^{ns}	165.74*	58.79^{ns}	23.20			20.04		-116.36*		-9.15^{ns}		26.22^{ns}
ITB													
7	60.30	245.43	97.93	83.60	1071.71	872.44	1011.41	-38.01	627.01	-23.42	774.50	-22.99	778.84
8	153.09	244.58	120.83	100.42	40	133.33	-113.09	N/A	111.25	N/A	12.50	N/A	32.92
9	13.23	125.35	34.74	9.36	8.10	18.23	-5.18	1967.95	-107.12	218.73	-16.51	N/A	8.87
10	24.88	101.42	39.96	20.96	49.8	57.54	24.91	N/A	-43.87	-29.43	17.58	46.85	36.58
Mean	62.88^{ns}	179.20^{ns}	73.37^{ns}	53.59			229.51^{ns}		146.82		197.02		214.30
IHB													
11	21.71	164.15	53.73	17.07	41.54	62.01	19.82	N/A	-102.14	-58.22	8.28	126.74	44.94
12	35.48	207.19	71.99	29.50	356.67	152.14	321.19	N/A	-55.05	-75.04	80.16	-61.81	122.65
13	52.69	167.20	50.53	59.95	184.11	215.40	131.42	-63.32	48.21	2.15	134.25	18.28	155.45
Mean	36.63	179.51*	58.75^{ns}	35.51^{ns}			157.48^{ns}		-36.33		74.23		157.48^{ns}

* - significantly greater (P < 0.05),

** - significantly greater (P<0.01), *** - signficantly greater (P<0.001),

ns - not significant; n\a = not available ~~in bodyweight at the last day of fingerlings collection under four production system.~~

Appendix 8 Freshwater aquaculture development in the Philippines: the case of tilapia

An overview of the Ph.D. thesis of the same title by Dr. Ruben C. Sevilleja

Two related aspects of the process of tilapia technology adoption in the freshwater aquaculture sector are analysed in this dissertation: (1) the factors which contribute or impede the adoption of tilapia culture technology; and (2) the economic and social consequences that follow the widespread introduction of the technology, within the context of the agrarian structure in terms of the financial benefits which accrue to the participants. Two villages were selected as study sites: Kabaritan, Sto. Domingo in the municipality of Bay, province of Laguna (Southern Luzon), where tilapia hatchery operation is the main culture system practised by farmers; and Partida in the municipality of San Miguel in the province of Bulacan (Central Luzon) where the technology of tilapia grow-out operation is widely adopted. Field investigations were made on the livelihood, social relations and economic behaviour of the different groups of farmers involved in tilapia production; functioning and make-up of the agrarian structure; and the operation of units of tilapia production.

Tilapia Farming Technology and the Farmer

This section discusses the first aspect of this research which is concerned with the motivations of the farmers and the dilemmas which confront them in the face of increasing technological change and market integration.

Motivations of Fish Farmers

The research considered the different motivational factors which influenced farmers to adopt tilapia farming technology. A range of factors combine to induce farmers to go into fish culture. In general, most of the factors are common among all farmers in both study sites. However, they vary in their degree of importance. What is apparent, however, is that the decision to adopt the technology is mainly based on the availability of resources to the farm household.

The overriding motivation in adopting tilapia culture is to earn more income. This connotes their strong desire to pursue means of providing a better and more decent standard of living for their family. It also means that they respond to prices and market forces.

Extent of Technology Adoption

The study reveals that tenure does not impede the adoption of tilapia farming. The adoption pattern of tilapia farming technology can be illustrated by differences in the size of landholding. Large farms tend to adopt early, but smaller farms follow suit. These results are consistent with that of other studies which find that farm size is not a serious barrier to adoption. However, what is apparent is the significant difference with respect to the absolute

size of fishpond and total land ownership among farmers. The biggest hatchery and grow-out farmers operate an average area of slightly over 1.0 hectare. This is about 13 times bigger than the average operational size of farmers within the smallest fishpond size category who also outnumber the former group. This unequal distribution of ownership means that the total benefits which can be derived from tilapia farming are biased in favour of large farms. Even if all farmers, large or small adopt the technology simultaneously, large farmers would still benefit in so far as benefits are proportional to holding size. Herein lies the problems and dilemmas which small farmer-adoptors of tilapia culture are confronted with.

Adoption Constraints and Problems

Results of this study show that the constraints and problems expressed by farmers with regards to the adoption of tilapia farming technology are economic, technical and social in nature.

The action and behaviour of individual farmers, whether big or small; rich or poor, is governed by a common denominator: the goal of attaining a certain standard of livelihood. But differences lie on the level of that standard being aimed at. Their decisions about technological practices are then based upon the urgency of the strategies which characterize this pursuit.

Central to this problem is the manner in which the elements of livelihood are produced or purchased. As can be deduced from the motivational factors which encourage farmers to take up tilapia farming, the possibility of doing this depends upon the cultivator's access to various factors of production; his knowledge level at adapting modern or scientific information to existing farming practices; and his capacity to integrate with the market.

Prospects of Adoption

Despite their constraints and problems, the overall attitude of the farmers toward tilapia technology is generally positive. Majority of them have expressed their desire to continue their operation. This attitude is buoyed mainly by their perception about the favourable growth of the industry and the availability of production technologies. Most farmers also expressed their willingness to culture genetically male tilapia (GMT).

Farmers' responses to the technology, however, are influenced by their economic objectives which relate to their conditions as consumers and producers. Constraints which relate to the availability of resources among farmers put small producers at a disadvantage. Based on the case studies, the following factors have, in general, contributed to and facilitated the adoption of tilapia farming technology: availability of credit; access to technology information; water supply; resource ownership; and favourable social environment.\

Farm Profitability and Income Differential

The second main aspect of freshwater aquaculture development which was investigated in this study is the potential economic and social consequences resulting from the introduction of tilapia culture technology. Two closely related issues were addressed. First, how do farms under different tenurial arrangements differ with one another in levels of productivity and use of resources? Second, how do small farms compare with large farms?

Effects of Land Tenure

Results of the study show that tenancy does not lead to allocative inefficiency. Evidence provided by this study shows that the intensity in the use of inputs is the major determining variable as far as farm productivity is concerned. This result illustrates a situation where tenancy does not limit output, but the tenure system pushes the cultivator to produce beyond the income level which satisfies the consumption needs of the farm family.

Effects of Farm Size

The inverse relationship between size and productivity commonly observed in agriculture does not seem implausible in the light of the case study data for hatchery operations in Laguna. The average annual land productivity of decreases as farm size increases. Correspondingly, the amount of net income follows a similar pattern. The pattern of decreasing productivity per hectare as farm size increases reflects the efficiency of small hatchery farmers in using their available resources. As far as can be ascertained, the difference in land productivity by size is mainly attributed to the higher cropping intensity and higher labour use in small farms than in large farms.

However, the inverse size-productivity relationship is not very evident under grow-out operation in Bulacan. Although the negative relationship is observed within the three smallest size categories, productivity per hectare is highest in the largest farm group. This~~group.This~~ pattern is explained by the more intensive use of non-labour input in large farms. In general, grow-out operation results in greater benefits from the technology by big farmers on account of their better access to production resources.

Implications of Results

Results of his study are significant in the light of the government's general objectives of enhancing the productivity of fisheries resources, and uplifting the socio-economic conditions of small-scale fisherfolk. Current production trends in aquaculture indicate that tilapia farming will play a significant role towards the realization of these objectives and the attainment of production targets. In~~b~~ this connection, government must develop and implement programmes which will promote its adoption and expansion. Such programmes, however, must not lose sight of one of the most important aspects of the government's overall development strategy: poverty alleviation and equitable income distribution.

Effects on Equity and Income Distribution

It is clearly demonstrated from the results of this study that tilapia farming is a profitable production activity. The financial returns obtained from hatchery and grow-out operation are higher than reported returns generated by other farming activities. Between the two systems, returns to the different factors of production show that tilapia hatchery is more profitable compared to grow-out operation.

Tilapia production has provided investment opportunities to farmers because of the high rates of returns to such investments. But while the benefits of increased income is possible with the adoption of tilapia production technology, the distribution of such benefits are influenced by the agrarian structure and nature of production relations. In terms of economic feasibility, and relative cost and returns to investment, the big farmers are placed in a superior position than their smaller counterparts for exploiting the benefits of tilapia farming. This underscores that, compared to the small farms, the big farms stand to gain more from the introduction of the technology. Consequently, the inequality among farm families in terms of farm income are bound to grow under the impact of tilapia technology adoption.

Strategies for Freshwater Aquaculture Development

The agrarian structure is of paramount importance in addressing the equity aspects of agricultural development. But issues of equity are often in conflict with development strategies and are never incorporated in technologies. In order to address the equity issues, distortions in the agrarian structure must first be tackled. Aquaculture can be a means to initiate changes in the existing structure by introducing tilapia farming as a cash crop into a subsistence agriculture system.

Results of the study indicate that a strategy to promote tilapia farming beneficial to both small and large operators should be adopted if the targets and goals of the fisheries and the government's overall development programme are to be met. The profitability of tilapia farming will serve as the encouragement to attract more investment. However, rapid expansion in freshwater aquaculture requires more than just higher and faster rate of capital accumulation. In order for investment to be effective, it must be accompanied by technical change. In other words, techniques of production must be changed, new species of fish or improved strains must be introduced, support infrastructures must be provided, aquacultural supply industries expanded, and institutional support strengthened.

With aquaculture, it is possible to reconcile the concept of profitable farming with that of Philippine traditional farming practices which are based generally on a system of self-sufficiency and to a large extent are too small to be in any way commercially viable. Because the essential problem is the lack of land and limited resources, freshwater aquaculture development in the future will rely more on technology improvements rather than on expansion

of areas. At present, tilapia farming practices are in general extensive in nature. Thus, productivity can be increased through technological innovations. However, the scope of land intensification and increase in productivity is limited if it is left entirely to the response of individual farmers.

The need for appropriate tilapia technology to enhance freshwater aquaculture development should be analysed with regards to the relevance of high productive technologies such as genetic manipulation which are now available. These technologies are vital to aquaculture in terms of productivity, stability and sustainability of production systems. Results of the study show that majority of the farmers have expressed their desire to grow genetically male tilapia. But the equity issues which are equally important in attaining aquaculture sustainability maybe left out by these technologies. Government efforts to promote these types of innovation, therefore, must address the equity aspects in order to enhance their adoption and harness their potentialities. Underlying this strategy is the implementation of programmes to help small farmers mitigate the advantages of large producers such as the building of infrastructures for greater production like irrigation facilities, provision of credit, and making technology information more accessible. Only then can the goal of increased production be achieved more successfully, without violating the principle of distributive justice.

Appendix 9 Copies of publications produced under the project

Appendix 10 A sample of media articles relating to the YY male technology in the Philippines.

Appendix 11 Details and supporting papers for the accreditation of private sector hatcheries under Phil-Fishgen's dissemination programme for the products of the YY male technology.

1. Details of Scheme 1 and Scheme 2
2. Copies of licences and certificates
3. Sample copy of MOA with accredited hatchery

Appendix 1 Capital equipment inventory for TAD project (financial Year 1988-1995)

Description	Date of Purchase	Cost (£)	Serial No.	Location	In-charge
386 computer	03/92	1,104.86	T6230R	FAC-UWS Office	G. Mair
Avid PIT tag reader 2 units	03/93	1,600.00	14109	FAC-UWS Field Office and AIT, Thailand	J. Abucay and P. Tuan
Notebook computer	03/05/94	2,362.00	97-D2AT-2	FAC-UWS Office	G. Mair
Weighing balance (Sartorius)	07/28/94	813.46		FAC-UWS Laboratory	J. Abalos
L-200	03/21/95	13,229.85	Q84/K34TJU NSL	FAC-UWS Office	G. Mair
Scanjet	09/27/95	1,400.00	SG511106D	FAC-UWS Office	G. Mair
Binary coded tagging applicator	23/02/97	3,000	No. 271	FAC-UWS Office	G. Mair

Includes items passed on from previous project (R. 5068A)

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