Genetic Improvement and Utilisation of Indigenous Tilapia in Southern Africa

DFID Aquaculture and Fish Genetics Programme (R 7284)

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Executive Summary

This Project is a collaborative, strategic research activity between the University of Stellenbosch (UoS) in the Western Cape Province of South Africa, and the University of Wales Swansea (UWS), U.K. All research and development activities were carried out in Southern Africa. The overall stated purpose of the Project was to **develop a higher yielding genetically male tilapia (GMT) developed for improved livelihoods in small-scale aquaculture in Southern Africa**. The Project was initiated in December 1998 under the DFID Fish Genetics Research Programme (FGRP) managed by UWS and continued under the DFID Aquaculture and Fish Genetics Programme (AFGRP) managed by the Institute of Aquaculture, Stirling University. Following a 6-month extension, the Project was completed in June 2002. The principal research objectives of the Project and progress towards these objectives are listed below:

1. *To develop an assembly of accessions of up to 15 strains of O. mossambicus from throughout Southern Africa. To conduct growth trials for a minimum of eight strains.* A total of 12 wild caught (indigenous and introduced) and domesticated strains were collected to create a unique live gene bank of *O. mossambicus* germplasm from across its natural range. Two comparative growth performance trials, including eight strains, were completed. Relative growth rates among the strains were consistent throughout all the stages of the culture cycle and four of the strains (all wild caught) were identified as possessing superior growth characteristics and it is recommended that they represent the basis for future breeding programmes do develop superior stocks for aquaculture.

2. *To undertake genetic characterisation of available strains of O. mossambicus using molecular markers to identify, where possible, levels of genetic variability, population structure and stock/species specific markers.*

   Twelve strains were characterised using five microsatellite DNA loci. Levels of genetic variation were higher in wild caught stocks than in domesticated fish and overall a strong population structure was identified providing support to the idea that individual populations may need to be conserved to preserve key reservoirs of genetic diversity in this species. Markers were also used to identify hybrid introgression between *O. mossambicus* and the introduced *O. niloticus* in the Limpopo river system, confirming the threat to the genetic integrity of stocks of indigenous tilapia, posed by the introduction of exotics

3. *The adaptation and development of the YY male technology for production of genetically male tilapia (GMT) to strains of O. mossambicus.*

   Significant progress was made in the development of the YY male technology for production of genetically male tilapia (GMT) in *O. mossambicus* with 20 YY males identified by the Project end. However, unanticipated delays, due primarily to excessive turnover of staff assigned to this work, lead the failure to achieve the planned output in the form of GMT to be used in performance evaluation trials. Sex ratio of GMT was also lower than expectations with a mean of 88% male, indicating that selection for sex ratio will be required in the further development of the breeding programme.

4. *On-farm trials of GMT*

   This activity was not possible due to failure to produce sufficient GMT by the last year of the Project. This activity was replaced by the initiation, in collaboration with the Western Cape Dept. of Agriculture (DA), of a series of pilot studies to more generally investigate key issues in the uptake of small-scale tilapia culture as a component of rural development programmes. Whilst limited data were available from this activity by the Project end, available results did confirm the significant potential for small-scale aquaculture to contribute to the food security and improved livelihoods of members of impoverished rural communities in Southern Africa.

5. *Evaluation of potential social and economic impact of GMT production by small-scale farmers*

   The activity towards this objective was intended to be based on surveys of participants in on-farm trials of GMT, which as again not possible due to absence of available GMT by the final year of the Project. The planned activity was thus replaced by two additional activities. The first of these investigated the sustainability of small-scale aquaculture practices being carried out under the UoS sponsored Small-Scale Aquaculture Programme. The study looked at biological, economic and social sustainability issues. The study indicated the economic viability of the practices although concern persisted over the long term profitability of some of the culture systems. Indicators demonstrated that participants in the programme gained significant improvements to their livelihoods through skills development and enhanced social capital, improved income and better nutrition. Awareness of benefits in the broader community was however not well established and needed to be addressed.

During the 6-month Project extension two supplementary objectives were added, namely:

6. *To the address the current demand for uptake of tilapia technologies, and in particular its extension to other regions of South Africa.*

   This was addressed, to some extent, through the conduct of a number of training courses and the development of some extension material. This training has contributed to significantly enhanced and widespread awareness and understanding of the potential of small-scale aquaculture, particularly of tilapia, as a component of rural development programmes and the livelihood benefits that can result from its adoption.

7. *To initiate the process of developing a strategy/policy for the sustainable development of indigenous aquatic resources, for aquaculture development in the Southern African region.*

   This objective was addressed through the conduct of a one day workshop on the topic in September 2002 attended by 47 concerned participants from nine SADC countries. The workshop highlighted key issues and adopted a modified version of the "Nairobi declaration" as a basis for formulating strategies and policy for sustainable development of indigenous aquatic resources.

Overall four of the five indicators (OVIs) for successful completion of activities were met as were three of the five OVIs for projected outputs. Where activities were not completed substitute activities were carried out, producing alternative but equally valuable knowledge outputs. Some additional, unplanned activities were carried out, particularly with regard to the genetic characterisation of stocks and species and the training and workshop activities completed during the 6-month Project extension.

The Project has had, and undoubtedly will make very significant contributions to developmental impact associated with the management and exploitation of indigenous aquatic resources in South Africa. The accession of strains being maintained as a live gene bank provides a unique resource to backstop this process. The Project has also identified four strains within the accessions of *O. mossambicus* with growth performance traits most valuable for aquaculture and which will form the base of future development of superior and improved breeds of the species. Progress in the development of the YY male technology provide for optimism that near monosex male seed will be available in the near future which will not only enhance production from aquaculture but also mediate to some degree the potential environmental impacts of widespread adoption of the culture of this indigenous tilapia. The molecular genetic characterisation work has provided baseline data on the levels of genetic variability in stocks and will provide a useful management tool for the future. The research also indicated strong population structuring of the species highlighting the need for caution with regard to conserving key reservoirs of genetic diversity and assessing the threat that aquaculture development might pose to these resources. The Project has also contributed significantly to human resource development through the graduation of two master's students, through training of its staff and those of its partners including extension officers from several Provinces, and the training in aquaculture of a small number of farmers.

Research into various aspects of the potential role of aquaculture of tilapia as a component of rural development projects has provided further evidence for the significant potential of small-scale tilapia based culture systems to sustainably enhance the livelihoods of the rural poor through benefits to income, food security, nutrition and social capital. Evidence was also presented for the acceptability of tilapia as a food source among rural poor. Alongside this potential for tilapia aquaculture to play a direct role in rural development there is also a growing interest in tilapia culture by SMMEs[1] with a number of operations being initiated by the Project end. Significant uptake of tilapia culture by this sector will provide indirect benefits to the poor through generation of employment opportunities and through local supply of affordable fish protein.

There is little doubt that, in addition to the potential direct benefits of project outputs, the presence of the Project in the region has acted as a catalyst for, and contributed very significantly to, what appears to be a paradigm shift in thinking concerning the potential for aquaculture of the indigenous tilapia *O. mossambicus* both as a component of rural development programmes and also for uptake by business enterprises. This new paradigm has been exemplified in a number of ways as illustrated by the following examples of impacts of the Project:

- An enhanced knowledge and understanding of aquaculture practices and the potential role it can play in rural development in the Western Cape Department of Agricultural which has now taken on a major role in promoting aquaculture in rural

[1] Small, Medium and Micro Enterprises, an acronym commonly used in South Africa

development and has worked closely with the Project, supported by a project sponsored VSO volunteer.

- The adoption of training programmes based on UoS project related training in several other Provinces in South Africa.
- The recognition of needs among developmental agencies (e.g. DA and Dept. of Economic affairs) and facilitation of specific funding for aquaculture skills training for rural communities
- The commitment of funding for future development of tilapia based aquaculture by various national Departments and Agencies
- The establishment of UoS as a major facilitator of informal national and regional networks of stakeholder partners (e.g. through the Aquaculture Association of Southern Africa) concerned with aquaculture development, further facilitated by UoS connections to international networks which have been greatly enhanced by Project activities.
- The initiation of a new project, funded by a significant grant from the DFID Business Linkage Challenge Fund (BLCF) on "The transfer of Tilapia Aquaculture Technologies for Rural Development in Southern Africa" involving the existing project partners. This project will move forward with a number of the continuing project activities and retain the existing momentum which will help to move the developmental impact from a strategic to an adaptive phase.
- The initiation of a number of new tilapia projects, in many cases supported by UoS and other UoS/project partners. These new projects include a hatchery and separate production facility and several community based tilapia farming system, all in Kwa-Zulu Natal; a regional tilapia hatchery and community based tilapia farming system in Limpopo Province; and a commercial based tilapia farm in Namibia. Other similar proposals are presently under development or submitted.
- Plans for new companies and SMMEs to be involved in the secondary phases of the development of tilapia farming systems (e.g. marketing and processing) with emphases on black empowerment.

For the reasons listed above, the authors believe that the Project will have a lasting development impact which should be assessed after 3-5 years. Project partners will continue to support many of the development processes listed above and the DFID BLCF funded project will greatly facilitate this process. In addition UoS and UWS will continue to work together on strategic research elements of the development process focussed on the further improvement and characterisation of *O. mossambicus* stocks to provide good quality tilapia seed for aquaculture development, whilst minimizing potentially deleterious environmental impacts.

Background and Introduction

Many inhabitants of Southern Africa have very low proportions of animal protein in their diets. For example the South African Medical Research Council has established that malnutrition is evident among both permanent and seasonal labour forces in the Western Cape, especially among children[2]. Also, as we write many parts of the region are suffering famine. Case studies have shown that tilapia is a very acceptable dietary item to such communities and is preferred to other low value fish such as carp. Very modest dietary supplements with fish protein are able to achieve striking results in most underweight children[3]. Also aquaculture remains extremely underdeveloped in the region and with stable or declining yields from once rich fisheries; aquaculture expansion is required in order to meet the growing demand for fish consumption and to provide alternative and diversified livelihood opportunities to resource poor rural farmers.

In many parts of Southern Africa the Nile tilapia (*Oreochromis niloticus*, the commonly preferred tilapia species for freshwater aquaculture) is not endemic but the Mozambique tilapia (*O. mossambicus*) is. Considerations of environmental protection of local biodiversity, which is being threatened by the introduction of exotic species such as *O. niloticus*, have led to the promotion of the Mozambique tilapia as a species that should be suited to small-scale aquaculture in southern Africa.

The species

The Mozambique tilapia, *O. mossambicus*, was one of the first species to be used in aquaculture, mainly in Asia (1940's and 1950's). *O. mossambicus* has subsequently been widely distributed for aquaculture resulting in the establishment of farm and feral populations in more than 61 countries. Establishment of feral populations throughout Asia, has hindered the local fisheries resulting in plans to eradicate unwanted populations in many areas. However, in Sri Lanka *O. mossambicus* became well established in lakes and reservoirs and actually improved the local fisheries.

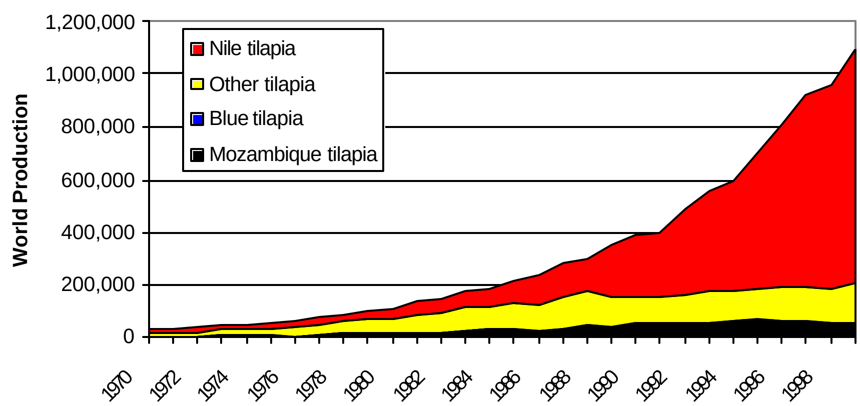
The performance of *O. mossambicus* in aquaculture in most Asian countries has been extremely poor. A small number of originators and lack of stock management led to

[2] Krige, M.U. and Senekal, M. (1997). S. Afr. J. Food Sci. Nut. **9**, 14-23.
[3] Steyn, N.P. et al. (1995). S. Afr. J. Food Sci. Nut, **7**, 158-162

inherent founder effects (loss of genetic diversity) and inbreeding (mating amongst relatives), and is probably responsible for the relatively poor performance of *O. mossambicus* in Asia. It appears probable that only four fish (three males and a single female), from an aquarium population in East Java, may have formed the founder population for the entire Asian *O. mossambicus* aquaculture industry[4]. As a result the Asian strains of *O. mossambicus* are known to be genetically depauperate[5].

A species shift in aquaculture to the better domesticated and faster growing North African tilapia *Oreochromis niloticus* (Nile tilapia) occurred in the 1960's displacing *O. mossambicus* from the majority of aquaculture. Current global tilapia production figures reflect a very strong preference for *O. niloticus* over *O. mossambicus* (Figure 1). Tilapia (predominantly *O. niloticus*) has been found to be an ideal species for low input aquaculture systems and together with carps it forms the mainstay of inland aquaculture in many tropical developing countries, particularly in Asia. Tilapia is also known as the poor man's fish and where it has been adopted for aquaculture it plays a vitally important role in the food security and livelihoods of many hundreds of thousands of rural poor.

Figure 1 World aquaculture production of tilapia species (FAO 2000).



With the possible exception of Egypt, Africa has not participated in what could be considered a "tilapia revolution" despite the species being indigenous to large parts of the continent. With the main aquaculture species being *O. niloticus*, one of the constraints to

[4] Pullin, R. S. V., 1988. Tilapia. Genetic Resources for Aquaculture. ICLARM, Philippines. P107

the development of tilapia aquaculture in Southern Africa are the risks associated with the introduction of this exotic species from Northern Africa. With the poor reputation of *O. mossambicus* as an aquaculture species arising from the unsatisfactory culture performance of the genetically depauperate domesticated strains, mainly in Asia, the species has seldom been considered to have any role to play in aquaculture development in the region to which it is indigenous. Fortunately, undisturbed wild populations of *O. mossambicus* tilapia still exist in their native range in Southern Africa. The potential of wild *O. mossambicus* from Southern Africa as a source of genetic material for developing and supplementing new aquaculture strains, was considered in 1988 as part of a review on wild tilapia genetic resources[6]. Growth performance and appearance of wild Mozambique tilapia in Southern Africa were quoted as being superior to that of Asian introduced populations but little data were presented to back up this hypothesis.

Aquaculture of *O. mossambicus* in Southern Africa

Aquaculture in Southern Africa is extremely undeveloped, contributing to less than 1% of world aquaculture production. There is however a very significant potential for aquaculture in the region at various different levels from intensive commercial production to more extensive, low-input and subsistence production with available and underexploited or unexploited resources. Previous attempts at aquaculture development have met with limited success either due to limited resources and or the extension of inappropriate or unsustainable aquaculture technologies and practices. In South Africa for example there is some intensive production of a high value species such rainbow trout and in the past there has be culture of the African catfish *Clarias gariepinus* which was not sustained due in large part to the lack of a sustainable market for the species.

There is little appreciation in the region, particularly in South Africa, for the potential of lower input, extensive or semi-intensive aquaculture, to which tilapia is particularly well adapted, and could stand to selectively benefit socially and economically disadvantaged sectors or rural and peri-urban communities.

Among the tilapias *Oreochromis mossambicus* has received some interest for aquaculture in Southern Africa due to its favourable growth compared to other indigenous tilapia species in the region. Early attempts at farming with tilapia in Southern Africa were

[5] Agustin, L.Q., Mather, P.B. & Wilson, J.C. (1997) Proceedings from the Fourth International Symposium on Tilapia in Aquaculture (Fitzsimmons, K. ed.), Vol. 1, pp. 75 - 86. Northeast Regional Agricultural Engineering Service (NRAES), New York.

hampered by technical and environmental factors. One of the main obstacles was the prolific breeding achieved through precocious maturity that occurred in dams stocked with both sexes leading to the production of small fish of little market value (see further below). A further limiting factor was the susceptibility of most tilapia species to cold temperatures experienced in the Western Cape and the Highveld during winter. The Department of Inland Fisheries of the Western Cape led a search for cold tolerant species for aquaculture in the Western Cape. A number of tilapia species including exotics such as *Tilapia aurea* (*Oreochromis aureus*), *Tilapia galilaea* (*Sarotherodon galilaeus*), and *Tilapia zillii* were imported from Israel in 1959 and Western Cape farm dams were stocked with tilapia offspring from the Jonkershoek species. Little came of this activity and *Oreochromis mossambicus* was introduced into Western Cape farm dams and coastal lagoons by the Cape Nature Conservation Department (the Western Cape governmental agency responsible for conservation) in the 1960's but again no significant aquaculture production arose although the populations did survive and establish themselves in several water bodies.

One major constrain in *O. mossambicus* mentioned above is that the species suffers from the same disadvantage as the Nile tilapia, namely precocious sexual maturity leading to diversion of energy from somatic growth to reproduction. In grow-out ponds this problem is further compounded by the production of numerous economically undesirable recruits, which compete with the stocked fish for available resources. However, the problem in the Mozambique tilapia would appear to be even greater due to higher fecundity and a greater tendency to stunting. In Mozambique tilapia females have only some 60% of the growth rate of males and males are therefore preferred as the sex for monosex culture. Thus, a reliable and environmentally friendly technology for production of monosex male populations for aquaculture such at the YY male technology (developed under the previous DFID Fish Genetics Research Programme) may be even more important for aquaculture in *O. mossambicus* than it is for *O. niloticus*.

Conservation Issues Surrounding *O. mossambicus* in Southern Africa

The history of *O. mossambicus* introductions in South Africa is documented from the early 1950’s to the 1980’s. Translocation in South Africa took place mainly for utilisation in aquaculture, recreational fishing, and biological control of chironomids and macrophytes. There have however been few studies on the impact that these introductions have had on

[6] Pullin, R.S.V. (ed) 1988. Tilapia Genetic Resources for Aquaculture. ICLARM, 107 pp.

local aquatic biodiversity. The potential negative effects of the local translocation of Mozambique tilapia into areas outside its natural range were considered to be more important than the potential advantages the species may bring in terms of aquaculture and fisheries production (de Moor and Bruton, 1988[7]). Translocation of cultured or non-local *O. mossambicus* populations was also described by the same authors as a threat to the genetic diversity present in local wild *O. mossambicus* populations. Genetic diversity in wild populations was stated as being worthy of protection due to unique qualities such as cold tolerance and high salt concentration tolerance characteristic of certain populations.

Furthermore, the introduction into South Africa of the popular *O. niloticus* species represents a potential threat to natural tilapia populations, since most tilapia species readily hybridise.

Present South African conservation laws restrict translocation of indigenous fish and introduction of exotic fish into natural water systems. One of the biggest potential threats to the genetic integrity of *O. mossambicus* populations, however, arises from the existence of exotic populations of *O. niloticus* already present within the natural range of *O. mossambicus* and some morphological and biochemical evidence of wild F_1 hybrids between the two species in the Limpopo river system has already been documented.

Oreochromis mossambicus is also likely to be threatened by habitat loss or fragmentation through interventions on natural water systems for power, transport, agriculture and water consumption and the effects of human encroachment. The extent of threat to *O. mossambicus* in this regard is not well established.

With the commercial potential of *O. mossambicus* in the southern African region and the knowledge and understanding of the importance of biodiversity that exists in the region (reflecting the importance of ecotourism to the economies of countries in the region) there is a pressing need to characterise the genetic diversity of the species before it is further compromised.

[7] de Moor, J. I. and Bruton, M. N., 1988. Atlas of Alien and Translocated Indigenous Aquatic Animals in Southern Africa. *South African National Scientific Programmes Report* 144. South Africa

At Project inception it was hoped that a project of this nature would not only provide appropriate and sustainable technology, in the form of improved tilapia germplasm suited to a wide range of aquaculture systems, but also act as a catalyst for an enhanced understanding and realisation of the potential for aquaculture of indigenous species such as O. mossambicus in the Southern African region and promote awareness of key issues related to the utilisation, management and conservation of this important indigenous genetic resource.

Project Purpose

The overall purpose of the Project, as defined in the project logical framework, was "**higher yielding genetically male tilapia (GMT) developed for improved livelihoods in small-scale aquaculture in Southern Africa**".

Overall Development Objectives

The broader overall development objective of the Project was the enhancement of livelihoods of rural poor in Southern Africa through:

- The enhancement of production of fish from existing aquaculture facilities
- The sustainable use of existing resources, infrastructure and expertise with the settlement of small scale farmers as independent fish producers
- Building entrepreneurial capacity and improving employment opportunities in an environment of decreasing demand for farm labour.
- The increased availability of affordable fish protein through the expanded culture of low input, low cost species and enhanced fish production.

Specific Project Research Objectives

- To develop an assembly of accessions of up to 15 strains of *O. mossambicus* from throughout Southern Africa. To conduct growth trials for a minimum of eight strains, under communal stocking in two environments, to include farm cages and earthen ponds to determine the relative culture performance of the strains.
- To undertake genetic characterisation of available strains of *O. mossambicus* (a minimum of eight) using molecular techniques (using one or more of the techniques of analyses of microsatellite DNA loci, amplified fragment length polymorphisms and/or restriction endonuclease analysis of mitochondrial DNA haplotypes). Strains were to

be characterised for strain specific markers, levels of genetic variability and population structure.

- The adaptation of the YY male technology to strains of *O. mossambicus* chosen on the basis of growth data and species purity. YY males were to be generated using two approaches: a) the standard breeding programme combining feminisation and progeny testing[8]; b) a more rapid means using androgenesis as developed in Project R4700 by the University of Stirling. It is estimated that androgenesis may enable production of a few YY males in 1.5 generations (1-1.5 years) compared to 2.5 (1.5 - 2.5 years) for the standard breeding programme.
- On-farm trials of GMT were to be conducted on at least 10 farms in the final year of the Project. It was intended that these would be both in cages and ponds. Comparisons were to be made with non-improved, locally available *O. mossambicus* as controls.
- Before and following completion of on-farm trials, participating farmers were to be interviewed to determine their response to the trials. Potential social and economic impacts of the technology were to be determined from participatory research with farmers and potential consumers.

Due to some unanticipated delays in the progress of some activities the Project was extended for six months from January – June 2002 (see proposal in Appendix 5). During this project extension two supplementary objectives were added, namely:

- To the address the current demand for uptake of tilapia technologies, and in particular its extension to other regions of South Africa.
- To initiate discussion that could contribute towards a strategy/policy for the sustainable development of indigenous aquatic resources, for aquaculture development in the Southern African region.

[8] Mair, G. C., J. S. Abucay, D. O. F. Skibinski, T. A. Abella, and J. A. Beardmore. 1997. Genetic manipulation of sex ratio for the large-scale production of all-male tilapia, *Oreochromis niloticus*. Canadian Journal of Fisheries and Aquatic Sciences 54:396-404.

Project Structure and Organisation

This Project was structured as collaboration between the Department of Genetics of the University of Stellenbosch (UoS) and the School of Biological Sciences of the University of Wales Swansea (UWS). The UWS was responsible primarily for coordination and technical expertise whilst all the research work was carried out in Southern Africa, predominantly on the premises of UoS.

Management Structure

Project coordination was carried out by the UWS PIs Graham Mair (GCM - research, reporting and overall coordination) and John Beardmore (JAB - administrative). GCM (based at AIT in Thailand) conducted six monthly review visits, and co-ordinated reporting with Dr. Hoffman (LH), with JAB visiting annually. Dr. R.I. Lewis (RIL) participated in the Project on a full time basis in his capacity as Research Officer (post-doctoral), seconded from UWS to UoS, from the beginning of the Project up to the time that he resigned from the position in February 2001. His responsibilities in project activities included that of management, liaison, reporting and direct responsibility for molecular work. RIL was replaced at UoS by Dr. M.E. D'Amato (MED), who took over responsibility for the molecular work whilst Danie Brink (DB), a UoS faculty assumed primary responsibility for project coordination at UoS.

The UoS staff contingent was supervised by LH (PI) and DB (Director, UoS Division of Aquaculture).

Management activities were co-ordinated at three hierarchical levels:

- International review meetings (6 monthly)
- Local (UoS) management meetings (monthly)
- Local (UoS) research meetings (bi-weekly)

In addition regular contact was maintained between UoS, UWS, and AIT by email, fax, and telephone. The management structure is subject to periodic review.

Personnel

Staff who worked under the Project in different capacities during its full term are listed in Appendix 4. A number of problems arose in the staffing of the Project at UoS with several key staff leaving the Project, most notably RIL. This caused a number of significant delays to some aspects of the Project. Most affected was the breeding programme for the development of YY males which was disrupted several times by staff departures during the Project, including during the project extension. This was a significant factor in failing to meet the projected outputs from this activity.

Project Duration

The Project was originally scheduled to run for a period of 3 years from January 1, 1999. Towards the end of the Project in December 2001 justification was presented for an extension to the Project with some supplementary funding for the UoS component only (see Appendix 5).

Physical Infrastructure

The infrastructure was utilised for the Project, much of which was developed during the Project using both DFID funds and parallel funds from UoS are listed in Appendix 6.

Research Results

Research Output Highlights

- An extensive collection consisting of 12 populations of *Oreochromis mossambicus* has been assembled and the accessions have been maintained and reared through several cycles in captivity. These strains are being maintained as a live gene bank at the facilities of the University of Stellenbosch and undoubtedly represent an important resource for the future in the southern African region and beyond. Molecular analysis has indicated that the average heterozygosity values for the respective strains have been maintained whilst being kept in captivity which has included a domestication process for some strains. However, some changes in allele frequencies are apparent.
- Excellent broodstock conditioning, egg incubation, and nursery facilities have been developed at UoS for tilapia breeding and for the conduct of various types of genetic work. Useful fish production and research facilities have also been developed with a research partner, the Western Cape Dept. of Agriculture.
- The relative growth performance of 10 of the *O. mossambicus* accessions was characterised through the different phases of the life cycle. Analysis of results indicated significant differences between the growth rates of strains with four strains identified as having superior strains growth performance. It is recommended that these strains for the basis of future breeding programmes for the improvement of the species for aquaculture. Three of these faster growing strains were incorporated into the programme for YY-male production and are bred to provide fingerlings for field trials and pilot projects.
- The transfer of YY technology from *Oreochromis niloticus* to *Oreochromis mossambicus* has been relatively successful at all stages of application although proceeding slower than planned. The delays were due in part to problems of continuity of responsible personnel and due to the failure, for technical and administrative reasons, to produce YY males by androgenesis. The delays in the implementation of the breeding programme were responsible for the Project not attaining the projected output of having YY males and GMT available for distribution. A total of 20 YY males were identified by the Project end producing a mean sex ratio

of 88.5% male. Sex ratios were found to be more variable and to deviate further from expectations than was observed in similar work on an Egyptian strain of *O. niloticus* and selection for sex ratio will need to be applied in the further development of this breeding programme. Nevertheless it can be considered that the Project has successfully demonstrated that the YY male technology may be transferred to *O. mossambicus*.

- The transfer of molecular technology from *O. niloticus* to *O. mossambicus* (from a published microsatellite primer database) has proved to be highly successful. Microsatellite loci are highly polymorphic, and show clear differences between strains both in levels of variation and in gene frequencies. DNA based techniques have been successfully applied facilitating non-destructive sampling. These techniques have been successfully used to elucidate the population structure of the species in Southern Africa, to identify the occurrence of hybrid introgression in wild stocks and to characterise the genetic status of base strains with potential for aquaculture.
- On-farm trials of GMT, planned for the last year of the Project, could not be implemented due to the lack of available GMT imposed by the delays in the breeding programme for the YY male technology.
- In the absence of growth trials for GMT a series of pilot studies evaluating the potential for and impact of tilapia aquaculture as a component of rural development projects was implemented with our research partner the Western Cape Department of Agriculture. Several pilot studies were implemented and fish was successfully produced in all. However, due to the coincidental, unexpected and abrupt departure of all personnel involved with these trials, prior to the end of the Project, important information on the relative success, uptake and impact of these pilot studies is not available at the time of writing.
- An assessment of the potential socio-economic contribution of the development of rural aquaculture was carried out through examination of biological, economic, social, production and marketing factors that may contribute to the sustainability of small-scale aquaculture systems. The important biological criteria will be the integrated use of water resources for aquaculture and crop irrigation, which provides the key resource of aquaculture and ample opportunity for sustainable development. Economic analysis of small farmer projects provides evidence (i.e. net present value, internal rate of return, etc) in support of economic sustainability. Long-term profitability is however

expected to come under pressure and emphasis should be placed on ways to reduce input costs (production systems, input commodities) and to improve productivity (skills development, training). The socio-economic expectations of the participants in pilot studies are being met in term of improvement in skills, nutritional status, per capita income and quality of life. An awareness of benefits to the broader community is however not well established and needs to be created in order for the programme to succeed in the long run. With regard to production related factors the study concluded that operational risks (theft, disease, natural, human error, etc.) was relatively low. The maintenance of extension services will however be required. The study revealed that small scale tilapia farming systems have the potential to contribute towards sustainable rural development and improvement of livelihoods of members of disadvantaged communities if a holistic development plan is implemented that takes into account all aforementioned factors related to sustainable development.

Collection and Maintenance of Strains

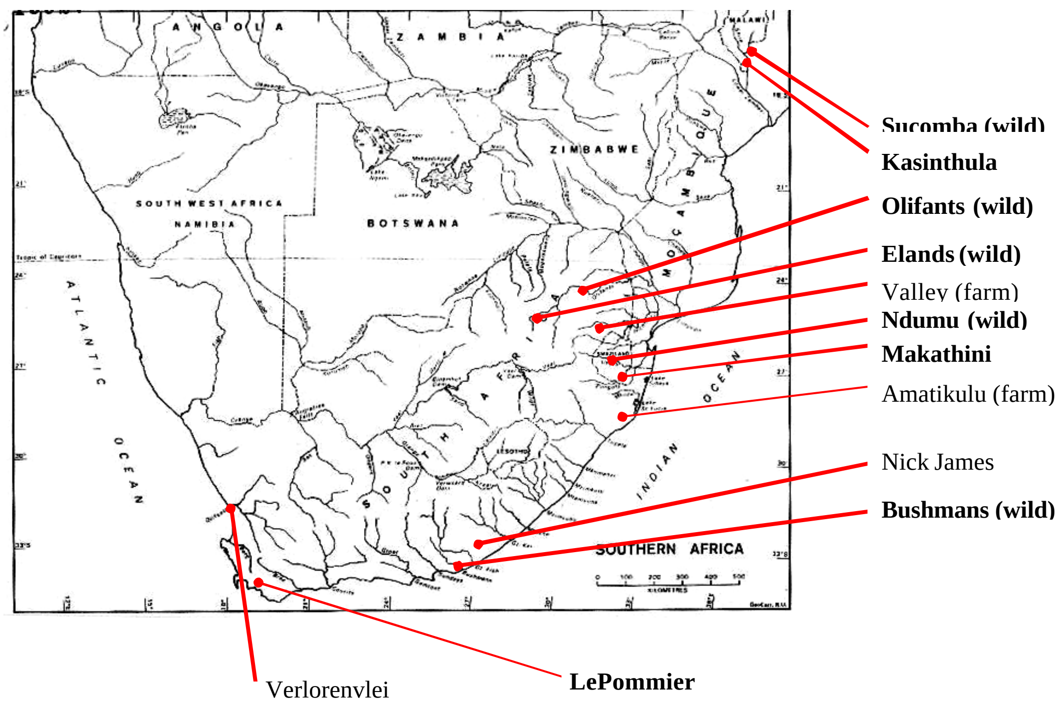
Sufficient live material was been obtained with 12 collections that were made by the UoS at the onset of the Project. In relation to molecular characterisation of stocks[9] there were no major omissions from the live collections in terms of sampling what are considered to be the main reservoirs of genetic diversity for the species.

The molecular characterisation work included tissue samples from four distinct populations that are not included in the live collections. Arrangements have been made to expand the current collection to include further live specimens from Malawi, as well as the Save drainage in Mozambique, and from the SE Natal region.

The collections have been maintained through 2-3 generations using up to 15-20 full-sib families per generation. The stocks have adapted well to conditions at the Welgevallen experimental farm. An analysis towards the end of the Project showed no significant reduction in heterozygosity values and allele frequencies of the current populations ($H_e = 0.731 \pm 0.025$), when compared to the original generations ($H_e = 0.724 \pm 0.017$) indicating that appropriate domestication practices were implemented. The locations of the live collections and the populations sampled for genetic characterisation are shown in Figure 2.

[9] Agustin, L.Q. (1999) Ph.D. thesis, Queensland University of Technology pp. 130 and this project.

Figure 2 Map showing locations for twelve *O. mossambicus* populations sampled for genetic characterisation and from which live gene banks were created at UoS (live gene banks were c reated only for stocks shown in bold).



Growth Characterisation of Strains

The objective of this part of the research programme was to evaluate the culture performance, focussing initially only on growth, of the available strains of *O. mossambicus* collected by the Project. It was initially planned to conduct two or three complete trials, with the first two trials being conducted on-station, under experimental conditions and with the third trial hopefully being conducted under conditions more closely resembling farm conditions. Unfortunately, due to limitations of time, facilities and staff (the staff assigned to the growth trials left the employ of UoS in the second year of the Project) it was not possible to implement the third trial. Also problems were encountered with the grow out phase of the first trial in which the retention rate of marks and tags applied to the fish stocked communally was very low and it was not possible to distinguish the strains and thus no comparative data was obtained.

The methods used in the growth characterisation studies are summarised below but included in more detail in Appendix 7.

- Two trials were conducted A and B

- In trial A, five strains were compared but data was collected only from the initial nursery phase of 3 months with fingerlings reared in $1m^3$ tanks
- In trial B, growth was not monitored during early nursing but during two subsequent growth phases. Eight strains were included in Phase I grow-out and all 10 strain in the Phase II grow-out
- Phase I growth was evaluated in 0.8 m^3 tanks within a recirculating system with two replicate tanks per strain, for a period of 147 days from a mean weight of 6g up to a mean weight of 116g
- Phase II growth was evaluated in 8 m^3 cages stocked in a cover earthen pond with two replicates per strain, for a period of 126 days during which time the fish grew from an average weight of 80g up to an average of 433g.
- Data on final weight, length and average daily weight or length gain were analysed using ANOVA.

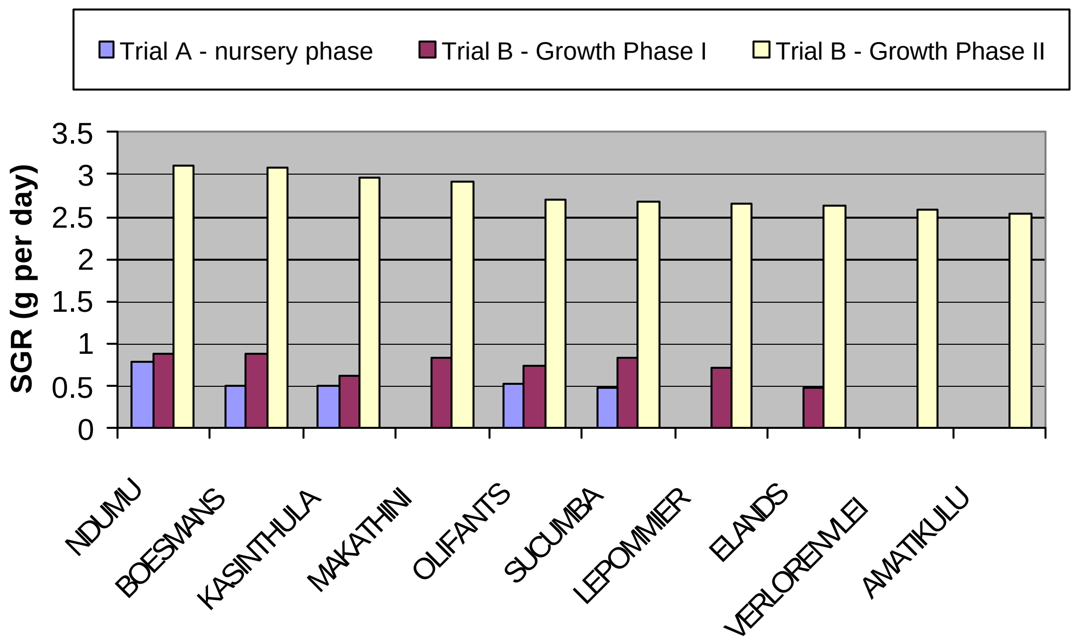
With the two trials considered together it was possible to evaluate the relative growth of most of the strains over three growth phases include an initial nursery phase. The analysis of the results of the trials is show in some detail in Appendix 7 and summarised below including a graph showing comparative specific growth rates in terms of average daily weight gain in the different stages of the two trials (Figure 3).

- In combining analysis of growth performance across the two trials, valid comparisons were obtained for five strains across all stages of the culture cycle.
- Good comparative data were obtained for 8 and 10 strains respectively for growth Phases I and II of Trial B.
- Significant differences in growth rates for the strains were seen at various stages of the production cycle, commonly for weight gain data.
- The trials did not represent common aquaculture environments so results should be interpreted in the context that genotype x environment interactions may change the rankings of the strains in different environments.
- The consistency of the comparative growth performance of the strains at all phases of the production cycle and the relative lack of importance of GxE interactions

seen in other tilapia studies, provide for some confidence in selection of superior strains for future development.

- The Ndumu, Kasinthula, Boesmans and Makathini strains were consistently the fastest growing strains at all stages of the culture cycle in which they were evaluated and should form the base of any future breeding programmes to develop strains for aquaculture.

Figure 3 Bar chart showing the comparative values for Average Daily Weight Gain for the different strains evaluated in the two trials, for different growth phases. Missing bars indicate those strains that were not included in the respective evaluation trials/phases.



The OVI for this activity was met.

YY-male Breeding programme

The objective of this part of the Project was to emulate the work done with *O. niloticus* (Mair *et al*, 1997) to develop YY males for the production of high yielding genetically male tilapia (GMT) in *O. mossambicus*. There were several stages to this process.

Development of XY-females

Feminisation Treatments

Feminisation treatments were undertaken using DES, which was included in the feed at a concentration of 100mg.kg^{-1} and fed to 13 half-sib families obtained from three strains (5-

Ndumu; 5-Kasinthula; 3-Boesmans). The effectiveness of treatments was assayed by determining sex ratios using the gonad squash technique. Sex ratios were highly significantly different (positively skewed to female) from those expected under a null hypothesis of no treatment effect ($P<0.001$), indicating that the DES treatment induced significant feminisation of progeny (Mean % female was 90.9 %, SD=10.53, N=13 families). Results are summarised in Table 1.

Table 1 Proportion of females found from sexing of families subject to hormonal sex reversal to generate XY females.

Strain and Treated Family Code Number	Proportion of females (%)
Boesmans 1	79
Boesmans 2	100
Boesmans 3	92
Kasinthula 1	100
Kasinthula 2	76
Kasinthula 3	100
Kasinthula 4	100
Kasinthula 5	76
Ndumu 1	78
Ndumu 2	98
Ndumu 3	100
Ndumu 4	92
Ndumu 5	92

Progeny testing of candidate XY Females

An initial phase of progeny testing of treated fish from the Ndumu, Kasinthula and Boesmans strains was completed in the first 24 months of the Project. A total 105 candidate XY females were selected and test crossed using normal XY-males, with the progeny being grown-on in glass aquaria. Sex ratios were highly variable and sex ratio frequency distribution did not follow the expected biomodal distributions around 50% and 75% male for XX and XY females respectively except to some degree for the Ndumu strain (Figure 4). Due to the lack of a clear trend in identifying XX and XY genotypes, fairly stringent criteria were applied in assigning genotypes (see Table 2).

Using these criteria, 43 females were considered to be sex reversed XY genotypes, representing 43% of all females progeny tested. Given the success of the original feminisation treatments, with an estimated 41% sex reversal rate, this proportion was in line with expectation. However, given the lack of clear trends in sex ratio frequency distributions, there remained significant probability of incorrect assignment of genotype.

Test crossing of candidate YY-males

A total of 274 potential YY males, derived from crosses of presumed XY females were stocked for progeny testing. A total of 183 males were effectively tested with only 20 (11%) being assigned the YY genotype based on a criteria of a sex ratio significantly different from 1:1, with >80% male progeny in a sample sized >30 (Table 3). This proportion of YY males is lower than would be expected assuming that all females used to produce them were indeed XY genotypes. It thus appears likely that some oestrogen treated females were incorrectly assigned the XY genotype (Table 2), particularly in the Kasinthula and Ndumu strains but it also seems likely that some YY male genotypes produced sex ratios less than 80% male.

Unfortunately, very few identified YY males remain at the end of the Project (only 5). It is clear, given the relatively low proportions of males in progeny of YY males, that it will be necessary to select for GMT sex ratios in the future development of the YY male technology on a commercial scale. Thus future breeding work should focus on this males producing the highest proportions of males in the GMT (circled in the scatter charts in Figure 5). In order to develop the technology on a commercial scale it will be necessary to identify further XY females and YY males by progeny testing in both the Kasinthula and Ndumu strains.

Figure 4 Family sex ratio frequency distributions from the progeny testing of oestrogen treated females for the purpose of identifying XY females expected to produce sex ratios of 75% male. The X-axis represents sex ratio frequency classes with the Y-axis representing no. of families.

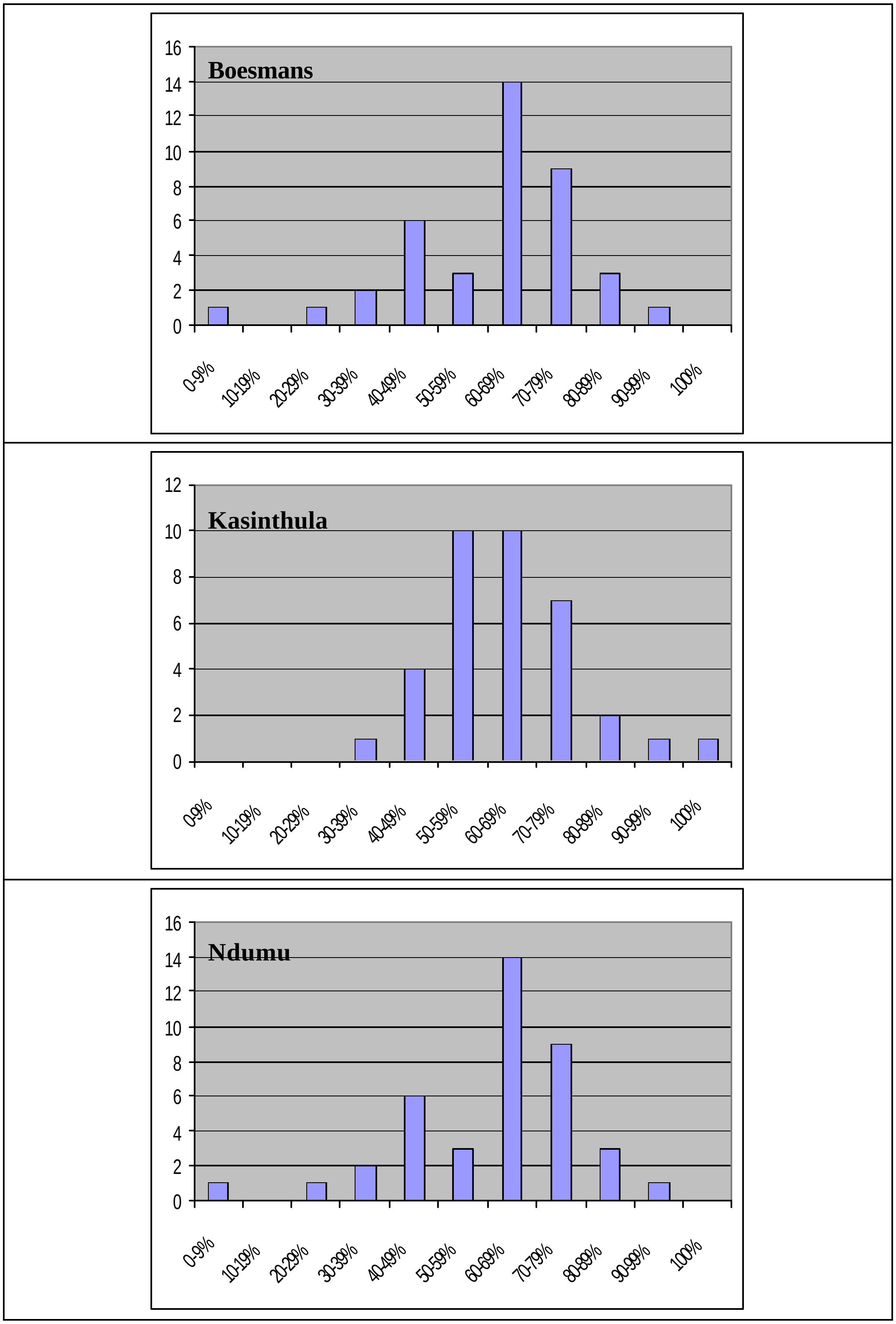


Table 2 Results from progeny testing of oestrogen treated females in three strains of *O. mossambicus* for the identification of sex reversed (XY) females

Strain (and treated family code no.)		Number of females spawned	Number of families sexed (mean and range of sample sizes sexed)			Broodstock assigned as XY		Broodstock assigned as XX	
			No.	Mean	Range	Number ID (surviving[10])	Mean sex ratio and range (% ?)	Number (proportion[11])	Mean sex ratio and range (% ?)
Boesmans	1	21	18	35.8	14 – 106	7 (1)	75 (68 – 86)	11 (61%)	54.4 (34 – 68)
	2	2	2	74.5	53 – 96	0	--	2 (100%)	46.5 (46 – 47)
	3	6	8	31.8	28 – 40	5 (0)	77 (68 – 89)	3 (37.5%)	48.3 (27 – 63)
	6	1	1	82		1 (0)	82	--	--
Sub-total		**30**	**29**	37.6	14 – 106	**13 (1)**	**78 (68 – 89)**	**16** (55%)	**49.7 (27 – 68)**
Kasinthula	**1**	14	6	45.7	25 – 64	2 (0)	68.5 (68 – 69)	4 (67%)	53.8 (50 – 58)
	2	4	6	83.2	29 – 148	2 (0)	78.7 (71 – 84)	3 (50%)	59.3 (53 – 63)
	3	10	9	132.8	69 – 199	3 (0)	76.3 (64 – 96)	6 (67%)	53.7 (44 – 61)
	4	18	4	69.8	30 – 141	2 (0)	75 (72 – 78)	2 (50%)	41.5 (39 – 44)
	5	11	11	41.8	25 – 110	4 (0)	74.8 (72 – 79)	6 (55%)	55.5 (43 – 63)
Sub-total		**57**	**36**	75.2	25 – 199	**13 (0)**	**74.7 (64 – 96)**	**21** (58%)	**52.8 (27 – 68)**
Ndumu	**1**	4	4	71.5	21 – 118	3 (0)	78.3 (70 – 95)	1 (25%)	52
	2	10	6	42.7	25 – 89	3 (0)	71.3 (65 – 78)	3 (50%)	50.7 (28 – 64)
	3	12	7	53.9	25 – 91	3 (0)	71.4 (69.2 – 73)	4 (57%)	54.2 (44 – 62)
	4	19	13	46.8	25 – 137	5 (2)	73.2 (68 – 84)	8 (61%)	56.5 (45 – 69)
	5	13	10	45.9	19 – 112	3 (1)	81.2 (77 – 85)	7 (10%)	41.1 (0..03 – 68)
Sub-t**otal**		**68**	**40**	49.7	19-137	**17 (3)**	**75.1 (65 – 95)**	**23** (57%)	**50.9 (0.03 – 69)**
Grand total		**105**	**105**	**55.1**	**14-199**	**43 (4)**	**75.9 (64 – 96)**	**60 (57%)**	**51.2 (0.03 – 68)**

Criteria for classification of XY genotypes: If n is available, conduct Chi-square test against a 1:1 ratio with XY classed as significant $?^2$ with $p < 0.001$

If n is not available XY = > 68% male with n > 30

[10] Number surviving at the end of the project.

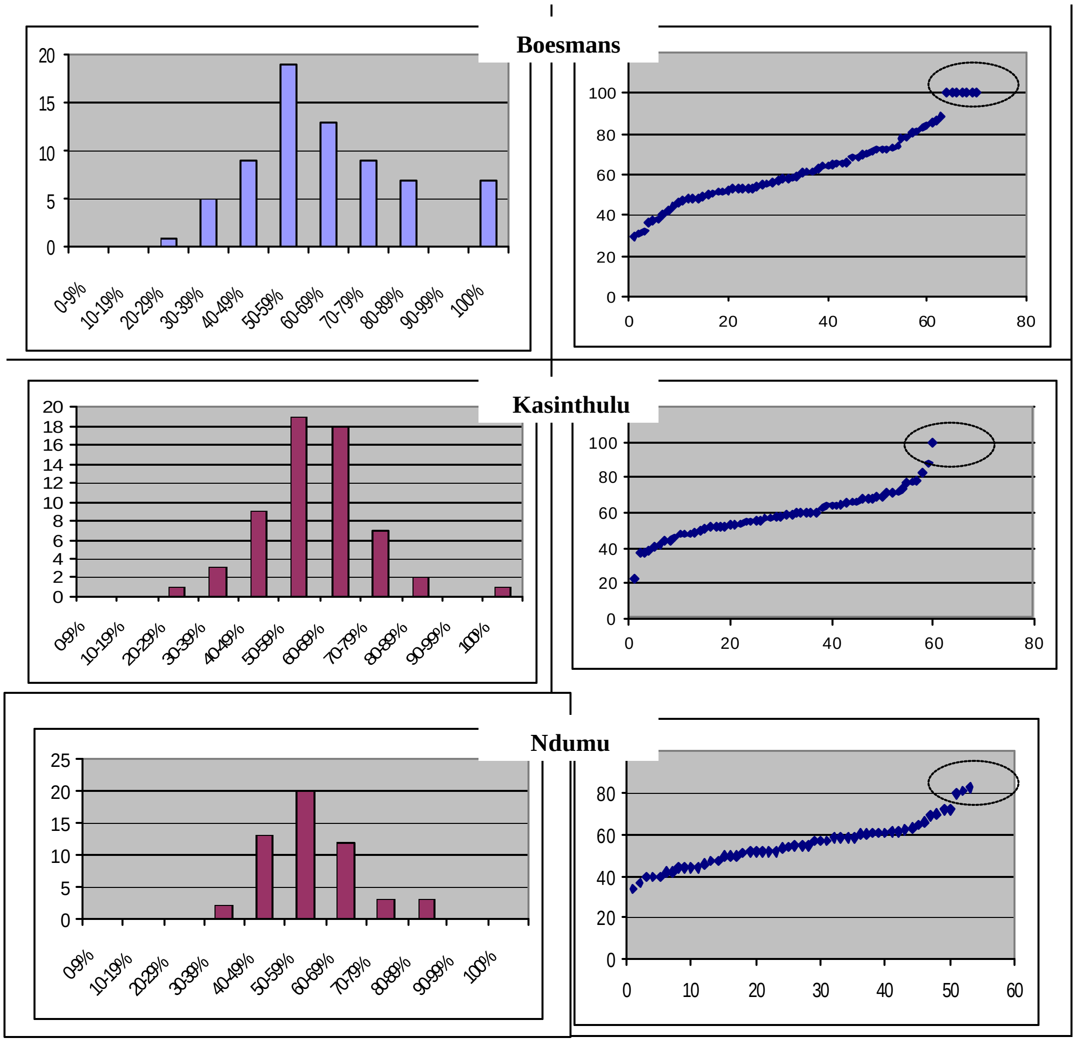
[11] Proportion (%) of normal XX females in those fish effectively tested. The remaining proportion were classed as sex reversed XY females.

Table 3 Results from progeny testing of males from crosses of probably XY females (XY x XY) in three strains of *O. mossambicus* for the identification of YY males

Strain		Num ber of males bred (tested)	Progeny results		Broodstock assigned as YY		Broodstock assigned as XY	
(original treated family number)			No. of families sexed	Mean and range of sample sizes sexed	Number (Surviving)	Mean sex ratio and range ((% ?)	Number (proporti on)	Mean sex ratio and range (% ?)
Boesmans	1	63	34	72.3 (23 – 276)	6 (1)	96.8 (80.5 – 100)	28 (82%)	54.8 (29.6 – 77.5)
	3	29	24	58.3 (25 – 168)	5 (1)	90.6 (83 – 100)	19 (79%)	56.1 (31 – 78)
	6	10	6	41.2 (25 – 68)	2 (2)	84.5 (80.9 – 88)	4 (67%)	59.9 (42 – 72)
Other		7	6	33.2 (26 – 45)	1 (0)	88.0	5 (83%)	65.3 (55 – 57)
Sub-total		**109**	**70**	**51.5 (23 – 276)**	**14 (4)**	**90.0 (80.5 – 100)**	**56 (80%)**	**59.0 (29.6 – 78)**
Kasinthula	1	6	3	31.3 (29 – 36)	0	0	3 (100%)	63.7 (53 – 72)
	2	34	25	83.3 (25 – 372)	1(0)	83.0	24 (96%)	58.8 (38 – 78.3)
	3	11	9	35.8 (25 – 69)	0	0	9 (100%)	50.3 (22 –64)
	4	8	4	44.1 (27 – 49)	0	0	4 (100%)	61.8 (41 – 71)
	5	19	9	43 (25 – 139)	0	0	9 (100%)	56.0 (42 – 67.8)
Other		6	10	44.4 (25 – 110)	2 (0)	94 (88 – 100)	8 (80%)	57.3 (44 – 71)
Sub-total		**84**	**60**	**47.0 (25 – 139)**	**3 (0)**	**88.5 (83 – 100)**	**57 (95%)**	**57.9 (22 – 78.3)**
Ndumu	1	19	14	49.9 (25 – 272)	3 (1)	81.3 (80 – 83)	11 (79%)	57.9 (46 – 70)
	2	11	9	68.1 (25 – 160)	0	0	9 (100%)	48.6 (34 – 62)
	3	4	4	38.8 (25 – 73)	0	0	4 (100%)	56.8 (52 – 63)
	4	22	22	30.5 (22 – 50)	0	0	22 (100%)	53.6 (47 – 72)
	5	24	3	107.7 (27 – 269)	0	0	3 (100%)	52.6 (50.9 – 55)
Other		1	1	112	0	0	1 (100%)	58.9
Sub-total		**81**	**53**	**67.8 (22 – 272)**	**3 (1)**	**81.3 (80 – 83)**	**50 (94%)**	**54.7 (34 – 72)**
Grand total		**274**	**183**	**54.9 (22 – 372)**	**20 (5)**	**88.5 (80 - 100)**	**163 (89%)**	**57.7 (22 – 78.3)**

Criteria for classification of YY genotypes: Sex ratio > 80 % male, with n > 30

Figure 5 Sex ratio frequency distribution and scatter charts of sex ratio in the progeny testing of potential YY males produced in crosses of sex reversed XY females in three strains of *O. mossambicus*.



Development of YY males by androgenesis

RIL attended training in androgenesis techniques at the Institute of Aquaculture, University of Stirling during the first year of the Project. Following this a comprehensive set of infrastructure for temperature control and androgenesis experiments were developed at the Welgevellen station in Stellenbosch which was completed in the early part of the second year of the Project. These consist of five CT rooms, each fitted with eight 160 l tanks as part of a recirculation system, as well as an incubation unit. A total of 16 females and 16 males were successfully conditioned with the females adopting two to three week spawning cycles. However, attempts at hand stripping were unsuccessful with very low fertilization rates achieved. This may have been associated with a combination of the inexperience of researchers and a lack of adaptation to the aquarium environment in the newly domesticated *O. mossambicus* stocks. Further delays in the androgenesis programme were encountered when efforts of staff were temporarily diverted to the YY male breeding programme in an attempt to make up time. Following this, the resignation of RIL from the Project in February 2001 resulted in the loss of the critical expertise gained from the original training. Given that the potential time advantage associated with producing YY males by androgenesis had already been obviated by this time and given the potential drawbacks of inbreeding in androgenetic fish, it was decided, at the beginning of the third year of the Project, to temporarily abandon this approach as part of the breeding programme for producing YY males. Following completion of his M.Sc. at UoS, EH completed a training programme in androgenesis at the University of Stirling during his British Council sponsored visit to the U.K. in June 2002, thus there remain options to resume this programme at a future date.

The next phase of the breeding programme

The next phase of the breeding programme is to increase the proportion of YY males in the progeny by crossing identified YY males with previously identified XY females in the same strain. Part of these progeny should then be feminised using oestrogen treatments. A total of 10 YY x XY matings were set up towards the end of the Project. Some of the broodfish were killed by fighting and only two spawnings were obtained. One spawning between a female which had produced 63% male progeny (against and expectation of 75%) with a YY male which had produced a progeny sex ratio of 88% male (against and expectation of 100%) produced progeny with a sex ratio of 100% male (n=110). This would appear to confirm the assumptions regarding the parental genotypes of this cross and it is expected that 50% of these progeny are YY male genotypes, which will require to be progeny tested. A second successful cross (repeated) between assumed XY x YY genotypes produced sex ratios of only 55 and 62.5% male indicating that the assignment of genotype on one of the parents (probably the YY male) was incorrect.

Several more XY x YY crosses are required to take the breeding programme forward with a reasonably broad genetic base.

Summary

The progress in the breeding programme towards the generation of YY males and the mass production of GMT was somewhat disappointing. The intended output from this part of the programme was to be GMT which would be produced in time (albeit from a limited number of YY males) that they could be used in growth evaluations in the final year of the Project. The main reasons for the slower than anticipated progress included the unexpected turnover of staff disrupting continuity and resulting in delays while new staff were trained. In addition the failure to effectively induced androgenesis meant that this faster path to producing YY males could not be explored. In addition, greater than expected variability in sex ratio of sex manipulated *O. mossambicus* making it more problematic to identify XY females and YY males with a high degree of confidence

Nevertheless a significant number of XY females and YY males were identified and UoS intends to continue with the breeding programme to develop the technology to the next stage whereby GMT could be produced on a commercial scale.

Points of note are:

- High rates of feminization was successfully achieved for all families treated
- XY females were identified by progeny testing in expected proportions
- The proportion of YY males identified was below expectation, indicating possibly lower viability of these genotypes or more likely incorrect assignment of the XY genotype to some of the oestrogen treated maternal parents and highly variable sex ratios in YY males.
- Results to date indicate that the technology can still be viable in this species but further breeding and selection against sex modifying factors will be required.

The OVI for this activity was met within the timeframe of the Project but later than projected in the logical framework.

Molecular Characterisation of Strains

A total of 12 populations of *O. mossambicus* from the Southern African region were included in the molecular genetic characterization of strains. These include all the strains that formed part of the growth characterization (see Figure 2).

This work was carried out primarily by Edward Hall (EH), an M.Sc. student at UoS, under the supervision of RIL. Full details of the analysis are given in the report in Appendix 8 and highlights are listed below.

Objectives

1. To evaluate molecular genetic diversity within and between populations of *Oreochromis mossambicus* throughout its natural range for both microsatellite, and mitochondrial DNA markers.

2. To undertake a phylogeographic interpretation of genetic diversity patterns in conjunction with known histories of population movements, to identify populations of greatest conservation value and of greatest potential for utilisation in aquaculture.

3. To evaluate the utility of microsatellite nuclear markers as a tool for informing an existing quantitative genetic breeding programme. To investigate such issues as parental contributions in communal spawning environments, and unambiguous parental identification in mixed family rearing experiments.

Microsatellite Analysis of Collected Stocks

Materials and Methods

- Some preliminary work was carried out using RFLP of mtDNA but microsatellite analysis was selected as the preferred methodology to meet the objectives

- Five microsatellite primers, originally developed for *O. niloticus* were used

- DNA was extracted from fin clips taken from a minimum of 9 individuals (average of 24, maximum 36) from the 12 strains. Four strains were domesticated farm stocks, whilst among the wild caught stocks six were indigenous and two were introduced

- PCR products were run on ABI sequencer with four loci run in Stellenbosch and the fifth from UWS.

- Locus Diversity, population diversity, genetic structure, gene flow, genetic distance and phylogeography, genetic drift in captivity, and Microsatellite allele inheritance and frequency of null alleles analyses were carried out using various software

Results

- The five loci had between 3 and 17 alleles with an average of 11.4. This is higher than average for freshwater fish.
- The average heterozygosity and allelic diversity of the wild stocks were slightly higher than the introduced (i.e. wild but non-indigenous) stocks with the domesticated farm stocks having much lower levels. This indicates loss of diversity through genetic drift and possibly inbreeding in domesticated stocks.
- There were observed heterozygosity deficiencies in all stocks which may be indicative of the presence of null alleles, inbreeding in some stocks and population sub-structuring.
- All five loci revealed significant allele frequency heterogeneity between the populations with the F_{ST} value of 0.27 and R_{ST} of 0.29 ($P < 0.001$) being typical of a high degree of population structuring.
- Interpretation of gene flow analysis provided some support to the hypothesis that northern populations are ancestral to southern ones with migration occurring in southward flowing coastal currents in southeast Africa.
- The Neighbour Joining trees (see Appendix 8) depicted population clusters related to but not exclusively linked with the geographic location of the source populations. Genetic drift during time spent in captivity may have obscured some of the original genetic relationships between the populations
- Some inferences can be made regarding the possible origin of domesticated and farm stocks
- The current level of resolution from the analysis of the twelve *O. mossambicus* populations is not sufficient to accurately classify evolutionary significant units (ESUs) or management units (MU's) which could inform conservation strategies, due to the lack of demographic history for the populations. However, the fact that clear population structuring exists supports the hypothesis that protection of habitats and river systems in which the wild

source populations of *O. mossambicus* occur is necessary and important to the conservation of genetic diversity in the species.

Conclusions

The analysis of genetic structure and diversity provided evidence for high levels of variation within and between the twelve *O. mossambicus* populations. The populations therefore represent genetic resources from a conservation perspective and a sustainable aquaculture standpoint. Genetic diversity within the populations varied according to origin. Farm populations had reduced levels of variation compared to populations from wild and introduced sources. Introduced populations had intermediate levels of variation. This emphasises the need for domestication to be carried out following good principles of broodstock management.

The reduced heterozygosity observed for most of the populations may merely be a consequence of a high prevalence of null alleles although it is recommended that the populations be monitored occasionally to detect possible reduction in heterozygosity through inbreeding and genetic drift. The data presented in this study provides a basis for conservation and exploitation issues pertaining to wild and captive populations of *Oreochromis mossambicus*.

Recommended areas for future research include the continued monitoring of genetic variation in Mozambique tilapia strains and molecular characterisation of additional populations from the wild Mozambique tilapia distribution to enhance phylogeographic interpretations.

Microsatellite Analysis – Species Specific Markers to Identify Hybrid Introgression

This was a supplementary study additional and complementary to the study on the collected stocks described above.

This study used molecular markers to examine the issue of hybrid introgression between the introduced *O. niloticus* and the indigenous *O. mossambicus*. The introduction of *O. niloticus* threatens the genetic integrity of indigenous stocks and may even threaten the survival of the species in freshwater environments if it successfully colonises Southern African rivers.

Objectives

The broad objectives of this study were to determine the status of the endangerment of indigenous *O. mossambicus* through introgression with the introduced *O. niloticus* using species specific genetic markers to identify hybrid introgression. The study was carried out by Marna Esterhuyse, an M.Sc. student at UoS under the supervision of DEM (UoS) and Filip Volkaert (Universit of Leuven) and the specific objectives were:

1. To identify microsatellite markers that can be diagnostic between species
2. To use the above markers to determine whether naturally occurring hybrids are present in an where *O. niloticus* has been introduced
3. To formulate recommendations on the use of genetic markers in the in the conservation of indigenous fish species

Materials and Methods

Nine stocks were analysed in this study, five stocks of indigenous *O. mossambicus*, including one from the Limpopo River, four stocks of *O. niloticus* and one from a hybrid zone where *O. niloticus* was known to have been introduced. The hybrid samples were collected from the Limpopo River by an experienced researcher who predicted, based on the colour and morphology of the fish, that they were indeed naturally occurring hybrids. (i.e. resulting from natural reproduction within the river system).

Table 4 Samples collected for this study on hybrid introgression. N refers to the number of individuals in that sample; date refers to the year in which the sample was taken. * indicates where information was not available

Species	Sample						
	Abbr.	Location	Drainage.	Co-ordinates	Wild/farm population	N	Date
Oreochromis mossambicus	MWLI	Samaria/Levuvhu	Limpopo river	22° 16' S 29° 16' E	Wild	21	2000
	MWBO	*	Boesmans river	33° 30' S 26° 30' E	Wild	10	1999
	MWND	Ndumu	Pongola/Usuthu river	26° 45' S 32° E	Wild	9	1999
	MILE	Le Pommier	*	34° S 18° 45' E	Introduced	7	1999
	MWKA	Kasinthula	Shire river	17° S 35° 30' E	Wild	14	1999
Hybrids	HWLI	Samaria/Levuvhu	Limpopo river	22° 16' S 29° 16' E	Wild	7	2000
Oreochromis niloticus	NFTH	Thailand	AIT	*	Farm	20	1999
	NFPH	Philippines	CLSU	*	Farm	20	2001
	NWE1	Egypt	Nile river	*	Wild	21	2001
	NWE2	Egypt	Nile river	*	Wild	16	2001

- In attempting to find genetic markers to distinguish between the two species of tilapia 20 microsatellite dinucleotide (CAn) repeats were tested during a preliminary study and of these five were found promising in that they exhibited little intra-specific genetic diversity but large genetic variation between species.
- These five loci were amplified in 145 individuals from the 10 populations, which included the two species and their indicated hybrids.
- Overall the *O. niloticus* stocks had higher allelic diversity, particularly among the wild caught Egyptian stocks. The domesticated stocks of *O. niloticus* from Thailand and the Philippines in particular had low levels of allelic diversity.
- Between the two species, allele sizes were overlapping, but when data were analyzed by statistical models, the differences could be seen for populations but not for individuals (see Figure 6 and Figure 7).

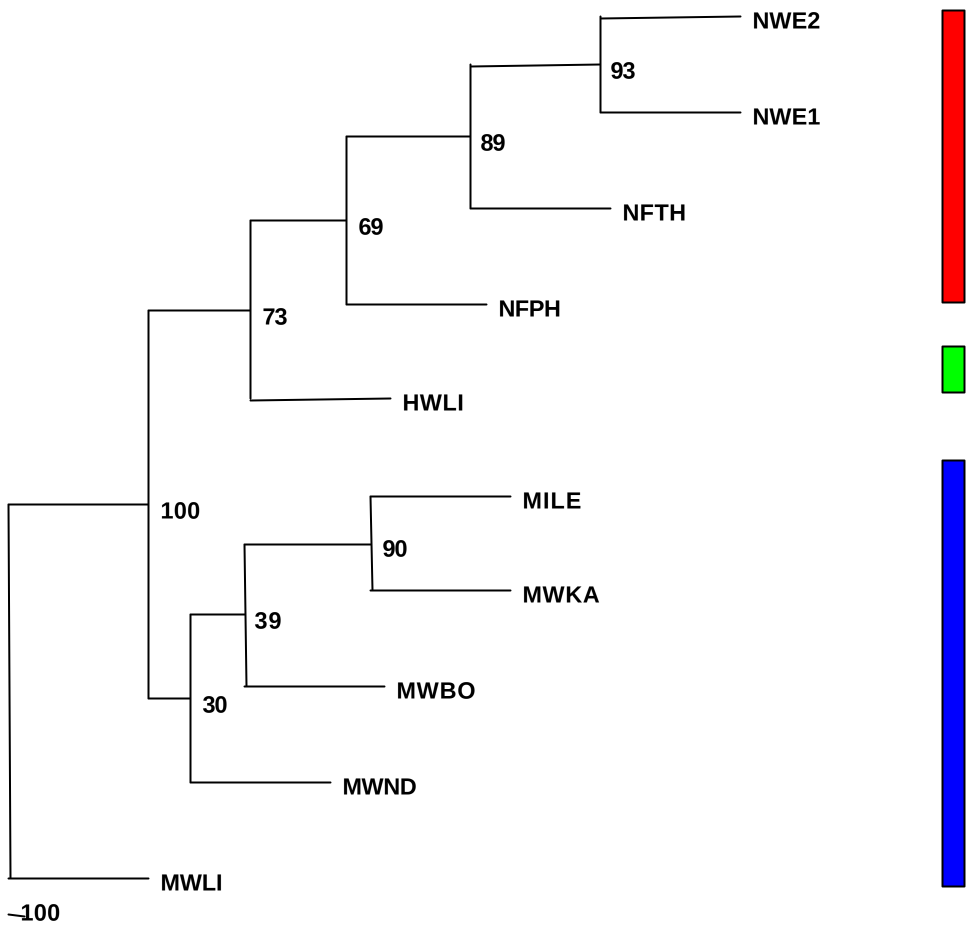
Table 5 Number of alleles for five microsatellite loci in *O. mossambicus,* *O. niloticus* and their putative hybrids. Values in parentheses indicate the number of individuals per sample amplified. Common alleles refer to the number of alleles with a total frequency of at least 5%. Numbers in brackets after these values indicate the total number of alleles over all populations.

Population		Total N	Number of alleles					Mean no.
			UNH102	UNH124	UNH129	UNH146	UNH192	of alleles
O.moss	1	21	4 (21)	4 (18)	3 (21)	3 (21)	5 (20)	3.8
	2	10	4 (10)	4 (10)	2 (8)	2 (10)	3 (10)	3.0
	3	9	2 (7)	3 (9)	2 (8)	3 (9)	1 (9)	2.2
	4	7	4 (4)	3 (5)	2 (6)	3 (7)	4 (7)	3.2
	5	14	6 (13)	4 (12)	3 (12)	3 (12)	3 (13)	3.8
	Total		**6 (55)**	**6 (54)**	**3 (55)**	**3 (59)**	**8 (59)**	**5.2**
Hybrid	6	7	2 (4)	5 (4)	2 (5)	2 (5)	4 (7)	3.0
O.nil	7	20	2 (17)	5 (18)	2 (17)	4 (17)	5 (20)	3.6
	8	20	1 (10)	2 (11)	2 (19)	2 (20)	3 (20)	2.0
	9	21	4 (13)	7 (12)	4 (16)	5 (15)	5 (17)	5.0
	10	16	1 (15)	7 (16)	5 (16)	4 (16)	5 (16)	4.4
	Total		**4 (55)**	**11 (57)**	**6 (68)**	**7 (68)**	**8 (73)**	**10**
Common alleles			4 (7)	8 (11)	4 (6)	4 (7)	7 (9)	

In conclusion, the five microsatellite loci, UNH102, UNH124, UNH129, UNH146 and UNH192 are enabling us to discriminate between populations from the species *Oreochromis mossambicus* and *O. niloticus*. However, it is not possible to use these markers to identify individual hybrids since there is still uncertainty of the position of hybrid populations in the various analyses which it is possible to perform.

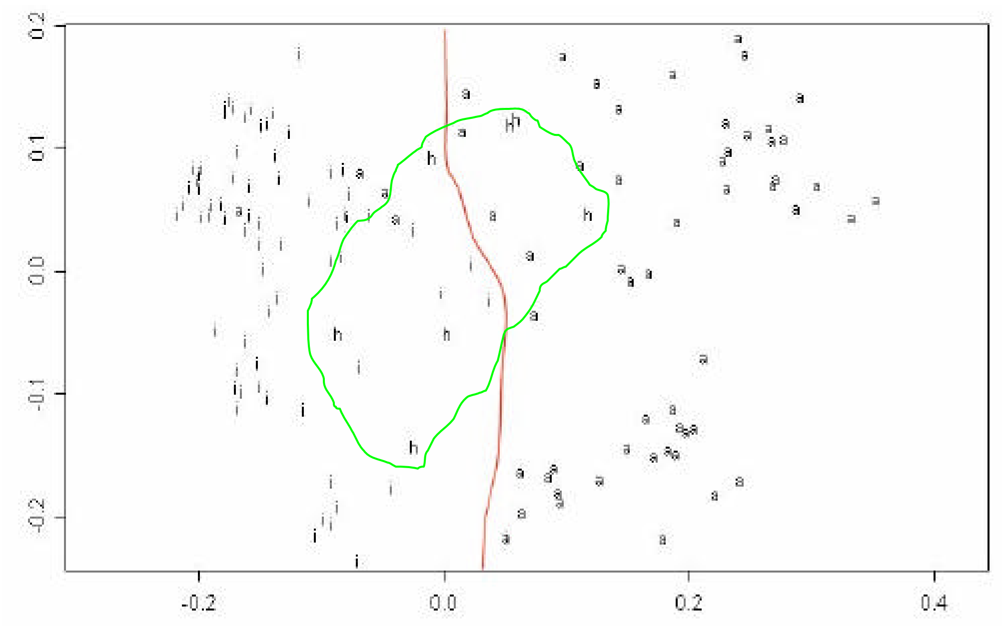
The results indicate that the natural drainage systems of Southern Africa, in particular the Limpopo, is being invaded by *O. niloticus* and that natural hybridisation and introgression is occurring. Some fish samples which morphologically resembled *O. mossambicus*, appear, based on our markets to be of hybrid (F_3 or F_4) origin, indicating quite long term introgression..

Figure 6 An UPGMA consensus tree (unrooted). The red line on the right of the picture indicates the populations of *O. niloticus*, the green that of the hybrids and the blue line indicates the populations of *O. mossambicus*.



Finally, it is recommended that the set of markers used in this study need to be expanded, in both the type of marker system and the number of markers used in order to improve the resolution of this method in species and hybrid diagnostics. Ideally species specific alleles should be identified.

Figure 7 Multidimentional scaling plot based on the genetic distance as proposed by Cavalli-Sforza[12] (1967). "a" Refers to individuals belonging to *O. mossambicus*, "h" refers to hybrids and "i" refers to individuals of *O. niloticus*. The green line crops the hybrid populations.



Microsatellite Analysis – To Monitor Impacts of Domestication

This was a further supplementary study additional and complementary to the study on the collected stocks described above.

This study was carried out by EH at UoS. This was a preliminary study to determine if microsatellite markers could be sensitive enough to detect changes in allele frequencies during the process of domestication of the strains at UoS.

At the time this analysis was conducted most of the populations of *O. mossambicus* had been in captivity for one and a half to two years. During this period the populations underwent periodic population size reductions due to natural deaths and unforeseen infrastructure failures. The original populations were also allowed to propagate according to a random mating protocol. This practice led to the presence of overlapping generations in the tanks and to the potential loss of genetic diversity over time due to the possibility of unbalanced sex ratios (decreasing effective population size and increasing the rate of genetic drift), and possible captive bottlenecks.

[12] Cavalli-Sforza, LL & Edwards, AWF (1967) Phylogenetic analysis: models and estimation procedures. *American Journal of Human Genetics*. 19:233-257.

Objectives

The broad objectives of this study was to identify any changes in allele frequencies in one generation of domesticated breeding in the facilities of UoS. Specifically to determine the changes in allele frequencies for two microsatellite primers in individuals from successive generations of maintenance of the live gene bank in the Boesmans strain of *O. mossambicus*.

Materials and Methods

The first sample of 25 fish were taken from the Boesmans population in March 1999, consisting of original fish collected from the wild. A second sample of 36 fish from six randomly chosen families bred using the wild caught broodfish, was taken in March 2000 for comparison with the sample of March 1999. Samples were compared for significant changes in allele frequencies and heterozygosity. This was done using fluorescent labelling of PCR products for the UNH104 and UNH123 loci on the ABI377 automated sequencer using methods described elsewhere in this report.

Results

Successive calculation of genetic variation and diversity between the years 1999 and 2000 for the Boesmans population showed significant changes in allele frequency at the UNH104 (P = 0.0007) and UNH123 (P = 0.0002) loci (see Figure 8 and Figure 9). One low frequency allele present in the 1999 sample was absent in the 2000 sample at the UNH104 locus. Six low frequency alleles present in the 1999 sample were absent in the 2000 sample at the UNH123 locus.

However, observed heterozygosity increased significantly between 1999 and 2000 based on a t-test for significant change in mean observed heterozygosity (P = 0.003). There were no significant differences in expected heterozygosity between the 1999 sample and the 2000 sample after using a t-test (P = 0.631) (Table 6).

Table 6 Table of *t*-test for change in mean expected heterozygosity and mean observed heterozygosity between the 1999 Bushmans and 2000 Bushmans samples

	Expected heterozygosity		Observed heterozygosity	
Locus	***1999***	***2000***	***1999***	***2000***
UNH104	0.6863	0.6771	0.0588	0.1111
UNH123	0.7422	0.7862	0.4762	0.5294
***t*-Test: Paired two sample test for change in expected heterozygosity**				
	1999	***2000***	***1999***	***2000***
Mean	0.71425	0.73165	0.2675	0.32025
Variance	0.001562	0.005951	0.087111	0.087487
Df	1		1	
Probability (*P*)	0.631221		0.002715	

These results indicate that the broodstock management practices at UoS are resulting in changes in allele frequencies, as might be expected. Focus on other elements of Project work and limitation of both space and staff time capacity impose some restrictions on the effective management of the stocks. It is clear from these results that greater attention should be taken to stock management of the live gene banks of *O. mossambicus* strains in the future in an effort to maximise effective populations sizes which will minimize further changes in allele frequencies and in particular minimize further loss of rare alleles.

Figure 8 Allele frequencies at the UNH104 locus for the 1999 and 2000 samples of the Boesmans population maintained in the UoS hatchery.

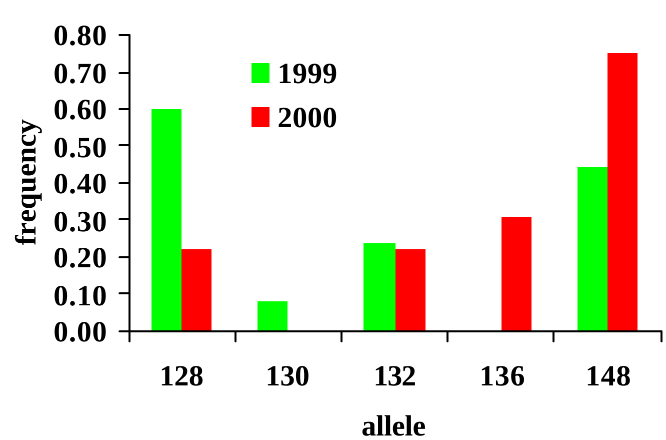
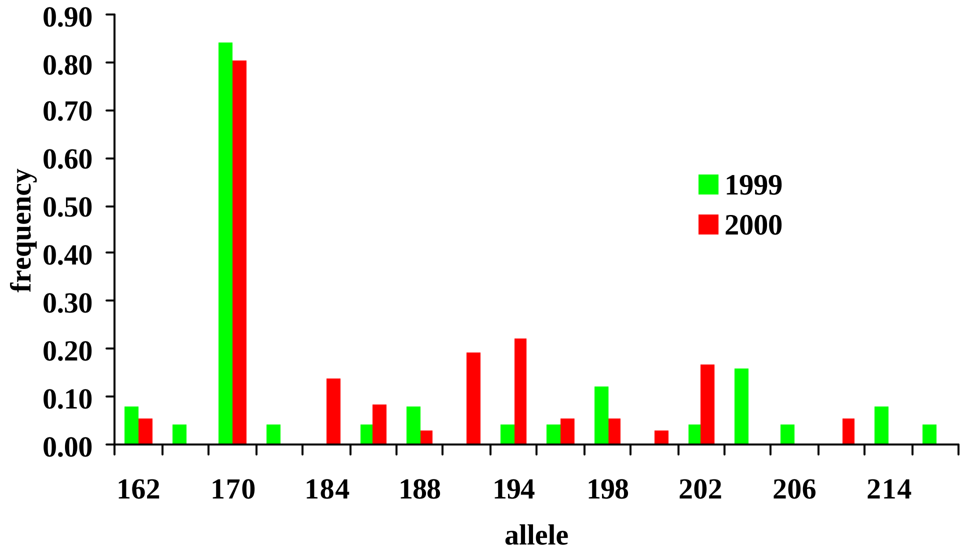


Figure 9 Allele frequencies at the UNH123 locus for the 1999 and 2000 samples of the Boesmans population maintained in the UoS hatchery.



The OVI for this activity was met and a number of additional activities were completed.

On-Farm Trials

It had been planned to conduct a serious of on-farm trials of GMT in the final year of the Project, most likely with farmers in the UoS Small Scale Farmers Project. However, given that the breeding programme for the production of YY males did not reach the point at which GMT could be produced in any significant numbers, this activity could not be implemented. In place of these trials the Project worked closely with the Department of Agriculture (DA) of the Western Cape in establishing a series of pilot studies on the culture of tilapia as a component of DA rural development activities. These activities had commenced by the Project end but we are not able to secure details of the progress of these activities as all personnel involved with the activity (Paul King, Jocob Swart of the DA Robert Hartnell, a VSO volunteer) by unfortunate coincidence, left their positions within a short period of time towards the end of the Project and without producing a report on this activity. We are endeavouring to determine the progress and will report any significant elements of this subsequently. All that is known at present is that the following pilot studies were implemented with minimal summary details of progress recorded. There is no information currently available on the relative success, continuation and sustainability of these projects.

1. Leliefontein, Namakwaland Northern Cape Province

Operating System: Open water system, irrigation dam

Data: 15 000 fingerlings were stocked in October 2000. Gillnet harvesting systems were introduced in March 2001. Fish in a size range of 180 to 400g grams were harvested. Local inhabitants managed to collect between 120 and 150kg of fish per week during the period March to April 2001.

2. Matjiesrivier, Oudshoorn, South Western Cape

Operating System: Pond systems, 4 x 600 m^3 ponds

Data: 6 000 fingerlings (15g) were stocked in September 2000. Pond fertilisation was applied with supplementary feeding of commercial feeds during the latter stages. Fish were harvested in March to May 2001. Fish in a size range of 250 to 450g grams were harvested with seine nets. A total harvest of 1,830 kg was recorded.

3. Worcester, Western Cape

Operating System: Cage farming system, irrigation dam (2 x 400 m^3 cages)

Data: 4 000 fingerlings (20g) were stocked in October 2000. Artificial tilapia feeds were used. Fish of a size range of 230 to 420g grams were harvested during March to May 2001. A total harvest of 1 460 kg were recorded.

4. Elsenburg, Stellenbosch, Western Cape

Operating System: Cage farming system, irrigation dam (2 x 400 m^3 cages)

Data: 4 000 fingerlings (18g) were stocked in October 2000. Artificial tilapia feeds were used at standard recommended rates (feeding table). Fish of a size range of 200 to 470g grams were harvested during March to May 2001. A total harvest of 1 350 kg were recorded.

5. Elsenburg, Stellenbosch, Western Cape

Operating System: Pond systems, 10 x 250 m^3 ponds

Data: 1200 fingerlings (2g) per pond were stocked in October 2000. Pond fertilisation were applied with supplementary feeding of commercial feeds from the early stages (>5g). Fish were harvested in April to May 2001. Fish in a size range of 160 to 380g grams were harvested. A total harvest of 2 130 kg were recorded.

6. Nitvoorbij, Stellenbosch, Western Cape

Operating System: Cage farming system, irrigation dam (2 x 400m³ cages)

Data: 5 000 fingerlings (12g) were stocked in September 2000. Artificial tilapia feeds were used at standard recommended rates (feeding table). Fish of a size range of 180 to 450g grams were harvested during March to May 2001. A total harvest of 850 kg was recorded. Some 1200 fish were lost in February 2001 due to damage of the cage net.

These pilot studies on the basis of information reported above seem to have been relatively successful but too little information is available to accurately evaluate this and a follow up study is urgently required and UoS will attempt to implement this.

The OVI for this activity was not met although alternative activities were implemented.

Determination of Social and Economic Impacts

The original objective was to attempt to determine the likely social and economic impacts of tilapia aquaculture and specifically improved tilapia such as GMT through surveys of farmers participating in on farm trials or pilot studies. However, the on-farm trials were postponed due to the delays in developing YY males. This study was then replaced by two separate studies.

Sustainability of small scale aquaculture development

This study was carried out as part of an evaluation of the potential impact of tilapia aquaculture as a component in rural development projects. A series of on-farm trials (see previous section above) were conducted in various rural communities of the Western Cape Province. These sites all formed part of the Small Scale Aquaculture Development Programme of the UoS and the DA. This programme was actually initiated in 1998 before this Project was underway and was already providing support to a small number of farmers growing fish in small-farm reservoirs. An investigation into the sustainability and socio-economic contribution of these projects towards rural development were conducted in 2001, the findings of which were presented in a full report (see Appendix 9).

The aim of the study was to investigate the sustainability of small-scale aquaculture systems in the Province of the Western Cape and their ability to contribute towards rural development programmes. Sustainability was evaluated in the broad context with cognisance of biological, economic, socio-economic, marketing, production factors that may contribute to or threaten the sustainability of small-scale aquaculture systems.

Biological sustainability was reliant mainly on the integrated use of water resources, namely for aquaculture and crop irrigation. Data generated by a three-year water-quality monitoring programme presents evidence of a negative impact of aquaculture systems on water quality, with reference to crop irrigation and fish farming standards. Possible long terms effects will however require the maintenance of an on-going water-quality monitoring programme.

Economic analysis of current small farmer projects provides firm evidence (i.e. net present value, internal rate of return, etc) in support of economic sustainability. Long-term profitability is however expected to come under pressure and emphasis should be place on ways to reduce input costs (production systems, input commodities) and to improve productivity (skills development, training).

Socio-economics evaluation indicates that the expectations of current participants are being met in term of improvement in skills, nutritional status, per capita income and quality of life. An awareness of benefits to the broader community is however not well established and need to be created in order for the programme to succeed in the long run. Given the recent nature of its introduction the programme has the potential to extend its benefits to also include the broader communities. Community participation is essential to ensure future support and sustainability of the projects, particularly on communal land.

With regard to production related factors the study concluded that operational risks (theft, disease, natural, human error, etc.) was relatively low with no threat to sustainability. Small farmers are however currently reliant on extension services that contributes largely to the reduction in risks. If the services can not be maintained it will inevitably lead to increased risk and reduced sustainability, particularly during the first phase of recruitment of new small farmers.

In conclusion the study revealed that the Small Scale Fish Farming Programme meets all the requirements of analysis of sustainability. It also confirms its potential to contribute substantially towards rural development and improvement of life of disadvantaged communities if a holistic development plan is implemented that take into account all aspects related to sustainable development (biological, economic, socio-economic, marketing, production factors).

The potential of tilapia as a food source for rural poor.

An additional investigation into the potential of Tilapia (*Oreochromis mossambicus*) as a food source for lower-income communities was conducted in 2001, the results of which are summarized in the form a draft report, Appendix 10.

Semi-structured questionnaires, individual interviews and informal group discussions were used for data collection. Some of the questionnaires (25%) were completed with the assistance of a researcher, while the majority (75%) was handed to farming households for completion. In the presence of a researcher, open-ended questions were asked to elicit or inspire respondents to freely raise their opinions. The questionnaires were also structured in such a way that respondents could relate easily without being intimidated by the science of information gathering.

The areas in which the market search was conducted were as follows:

1. Informal fish market in Grabouw, Western Cape
2. Thembalethu black township in George, Southern Cape
3. Local community in Oudtshoorn, Southern Cape
4. Farming community households at Nietvoorbij, Stellenbosch, Western Cape
5. Local community in Zwelathemba township in Worcester, Western Cape
6. Local community at Leliefontein, Namakwaland, Northern Cape

The approach in each of the 6 areas differed according to the local socio-economic environment, for example literacy, awareness of aquaculture, knowledge of tilapia, etc.. In each of the research areas, emphasis was placed on the following aspects:

- general consumer purchasing habits with regards to fish (marine and fresh water)
- importance of fish in their diets and frequency of household consumption
- methods and short recipes of preparation
- consumer attitude towards this new species of fish
- the general perceptions of fresh water fish as a food source, oppose to marine fish

A summary of the results includes:

- Generally all the respondents in the survey showed a liking to the taste, texture and appearance of tilapia. A few respondents felt it was difficult to clean.
- Preferred fish sizes were not recorded for all the areas, but the some results (Grabouw) indicate that the respondents found fish of 250g – 300g to be small compared to marine species with which they were familiar, while other (Matjiesrivier, Oudsthoorn) indicated the willingness to buy fish of sizes 256-450g. Matjiesrivier prefer the mixing of sizes in bunches. Worcester preferred larger fish (>500g).
- Most the respondents (>85%) felt that tilapia has a pleasant taste after it had been prepared and cooked.
- The main marine fish competitors are hake (fillets and whole), snoek, maasbanker and processed products (canned fish). The indications were that tilapia would be able to compete with these species on local markets in terms price, quality and taste.
- From this survey, it can be extrapolated that tilapia has a greater market potential within the lower income group compared to the higher income group (>R1500 per household per month) if it is sold in the whole fresh form.
- Proposed prices by respondents were in the region of R8.00-R12.00 per kg of fish

The overall response with regards to consumer acceptance of tilapia was good. More research needs to be conducted into promotion and awareness of fresh water fish amongst these communities.

The OVI for this activity was met.

Additional Objectives

As part of the 6-month extension to the Project two new objectives were added to the activities of the Project.

To the address the current demand for uptake of tilapia technologies, and in particular its extension to other regions of South Africa.

The Project, through the allocation of additional funds during the 6-month extension, has made a valuable contribution towards the dissemination and uptake of tilapia technologies.

Two five-day training courses for small-scale fish farmers were presented during the first half of 2002. One of these courses was presented from the 22nd to 26th April 2002, at Elsenburg in collaboration with the Department of Agriculture, Western Cape. The course was attended by a total of 29 participants, consisting of existing (x11) and prospective (x18) small-scale fish farmers, from various districts throughout the region. The course included both theory in the form of presentations and handouts, as well as practical training sessions at the aquaculture facilities of the DA at Elsenburg, as well as that of the UoS at Welgevallen and Jonkershoek. A "Training manual for small-scale fish farming systems" was produced by Division of Aquaculture, UoS (see Appendix 14).

A second course was presented on the 10th to 14th of June 2002, at the Makatini Research Station of the Department of Agriculture in Northern Kwa-Zulu Natal Province. The course was attended by a total of 21 participants, including three extension officers, from the greater Makatini Flats area. The area has been identified a one of the primary areas for small-scale aquaculture development in the Province. A tilapia demonstration project has also been initiated on the research station. The course was structured similarly to the one presented at Elsenburg, and the same training materials were used.

In order to facilitate the work of extension officers various aids were developed including a "Guide for extension services on the development of SMMEs for aquaculture" and a "Procedural Manual for the implementation of a small scale aquaculture project" (see Appendix 13 and Appendix 15). A "Statute for the formation of a Farming Co-operative for small-scale fish farmers[13]" has also been developed. Both the training program and extension materials have been made available to the DA in other provinces, as well as other development agencies for use in the own constituencies.

A specific briefing was also made to the respective Offices of the DA in the provinces of the Western Cape (Elsenburg) and Kwa-Zulu Natal (Pietermaritzburg) the contribution of aquaculture, and tilapia in particular, towards rural development projects. Their respective delegations have included representatives of the Directorates of Farmer Settlement, Animal Production and Technology Transfer (extension). The presentations were well received, and both the departments have

[13] Available from Danie Brink, upon request

since accepted the roles as lead agency for rural aquaculture development in their respective provinces. Additional presentations have also been made to the Northern Aquaculture Workgroup, with representation from four provinces (Limpopo, North West, Gauteng and Mpumalanga) as well as various development and research institutions (Agricultural Research Council, Department of Water Affairs and local Universities).

A total of 50 individuals, representing 23 communities, in two provinces have received training and orientation. The improved awareness amongst the participants, together with the development of demonstration units in these provinces, has made an important contribution towards the demand for uptake of tilapia technologies and extension. Representatives from four other provinces and various institutions have also been exposed to information on the contribution of tilapia technologies towards rural development projects. A further three new farming projects at George, Grabouw and Paarl has since been established in the Western Cape Province. Two projects have been established at Makatini and Riverview in Kwa-Zulu Natal, with a further two projects to be added at Joshini and Tukela during the second half of 2002.

A community based survey of the potential of tilapia (*Oreochromis mossambicus*) as a food source for lower-income communities (Appendix 10) has confirmed the acceptance, affordability and contribution of tilapia towards improved livelihoods in terms of income, food security and the socio-economic status.

To initiate actions towards a strategy/policy for the sustainable development of indigenous aquatic resources, for aquaculture development in the Southern African region.

The utilisation of tilapia technologies as part of rural and economic development programmes within the Southern African region forms part of a broader concept of the promotion of the use of indigenous species in aquaculture development. Aquaculture developments in the region are characterised by the introduction of exotic species and related technologies, with the associated detrimental environmental and social effects. The idea to base future development of aquaculture within the region on the sustainable utilisation of its indigenous aquatic resource, as opposed to exotic species, has become a popular topic of debate, in both local and international fora. The nature of the debate, in particular amongst regional role players, was that issues were often seen in isolation, it was conflict orientated and destructive in nature with little impact.

To address these issues, the Project sponsored a workshop on "Sustainable Development of Indigenous Resources, for Aquaculture Development in the Southern African Region", funded in part by funds from DFID AFGRP and in part by the Aquaculture Association of Southern Africa (AASA). The objective of the workshop was to initiate discussion that could contribute towards a strategy/policy for the sustainable development of indigenous aquatic resources, for use in aquaculture in the Southern African region. The workshop, again through funding assistance from DFID AFGRP, achieved regional representation from 47 delegates, representing nine countries from the Southern African region, as well as equitable representation from the various sectors including government, conservation, regulatory, research, development and commercial institutions. A detailed report, including a list of participants, is presented in Appendix 12.

The following outputs were achieved by the workshop:

- An exchange of information on the genetic status of indigenous aquatic resources of the region.
- An enhanced awareness among candidate beneficiaries of the potential of indigenous aquatic resources, for aquaculture development in the Southern Africa.
- An enhanced awareness among stakeholders of the potential impact of commercial species/stocks and improved breeds on indigenous aquatic resources and genetic diversity in the Southern African region.
- The formulation and adoption of a framework for a strategy/policy for the sustainable development of indigenous aquatic resources, for aquaculture development in the Southern African region based on the Nairobi declaration[14] (see modified version in Appendix 12).
- A report summarising discussion and recommendations that could form the basis for future proposals/policies on the utilisation of indigenous species for aquaculture development in Southern Africa (Appendix 12).

The workshop report is currently been circulated amongst the participating delegates and their respective constituencies for final comment and conformation. Various provincial, national and regional authorities in the SADC-region have indicated their

14 Original Nairobi declaration can be found at http://www.iclarm.org/news/Nairobi%20Declaration.pdf

support towards a process whereby the recommendations of the workshop could be incorporated into provincial, national and regional policies and protocols aimed at sustainable development of indigenous resources, for aquaculture development. These authorities include amongst other:

- Various industry related sectors through the Aquaculture Association of Southern Africa.
- Provincial and national conservation bodies, such Cape Nature Conservation, Kwa-Zulu Natal Parks Board, Mpumalanga Parks Board, the National Aquatic Forum, Department of Environmental Affairs, Aquatic Science Forum and the Directorate for Indigenous Resources Conservation.
- Development agencies, such as Provincial (Western Cape, Kwa-Zulu Natal, Mpumalanga, Limpopo) and the National Department Agriculture

Future steps will include the official presentation of the recommendations of the Workshop to provincial, national and regional authorities in the SADC-region, including the Aquaculture Workgroup of the Southern African Development Community (SADC), for uptake into policies and protocols aimed at sustainable development of indigenous aquatic resources.

Progress towards outputs

Rational choice of most promising strains of O. mossambicus for use in small-scale aquaculture and for GMT production.

The successful completion of the nursery phase of Trial A and the two grow-out Phases of Trial B provided consistent and reliable information on the comparative growth performance of the various accessions of *O. mossambicus* in on-station environments. It is reasonable to extrapolate these results to more realistic culture environments, particularly low input systems although a farm trial of at least some of the strains would have been preferred. Four strains were identified as superior in terms of growth performance and are considered the most appropriate for the development of aquaculture in Southern Africa. Three of these superior strains were used in the development of the YY male technology breeding programme.

The OVI for this output was met.

Recommendations on choice of strains, future strategies for further genetic improvement, and management of genetic diversity of O. mossambicus

Quantitative data and the molecular characterisation of strains in terms of genetic variation and relationships have provided important information with regard to the choice of strains, and strategies for further genetic improvement, as well as for the management of genetic diversity of *O. mossambicus*. During the workshop on "Sustainable Development of Indigenous Resources for Aquaculture Development in the Southern African Region" it was apparent that valid concerns exist among stakeholders over the threats to genetic diversity of indigenous species that will be posed by aquaculture development. The research demonstrated clear population structuring in *O. mossambicus* in southern Africa and thus demonstrated the utility of genetic markers as a tool for managing genetic diversity of commercially important species. Recommendations on strategies for further genetic improvement of the species for aquaculture development in southern Africa is that selecting breeding programme should be initiated utilising a base strain comprising of the Ndumu, Boesmans, Kasinthula and Makathini strains. A proposal has already been developed for such a breeding programme. However, with regard to conservation of key pools of genetic diversity, strains should also be maintained in isolation and these strains should be used for aquaculture locally under conditions where environmental impact assessment indicates significant threats to indigenous genetic diversity would be posed by tilapia aquaculture development.

The OVI for this output was largely met as the results have been presented in various fora. It is apparent that UoS and its partners will be the main responsible agencies for promoting and disseminating superior and improved strains.

Reliable genetic technology for the production of all-male O. mossambicus developed

The progress in the breeding programme towards the generation of YY males and the mass production of GMT was significant, is somewhat slower than projected. Results indicated that transfer of the technology to southern African *O. mossambicus* is viable although further work is required, including an element of selection for sex ratio, in order for the technology to be developed to on a commercial scale. It seems probable that availability of monosex seed in the best strains of the species would represent and important catalyst to tilapia aquaculture development in southern Africa.

The OVI for this output was not met but it is anticipated that YY males and GMT will be available for distribution within 18 months.

Benefits of application of GMT to small-scale aquaculture quantified

The socio-economic benefits of GMT technology to rural development could not be demonstrated or assessed, due delays in GMT production. The assessment of the contribution of tilapia technology in general to small-scale aquaculture was however evaluated. The assessment concluded that tilapia has the potential to sustainably contribute towards small-scale aquaculture amongst rural communities, with significant benefits towards improvement of the quality of life of these communities in terms of per capita income, risk diversification, skills development and food security. The additional of availability of improved breeds including the GMT technology is expected to make further contributions towards small-scale aquaculture in terms of improved productivity, resource utilisation and bio-security.

The OVI for this output was not met but alternative and equally valuable outputs were generated.

Social and economic impacts derived from application of GMT to small-scale aquaculture described and quantified.

The assessment of the application of tilapia technology in general, (and almost certainly to a superior GMT when available), to small-scale aquaculture amongst rural communities gave indications of a substantial contribution towards social and economic improvement and benefits to rural livelihoods in the form of improved nutrition, food security, improved per capita income and skills development.

The OVI for this output was met although knowledge gained was related more to the culture of indigenous tilapia in general rather than specifically the culture of GMT.

Contributions of Outputs to Developmental Impact

This Project is clearly more strategic, rather than adaptive, in nature, and thus outputs might not be expected to produce developmental impact within the timeframe of the Project itself. Nevertheless the various outputs have and will generate meaningful contributions towards developmental impact:

Collection of strains of *O. mossambicus* from Southern Africa

The collection of 12 strains of *O. mossambicus* from Southern Africa represents the most comprehensive assembly of genetic material of the species in the region and in fact worldwide. The molecular and quantitative assessment of these strains has further contributed to the establishment of these stocks as reference strains for the species. This has generated wide interest in the application of the strains in research, developmental and commercial projects, both within the region and abroad. It has also laid the foundation for the development of comprehensive and well describe gene bank for the species.

The collection has also contributed towards the formation of a collaborative exchange network of material and results in connection with tilapia (*O. mossambicus*) genetics, extending throughout the Southern African region, as well as to internationally, including such as the USA, UK, East Asia and North Africa.

Characterisation of growth performance of strains of *O. mossambicus*

The characterization of the growth potential of 10 strains of *O. mossambicus* was the first work of its kind on strains and populations indigenous to the region. The results indicated significant differences between the growth rates of strains, with four strains identified as having superior strains growth performance. The results, through their presentation and publication, have also generated wide interest for application in current developmental and commercial projects on tilapia (*O. mossambicus*) in the region and internationally. These results have been instrumental in stimulating other research groups, such as at the University of the North, to conduct complementary characterization of strains in their area. The results have also provided information for the identification of the faster growing strains for incorporated into the programme for YY-male production and GMT fingerling production and to form the basis of future selection. The outputs also included the development of human resources, in the form of advanced training of three researchers in improvement of tilapia through means of quantitative genetic procedures. This will make and important contribution towards the domestication selection and further genetic improvement of biological and commercial characteristics of the species. It has also introduced the UoS and its institutional partners into a wider network of genetic improvement of tilapia that include that of CRSP/USAID, ICLARM/GIFT, INGA[15] and others (see Appendix 11).

Genetic characterisation of Southern African *O. mossambicus* strains

The molecular genetic characterization of *O. mossambicus* was the first work of its kind on strains and populations indigenous to the region. The results emphasized the wealth of genetic diversity within the species that has been largely overlooked and underutilized, until this point in time. The results of the study are providing valuable contributions towards the management and conservation of biodiversity within the species and the region, in the form of reference stocks and baseline data. It has also contributed towards an increased awareness amongst interest groups and role players about the genetic diversity within the species and the conservation thereof. This culminated in a workshop on "Sustainable Development of Indigenous Resources, for Aquaculture Development in the Southern African Region" that was held at the UoS, with Project (i.e. DFID) support, on the 10th September 2002. The workshop has laid the framework for national and regional policies aimed at sustainable development of indigenous resources (Appendix 12). This component of the Project has also made invaluable contributions towards human resource development, particularly with regard to local students and scientists, in the field of molecular and conservation genetics. Two M.Sc.-theses have been completed, whilst two researchers has obtained advanced training at associated overseas institutions. A total of five conference presentations have also been generated by these outputs. An extensive collaborative network has been established between local and regional interest groups in the areas of research (Universities of Stellenbosch, The North, Venda, RAU, Western Cape, Cape Town, Pretoria), regulatory, conservation and industry. Also with international groups such as the University of Stirling, UWS, University of Leuven, Belgium,

[15] UoS has applied to join the INGA which will further enhance international linkages

Asian Institute of Technology, University of Malawi and ICLARM, has been established (see Appendix 11 for details of various partners).

The transfer of YY male technology to *O. mossambicus*

The transfer of YY technology from *Oreochromis niloticus* to *Oreochromis mossambicus* has been relatively successful at all stages of application. Sex ratios were found to be more variable and to deviate further from expectations and selection for sex ratio will need to be applied in the further development of this breeding programme. The Project has successfully demonstrated the transfer YY male technology from *Oreochromis niloticus* to *O. mossambicus*. GMT and the derived benefits has also made an important contribution towards awareness of the benefits of tilapia technologies for aquaculture development within the region, particular through the use of indigenous species, as opposed to the continuous introduction of exotic species and related technologies. Availability of higher yielding GMT in *O. mossambicus* will enhance the efficiency of most tilapia production systems, particularly in low input systems where reproduction represents a major constraint, improving their productivity and profitability. Culture of GMT should also enhance competitiveness of commercial aquaculture targeted at higher value domestic, and export markets.

Socio-economic and livelihoods related outputs

During a recent workshop of stakeholders in rural aquaculture development (Appendix 12) it was reconfirmed that the objectives of the Project fall within the directives and priorities of both the National and Provincial Governments in terms of:

- Support of the Land Redistribution and Agricultural Development Programme (LRAD)
- The socio-economic development of rural communities through formal & non-formal training, farmer establishment and support, infrastructure, development plans, etc.
- The participation of disadvantaged rural communities in the local, regional and national economy.
- A contribution towards food security, job creation and human resources development amongst disadvantaged rural communities.

- Resource conservation through the integrated use of water, the use of indigenous species, the development of environmentally friendly production systems, etc.

The Project has also made significant contributions towards current and future developmental impact in the areas of social and economic development and improved livelihoods, both upstream and downstream, with particular reference to:

Human resource development

The Project has made a particularly important contribution towards human resource development. Upstream impacts include:

- The acknowledgement by various developmental agencies, including the DA and the Department of Economic Affairs, of the need for adequate policies, programs and provisions for aquaculture skills training amongst rural communities.
- The facilitation of specific funding by development agencies, such as the Land Reform and Development Programme (LRAD), the Land Bank, the Department of Economic Affairs and the Department of Agriculture, for aquaculture skills training amongst rural communities.
- The inclusion of training of small-scale fish farmers into the developmental framework of the Directorate of Farmer Settlement, of the Department of Agriculture.

Downstream impacts include:

- An enhanced knowledge and understanding of aquaculture practices and the potential role it can play in rural development among DA extension officers. A three day course run under the auspices of the Project was attended by a total of 35 extension officers representing districts throughout the Western and Northern Cape Province. This activity has the potential to reach at total of 1800 commercial and 600 emerging small-scale farmers in the respective provinces. Positive feedback was observed through this particular activity, and four small-scale scale projects (Leliefontein, Oudshoorn, George, Vredendal) have since been established under supervision of regional extension officers.
- Three training courses for small-scale fish farmers were presented. Two of these five-day courses were presented in the Western Cape and attended by a total of 48 participants, and a third in Kwa-Zulu Natal attended by 21 potential

small farmers. These courses have made a significant contribution to both awareness and skills training in aquaculture amongst rural communities. The participants were representative of the primary target areas/districts for future small-scale aquaculture development in the respective provinces.

- The structured training programme and material has also been made available to other provincial departments (Northern Cape, Kwa-Zulu Natal, Mpumalanga, North West) and development agencies, for presentation in their respective constituencies

Further direct impacts of the Project include the training and orientation of local researchers (x5), technical staff (x3) and students (x5) in a wide range of field, including molecular and quantitative genetics, data analysis, husbandry, systems design and management. A total of two staff members and three students have since acquired permanent jobs in related fields with the Department of Agriculture as other employment agencies in the field of commercial and developmental aquaculture.

- A network for future collaboration with regard to staff training has also been established through the participation of eight Project members in training programmes at the University of Wales, Swansea, University of Stirling, University of Leuven, Belgium, Asian Institute of Technologies and at ICLARM: World Fish Center in the Philippines and Abassa, Egypt. .
- The outputs has also been acknowledged by other interest groups such as the US-based Claude Harris Leon Foundation, which are sponsoring the post-doctoral fellowship of Dr. Eugenia D'amato for a further two years, allowing here to continue with research on molecular genetics of tilapia (*O. mossambicus.*
- The UoS has also created an additional permanent position within the aquatic genetics research group to provide support to the development of the current research program, focusing on sustainable development and utilization of indigenous aquatic resources...

Enhanced Awareness

The Project has made a very marked contribution towards improved awareness amongst government, industry and development agencies, of the potential contribution of aquaculture, and tilapia culture in particular, to rural development, economic growth the livelihoods of poor farmers and food security. This has been achieved through demonstration projects, publications, reports, presentations, consultations and delegations in various fora, throughout the duration of the Project. UoS now has an extensive informal network of collaborative partners within Southern Africa, all with an interest in developing the aquaculture of indigenous tilapia (see Appendix 11). The improved awareness is demonstrated by:

- The spread of initial awareness and expansion of activities from the Western Cape Province to include other provinces of the Northern Cape, Eastern Cape, Kwa-Zulu Natal, Mpumalanga and the North West, as well as that of neighboring countries suc h as Namibia, Mozambique and Botswana.
- The commitment of funding towards future developments in tilapia based aquaculture by various Departments such as Agriculture, Water Affairs, Trade and Industry, and agencies such as Land Reform and Development (LRAD), Industrial Development Corporation (IDC), Rural Development Initiative (RDI), Water Research Commission (WRC) and the Agricultural Research Council (ARC).
- The DA has also extended its commitment towards aquaculture development through the appointment of an additional researcher in the Aquaculture Research Unit, together with a dedicated extension officer for rural aquaculture development in the Directorate of Farmer Settlement.
- The recent Conference of the Aquaculture Association of Southern Africa, (AASA) held at Stellenbosch in September 2002, has provide further proof of the growing awareness of the potential of tilapia to contribute towards rural development of the region. This has been demonstrated by the number of presentation on tilapia as well as the general interest from delegates in the species and related matters.

There is little doubt that the very presence of the Project at UoS, in combination with a number of other smaller activities, has acted as a major catalyst for an expansion of interest in the culture of the indigenous tilapia. This expanded interest appears likely to bring about what looks like being a paradigm shift in thinking about the role of aquaculture throughout the southern African region among farmers and policymakers alike. This change in thinking has however yet to be reflected in any significant way in the uptake and impact of aquaculture and it is thus important that the current momentum is maintained and enhanced. A number of new initiatives have been spawned, largely as a result of the presence of the Project in the region:

New Initiatives

The sections above have highlighted how the Project as stimulated substantial interest in tilapia-technologies as a mechanism to contribute towards socio-economic development and improved livelihoods in the Southern African Region, over a wide range of areas, including:

- Food security, human resource development and socio-economic development in rural communities.
- Establishment of SMMEs contributing towards both the local and regional economy.
- Establishment of a regional industry with a potential for job creation and foreign exchange earnings.
- The use of indigenous species to contribute towards food security and socio-economic development.

A number of new initiatives have arisen which exemplify the above and which are likely to provide important contributions to ensure continuation as well to contribute towards future developmental impact and benefits to rural communities incorporating the outputs of this Project. These initiates include:

DFID Business Linkage Challenge Fund Project

The DFID Business Linkage Challenge Fund has awarded a significant grant in support of the Project "The transfer of Tilapia Aquaculture Technologies for Rural Development in Southern Africa". The grant was awarded to the current Project team members from the UoS and UWS, through respective spin-off companies, together with other linkage partners from South Africa, have submitted the application. The overall objectives of the project are:

- Improvement of the income, livelihoods and opportunities of poor people in rural areas of Southern Africa through the development of tilapia fish farming systems, that will contribute towards food security, job creation and small business entrepreneurship and development.
- Enhancement of the competitiveness of participating businesses partners and the development of markets, through the commercialization and application of

improved varieties of the indigenous tilapia, *O. mossambicus*, and associated technologies.

- To contribute towards the development of a sustainable tilapia farming industry in Southern Africa, directed towards both local and export markets.
- To establish a sustainable complementary network between the linkage partners with the realization of benefits to all members.

The main intended beneficiaries are:

- Rural communities/households through improved per capita income (job creation), human resource development (skills training) and food security (access to affordable quality fish protein).
- The commercial and rural development component of the project hopes to deliver benefits in terms of employment for ~260 and ~1,300 people respectively and social/economic benefits to a further 5,000 people over its 3-year duration.
- The project should also contribute to food security in rural areas in which it operates.
- Companies participating in the secondary phases of the development of tilapia farming systems. New companies and SMME's will be incorporated into the production network, with emphasis on black empowerment.
- Companies providing upstream services (feed suppliers, transport, construction, equipment, etc.) and downstream services (processing, packaging, distribution, etc.) to the tilapia farming systems.

Department of Agriculture as Lead Agency for Rural Aquaculture Development

Since the initiation of the Project, the Department of Agricultural of the Western Cape has taken on the role as lead agency, responsible for rural aquaculture development and small-farmer farmer settlement. This will have a marked improvement on current extension capacity in aquaculture. The Department of Agriculture has created additional posts in the Directorate of Farmer Settlement, for this purpose. A specific action plan is also being developed to address the issues with regard to extension services and uptake of tilapia technologies throughout the Southern African Region. It is perceived that the activities of the DA in its role as lead agency, will contribute towards accelerated uptake of tilapia aquaculture and the delivery of benefits to rural communities in all provinces of South Africa.

Sustainable utilization of *O. mossambicus* as an indigenous aquatic resource

- The work on the genetic characterisation of *O. mossambicus*, initiated by the Project, has received recognition by the National Research Foundation of South Africa (NRF) as well as conservation authorities such as the Regional Aquatic Conservation Forum. This is the first project of its kind where technology has been transferred and applied to the development of an indigenous aquatic species of the Southern African region, in order to ensure its utilisation in both rural development programmes as well as for commercial application. The Forum has representation from all countries in the Southern African Region (SADC) and is responsible for the formulation of conservation policy for the region as a whole.
- Outputs of the Project, such as the workshop on "Sustainable Development of Indigenous Resources, for Aquaculture Development in the Southern African Region" (Appendix 12) and other publications (Appendix 8), have been well received and will contribute to an improved awareness and regulatory framework governing the management and utilisation of tilapia genetic resources in the region. The AASA, as an outcome to the DFID Workshop, has launched a formal initiative to develop a regional policy framework for sustainable development of indigenous aquatic resources.
- These molecular characterisation activities have received further support from the US-based Claude Harris Leon Foundation in the form of a two-year sponsorship of a post-doctoral fellowship to continue with research on molecular genetics of tilapia (*O. mossambicus*), as well as from the Flemish Bilateral Scientific Collaboration Programme. Through the aforementioned funding, molecular characterisation work is now being extended to an indigenous abalone (*Haliotis midae*) used in aquaculture.

New Tilapia Projects

The Project was instrumental in the initiation of a number of new tilapia farming projects, based upon *O. mossambicus,* which are currently being developed. These projects range from extensive rural development programmes to intensive commercial based systems (including the BLCF project mentioned above), as well as collaborative projects between communities and industry, or so-called Community Private Partnerships (CPP's). Many of these projects form part of a structured initiative, co-ordinated by a group called AquaStel, to promote the development of a tilapia aquaculture industry in Southern Africa. All previous development related to tilapia in the Southern African region (Zimbabwe, Zambia, Namibia) have been based on the utilisation of *O. niloticus*, a species exotic to Southern Africa.

Some of the new projects include the development of:

- A regional tilapia hatchery unit at Monzi, Kwa-Zulu Natal.
- Commercial tilapia farming systems at Matubatuba, Kwa-Zulu Natal.
- Community based tilapia farming systems in Kwa-Zulu Natal, at Makatini, Joshini and Tukela.
- A regional tilapia hatchery unit at Polokwane, Limpopo Province.
- Community based tilapia farming systems in the Limpopo Province, Northern region.
- Commercial tilapia farming systems at Hardap Dam, Namibia

Similar proposals have been submitted to and are currently being considered by various institutions with an interest in development such as government departments, mining houses, electricity and energy suppliers, rural development forums, etc. Preliminary investigation has also been initiated for the development of tilapia farming systems in Botswana and Mozambique.

Continued development of improved breed(s) in *O. mossambicus*

UoS has stated its commitment to continue with the research initiated under the Project on the development of the YY male technology in *O. mossambicus*, after the end of the Project, due to the confirmed demand for the technology. Continuation of the work will be funded through the use of local funding from national and industrial sources with technical support being provided through the BLCF project. In addition plans are being developed to apply GIFT style selective breeding using the superior strains identified by the Project as the base for this breeding programme. The genetic characterisation data already obtained under the Project will provide novel baseline information on genetic variability and will undoubtedly add value to the GIFT type selection programme. A pre-proposal for this work has been submitted to ICLARM.

Publications and other outputs:

Accepted Publications

Peer reviewed papers:

Beardmore, J.A., Mair, G.C. and Lewis, R.I. (2001) Monosex male production in finfish as exemplified by tilapia: Applications, problems, and prospects. Aquaculture 197: 283-301

Edited Conference Proceedings and book chapters

Brink, D and L.C. Hoffman. (2001) Aquaculture Production in South Africa. Proceedings of the Eighth Conference of the Animal Feed Manufacturers Association of Southern Africa. AFMA, Pretoria, South Africa: 27-35.

Mair, G.C. (2001) Genetics in Tilapia Culture (2001) In: Subasinghe, S. and Singh, T. (eds) Proceedings of the Tilapia 2001 International Technical and Trade Conference. 28-30 May 2001, Kuala Lumpur, Malaysia. INFOFISH, Kuala Lumpur, Malaysia: 136-148.

Mair, G.C. (2001) Tilapia Genetics in Asia. Sixth Central American Aquaculture Symposium. Proceedings: Tilapia Session (ed. D.E. Meyer). PD/A CRSP, Tegucigalpa, Honduras: 9- 34.

Mair, G.C. (2002) Tilapia Genetics and Breeding in Asia. In: Guerrero III, R.D. and M.R. Guerrero-del Castillo (eds.) 2002. Tilapia farming in the 21st century. Proceedings of the International Forum on Tilapia Farming in the 21st Century (Tilapia Forum 2002) 184 p. Philippine Fisheries Association, Inc.,Los Baños, Laguna, Philippines. p100-123.

Popular articles

Mair, G.C. (2001) Monosex tilapia: Factors, Effects, and Methods for Male-Only Production. Global Aquaculture Advocate 4(4): 79-81

Mair, G.C. (2001) Tilapia Genetics – Application and Uptake. Global Aquaculture Advocate 4(6): 40-43

Mair, G.C. (2002) Coded Wire Tags for Batch Marking in Tilapia. NMT Network News, Spring 2002. Northwest Marine Technology Inc. Seattle, U.S.A. p3.

Stander, H. (2002) Opportunities for small scale fish farming in South Africa. Farmers Weekly, 3 May 2002, pp. 6-8.

Theses:

Esterhuyse, Marna, 2002. Microsatellite markers to identify two species of Tilapiine fish, *Oreochromis mossambicus* (Peters) and *O. niloticus* (Linnaeus). M.Sc.-thesis. University of Stellenbosch, Stellenbosch, South Africa. 132pp

Hall, E.G., 2001. Levels of intraspecific variation (biochemical and genetic) in *O. mossambicus* with relevance to aquaculture species. M.Sc.Agric. March 2001. University of Stellenbosch, Stellenbosch, South Africa. 104pp

Other non-peer reviewed publications

Salie, K. (2001) Potential of Aquaculture in Haarlem, Eastern Cape Province. Report for Land Development Unit, University of Western Cape, South Africa. September 2001. University of Stellenbosch, Stellenbosch, South Africa. 21pp.

Salie, K and Karaan, A.S.M. 2001. An assessment of the economic and institutional sustainability of small-scale fish farming enterprises in the Western Cape Region of South Africa: From research to sustainable enterprises. University of Stellenbosch, Stellenbosch, South Africa. 34pp.

Salie, K. and Karaan, A.S.M. 2001. An analysis of CIRD-implemented SMMEs activities in the Western Cape Province: The small-scale farming development programme for Aquaculture. Report for the Centre for Integrated Rural Development. Centre for Integrated Rural Development, Cape Town, South Africa. 43pp.

Extension and Training Manuals

Guide for extension services on the development of SMMEs for aquaculture. Department of Agriculture: Western Cape, Private Bag X1, Elsenburg, 7602, South Africa. 50pp. (see Appendix 13)

Training manual for small-scale fish farming systems. Division of Aquaculture, University of Stellenbosch, Private Bag X1, Matieland, 7602, Stellenbosch, South Africa. 15pp. (see Appendix 14)

Procedural Manual for the implementation of a small scale aquaculture project. Division of Aquaculture, University of Stellenbosch, Private Bag X1, Matieland, 7602, Stellenbosch, South Africa. 8pp. (see Appendix 15).

Statute for the formation of a Farming Co-operative for small-scale fish farmers. Division of Aquaculture, University of Stellenbosch, Private Bag X1, Matieland, 7602, Stellenbosch, South Africa. 30pp.

Workshops and Training Courses Held

Small-scale Aquaculture Programme – Strategy Workshop. 23 & 27 June 2001. DA and UoS, Stellenbosch. (details available upon request)

Aquaculture Training Course for Small Scale Fish Farmers in the Western Cape Province. Elsenburg Agricultural Institute, Stellenbosch, South Africa. 16-19th October 2001. (attended by 28 small farmers from the Western Cape Region)

The role of tilapia farming systems in socio-economic development of rural communities of Kwa-Zulu Natal. Richardsbay, Kwa-Zulu Natal, South Africa. 24-25th January, 2002.

Workshop on Sustainable Development of Indigenous Resources, for Aquaculture Development in the Southern African Region. University of Stellenbosch, September 10, 2002. (attended by 47 delegates from 9 SADC countries). For full details of workshop outputs see Appendix 12).

Presentations (conferences, workshops, etc.)

More than 25 national, regional and international conferences were attended by Project representatives for the purposes of presenting work either directly related or closely related to Project activities. Full details of presentations made are given in Appendix 16.

Finances & Inventory

All financial aspects of the Project were in line with projections and budgets were spent up without overspend or underspend. Items of capital expenditure and inventory are shown in Table 8.

Table 7 Inventory of major capital items including cost and location.

Item	DFID contribution	UoS contribution	Location
Paddlewheel Aerators (x4)	1 500		Elsenburg
Net pens for rearing of progeny groups in YY-male breeding programme	1 000		Elsenburg
Subdivision of earth ponds for GMT/SRT growth trials		3 000	Elsenburg
Analytical apparatus for molecular laboratory	650		JC Smuts Biology Building
Pipettes for molecular laboratory	850		JC Smuts Biology Building
Egg incubation unit and androgenesis equipment [R 52G UV lamp, s/n 94-0012-06]	1300 [£623+VAT]		Welgevallen
Installation of four aquarium rooms each fitted with 8 x 120 litre tanks with biofilter and recirculation system	1 200	800	Welgevallen
Laptop Computer (RIL) – Sony PCG-505fx, s/n 28983850 5200205	2200		
New aquarium facility consisting of 90 x 120 litre tanks with biofiltration and recirculation	1175	3000	JC Smuts Biology Building
Installation of isolation structures over existing earth pond system consisting five 12x40m earth ponds		8 500	Elsenburg (DA)
Polyethylene net pens for holding of families groups during growth evaluation (x60)	800		Welgevallen & Elsenburg
Oxygen meter	450		Welgevallen
HP/DJ 610 printer	75		Welgevallen
Air-blower for supply of compressed air to earth pond system	1000	1000	Elsenburg (DA)

Appendices

Appendix 1 List of Acronyms Used in the Report

People

DB	D Brink, UoS
EH	E Hall, UoS
GCM	Dr. GC Mair, UWS, PI
JAB	Professor JA Beardmore, UWS, PI
JS	J. Swart, DA
KS	Khalid Salie, UoS
LCH	Dr. LC Hoffman, UoS PI
LFDW	Lourens de Wet, UoS
MED	Dr. ME D'Amato
PK	P. King, DA
PM	P Marais, UoS
RH	Dr. Robert Hartnell, VSO/DA
RIL	Dr. RI Lewis, UWS/UoS

Others

AASA	Aquaculture Association of Southern Africa
ADLG	Average of daily length gain (b-value, mm/day);
ADWG	Average of daily weight gain (b-value, g/day)
AFGRP	DFID Aquaculture and Fish Genetics Research Programme
AIT	Asian Institute of Technology
BLCD	DFID – Business Linkage Challenge Fund
CRSP	Collaborative Research Support Programme (of USAID)
CT Room	Controlled temperature or controlled climate room
DA	Western Cape Province, Department of Agriculture
DES	Diethylstilboestrol, an oestrogen hormone used for feminization
FGRP	DFID Fish Genetics Research Programme (managed by UWS)
GIFT	Genetic Improvement of Farmed Tilapia (an ICLARM project)
ICLARM	International Center for Living Aquatic Resources Management
INGA	International Network for Genetics in Aquaculture
OVI	Objectively Verifiable Indicators (from the logical framework)
RAU	Rand Afrikaans University
SADC	Southern African Development Community
SFDP	Small farmer development programme
SMME	Small, Medium and Micro-enterprises
UoS	University of Stellenbosch
USAID	United States Agency for International Development
UWS	University of Wales, Swansea, UK.

Appendix 2 Project Logical Framework

Project Title: Development of tilapia genetic resources for aquaculture in Southern Africa
Brief Description of Project: The development of improved strain(s) of *O. mossambicus* for use in small-scale aquaculture in Southern Africa. Improvements will be generated through evaluation and selection of the best performing strains from among those collected in the region and the application of the YY male technology to these appropriate strains of *O. mossambicus*.
His Code No.: R. 7284

Period of DFID funding:
From December 1, 1998 to December 31, 2001
Total DFID funding:
£ 294,247
Date Framework Prepared
October, 1998

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
GOAL: Performance (productivity) of fish in aquaculture and enhancement fisheries improved through selection, genetic manipulation, and improved broodstock maintenance.	Widespread, sustainable and profitable adoption of genetic based aquaculture technologies.	Census statistics supplied by government and development agencies	
PURPOSE: Higher yielding genetically male tilapia (GMT) developed for improved livelihoods in small-scale aquaculture in Southern Africa.	Improved production performance and economic benefits of small-scale aquaculture, through the introduction of GMT, demonstrated.	Project reports/publications and Dept. of Agriculture statistics.	There will be uptake of improved fish by target communities.

OUTPUTS: 1. Rational choice of most promising strains of *O. mossambicus* for use in small-scale aquaculture and for GMT production. 2. Recommendations on choice of strains, future strategies for further genetic improvement, and management of genetic diversity of *O. mossambicus* 3. Reliable genetic technology for the production of all-male *O. mossambicus* developed 4. Benefits of application of GMT to small-scale aquaculture quantified 5. Social and economic impacts derived from application of GMT to small-scale aquaculture described and quantified.	1. Reliable growth performance data obtained 2. Draft recommendations for submission to relevant Govt. and development agencies 3. YY males and GMT available for distribution 4. Growth performance data from on-farm trials of GMT 5. Surveys of participating small-scale farmers completed	1-5 Project final report and publications 3. Sex ratio data from progeny testing	Livelihoods are improved through increased production from aquaculture and/or availability of cheaper fish protein.
ACTIVITIES: 1. Characterisation of growth performance of strains of *O. mossambicus* 2. Genetic characterisation of strains of *O. mossambicus* using molecular techniques 3. Adaptation of the YY male technology to, and production of GMT in, chosen strains of *O. mossambicus*. 4. Evaluation of the production performance of GMT in small scale aquaculture systems in South Africa 5. Evaluation of social and economic impact of improved tilapia in small-scale aquaculture systems in South Africa.	1. Growth performance trials of at least 8 strains of *O. mossambicus* completed by May 1999. 2. Collection of data from molecular markers completed in at least 8 strains by August 2001 3. YY males and GMT produced in at least two strains by August 2000 4. On-farm trials of GMT completed in at least 10 sites by August 2001 5. Surveys conducted of families and communities participating in trials by August 2001 Budget: £294,247	1-5 Annual and Final reports.	3. Genetic inheritance of sex in *O. mossambicus* is similar to that in *O. niloticus* and YY male technology can be successfully adapted in this species.

Appendix 3 Original Project Proposal

DEPARTMENT FOR INTERNATIONAL DEVELOPMENT (DFID)

FISH GENETICS RESEARCH PROGRAMME
UNIVERSITY OF WALES SWANSEA (UWS)

File Number	Project number (to be completed by UWS)
	R. 7284

Project Title

Development of tilapia genetic resources for aquaculture in Southern Africa.

FISH GENETICS RESEARCH STRATEGY PROGRAMME
MANAGED BY UWS, UNDER THE DFID
NATURAL RESOURCES STRATEGY PROGRAMME

RESEARCH AND DEVELOPMENT
GRANT APPLICATION FORM

Notes

1. UWS is the designation used throughout this proposal for the University of Wales Swansea, a constituent institution of the University of Wales.

2. The form must be completed taking **fully into account** the attached 'Notes on Completion of UWS Project application form'.

3. The conditions attached to grants (section 15) should be studied before the proposal is completed.

Please complete in type

1. Project Title

Development of tilapia genetic resources for aquaculture in Southern Africa.

2. Full name and title of applicant(s)

Prof. John .A. Beardmore	Dr. Louw Hoffman	Dr. Graham C. Mair

Post(s) held and Department(s)

Consultant	Senior Lecturer	Research Fellow (seconded)

3. Name, address, telephone, fax and e-mail numbers of applicant(s)

School of Biological Sciences University of Wales Swansea Singleton Pk. Swansea SA2 8PP Wales, U.K. Tel. 44 1792 295382 Fax. 44 1792 513030 j.a.beardmore@swansea.ac.uk	Aquaculture Program Faculty of Agricultural Sciences University of Stellenbosch P.O. Box 3318, Matieland, Stellenbosch 7602 South Africa Tel.: 27 21 8084747 **Fax: 27 21 8084750** lch@maties.sun.ac.za	Aquaculture & Aquatic Resources Management Program (AARM) Asian Institute of Technology PO Box 4, Klong Luang Pathumthani 12120 Thailand Tel: 66 2 5245463 Fax: 66 2 5245463 gcmair@ait.ac.th

4a Project Location

Faculty of Agricultural Sciences
University of Stellenbosch
P.O. Box 3318, Matieland,
Stellenbosch 7602
South Africa

b Name, address, telephone, fax or telex number and e-mail number of any overseas collaborator(s)

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c If the project is located overseas or if there is an overseas collaborator, has the approval of the overseas government been obtained?

This Project have been approved by a General Aquaculture Forum of all the relevant government departments and other interest groups and does therefore does not need any further approval on implementation. This Forum known as the Aquaculture Working Group acts as an adviser to the relevant authorities.

5 Starting and finishing dates (month and year)

December 1 1998 to December 31, 2001

6a Total financial support requested from UWS.

£ 294,247

b Summary of financial support requested from UWS to be shown by Treasury financial years 1 April to 31 march (also complete Appendix 1).

	Year	1998/99	1999/00	2000/01	2001/2
Payroll Costs	£	13,589	44,622	56,814	42,898
Capital Equipment	£	5,500	8,000	6,000	1,000
Consumables	£	4,140	11,000	11,000	6,050
Travel & Subsistence	£	8,500	9,250	9,250	9,000
Overheads	£	3,126	8,903	11,805	9,255
Other	£	10,145	1,000	1,000	2,400
Total Costs	£	45,000	82,775	95,869	70,603

c Contributions from other organisations towards the costs of the project

The existing field and laboratory facilities of the University of Stellenbosch (UoS) will be used for much of the project work. One full time research assistant of UoS will be assigned to project activities. In addition UoS is co-ordinating the Small Farmer Aquaculture Programme (SFAP), in collaboration with local NGOs, the Agricultural Research Council and the Dept. of Agriculture. It is intended that representatives of these collaborating institutions will assist in promoting and disseminating the outputs of this project in the context of the SFAP.

d If this proposal is being submitted elsewhere for funding IN FULL state the organisation and when a decision is expected

No

7a At which development problems or needs is the project aimed?

Many inhabitants of South Africa have very low proportions of animal protein in their diets. The South African Medical Research Council has established that malnutrition is evident among both permanent and seasonal labour forces in the Western Cape, especially among children. ([16] Case studies have shown that tilapia is a very acceptable dietary item to such communities and is preferred to carp. Very modest dietary supplements with fish protein achieve striking results in most underweight children[17]

In many parts of Southern Africa the Nile tilapia (O. niloticus, the commonly preferred tilapia species for freshwater aquaculture) is not endemic but the Mozambique tilapia (O. mossambicus) is. Considerations of environmental protection of local biodiversity have led to the promotion of the Mozambique tilapia in small-scale aquaculture in S. Africa. However, this species suffers from the same disadvantage as the Nile tilapia, namely precocious sexual maturity leading to diversion of energy from somatic growth to reproduction. In grow out ponds this problem is further compounded by the production of numerous nutritionally and economically undesirable recruits which compete with the stocked fish for available resources. However, the problem in the Mozambique tilapia would appear to be even greater due to higher fecundity and a greater tendency to stunting. In Mozambique tilapia females have only some 60% of the growth rate of males and males are therefore preferred as the sex for monosex culture.

Whilst O. mossambicus has been widely introduced for aquaculture throughout the tropics and sub-tropics little is known of the commercial properties of different strains of the species and it appears quite probable that some indigenous strains will have better properties than those currently used in aquaculture. A characterisation of strains of O. mossambicus throughout its natural range is thus required in order to identify the important sources of genetic diversity for appropriate and sustainable conservation and exploitation.

In sub-tropical parts of Southern Africa temperate fish species can be cultured during the cool season. However, there is at present no suitable tropical species that can be stocked in the same culture facilities and grown to a marketable size in the time available during the warm season.

Within the FGRP a successful genetic technology has been developed in the Nile tilapia (O. niloticus) for the mass production, and use in aquaculture, of monosex males known as Genetically Male Tilapia (GMT). Furthermore UWS has developed the expertise in utilisation of molecular markers in strain identification and characterisation of tilapias. It is proposed to build upon these technologies to extend the benefits of the GMT approach to the appropriate strains of Mozambique tilapia, to evaluate the existing genetic resources and to assess the potential for other forms of genetic improvement in this species.

[16] Krige, M.U. and Senekal, M. (1997). S. Afr. J. Food Sci. Nut. **9**, 14-23.
[17] Steyn, N.P. et al. (1995). S. Afr. J. Food Sci. Nut, **7**, 158-162

7b What will the project contribute to resolving those problems or needs and over what timescale?

The project will characterise a large number of O. mossambicus strains collected throughout the region (with emphasis on S. Africa strains) for genetic variation and growth to determine the strains with greatest potential for aquaculture. Genetic improvement programmes will be initated with these strains. The project will also develop the YY male technology in two strains of the species to enable the production of monosex male stocks. It is anticipated that the results of this research will increase the growth performance of cultured O. mossambicus by 30-60% compared to currently available stocks within the time frame of the project. A long-term genetic improvement programme initiated by the project will be expected to improve the performance of the cultured stocks by a further 50% over the subsequent decade. These improvements will lead to very significant increases in aquaculture productivity and thus to the availability of cheap fish protein. Production costs of O. mossambicus are expected to be significantly lower than alternative cultured species and will thus be more accessible and affordable to the poor. In the time frame of the project, the improved strain(s) will be tested and made available in the Western Cape region through the SFAP. Following this appropriate dissemination of the outputs of the project will be made using existing contacts in S. Africa and by building contacts throughout the region.

7c Who will the beneficiaries be and are there any groups who will be disadvantaged by the application of the research findings?

The main immediate beneficiaries would be small scale ("previously disadvantaged") farmers working within community based aquaculture projects based on irrigation dams Using the Philips Beveridge model it is estimated that there is a sustainable production capacity of 6000 tonnes of fish p.a. from ±2,500 irrigation dames with a total area of 30,000 ha in the Western Cape. The UoS has been running, since 1995, a Small Farmer Aquaculture Programme (SFAP) for Aquaculture, which provides direct connections with rural communities. The programme embraces eight operational units with c. 4,000 direct beneficiaries. The fish farmers have a formal lease with the owners of the water bodies, the operation of which is overseen by UoS. Leases are for 5 years in the first instance. This Programme aims to embrace 80 farming communities by 2000 and in the longer term up to 500 communities. It is thought that each of these would produce about 16 tonnes of tilapia p.a. (using unimproved fish). No additional water resources would be required. Elsewhere in South Africa at least 100,000 ha. of utilisable water bodies exist. Ultimately it is believed that fish farmers throughout the region would benefit from the availability of genetically improved O. mossambicus with future dissemination programmes targeted at poorer regions such as North of South Africa, based on contacts with programmes similar to the SFAP.

The SFAP includes among its objectives the improved involvement of women in enterprise management and women will be encouraged to become involved in aquaculture development. It is not anticipated that any group would be disadvantaged by the planned programme of research and dissemination.

8 Objectives

8a What are the scientific/technical objectives of the project?

1. A reliable technology for mass production of genetically male tilapia (GMT) in two strains of the Mozambique tilapia through the adaptation of the YY male technology previously developed for O. niloticus.

2. An analysis of the genetic resources in Southern Africa strains of Mozambique tilapia available for exploitation through breeding programmes.

8b What are the developmental objectives of the project?

To enhance the livelihoods of rural poor in Southern Africa through:

- The enhancement of production of fish from existing aquaculture facilities
- The sustainable use of existing resources, infrastructure and expertise with the settlement of small scale farmers as independent fish producers
- Building entrepreneurial capacity and improving employment opportunities in an environment of decreasing demand for farm labour.
- The increased availability of affordable fish protein through the expanded culture of low input, low cost species

9a Describe the plan of work and complete the bar chart at Appendix 4 showing the planned activities over the life of the project.

1. Develop an assembly of accessions of up to 15 strains of O. mossambicus from throughout Southern Africa. Conduct growth performance trials of a minimum of eight strains, under communal stocking in two environments, to include farm cages and earthen ponds.
2. Genetic characterisation of available strains of O. mossambicus (a minimum of eight) using molecular techniques (using one or more of the techniques of analyses of microsatellite DNA loci, amplified fragment length polymorphisms and/or restriction endonuclease analysis of mitochondrial DNA haplotypes). Strains will be characterised for strain specific markers, levels of genetic variability and population structure.
3. Adaptation of the YY male technology to strains of O. mossambicus chosen on the basis of growth data and species purity. YY males will be generated using two approaches:
   i) the standard breeding programme combining feminisation and progeny testing (Mair et al. 1997) and;
   ii) a more rapid means using androgenesis as developed in project R4700 by the University of Stirling. It is estimated that androgenesis may enable production of a few YY males in 1.5 generations (1-1.5 years), compared to 2.5 (1.5 - 2.5 years) for the standard breeding programme.
4. On-farm trials of GMT will be conducted on at least 10 farms in the final year of the project. It is intended that these will be both in cages and ponds. Comparisons will be made with non-improved, locally available O. mossambicus as controls.
5. Before and following completion of on-farm trials, participating farmers will be interviewed to determine their response to the trials. Likely social and economic impacts of the technology will be determined from participatory research with farmers and potential consumers.

The project will be coordinated by a UWS post-doctoral research assistant who will be based virtually full time at the University of Stellenbosch (UoS). The post-doctoral researcher will work closely with local staff at Stellenbosch and with the collaborators under the SFAP but will take principal responsibility for the genetic characterization of the strains. UoS staff will take principal responsibility for the collection and growth performance characterisation of the O. mossambicus strains.

9b Logical Framework

Complete the logical framework (Appendix 5)

10a What will the outputs of the project be?

6. A rational choice of the most promising strains of O. mossambicus for use in small-scale aquaculture and for GMT production.
7. Recommendations on choice of strains, future strategies for further genetic improvement, and management of genetic diversity of O. mossambicus
8. A reliable genetic technology for the production of all-male O. mossambicus developed and broodstock for the production of GMT

9. A quantification of the benefits of application of GMT to small-scale aquaculture

10. A description and quantification of the potential social and economic impacts to be derived from the application of genetically improved O. mossambicus to small-scale aquaculture.

The aforementioned outputs will be published and presented in international, regional and national media in the form of oral presentations at scientific meetings and appropriate non-technical fora, peer reviewed and popular articles and progress reports to various parties.

10b Who will use the outputs of the project?

The outputs of this project will feed directly into the on-going Small Farmer Aquaculture Programme being coordinated by the University of Stellenbosch. Infrastructural and technical support to the project and to the SFAP will be provided by the Provincial Dept. of Agriculture. (PDA). The PDA will also assist the staff of the SFAP and the UoS Agricultural Extension service in provision of extension support to the beneficiaries. The beneficiaries will also receive business advice and credit services through a number of local NGOs.

In the longer term, the outputs of the project will be extended to other parts of S. Africa and the region via appropriate agencies such as the University of the North, with emphasis on poorer areas.

10c How will the outputs of the projects be disseminated to reach their users?

The mechanism for dissemination is outline in 10b above and in the attached information brief on the Small Farmer Aquaculture Programme (SFAP) from the University of Stellenbosch. A pilot project for the SFAP has been initiated (see brief).

UoS has conducted basic economic analysis of the potential for inter-cropping of trout and tilapia as outlined below:

The current reported annual production of tilapia in the Western Cape is 6 tons but is estimated at 25 tons for 1998-99. A commercial feed is used, though the UoS is attempting to develop feeds based on locally available products, especially agricultural and industrial waste. In the UoS project, tilapia are intended to be grown during the Summer in cages which are used during the winter to culture rainbow trout. The cages are maintained and harvested by the community as a whole although one or two of the farm workers take principal responsibility for day to day management.

A typical production cycle at full utilisation would be based on the rotational production of 6 tons of trout during the winter followed by 6 tons of tilapia during the summer. The same production systems would be used for the production of trout and tilapia. In the example provided below the capital structures required to accommodate this system include a set of two cages of 400m^3 each, and a store room for feed and equipment to the net value of R18 000. Financial assistance in the form of a R15 000 government subsidy is provided to each family unit of the small farmers as part of a scheme to empower previously disadvantaged communities. A typical annual budget of a rotational small farming unit is presented below, in line with actual returns of small farmers[18)].

The gross annual profit reported, which is substantially improved by the supplemenation of

[18] Salie, K. et al. (1998). Small scale fish farming in the Western Cape Province, South Africa: An analysis of technical and socio-economic outputs. Fourth African Farm Management Conference. University of Stellenbosch, January 1998.

trout with a tilapia crop, is equivalent to three times that of the average annual wage of rural farm workers in the region. This gross profit would be further improved by

- the genetic improvement of farmed stocks (particularly with regard to undomesticated O. mossambicus). In some cases this may be essential to produce a crop of marketable sized fish in the available time period.
- improvement in the efficiency and costs of feed;
- further training of small farmers, and
- the completion of loan repayment (after 4 years)

Annual budget for a small farming unit (SA Rand)

	Trout	Tilapia	Total
Income			
Sales of 6 tons of trout (R14.5.00/kg)	87000		
Sales of 6 tons of tilapia (R7.00/kg)		42000	
Annual total income			129,000
Running costs			
Loan repayment (over 4 years)	-15000		
Fingerlings	-21600	-5000	
Feed	-25200	-29000	
Transport	-2400	-1000	
Labour	-2400	-1000	
Water analysis	-600	-500	
Other	-1800	-500	
	-69000	-37000	-106,000
Gross profit (%)	26%	14%	22%
Gross profit after loan repayment (%)	61%	14%	42%
Net Annual Profit			23,000

11 What are the criteria that will be used to measure the outputs of the research?

Specific Criteria (numbered according to outputs specified in 10a above):

1. The choice of most promising strains will be made based on the completed analysis of reliable growth performance data and full analysis of data from the studies on genetic characterisation of the strain accessions.
2. Draft recommendations for submission to relevant Government. and development agencies will be drawn up for discussion by concerned parties. Recommendations will again be based on data from growth trials and genetic characterisation.
3. Criteria for determining the outputs related to the YY male technology will be the availability of significant numbers of YY males producing GMT with sex ratios in excess of 90% male.
4. Production benefits of application of the YY male technology will be determined through growth performance data from on-farm trials of GMT.
5. Preliminary evidence for the social and economic impact of the improved fish will come from the completed analysis of surveys of participating small-scale farmers.

General criteria:

The number of small farmers that are established and utilizing outputs from the project.

The improvement in biological productivity and economic performance of the production systems as indicated by the annual economic evaluation of the small farmer projects.

The contribution towards socio-economic development of rural communities as indicated by the annual socio-economic evaluation of the small farmer projects

12 What factors could prevent the attainment of;
a) Planned Inputs?

The loss of key staff members within the UoS Aquaculture Program although support should be available from co-workers in this unlikely event.

Difficulty in hiring appropriately qualified post-doctoral research assistant by UWS with necessary experience in fish breeding and molecular genetics.

Loss of strains of O. mossambicus already collected by UoS and difficulty or failure in collecting further strains

Loss of polictical stability in the region could impact upon all aspects of the project.

b Scientific/technical objectives?

Initially a lack of experience of research and technical staff with regard to tilapia may cause some difficulties but considerable experience of tilapia work on behalf of UWS should mitigate this.

Unforeseeably cold conditions might also have a negative influence on technical/scientific outputs in terms of a delay in growth characteristics, and shortening of breeding season reducing the number of generations that can be produced during the course of the project.

Failure to successfully induce androgenesis adopting techniques developed for O. niloticus would slow down the development of YY males in O. mossambicus.

If the assumptions that sex determination in O. mossambicus is similar to that in O. niloticus is not validated in the key strains of O. mossambicus to be used in this project the development of the YY male technology in this strain may be difficult.

Low levels of genetic variation in the accessions of O. mossambicus may make lessen the resolution of molecular markers for strain identification but alternative methods of analysis are available to increase the resolution.

c Intended outputs?

These are largely common to the points outlined above in 12b

Failure to achieve the technical objectives in sufficient time would prevent large scale evaluation of improved fish on farm which would in turn result in the failure to obtain sufficient data on the likely social and economic impacts of the introduction of small scale aquaculture (output 5) by the end of the project.

d The contribution or the project to the wider developmental problems or needs identified in section 7?

Inefficiency with regard to extension, training and technical support services towards new small farmers. (Currently extension workers of the Department of Agriculture have limited aquaculture experience). Training for DA staff will probably be needed at a stage when the number of small farmer increase beyond the capacity of the UoS.

Again the lack of experience in the existing extension network might hamper the wider implementation of the project into region outside the Western Cape into the Northern region of South Africa and the neighboring countries (Training might be necessary at that stage) The outcome of the sustained economic viability of the small farmer projects might have a negative influence on the wider developmental objectives, if the prospects are found to be limited for example if the land owners begin to restrict access of the farm worker to the water resources.

The market for tilapia in the region is not well established, it may be necessary to actively promote tilapia if market demand is not high or the production costs result in prices that are too high for local markets.

13a What work has been, or is currently being, done on the objectives of the project?
A review of literature should be attached.

Through work within projects R4452, R4803 and R6058, successful development of the GMT technology has been achieved in Nile tilapia with the key results summarised by Mair et al, 1997[19]. The essential protocols used in the production and identification of YY males including feminization and progeny testing have been developed and are described in Mair and Abella (1997) [20] Furthemore a technique of andorogenesis has been developed for Nile tilapia under a project partially funded by DFID which can enable more rapid production of YY males which will be homozygous[21] . Thus much has been learned about the factors influencing the determination of the sexual anatomy of tilapia. As the Nile and Mozzambique tilapias appear to possess similar genetic systems controlling sexual phenotype, much of the information already available is likely to be of direct help in this project.

In projects R6070 and R5068 the considerable practical value of the YY male technology have been demonstrated in terms of enhanced yields under culture[22] and economic benefits to farmers. Implementation of the research findings in the Philippines and Thailand have been demonstrated and the outputs of the technology are being broadly adopted in these countries. In R6938 the work is being extended to Vietnam.

[19] Mair, G.C., Abucay, J.S., Skibinski, D.O.F., Abella, T.A., Beardmore, J.A. (1997) Genetic manipulation of sex ratio for the large scale production of all-male tilapia Oreochromis niloticus L. Canadian Journal of Fisheries and Aquatic Sciences, 54(2): 396-404.

[20] Mair, G.C. and Abella, T.A. (eds) (1997) Technoguide on the production of genetically male tilapia. Freshwater Aquaculture Center, Central Luzon State University, N. Ecija, Philippines. 68p

[21] Myers, J.M., Penman, D.J., Basavaraju, Y., Powell, S.F., Baoprasertkul, P., Rana, K.J., Bromage, N., McAndrew, B.J., 1995. Induction of diploid androgenetic and mitotic gynogenetic Nile tilapia Oreochromis niloticus (L.). Theoretical and Applied Genetics, 90: 205-210.

[22] Mair, G.C., Abucay, J.S., Beardmore, J.A., Skibinski, D.O.F., 1995. Growth performance trials of genetically male tilapia (GMT) derived from YY - males in Oreochromis niloticus L.: On station comparisons with mixed sex and sex reversed male populations. Aquaculture, 137: 313-322.

To the best of our knowledge, no systematic work on genetic approaches to monosex production in the Mozambique tilapia for aquaculture has been carried out although it is believed that some YY males were produced in this species in China in the 1970s [23]. There is some interest in Israel in work of this kind and appropriate contact with Dr. G. Hulata and his group will be maintained.

The University of Stellenbosch (UoS), with funding from agencies, including the British High Commission in S. Africa and the Belgian Ministry of the Flemish Community, has recently established a project to develop small scale aquaculture based on the Mozambique tilapia and trout in the Western Cape. The communities targeted by the Small Farmer Aquaculture Program (SFAP) are rural and consist of small scale farmers and farm workers. In this project work has been done on the economics of trout and tilapia culture, production performance of O. mossambicus, effects on water quality, acceptability of tilapia in human diets, and work has been initated on the comparisons of production in different strains of O. mossambicus through the collection of a number of strain accessions from different parts of S Africa. As part of the project three pilot small farming operations are currently being conducted and a further eight are to be implemented during the 1998-99 season. These projects together with the eight existing small farming units are carefully monitored and evaluated with regard to the set objectives of the Project.

Furthermore, a detailed socio-economic evaluation of the SFAP is also currently planned for implementation during the first phase of the Project, in order to provide essential baseline information on the economic viability and sustainability of the SFAP project with regards to its future planning and implementation. This survey will also provide valuable data on the current social and economic status of the intended beneficiaries of the SFAP project and of the improved fish to be developed under this research project.

b Is this proposal a continuation or extension of work already funded by UWS under the Fish Genetics Research Strategy Programme? If so, please state Project Reference Number and Title.

This project is not a direct continuation of an ongoing DFID FGP project but it could be regarded as a logical extension into new regions and new species of previous projects working with Nile tilapia, focussed on S.E. Asia (R4452, R4803, R5068, R6058, R6070 and R6938).

14 What are the expected environmental impacts, (beneficial, harmful, neutral) of:
a The project itself

The project will bring in tilapia germplasm from several sources including some outside of S. Africa. It is not thought that there distinct are local populations of the species in the Western Cape region that would be at risk from contamination by the introduced populations. It is also intended that the introduced strains will be held in secure facilities with no connections to natural waterways or water bodies thereby minimizing any risk that the introduced fish will impact upon natural populations of other aquatic species.

The project will be initially be using DES for feminization of O. mossambicus. This compound is known to be partially toxic although relatively quickly broken down. To minimize any prospect of contamination of natural waters, water containing residues of the hormone, post treatment, will be disposed off by draining into the ground.

[23] Xia, D., Wu, T., 1993. A review of fish breeding research and practices in China. In: Main, K.L., Reynolds, E. (Eds.), Selective Breeding of Fishes in Asia and the United States. The Oceanic Institute, Honolulu, Hawaii, pp. 214-225.

In general the environmental impacts of the project itself should be minimal

b The implementation of the project?

Beneficial environmental aspects include the sustainable utilization of existing agricultural infrastructure and water resources (for example the introduction of fish may minimize chances of eutrophication). Irrigation systems are isolated from natural water bodies and no negative environmental influences from fish escaping from aquaculture into surrounding natural waterbodies and waterways are anticipated.

A continuous water quality monitoring program is being implemented on all farming systems in order to quantify any possible environmental impact of the aquaculture and to assess the accuracy of models for predicting sustainable production levels for these small farming systems.

Possible harmful aspects include the impact of O. mossambicus on ecosystems to which it is non endemic. This is one of the key aspects why the supermale technology is of utmost importance in order to minimise or prevent the uncontrolled spreading and breeding of tilapia in local aquatic ecosystems. Over enrichment of water bodies due to an unsustainable organic loading from the intensive fish farming systems in enclosed water bodies will also have to be monitored very carefully. This necessitates the accurate assessment of initial carrying capacities of these water bodies.

15 THIS APPLICATION IS MADE AND ANY GRANT SHALL BE AWARDED SUBJECT TO THE FOLLOWING CONDITIONS;

Note: This application should be submitted through (i) the Head of Department and (ii) the officer who will be responsible for administering any grant that may be awarded (eg Bursar, Registrar); (iii) Collaborators named in section 4(b).

i Project costs provided in Section 6 and appendices 1 (a), (b) and (c) shall be based on the best estimates of the proposers and allow for inflation. Any provision for increase in UK salary to offset inflation shall be based on HM Treasury estimates of inflation.

ii Where provision for overhead charges is appropriate, it will only be considered on the basis of payroll costs.

iii **Unless agreed in advance and recorded in writing** UWS shall not be responsible for any cost or expense of any kind, incurred for any reason, in excess of the agreed project funding. Sponsoring institutions will be expected to absorb increases in costs caused by inflation above the estimated levels.

iv Claims shall be submitted quarterly to UWS by 13 July, 13 Cctober, 13 January, respectively for the quarters 1-3. These are to include a statement of actual expenditure in the previous quarter and an estimate of expenditure for the next quarter. For quarter 4 the claim must be submitted by 5 March. This must include actual expenditure to date plus estimated expenditure for 5-31 March.

v Any part of any advance remaining unspent at the close of a financial year will be offset against the following year's allocation and cannot be added to the new financial year's funding provision.

vi The applicant shall submit to UWS progress reports in respect of the project. Unless UWS otherwise directs annual progress reports shall be submitted to be received by UWS **not later than 10 March** for all projects. Failure to submit the annual progress report on time may, at the discretion of UWS, result in the withholding of funds or the termination of the project. In particular the applicant's attention is drawn to paragraph 15 (v) above. Quarterly reports incorporating all information required (detailed separately from time to time) should be submitted no later than respectively June 10 (Q1), Sept. 10 (Q2), Nov. 10 (Q3 and 14 Jan. (Q4) respectively.

(b) UWS may request and the applicant shall provide, reports at any time while the project is in progress. Failure to comply with the request by the date specified by UWS may result in the withholding of funds or the termination of the project.

(c) The applicant shall submit to UWS **a draft final report to be received by UWS within 21 days of the project completion date.** Except in exceptional circumstances the final quarterly payment or 10 per cent of the total project cost, whichever is the greater, will be retained until the final report is approved by UWS. **The definitive final report should be submitted to UWS within 42 days of the project completion date.**

(d) **All** reports shall be made in the format specified in Appendix 3(A) & (B). Each final and annual report shall be accompanied by an executive summary of 300 - 500 words in the same format.

vii **Necessary time for final report writing must be included in the time scale of the project**. Any costs of publishing and dissemination of such reports, including essential translation for which UWS funding may be requested, shall be included in the application.

viii UWS shall regularly receive and retain copies of the Annual Audited Accounts of the Institute of the applicant.

ix UWS reserves the right at any time to visit its subcontractors giving reasonable notice of its intention, with full access to relevant personnel, offices, documents, papers and computers and computer software relating to the research. Such rights of access must also be afforded to the DFID, as sponsoring body for the programme, and the DFID's agents and servants.

x Any publications or unpublished papers arising from the Project shall acknowledge the DFID's sponsorship of the research. Copies of the manuscripts intended for publication should be submitted to UWS before they are submitted to the editors. The DFID shall not unreasonably withhold its approval or cause delay in publication.

xi It is a condition of this contract that the rights of the DFID as the sponsoring body of UWS's research programme, retains its rights as follows:

(a) "All rights, save for copyright, in any inventions, designs and technical information, arising under the project including the results of any work performed by any employee engaged to perform under the project, shall vest in and be the property of the Secretary of State for the Department for International Development, London, SW1; as regards copyright, this shall vest in the Crown. The applicant shall take all measures necessary to secure such vesting.

(b) The applicant undertakes that he has not entered into nor will enter into any agreement, understanding or arrangement with a third party which will or could affect all or any of the rights of the Secretary of State for the Department for International Development or the Crown arising out of this project except with the prior written consent of the Secretary of State, or Crown as the case may be.

(c) The Department for International Development shall have the sole right to determine whether the results of work under this Project shall be protected by patent or like protection. In the event that the Department for International Development wished to file a patent application the applicant shall provide all reasonable assistance at the expense of the Department for International Development necessary in connection with such patent or other applications.

(d) The applicant shall not either directly or through its servants or agents during or after the termination of the Project disclose to any third parties any information arising from this engagement relating to but not limited to the Project itself or the DFID unless the DFID has given its prior written approval of such disclosure.

(e) The Crown shall have the sole right to publish or reproduce in any form any results of, or arising from, work on the project.

xii UK airlines shall be used for travel abroad by those whose fares are paid from British public funds unless it is uneconomic, highly inconvenient or impractical to do so. Economy or APEX type fare will be used.

xiii C.V.'s of all staff participating in research projects must be submitted to UWS before their participation commences regardless of the stage at which they are recruited.

xiv UWS shall be informed immediately of any likely shortfall in the project time scale for whatever reason.

xv

(a) UWS reserves the right to terminate a grant where it is dissatisfied with the conduct of the project, including the administration of its grant, or where, in its judgement, the project objectives are unlikely to be achieved or are no longer of relevance to it. Before terminating a project UWS will set out in writing its reasons to the applicant, give the applicant 30 days in which to respond and if the applicant is in the United Kingdom or will travel to the UK at his own expense, will offer the applicant a hearing on a date of UWS's choosing. Where the reason for the termination of the project is, in UWS's judgement, outside the control of the applicant and the applicant's institution, UWS will give 3 months notice of termination of its grant; where the reason is considered to be within their control, UWS will give 30 days notice. UWS would expect, if it wishes, to reclaim capital equipment and any funds paid to the applicant or the applicant's institution which have not been spent in accordance with the project Memorandum after the period of notice has expired.

(b) In the event that the applicant has failed or fails to comply with the provision of sub-paragraphs ix hereof UWS reserves its rights to withdraw unspent project funds forthwith and the applicant will return unspent funds in their possession immediately upon UWS's first demand and provide evidence of UWS's satisfaction that all such unspent monies have been returned. For the avoidance of doubt the obligation of the applicant to return all unspent monies does not extinguish any further liability and UWS on behalf of the Secretary of State or the Crown reserves the right to bring claims in respect of any loss of rights or other loss or damage suffered by the Secretary of State or the Crown as a Consequence of such non-compliance by the applicant.

If my application is successful, I agree to accept and be bound by the conditions set out in paragraphs 15(i) - 15(xiv) of this application.

	1.
Signature of applicant(s)	2.
	3.
Date	

APPENDIX 4

R AND D BAR CHART

PROJECT NUMBER | **R 7284**

PROJECT TITLE: Development of tilapia genetic resources for aquaculture in Southern Africa

DATE: Dec 1 1998 to Dec. 31, 2001

ACTIVITY	**YEAR 1998/99**											
	A	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**	**M**
Collection of new germplasm											■	■
Collect baseline socio economic data											■	■
Molecular characterisation of strains												
Growth characterisation of strains									■	■	■	■
Production of YY ♂ breeding programme.												
Feminization of normal progeny									■	■	■	■
Progeny testing to identify XY Δ♀												
Feminize progeny from XY x XY crosses Progeny test ♂ & ♀ from XY x XY crosses												
Cross identified YY Δ♀ x YY ♂												
Production of YY ♂ by androgenesis												
Androgenesis training												■
Androgenesis on normal males												
Feminize 50% of androgenetics												
Progeny test androgenetic ♂ and Δ♀												
Cross YY Δ♀ x YY ♂ androgenetics												
Production of all females												
Masculinize normal progeny											■	■
Progeny testing to identify XX Δ ♂												
Masculinize progeny from XX x XX crosses												
Mass produce all females for broodstock												
Produce GMT for limited trials **Produce GMT for on-farm trials**												
On-farm trials of GMT												
Socio-economic survey on trial farms												
Formulation of recommendations/outputs												
OVERSEAS TRAVEL By / To / Duration (days)												
RO / UK / 14-21											■	■
GCM / SA / 10												■

NOTES

1. **Please tick appropriate box for activity/travel month.**
2. **A separate Bar Chart should be shown for each year of the project.**
3. **Overseas travel: give name of travellers, length of stay and the countries visited.**

APPENDIX 4

R AND D BAR CHART

PROJECT NUMBER R 7284

PROJECT TITLE: Development of tilapia genetic resources for aquaculture in Southern Africa

DATE: Dec 1 1998 to Dec. 31, 2001

ACTIVITY	YEAR 1999/2000											
	A	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**	**M**
Collection of new germplasm		■	■									
Collect baseline socio economic data												
Molecular characterisation of strains	■	■	■	■	■	■	■	■	■	■	■	■
Growth characterisation of strains	■	■						■	■	■	■	■
Production of YY ♂ breeding programme.												
Feminization of normal progeny												
Progeny testing to identify XY Δ♀	■	■	■	■	■							
Feminize progeny from XY x XY crosses				■	■	■	■					
Progeny test ♂ & ♀ from XY x XY crosses									■	■	■	■
Cross identified YY Δ♀ x YY ♂												
Production of YY ♂ by androgenesis												
Androgenesis training												
Androgenesis on normal males	■	■	■	■	■	■	■					
Feminize 50% of androgenetics			■	■	■	■	■	■	■			
Progeny test androgenetic ♂ and Δ♀									■	■	■	■
Cross YY Δ♀ x YY ♂ androgenetics												
Production of all females												
Masculinize normal progeny	■	■										
Progeny testing to identify XX Δ ♂					■	■	■	■	■			
Masculinize progeny from XX x XX crosses									■	■	■	■
Mass produce all females for broodstock												
Produce GMT for limited trials												
Produce GMT for on-farm trials												
On-farm trials of GMT												
Socio-economic survey on trial farms												
Formulation of recommendations/outputs												
OVERSEAS TRAVEL By / To / Duration (days)												
RO / UK / 21						■						
JAB / SA / 7				■								
GCM / SA / 10											■	

NOTES

1. **Please tick appropriate box for activity/travel month.**
2. **A separate Bar Chart should be shown for each year of the project.**
3. **Overseas travel: give name of travellers, length of stay and the countries visited.**

APPENDIX 4
R AND D BAR CHART

PROJECT NUMBER R 7284

PROJECT TITLE: Development of tilapia genetic resources for aquaculture in Southern Africa

DATE: Dec 1 1998 to Dec. 31, 2001

ACTIVITY	YEAR 2000/2001											
	A	M	J	J	A	S	O	N	D	J	F	M
Collection of new germplasm												
Collect baseline socio economic data												
Molecular characterisation of strains	■	■	■	■	■	■	■	■	■	■	■	■
Growth characterisation of strains	■	■						■	■	■	■	■
Production of YY ♂ breeding programme.												
Feminization of normal progeny												
Progeny testing to identify XY Δ♀												
Feminize progeny from XY x XY crosses												
Progeny test ♂ & ♀ from XY x XY crosses	■	■										
Cross identified YY Δ♀ x YY♂	■	■	■	■	■	■	■	■	■	■	■	■
Production of YY ♂ by androgenesis												
Androgenesis training												
Androgenesis on normal males												
Feminize 50% of androgenetics												
Progeny test androgenetic ♂ and Δ♀	■	■										
Cross YY Δ♀ x YY ♂ androgenetics	■	■	■	■	■							
Production of all females												
Masculinize normal progeny												
Progeny testing to identify XX Δ ♂												
Masculinize progeny from XX x XX crosses										■	■	■
Mass produce all females for broodstock			■	■	■	■	■	■	■	■	■	■
Produce GMT for limited trials	■	■	■	■								
Produce GMT for on-farm trials											■	■
On-farm trials of GMT												
Socio-economic survey on trial farms										■	■	■
Formulation of recommendations/outputs												
OVERSEAS TRAVEL												
By / To / Duration (days)												
RO / UK / 21	?											
JAB / SA / 7	?											
GCM / SA / 10	?											

NOTES

1. **Please tick appropriate box for activity/travel month.**
2. **A separate Bar Chart should be shown for each year of the project.**
3. **Overseas travel: give name of travellers, length of stay and the countries visited.**

APPENDIX 4

R AND D BAR CHART

7284

PROJECT NUMBER | **R**

PROJECT TITLE: Development of tilapia genetic resources for aquaculture in Southern Africa

DATE: Dec 1 1998 to Dec. 31, 2001

ACTIVITY	**YEAR 2001/2002**											
	A	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**	**M**
Collection of new germplasm												
Collect baseline socio economic data												
Molecular characterisation of strains	■	■	■									
Growth characterisation of strains	■	■	■									
Production of YY ♂ breeding programme.												
Feminization of normal progeny												
Progeny testing to identify XY Δ♀												
Feminize progeny from XY x XY crosses												
Progeny test ♂ & ♀ from XY x XY crosses												
Cross identified YY Δ♀ x YY ♂	■	■	■	■	■	■	■	■	■			
Production of YY ♂ by androgenesis												
Androgenesis training Androgenesis on normal males												
Feminize 50% of androgenetics												
Progeny test androgenetic ♂ and Δ♀												
Cross YY Δ♀ x YY ♂ androgenetics	■	■	■	■	■	■	■	■	■			
Production of all females												
Masculinize normal progeny												
Progeny testing to identify XX Δ♂												
Masculinize progeny from XX x XX crosses				■	■	■						
Mass produce all females for broodstock	■	■	■	■	■	■	■	■	■			
Produce GMT for limited trials												
Produce GMT for on-farm trials	■	■	■									
On-farm trials of GMT								■	■			
Socio-economic survey on trial farms	■	■										
Formulation of recommendations/outputs					■	■	■	■	■			
OVERSEAS TRAVEL By / To / Duration (days) RO / UK / 21	?											
JAB / SA / 7	?											
GCM / SA / 10	?											

NOTES

1. **Please tick appropriate box for activity/travel month.**
2. **A separate Bar Chart should be shown for each year of the project.**
3. **Overseas travel: give name of travellers, length of stay and the countries visited.**

Appendix 4 Project Staff and Affiliated Institutions

University of Wales Swansea

Dr. Graham C. Mair, Research Fellow (seconded to and based at the Asian Institute of Technology, Bangkok, Thailand). Principal investigator, Overall Project Coordination and Reporting

Prof. John A. Beardmore, Consultant. Principal Investigator and UWS Component Administration

University of Stellenbosch

Academic staff

Louw Hoffman - Principal Investigator, Responsible for Project Coordination

Danie Brink – Responsible for project management, administration, liaison and reporting

Rupert Lewis* - Research Officer (Feb., 1999 - March 2001) – Responsible for project management, liaison, reporting and direct responsibility for molecular work.

Eugenia D'Amato* (March 2001 – Dec 2002) – Research Officer (March 2001 to December 2001). Responsibility for molecular work and reporting.

Mariette Du Plessis – Researcher (Jan to Dec 2001) Responsible for the production and progeny testing of YY-males and the execution of comparative growth trials between GMT and mix-sex strains of tilapia (*O. mossambicus*). YY breeding programme and complementary research on sex determination

Paul Marais – Research assistant (Jan 1999 to Dec 2000) Responsible for the production and progeny testing of YY-males and the execution of comparative growth trials between GMT and mix-sex strains of tilapia (*O. mossambicus*). Growth trials and YY breeding programme

Kobus Swart* - Research assistant. (Feb to Dec 2001). Assistance with the production and progeny testing of YY-males.

Henk Stander (dates) – Technical assistant. (Jan 2000 - Dec 2001). Management of fish keeping faciities (P/T)

Khalied Salie - Development officer. (Jan 2000 - Dec 2001). Assistance with extension services, training and surveying amongst rural communities.

Edward Hall - Student (Jan 1999 - Dec 2001) Molecular characterisation of *O. mossambicus* strains

Marna Esterhuyse – Student (Jan 2000 to Dec. 2001) Genetic characterisation of tilapia species and their hybrids

Technical Support

Syster van Wyk* (Welgevallen) P/T	Filip Fortuin* (Elsenburg) P/T
Isaac Sinden (Elsenburg)	Werner Petersen (Welgevallen)
Aletta Bester (Molecular lab)	Aletta Bester (Molecular lab)

* Staff members funded by DFID Project Funds. Other members funded by the Univ. Stellenbosch
The Project has significant interaction with personnel of the Western Cape Department of Agriculture, who are providing inputs regarding extension work and field trials.

Department of Agriculture of the Western Cape

Paul King – Researcher (Jan 1999 to Dec 2001). Responsible for Project coordination and integration of activities into the objectives of the Department of Agriculture.

Robert Hartnell[24] - VSO volunteer assigned to the Department of Agriculture (July 1999 to December 2001). Assistance with extension services, training and surveying amongst rural communities.

Jaco Swart - Researcher. (Jan 1999 to Dec 2001). Assistance with research on strain evaluation as well as with extension services to rural communities

[24] The VSO volunteer was assigned to DA as a result of a project initiative in which we requested assistance from VSO in evaluating the potential of tilapia culture as a component of rural development programmes.

Appendix 5 Application for extension to Project R 7284.

20th February 2002

Prof. James Muir
Institute of Aquaculture
University of Stirling
Stirling FK9 4LA
Scotland
UK

Dear Prof. Muir

Application for additional funding: DFID Project R7284: Improvement and Utilisation of Indigenous Tilapia Genetic Resources in Southern Africa

The overall development objective of the project is the enhancement of livelihoods of rural poor in Southern Africa through:

- The sustainable use of existing resources, infrastructure and expertise with the settlement of small scale farmers as independent fish producers:
- The increased availability of affordable fish protein through the expanded culture of low input, low cost species enhanced fish production.
- Building entrepreneurial capacity and improving employment opportunities through the enhancement of production of fish from existing aquaculture facilities.

Good progress have been made with regard to various of these **overall objectives**, including:

- ♦ Tilapia farming systems has been accepted as a set component of the Small Scale Aquaculture Programme of the National Department of Agriculture. Twelve tilapia farming systems and three demonstration units have been introduced in various districts of the Western Cape Province (see Figure 1). The programme is currently being expanded throughout the Province as well as to other provinces (Mpumalanga, Northern Province, Kwa-Zulu Natal; see Figure 2). The current demands for the uptake of tilapia technologies however far out-way the institutional capacity Department of Agriculture and the University of Stellenbosch. The continued facilitation of uptake tilapia technologies will make an important towards ensuring continuation of project.

- Tilapia (*O. mossambicus*) has been well accepted by consumers at all levels of society, from rural communities to retail outlets. Tilapia was able to compete well with other low cost/affordable protein sources in rural communities and lower income groups. The potential of the specie to contribute towards food security and improved nutritional status in these communities has been confirmed. More work needs to be done to further quantify the social and economic impacts of the technology on the target communities, in particular to expand the surveys to include other regions of the country that could also benefit.

- The commercial fisheries and agricultural sectors are showing distinct interest in the development of commercially orientated tilapia farming systems. This was mainly due to an improved awareness of the potential for tilapia culture created by the DFID project. The envisaged commercial developments will play an important role in facilitating the future application of tilapia technologies, in as far as that it will assist with the distribution of technologies and related opportunities throughout the Southern African Region. Viability studies for commercial development of tilapia farming systems include all regions of South Africa, extending into Namibia, Botswana and Mozambique. The secondary distribution of benefits to the surrounding communities through job creation, training, services and food supplies form an integral part of the proposed commercial ventures.

The specific research objectives of the Project include:

- The collection of up to 15 populations of *O. mossambicus* representative of the geographical distribution of the specie through Southern Africa. To be followed by the growth performance under local conditions.
- The genetic characterisation of available populations of *O. mossambicus* in terms of levels of genetic variability and population structure.
- The application of YY male technology to select strains of *O. mossambicus*.
- Comparative growth trials between GMT, sex-reversed and non-improved *O. mossambicus*.
- The determination of social and economic impacts of the technology will be determined from participatory research with farmers and potential consumers.

Significant progress have been made with regard to various of the research objectives, including:

- A valuable collection of genetic material representative of the genetic resources of the region has been constituted in the form of 15 populations.

- The genetic characterisation of the populations has been completed in terms of growth performance, genetic variability and population structure.

- Conservation authorities and institutions engaged in rural development programmes have indicated a specific interest in the sustainable utilisation of indigenous aquatic resources for rural development. Tilapia, and more specifically the outputs from the project, has given a clear indication how such resources could be developed to contribute to the objective of socio-economic development of rural livelihoods.

- Good progress has been made with regard to the application of YY male technology to select strains of *O. mossambicus*. The project is currently engaged in the final stage of progeny testing and identification of YY males. Various technical matters (i.e. facilities, environmental, human resources) as mentioned in quarterly reports have delayed progress, causing this facet of the project to exceed the initial dates for completion.
- Good baseline data has been obtained with regard to the social and economic impacts of the technology on rural communities in particular, giving indication of meaningful contributions towards improved livelihoods of rural communities. This component of the study however needs to be expanded to include a wider regional representation as well as a further focus on secondary benefits to communities.

Conclusion

On the basis of these developments we would like to forward an application for the allocation of additional funds in order to allow us to completed the following essential components of the original proposal:

- Completion of the final stage of progeny testing and identification of YY males.
- Completion of the determination of social and economic impacts of the technology on improved livelihoods of rural communities. Also to respond to specific opportunities that will make a meaningful contribution towards ensuring continuation of the project and the achievement of its overall objectives such as:
- To the address the current demand for uptake of tilapia technologies, and in particular its extension to other regions of South Africa.
- To initiate discussion that could contribute towards a strategy/policy for the sustainable development of indigenous aquatic resources, for aquaculture development in the Southern African region.

Proposed Budget for Application for Additional Funding:

The details for the further application of funds within the framework of the project are:

Completion of the technical trials on the YY identification and progeny testing

Objective: The further acquisition of data to provide conclusive prove of the status of YY individuals in the current strain of O. mossambicus.

		Pound Stirling
Costs:	**Staff costs:**	**1 300**
	Consumables:	**800**
	Travel & Subsistence:	**400**
	Total	**2 500**

An extended evaluation to determine the social and economic impacts of the technology on improved livelihoods of rural communities.

Objective: To determine the social and economic impacts of low input aquaculture technology on improved livelihoods of target communities through participatory surveys.

		Pound Stirling
Costs:	**Staff costs:**	**800**
	Consumables:	**120**
	Travel & Subsistence:	**1 000**
	Total	**1 920**

Addressing the immediate demand for uptake of tilapia technologies

Objective: To facilitate the dissemination, awareness and uptake of tilapia technologies to rural communities of the Western Cape and the Kwa-Zulu natal. This will be done in the for of site visits, workshops, short courses and demonstration units.

		Pound Stirling
Costs:	**Staff costs:**	**1 500**
	Consumables:	**1 000**
	Travel & Subsistence:	**1 400**
	Total	**3 900**

A strategy/policy for the sustainable development of indigenous resources, for aquaculture development in the Southern African region.

Objective: To initiate discussion that could contribute towards a strategy/policy for the sustainable development of indigenous aquatic resources, for aquaculture development in the Southern African region. This would be done in the form of a workshop with representation from government, conservation, research and commercial institutions.

		Pound Stirling
Costs:	**Staff costs:**	**1 500**
	Consumables:	**500**
	Travel & Subsistence:	**2 500**
	Total	**4 000**

Total Application: 12 320 (Pound Stirling)

Summary

The application for additional funds is intended to conclude outstanding aspects related to the research component of the existing project (R7284), as well as to attend to specific opportunities that will make a meaningful contribution towards the overall objectives of the project and to contribute towards its continuation.

We trust that you will be able to support our application.

Yours sincerely

Dr. L.C. Hoffman

Principle Investigator: University of Stellenbosch

Fig 1 Location of Tilapia Small Farming systems in the Western Cape

Figure 2 Provinces where tilapia farming systems are being introduced

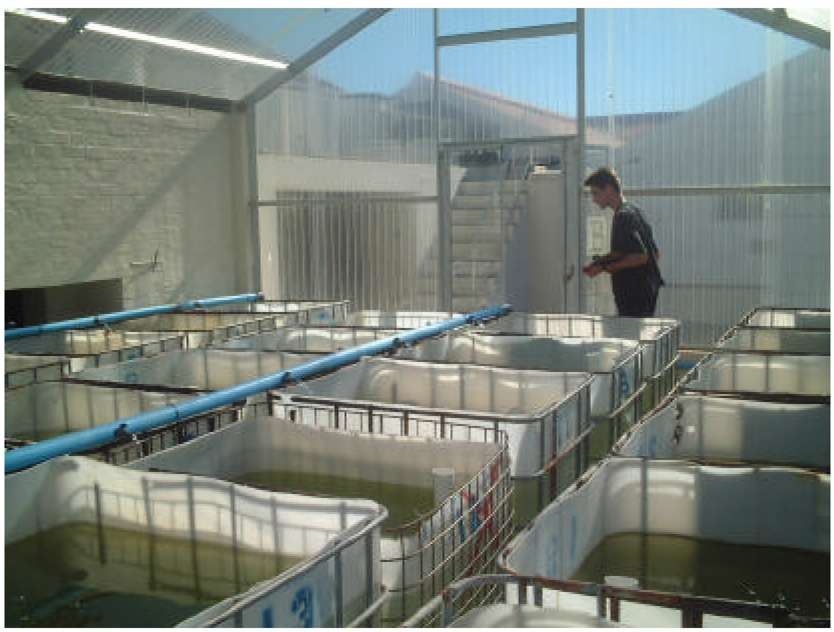
Appendix 6 Physical facilities developed and/or utilised by the project

Welgevallen Experimental Farm (nearby UoS campus)

Glasshouses

- Two recirculating systems in glass houses with 55 x $1m^3$ square tanks for breeding of family groups
- Three recirculating systems in glass houses with a total of 108 x $.2m^3$ circular tanks for rearing single families
- Temperature controlled recirculation unit with 24 x $1m^3$ concrete tanks for holding of live tilapia germplasm.

Figure 10 Tanks for breeding *O. mossambicus* at the Uos Welgevellen Agricultural Research Facility.



Climate controlled rooms

- Five climate controlled rooms each equipped with 8 x .18 m^3 glass tanks as part of a recirculation system, used for holding and spawning broodfish prior to chromosome manipulation work and for rearing of family groups.

Tunnel and Pools

- One large polythene tunnel with four self-contained $20m^3$ circular pools (for nursing or grow-out).

Elsenburg Experimental Unit (Co operated with the Dept. of Agriculture)

- A total of 10 x 200 m² open earthen ponds, used for growth trials.
- A total of 5 x 350m² covered earth ponds, used for growth trials and fingerling production.
- A nursery facility with 15, 2m diameter circular tanks

Figure 11 A hatchery and nursery under construction by the DA at Elsenberg. The DA began to develop these facilities to support aquaculture development in response to initiatives made by UoS under the project.

JC Smuts School of Biological Sciences (UoS campus)

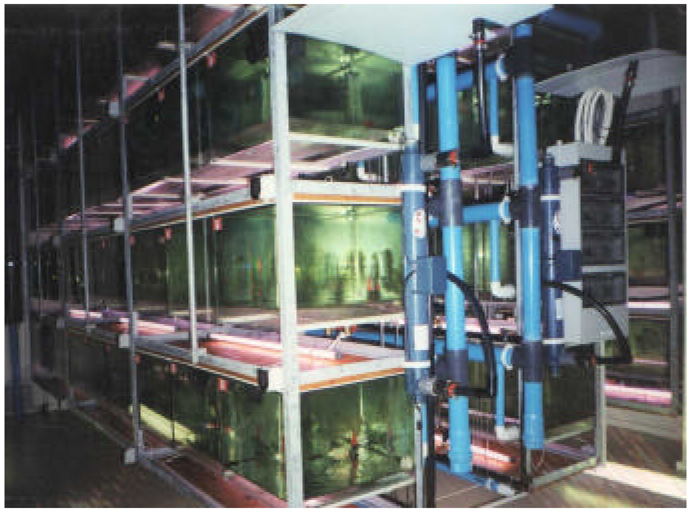
Molecular Laboratory

A fully equipped molecular laboratory is at the disposal of the Project research group. The laboratory is well supported by centralised facilities such as DNA-sequencing, PCR, centrifuges, etc.

Aquarium Facility

A climate controlled aquarium facility consisting of a total of 90 x 120 liter tanks was developed for use by the project. This facility was used primarily for progeny testing in the YY male breeding programme.

Figure 12 Photograph of climate controlled aquarium facility constructed largely using project funds in the J.C. Smuts building at UoS.



Appendix 7 Preliminary report on growth performance evaluation of strains of *O. mossambicus*.

Draft Report

Characterisation of Growth Performance of Strains of the Indigenous Tilapia (*Oreochromis mossambicus*) of Southern Africa

Paul Marais, Danie Brink and Graham Mair

Division of Aquaculture, University of Stellenbosch

University of Wales Swansea

with support of

DFID Fish Genetics program, Project R 7284

"Improvement and Utilisation of Indigenous Tilapia Genetic Resources in Southern Africa"

December 2001

Introduction

Under DFID funded research project R. R 7284 "Improvement and Utilisation of Indigenous Tilapia Genetic Resources in Southern Africa" a range of indigenous and introduced wild and domesticated farm stocks of *O. mossambicus* were sampled with live gene banks being established at the facilities of the University of Stellenbosch. The objective of these collections was to conserve and evaluate *O. mossambicus* germplasm (see Table 8 for details of germplasm available) for its potential in aquaculture. This report presents the preliminary analysis of the results from two separate, dual phase growth performance trials under artificial conditions at the aquaculture facilities of the University of Stellenbosch.

Table 8 A summary of the various populations of tilapia (*O. mossambicus*) collected from the Southern African Region, for inclusion in comparative growth trials.

Population	Source	Type
Kasintula	Kasinthula Research Station – Shire river - Malawi	WT
Sucomba	Sucomba, commercial sugarcane enterprise with river catchment – Lower Shire river- Malawi	WT
Ndumu	Ndumu Game reserve – series of small lakes - Pongola river basin — South Africa/Mozambique border	WT
Makathini	Makatini Research Station – Pongola river basin – South Africa	F
Olifants	Mpumalanga Nature Conservation - Olifants river – Loskopdam irrigation scheme – South Africa	WT
Elands	Mpumalanga Nature Conservation - Elands river – Loskopdam irrigation scheme – South Africa	WT
Bushmans	Rivendell Hatchery - Rufanus river – Boesmans river basin – South Africa	WT
Lepommier	Le Pommier - On-farm irrigation reservoirs- -South Africa	INT
Verlorenvlei	Verlorenvlei - Introduced population in a brackish water estuarine lake in the Western Cape, South Africa	INT
Amatikuli (red)	Amatikulu - A red coloured population at the Amatikulu ornamental fish farm in Kwa-Zulu Natal - South Africa	F
WT = Wild Type natural population	**INT** = Introduced population	**F** = Farm population

Trial A

A preliminary trial was intended to compare the culture performance of five of the nine strains available for breeding at the start of the project. These fish were produced and nursed and marked (with a combination of fin clipping and dye marks) and stocked communally in cages in a reservoir and in two earthen ponds failed. After 6 weeks of the grow-out phase, the majority of tags/marks which distinguished the strains were no longer visible and the trial had to be abandoned. However, some early growth data from the nursery phase could be utilised.

Materials and Methods

The trial fish bred from first generation broodstock were stocked separately in $1m^3$ concrete tanks, and a red strain was used as an internal reference group (IRG) to control for differential environmental effects between tanks, and between half-sib components of full-sib families (derived from a common male). The objective of the trial was to investigate variation in average daily growth rates over three successive time intervals (28 days each) during the nursing of five strains of Oreochromis mossambicus consisting of two half-sib families from each strain.

Each half-sib family comprised 40 individuals (20 individuals/full sib family). Thus two groups per half-sib family were used. The IRG was composed of one half-sib family of the particular red strain, 20 individuals per tank. Mean body weights taken before and after successive growth intervals, were composed of weighted contributions from the full sib replicate such that the IRG was standardised. Growth rates were transformed prior to analysis, which consisted of a two-way analysis of variance (ANOVA). A feature of fish growth studies is the change in rank order of growth rates that commonly occurs between families between successive time intervals. From this point of view it is useful to consider a null hypothesis of independence of growth rates within families between time intervals, in which case an ANOVA model is appropriate (multiple regression would be the alternative given an assumption of dependence).

Results

The growth rates recorded during the first inter-sampling interval revealed significant differences between the strains ($P < 0.05$). However, analysis of the growth rates from the subsequent study over a longer growth period, however, did not reveal significant inter-strain growth rate differences, although strains exhibited markedly different performance between time intervals.

Table 9 shows the growth (transformed for the relative growth of the internal reference group) for the three sample period.

Table 9 Transformed Relative Specific Growth Rates- SGR ($g.day^{-1}$) of Five Strains of *Oreochromis mossambicus* over three successive growth intervals (N = 20 per family) during the nursing phase of Trial A.

Family	Interval 1	Interval 2	Interval 3	Mean SGR	SE
Ndumu 1	1.57	0.25	0.38	0.79	0.32
Ndumu 2	1.99	0.41	0.17		
Kasinthula 1	1.37	0.23	0.24	0.51	0.20
Kasinthula 2	0.81	0.21	0.22		
Sucomba 1	0.96	0.24	0.31	0.48	0.14
Sucomba 2	0.89	0.23	0.28		
Boesmans 1	0.96	0.13	0.28	0.50	0.17
Boesmans 2	1.10	0.20	0.31		
Olifants 1	0.96	0.24	0.28	0.54	0.18
Olifants 2	1.21	0.22	0.33		

An infrastructure failure during the experiment (heat exchanger malfunction) caused a temperature drop, which accounts for growth rate reductions during the second growth interval. This may also have masked growth performance differences between strains during the 2nd month growth slow-down, since there are significant differences in growth rates during the 1st month. The growth rates during the nursery phase of Trial A has revealed significantly higher growth rates in the Ndumu and Kasinthula strains ($P < 0.05$) during part of the period and overall higher SGRs in these strains. These represent two of the three strains chosen for the focus of development of YY technology in *O. mossambicus* (the third being the Boesman's strain).
It is worthy of note that these growth rates generally compare very favourably with production data from commercial producers of *O. niloticus*. This provides some support to the contention that feral and cultured *O. mossambicus* stocks in Asia are from a very small gene pool, and are not representative of growth rates, even from our first generation unselected African *O. mossambicus* stocks.
It is unfortunate that the grow-out phase of this experiment failed to poor choice and implementation of marking techniques for the strains.

Trial B

Following the failure of the grow-out phase of Trial A, a second trial was initiated, this time to compare the growth performance of eight of the strains of *O. mossambicus*. In this experiment, the nursing phase was carried out in a similar environment to that for Trial A and the grow out phase was completed in cages in ponds under plastic tunnels and the DA's Elsenberg complex. The strains selected for this evaluation included those use in Trial A, plus LePommier, Makathini and Elands.

Materials and Methods

Nursing and Growth Phase I

First generation breeders (i.e. those actually collected from their respective locations) were bred to produce fry and offspring from 15-20 full-sib families per strain were obtained from 1m³ spawning tanks. The fry of each strain were reared separately for a period of eight weeks after which they were randomly selected and divided into two replicates of 160 individuals each. A semi-closed recirculation system comprising of 24 tanks of 0.8m³ each, was used. The fish were fed on a standard commercial tilapia diet (WPK Aquafeeds), according to a standardized daily feed table. Growth rates were recorded as total length (TL) and weight (Wt) over seven successive growth intervals, each interval with a duration of 21 days each. The initial period of 147 days was designated as growth phase I or nursery stage.

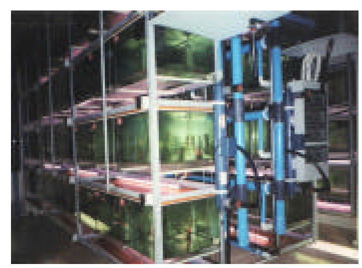
The Use of an Internal Reference Group as a Control
Experiments for the genetic evaluation of aquaculture species are often restricted by the difficulty of identification of individual small fish through means of mechanical or other marking devices. Tag loss can also occur which require multiple replications in order to reduce the variation caused by common environmental effects. A local strain of Red Tilapia (*O. mossambicus)* was obtained from a commercial hatchery, to be used as a distinguishable internal reference group (Doyle & Talbot 1986). Offspring were obtained from the spawning of 10 full sib families which were also reared

separately for a period of 8 weeks. Eighty of the red tilapia were then randomly allocated to each of the strains to be compared during the growth trial. The internal reference group was size matched to each test strain replicate in order to further reduce the environmental variation in growth caused by asynchronous spawning (Doyle & Talbot 1986; Basiaio & Doyle, 1990a). Each of 0.8m³ tank thus accommodated a total of 240 individuals, 80 from the red strain as an internal reference group and 160 from the respective strains under evaluation. Growth rates were recorded according to the same procedure as followed in the case of the test strains.

Growth Phase II

Ten strains of tilapia (*O. mossambicus*) were included in a second phase of the comparative growth trials run during the period of January to May 2001. These 10 strains include all eight of the wild type strains included in the nursery stage growth trials, as reported above, with the addition of two further strains, namely Verlorenvlei (introduced, wild type) and Amatikulu (a farmed, red variety). These two strains were excluded during the Phase I growth trials due to a lack of adequate number at that stage of the trial. The fish were however raised in a very similar environment during the first Phase. Due to the limited numbers of fish available following Phase II it was necessary to produce more fish for these trials so fry production continued after the Phase I comparison was initiated.

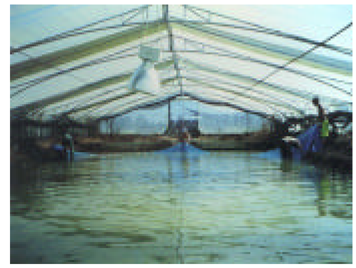
Sixteen females and eight males of each strain were placed in 1m³ spawning tanks in a temperature controlled (±25°C) recirculation system. Fry were collected on a weekly basis and placed in 120 litre glass aquarium tanks in a temperature controlled (±26°C) recirculation system. The fry were fed an *ad libitum* ration of a standard commercial tilapia starter diet, supplied by WOK Aquafeeds. Approximate 2400 fry from each strain were collected over a chosen period of two weeks, in order to minimise age effect during the later growth trial period.



Progeny groups were transferred to 1000 litre PVC tanks in a temperature controlled recirculation glass house systems (±25°C) at an age of 6 weeks, (average weight of 1.5 to 2 grams). The fish were fed on a standard commercial tilapia starter diet (WPK Aquafeeds), according to a standard daily feed table. The progeny groups were kept in the tanks for a period of 5 weeks, reaching an average weight of 15-20g, after which they were transferred to 6m^3 concrete tanks and their numbers standardised on a random basis at ±1000 fish per progeny group. Here they were kept for a further period of 11 weeks on a standard commercial tilapia starter diet (WPK Aquafeeds), until an average weight of approx. 80g were reached. The groups were fed according to a standard daily feed table, although amendments were made during the last three weeks in an effort to standardise group average weights before the onset of the growth trials.

Lack of facilities prevented accurate assessment of growth during these nursing phases and thus insufficient data were available to include in the description of early phase growth outlined above for the separately reared eight strains.

The grow-out stage growth trials were then initiated at an age of 22 weeks, by the random allocation of two groups of 300 fish per strain to separate 8m^3 cages in an enclosed earth pond system. The earth pond system operated at ambient temperatures, enhanced by the enclosure. Temperatures during the growth trial period fluctuated between 18 to 34°C, with an average of 23.4°C over the 18 week period. Each hapa was aerated by two internal air stones. Oxygen levels above 60% of saturation were maintained throughout the trial period. The fish were fed on a floating tilapia grower diet (WPK Aquafeeds), according to a standard daily feed table. Growth rates were recorded as total length (TL) and weight (TW) at the beginning and the end of an 18 week growth period. Sample weights were recorded every three weeks, according to which the feeding levels were calculated. Corrections were made for mortalities. Water quality in the pond was monitored on a daily basis for temperature and oxygen, and on a weekly basis for ammonia, nitrite and nitrate.



Results

Phase I growth comparisons

The data are summarised in **Error! Reference source not found.** indicating that during this "nursing" stage of 147 days the fish grew from an average of 6g to 116.2g or from an average of 59.2mm to 170.2mm in TL. Data were analyzed by the SAS General Linear Model Procedure (SAS Institute Inc., 1996). Student's t-LSD (Least Significant Difference) was calculated on the 5% level to compare treatment means. **Error! Reference source not found.** presents an ANOVA for Average Daily Weight Gain (RDWG, g/day) and the Average Daily Length Gain (RDLG, mm/day) of the respective populations.

Table 10 Summary data showing the mean initial and final weights & lengths for the eight strains of tilapia stocked under Trial B after 147 days of grow-out in nursery facilities (grow-out Phase II). Data are corrected for the data from the internal reference strain (red).

	Mean	Mean	Mean	Mean	Percentage increase	
	Stocking weight (g)	Final Weight (g)	Stocking Length (mm)	Final Length (mm)	Weight	Length
Ndumu	4.82	167.77	59.15	214.48	3380%	263%
Kasinthula	8.04	180.20	72.08	216.97	2141%	201%
Makathini	9.42	150.63	74.65	203.97	1499%	173%
Olifants	7.01	140.31	69.32	193.89	1902%	180%
Boesmans	5.31	153.10	62.38	205.84	2782%	230%
Sucomba	6.04	52.85	63.87	144.98	775%	127%
Lepommier	5.60	87.08	63.94	170.64	1454%	167%
Elands	7.55	113.68	67.21	181.08	1406%	169%
Mean	**5.98**	**116.18**	**59.18**	**170.21**	**1704%**	**168%**

Table 11. Analysis of Variance Table of ADWG and ADLG of 8 strains of tilapia (*O. mossambicus*) grown in duplicated 0.8 m³ tanks in a recirculating system (grow-out Phase I).

Source	Average Daily Weight Gain			Average Daily Length Gain		
	d.f.	Mean Square	P	d.f.	Mean Square	P
Group	1	0.5293	0.0010	1	0.2393	0.0048
Growth Stage	7	0.0894	0.0262	7	0.0273	0.2122
Error	7	0.0182		7	0.0145	
Total Corrected	15			15		
Non-Normality			0.8991			0.6566

ADWG = Average daily weight gain (g/day); ADLG = Average daily length gain (mm/day);

The analysis indicates significant differences between the growth rate of the various populations with regard to ADWG (P=0.0262), but not with regard to ADLG (P=0.2122).

The LS Mean values, rank order and t-test for the populations with regard to ADWG and ADLG are presented in **Error! Reference source not found.**, as well as the average heterozygosity (Ho) of the respective populations (data from the molecular analysis reported below). A significant correlation was observed between the respective LS Mean values for ADWG and ADLG (r=0.8237±0.1313), as well as between the rank order for ADWG and ADLG (r=0.7619±0.1713). No significant correlation was however observed between the LS Mean and heterozygosity values for either ADWG (r=-0.1297±0.4014) or ADLG (r=-0.3882±0.3467).

The results confirm the expected relation between weight and length gain of the different populations, though no significant relationship between weight or length gain and the levels of genetic variance of the respective strains could be established. This indicates that although the average heterozygosity (H_e) of populations is of importance to the procedures involved in a quantitative breeding program, the parameter (H_e) should not be used alone for selection of populations/strains/ of tilapia for inclusion in a breeding programme.

Table 12. LS Mean for ADWG and ADLG of eight strains of tilapia (*O. mossambicus*) grown in duplicated 0.8 m³ tanks in a recirculating system (grow-out Phase I).

	ADWG			ADWG			**Heterozygosity**
Population	**LS Mean**	**Rank order**	**t-test**	**LS Mean**	**Rank order**	**t-test**	**He**
Ndumu	1.0000	1	a	0.8940	1	a	0.4100
Boesmans	0.9105	2	a,b,	0.8841	2	a,b	0.7313
Makatini	0.8453	3	a,b,c	0.8267	4	a,b,c,d	0.4894
Kasinthula	0.8009	4	a,b,c,d	0.6372	7	a,b,c,d,e,f,g	0.6553
Olifants	0.7703	5	a,b,c,d,e	0.7381	5	a,b,c,d,e	0.4686
Sucomba	0.6975	6	a,b,c,d,e,f	0.8325	3	a,b,c	0.5125
Lepommier	0.5399	7	d,e,f,g	0.7330	6	a,b,c,d,e,f	0.6319
Elands	0.3071	8	f,g	0.4760	8	c,d,e,f,g	0.6484

ADWG = Average daily weight gain (g/day); ADLG = Average daily length gain (mm/day);

The Ndumu, Boesmans and Kasinthula strains were originally selected for inclusion into the YY-male breeding programme on the basis of preliminary results obtained from the first comparative growth trial (trial A – reported previously). Although Kasinthula has since dropped one position, from 3 to 4, in the rank order based on RDWG, an early decision had to be made on the inclusion of strains into the YY-male breeding programme due to the extended duration of this programme. A decision on which strains to include could not have been delayed until the conclusion of Trial B, which might have led to the inclusion of Makatini instead of Kasinthula in the breeding programme.

Phase II growth comparison

A summary of the growth statistics the 10 strains of tilapia (*O. mossambicus*) during a grow-out trial phase are presented in Table 13.

Table 13 Summary of growth statistics of 10 strains of tilapia (*O. mossambicus*) during a grow-out trial phase in cages in pond (grow-out Phase II).

Strain	Rep	Duration (days)	Starting number	Average starting weight (g)	Average starting length (mm)	Ending number	Average end weight (g)	Average end length (mm)
Amatikulu[RED]	1	126	305	69	131	290	385	265
	2	126	302	68	131	291	393	273
Boesmans	1	126	298	84	157	273	469	349
	2	126	300	82	153	282	473	353
Elands	1	126	305	71	140	290	415	295
	2	126	301	71	138	279	389	269
Kasinthula	1	127	295	87	159	277	458	338
	2	127	298	83	154	275	463	343
Lepommier	1	127	310	81	155	284	413	293
	2	127	307	83	163	290	427	307
Makathini	1	127	280	89	174	248	465	345
	2	127	283	85	163	264	448	328
Ndumu	1	128	307	74	137	282	485	365
	2	128	304	76	138	293	458	338
Olifants	1	128	311	91	181	273	443	323
	2	128	314	87	171	281	428	308
Sucomba	1	128	300	77	142	285	428	308
	2	128	301	81	147	289	419	299
Verlorenvlei	1	128	306	75	155	278	402	282
	2	128	302	73	150	274	406	286

Data were analysed by means of the SAS General Linear Model Procedure (SAS Institute Inc., 1996). Student's t-LSD (Least Significant Difference) was calculated on the 5% level to compare treatment means. Table 14 presents an ANOVA for Average Daily Weight Gain (ADWG, g/day). The results confirm the presence of significant differences between the strains in terms of Average Daily Weight Gain.

Table 14 Analysis of Variance Table for Average Daily Weight Gain (ADWG, g/day) for 10 strains of tilapia (*O. mossambicus*) during grow-out in cages in pond (grow-out Phase II).

Source	df	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.77061120	0.08562347	11.75	0.0003
Error	10	0.07284800	0.00728480		
Corrected Total	19	0.84345920			

The LSMean values for ADWG of the 10 strains are presented in Table 15 together with the t-groupings. The t-groupings for ADWG indicate the presence of two distinct, non-overlapping groupings, with Ndumu, Boesmans, Kasinthula and Makathini being in the fastest growing group.

Table 15 LSMean and t-Tests (LSD) for Average Daily Weight Gain (ADWG, g/day) for 10 strains of tilapia (*O. mossambicus*) during grow-out in cages in pond (grow-out Phase II).

Strain	LS Mean for ADWG	N	t-Grouping
Ndumu	3.0975	2	A
Boesmans	3.0795	2	A
Kasinthula	2.9565	2	A
Makathini	2.9095	2	A
Olifants	2.7070	2	B
Sucomba	2.6915	2	B
Lepommier	2.6615	2	B
Elands	2.6270	2	B
Verlorenvlei	2.5785	2	B
Amatikulu[red]	2.5435	2	B
Alpha value			0.05
Least Significant Difference			0.1902

Table 16 presents an ANOVA for Average Daily Length Gain (ADLG, mm/day). The results confirm the presence of significant differences between the strains in terms of Average Daily Length Gain.

Table 16 Analysis of Variance Table for Average Daily Length Gain (ADLG, mm/day) for 10 strains of tilapia (*O. mossambicus*) during grow-out in cages in pond (grow-out Phase II).

Source	df	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.40305625	0.04478403	5.89	0.0053
Error	10	0.07604750	0.00760475		
Corrected Total	19	0.47910375			

The LSMean values for ADLG of the 10 strains are presented in Table 17 together with the t-groupings. The t-groupings for ADLG indicate the presence of non-four overlapping groupings, with Boesmans, Ndumu, Makatini and Sucomba showing the highest rate of length gain during the trial period.

Changes in the rank order of the strains in terms of ADLG has occurred, compared to ADWG. The data for both ADWG and ADLG fitted a normal distribution.

Table 17 LSMean and t-Tests (LSD) for Average Daily Length Gain (ADLG, mm/day) for 10 strains of tilapia (*O. mossambicus*) during grow-out in cages in pond (grow-out Phase II).

Strain	LS Mean for ADLG	N	t-Grouping
Boesmans	1.8735	2	A
Ndumu	1.8570	2	A
Makathini	1.8380	2	A B
Sucomba	1.7790	2	A B
Kasinthula	1.7420	2	A B
Lepommier	1.6610	2	B C
Elands	1.6555	2	B C
Verlorenvlei	1.5390	2	C D
Olifants	1.5175	2	C D
Amatikulu[red]	1.4600	2	D
Alpha value			0.05
Least Significant Difference			0.1943

Summary

Although the trials did not go exactly as originally planned, the three trials that were successfully completed did effectively cover all three phases of a culture cycle, early nursing (Trial A), nursing/early grow-out and final phase grow-out (Trial B). The results in terms of comparative growth rates (especially weight gain) were very consistent through all phases of growth, with the Ndumu, Boesmans and Kasinthula strains having the faster growth rates, with the newly introduced strain Makathini also performing well during the final growth phase. In interpreting these results it must be considered that these comparisons were not made in culture environments that closely resembled those that might apply in aquaculture, and thus genotype x environment (GxE) interactions might modify the rankings of the strains somewhat in aquaculture environments. For this reason it is important that further trials of the different strains are conducted in aquaculture environments and this should be carried out ideally in on-farm trials. However, the fact that GxE interactions have been shown to not to be highly significant in other studies on tilapia, and the important consistency of the results from all phases can give us some confidence in selecting strains to form the basis of aquaculture activities and in particular to form base populations for a future breeding programmes.

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Appendix 8 Report on the Evaluation of Genetic Diversity for Sustainable Utilisation of Natural Genetic Resources in the Southern African Tilapia, *Oreochromis mossambicus*

AUTHOR: E. G. HALL (University of Stellenbosch Division of Aquaculture)

ABSTRACT:

Southern African populations of *Oreochromis mossambicus* have been classified as genetic resources in aquaculture and conservation. Twelve populations, collected for evaluation and strain development, were characterised using fluorescent microsatellite DNA analysis. Five, dinucleotide repeat, microsatellite loci conveyed significant genetic structure (F_{ST} = 0.27, R_{ST} = 0.29, $P < 0.001$) between the six wild, four farmed and two introduced populations. Genetic diversity differed for farm populations (Ho = 0.26, α = 1.75), wild populations (Ho = 0.39, α = 5.3) and introduced populations (Ho = 0.39, α = 4.4). Individual populations indicated suitable levels of genetic diversity for use in selective strain improvement programmes. Phylogenetic trees contained population clusters related to but not exclusively linked with geographic distribution. Phylogeographic interpretations were made to identify populations of conservation value. The twelve captive populations represent genetically distinct units that require management to preserve genetic material for strain development and conservation of natural populations.

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General Introduction

The African tilapias constitute about 870 species of the Cichlidae family, a diverse family of freshwater and brackish water fishes found in Africa, Central and Southern America, India, Arabia and Madagascar (Skelton, 1993). Species of the *Oreochromis*, *Sarotherodon*, and *Tilapia* genera have been widely exploited in aquaculture and natural fisheries. Within the *Oreochromis* genus *O. mossambicus*, *O. niloticus* and *O. aureus* are considered the most important species for aquaculture.
The Mozambique tilapia, *O. mossambicus*, was one of the first species to be used in aquaculture, mainly in Asia (1940's and 1950's), (de Moor and Bruton, 1988; Pullin, 1988; Agustin, 1999). *O. mossambicus* has subsequently been widely distributed for aquaculture resulting in the establishment of farm and feral populations in more than 61 countries (Agustin, 1999). Feral establishment in most Asian countries, has hindered the local fisheries resulting in plans to eradicate unwanted populations, however, in Sri Lanka *O. mossambicus* actually improved the local fisheries (Pullin *et al.*, 1988).

The performance of *O. mossambicus* in aquaculture in most Asian countries has been extremely poor. A small number of originators and lack of stock management led to inherent founder effects (loss of genetic diversity) and inbreeding (mating amongst relatives), and was probably responsible for the relatively poor performance of *O. mossambicus* in Asia. Four fish (three males and a single female), from an aquarium population in East Java, may have formed the founder population for the entire Asian *O. mossambicus* aquaculture industry (Pullin *et al.*, 1988). Agustin (1999) provides a detailed description of the introduction and distribution of *O. mossambicus* in tropical and subtropical countries, outside its natural range, and how the ancestry of many of these introduced populations can be traced back to the four original East Java aquarium fish. A species shift in aquaculture to the better domesticated North African tilapia *Oreochromis niloticus* (Nile tilapia) occurred in the 1960's literally displacing *O. mossambicus* from aquaculture. Current global tilapia production figures reflect a preference for *O. niloticus* over *O. mossambicus.* Total world production of *O. niloticus* increased from 12000 tons in 1970 to 888000 tons in 1999. Total world production of *O. mossambicus* in 1970 was 1100 tons. Production peaked in 1995 at 58500 tons however subsequently decreased to 46000 tons in 1999 (FAO, 2001). Fortunately wild populations of *O. mossambicus* tilapia still exist in their native range in Southern Africa (Van der Bank and Ferreira, 1987; Agustin, 1999). The potential of wild *O. mossambicus* from Southern Africa as a source of genetic material for developing and supplementing new aquaculture strains, was considered in 1988 as part of a review on wild tilapia genetic resources. Growth performance and appearance of wild Mozambique tilapia in Southern Africa were quoted as being superior to that of Asian introduced populations (Pullin, 1988). The implementation of a quantitative and population genetic evaluation of *O. mossambicus* at the University of Stellenbosch in 1998 aims to provide a genetic component to the development of aquaculture strains and the conservation of natural Mozambique tilapia populations.

Aquaculture of O. mossambicus in Southern Africa

Oreochromis mossambicus has received the most interest in aquaculture in Southern Africa due to its favourable growth compared to other tilapia species in the region (Jubb, 1967; Bruton, 1983; Ferreira and Schoonbee, 1983; Gaigher, 1983; Pike, 1983; McVeigh, 1995; McVeigh, 1998). Early attempts at farming with tilapia in Southern Africa were hampered by technical and environmental factors. One of the main obstacles was the prolific breeding achieved through precocious maturity that occurred in dams stocked with both sexes leading to the production of small fish of little market value. The technology needed to breed monosex, all male, fry or sterile hybrids was not available or too expensive for the average farmer. Another limiting factor was the susceptibility of most tilapia species to cold temperatures experienced in the Western Cape and the Highveld during winter (Jubb, 1967). The Department of Inland Fisheries, Stellenbosch, therefore spearheaded a search for cold tolerant species for aquaculture in the Western Cape. A number of tilapia species including *Tilapia aurea* (*Oreochromis aureus*), *Tilapia galilaea* (*Sarotherodon galilaeus*), and *Tilapia zillii* were imported from Israel in 1959 and reared at the Jonkershoek fish hatchery in Stellenbosch. Western Cape farm dams were stocked with tilapia offspring from the Jonkershoek species.

Oreochromis mossambicus was introduced into Western Cape farm dams and coastal lagoons by the Cape Nature Conservation Department (the Western Cape governmental agency responsible for conservation) in the 1960's (de Moor and Bruton, 1988). In 1998, Stellenbosch University Division of Aquaculture, in collaboration with University of Wales (Swansea), began developing the technology required to solve the problems surrounding *O. mossambicus* tilapia farming in Southern Africa. The technology developed for aquaculture of *O. niloticus* was adapted for use on *O. mossambicus*. This included research into nutrition, recirculation technology, rearing in tunnel enclosed ground dams, and development of monosex fry. A small-scale tilapia farming co-operative was established by encouraging local farm labourers to rear caged tilapia in existing wine farm dams.

Conservation Issues Surrounding O. mossambicus in Southern Africa

Introduction and Translocation

The history of *O. mossambicus* introductions in South Africa is documented from the early 1950's to the 1980's. Translocation in South Africa took place mainly for utilisation in aquaculture, recreational fishing, and biological control of chironomids and macrophytes (de Moor and Bruton, 1988). There have been few studies on the impact that these introductions have had on local aquatic biodiversity (de Moor and Bruton, 1988) despite the insinuation of *O. mossambicus* in the damaging of many international watercourses (Trewavas, 1983). *O. mossambicus* individuals of Transvaal origin were introduced into farm dams in Malmesbury (Cape Province) in 1936. Some of these fish were transferred to the Jonkershoek Hatchery to act as breeding stock for further distribution. A number of other hatcheries took part in the breeding and distribution of *O. mossambicus* in other parts of South Africa: the Umgeni Warmwater Hatchery (Kwazulu Natal), the Lydenburg Hatchery (Mpumalanga), the Amalinda Hatchery (East London) and the Lowveld Fisheries Research Station (Mpumalanga). The local translocation of Mozambique tilapia into areas outside its natural range was considered more of a disturbance to natural aquatic ecosystems than an advantage (de Moor and Bruton, 1988). Translocation of cultured or non-local *O. mossambicus* populations was also described as a threat to the genetic diversity present in local wild *O. mossambicus* populations (Pike, 1983; de Moor and Bruton, 1988). Genetic diversity in wild populations was stated as being worthy of protection due to unique qualities such as cold tolerance and high salt concentration tolerance characteristic of certain populations (de Moor and Bruton, 1988).

Hybridisation

The introduction into South Africa of the popular *O. niloticus* species that performs well in aquaculture in other countries represents a potential threat to natural tilapia populations, since most tilapia species readily hybridise (Wohlfarth, 1994; Van der Waal and Bills, 2000). Hybridisation is more likely between tilapia with close genetic relationships. Starch gel electrophoresis of 24 allozyme loci provided evidence of a close relationship between *O. mossambicus* and *O. niloticus* (Pouyad and Agnese, 1995). Present South African conservation laws restrict translocation of indigenous fish and introduction of exotic fish into natural water systems. One of the biggest potential threats to the genetic integrity of *O. mossambicus* populations, however, arises from the existence of exotic populations of *O. niloticus* already present within the natural range of *O. mossambicus* (de Moor and Bruton, 1988; Van der Waal, 2000). Indeed, Moralee and Van der Waal (2000) have already presented morphological and biochemical evidence of wild F_1 hybrids between the two species in the Limpopo river system. Ironically, in the 1950's, *O. niloticus* was imported into and distributed in South Africa by local companies and government agencies (reviewed by de Moor and Bruton, 1988). In 1955, the Cape Nature Conservation Department established a population of *O. niloticus* at the Jonkershoek Hatchery for breeding experiments. In 1978, the Natal Fisheries Development Corporation stocked a small dam in Northern Kwazulu Natal with a population of *O. niloticus* from the Amatikulu Hatchery. The population was later destroyed. The Tongaat Sugar Company introduced *O. niloticus* into the Dudley Pringle dam (Wewe river catchment in Kwazulu Natal) in 1982. The Stellenbosch University Aquaculture Division is, currently investigating DNA markers, to characterise parental and hybrid genotypes in *O. mossambicus* and *O. niloticus*.

Habitat Encroachment

Oreochromis mossambicus forms part of the rich fish diversity found in South African aquatic habitats (Skelton, 1993; Van der Waal, 2000). Habitat loss or fragmentation occurs through interventions on natural water systems for power, transport, agriculture and water consumption. The effects of human encroachment, on aquatic habitats are often detrimental, leading to the extinction of aquatic species (Crass, 1969; Avault, 1994). Aquaculture also constitutes a form of encroachment. In the vast majority of cases of habitat loss, the evidence suggest that species diversity has reduced, often dramatically so (Lande, 1998). The extent of habitat fragmentation for *O. mossambicus* needs to be ascertained.

Loss of Genetic Diversity

Genetic diversity within populations represents a fundamental level of biological diversity (Biodiversity) from which more commonly utilised estimates of biological diversity (such as numbers of species, families, and phyla) arise (Harper and Hawksworth, 1994, Beardmore *et al.*, 1997). Conservation genetics generally deals directly with the genetic diversity present in populations, how this affects the fitness of the populations and their demographic history (Beaumont, 2001). Genetic factors that can reduce genetic diversity in wild populations include founder events, bottlenecks and artificial supplementation with large numbers of organisms with low overall genetic diversity (Ryman *et al*., 1995; Ryman, 1997). Agustin (1999) provided evidence of dramatically reduced genetic variation, through founder effects and bottlenecks, in four feral *O. mossambicus* stocks in the Australasian Pacific region when compared to wild populations from the natural range of the species in South Africa. A single mitochondrial DNA (mtDNA) haplotype was found in all the feral stocks. Genetic diversity for mtDNA was reported as being highly diverse between eight wild South African populations that contained 26 different haplotypes. Microsatellite diversity was also considerably reduced in feral stocks (Agustin, 1999). de Moor and Bruton (1988) cautioned that the translocation of Mozambique tilapia within South Africa could jeopardize the unique genetic variation present in the wild populations of *O. mossambicus*. If this occurred on a large scale, the influx of non-locally adapted genes could reduce the fitness in the wild populations.

Molecular Markers for Population Genetics

The comprehensive characterization of genetic variation in the tilapias includes the use of morphometric characters, proteins (in particular allozymes), mitochondrial DNA (mtDNA) and nuclear DNA. These markers have been used to assess population and species level genetic variation for tilapias. Each method has noticeable advantages and disadvantages. Nuclear microsatellite DNA has the most valuable qualities for use in fish population genetics since the relatively high mutation rate of microsatellite DNA offers a high level of resolution most useful for population studies (O'Reilly and Wright, 1995) The use of the polymerase chain reaction (PCR) to amplify microsatellite DNA from small quantities of sample tissue facilitates a high throughput of genetic data. Broad conclusions from the research on microsatellite loci describe microsatellites as very useful for intraspecies studies involving populations that have not become too genetically diverged (Takazaki and Nei, 1996).

ORIGINAL OBJECTIVES:

1.1 To evaluate molecular genetic diversity within and between populations of *Oreochromis mossambicus* throughout its natural range for both microsatellite, and mitochondrial DNA markers.

1.2 To undertake a phylogeographic interpretation of genetic diversity patterns in conjunction with known histories of population movements, to identify populations of greatest conservation value and of greatest potential for utilisation in aquaculture.

1.3 To evaluate the utility of microsatellite nuclear markers as a tool for informing an existing quantitative genetic breeding programme. To investigate such issues as parental contributions in communal spawning environments, and unambiguous parental identification in mixed family rearing experiments.

OBJECTIVES ACHIEVED:

1.1 Molecular genetic diversity was evaluated within and between twelve populations of *Oreochromis mossambicus* collected from the natural distribution of the species using five microsatellite markers.
1.2 Interpretation of population phylogeography was conducted.

MATERIALS AND METHODS:

POPULATION SOURCE MATERIAL

The twelve populations used in this research work were initiated from six wild, four farmed and two introduced sources of *O. mossambicus*, located in the South Eastern area of Southern Africa. Between 50 and 100 individuals were collected for each population between 1997 and 1999, and returned for captive breeding at the University of Stellenbosch.

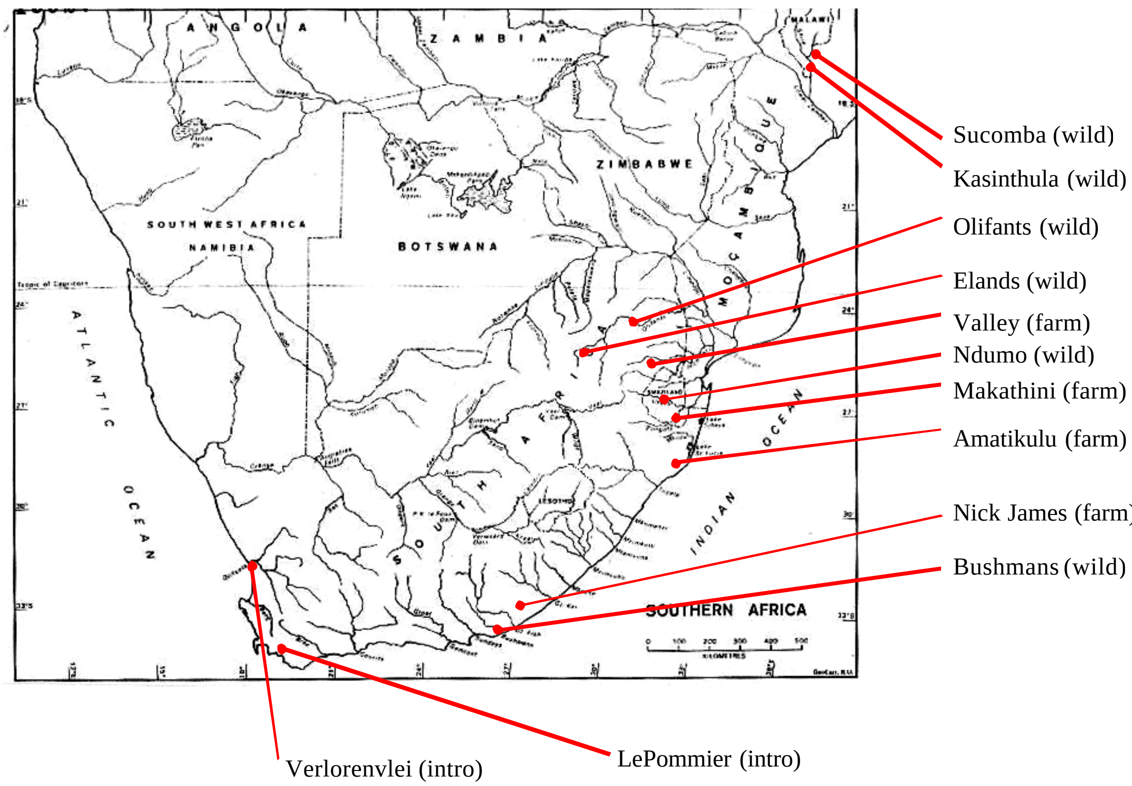

Figure 1: Diagram of collection locations for the twelve *O. mossambicus* populations in Southern Africa

MICROSATELLITE MARKER INFORMATION

Microsatellite locus primer data was obtained from the web site: (http://tilapia.unh.edu/WWWPages/TGP/CA-Tilapia.html)
Forward and reverse primer sequences for the five loci used in this study appear in Table 1.

Table 1: Sequence of forward and reverse primers for the five microsatellite loci

Locus	Forward Primer (5' – 3')	Reverse Primer (5' – 3')
UNH104	GCAGUATUGTGGTCACTA	GGTATATGTCTAACTGAAATCC
UNH111	TGCTGTTCTTATTTTCGC	ATAAGAGTGTATGCATTACTGG
UNH123	CATCATCACAGACAGATTAGA	GATTGAGATT TCATTCAAG
UNH146	CCACTCTGCCTGCCCTCTAT	AGCTGCGTCAAACTCTCAAAAG
UNH192	GGAAATCCATAAGATCAGTTA	CTTTTTCAGGATTTACTGCTAAG

The forward primer of UNH146 was labelled with HEX (Green) ABI sequencer compatible dye. The forward primer of UNH192 was labelled with NED (Yellow) ABI sequencer compatible dye. Addition of these labelled primers to the PCR reaction mix enabled the amplification of fluorescent PCR products for these two loci. The forward primers of the remaining three loci, UNH104, UNH111 and UNH123 were not labelled. Fluorescently labelled deoxycytosine triphosphates (FdCTPs), added to the PCR reaction mixes, were used to label the amplified PCR products at these loci.

LABORATORY TECHNIQUE

DNA Extraction

Genomic DNA extraction from the population samples (Table 2) made use of a modified commercial DNA kit extraction procedure (Nucleon 1, SCOTLAB, Scotland). Sections of fin clippings ($4mm^2$) were lysed in 1.5ml microcentrifuge tubes containing 40µl of lysis buffer (400mM TrisHCL, 60mM EDTA, 150mM NaCl, 1% SDS). Sodium Perchlorate was added to each tube to end the lysis stage and precipitate proteins. A chloroform extraction procedure was then applied. Isopropanol was used to precipitate the DNA followed by two ethanol washes. Extracted DNA samples were standardised to a concentration of 100ng/µl using a GENEQUANT spectrophotometer followed by storage at 4ºC.

Table 2: Population abbreviations, type and sample size

Population	Type	Sample size
A – Amatikulu	red farm strain	36
B – Bushmans	grey wild	36
E – Elands	grey wild	9
K –Kasinthula	grey wild	36
L- LePommier	grey introduced	36
M – Makathini	grey farm	10
N – Ndumo	grey wild	36
NJ – Nick James	red farm strain	36
O – Olifants	grey wild	10
S – Sucomba	grey wild	10
V – Valley	red farm strain	17
VE – Velorenvlei	grey introduced	16
		288

Fluorescent Nucleotide Polymerase Chain Reaction

Three, different coloured, FdCTPs were available for labelling the PCR products. Each locus was assigned a specific FdCTP for analysis on the ABI377. Tamra (yellow) was used for UNH104 PCR products, R6G (green) for UNH111 products and R100 (blue) for UNH123 products. Each FdCTP, at an original concentration of [100µM], was used at a ratio of 500:1 unlabelled dCTP to labelled FdCTP. A master-mix of PCR reagents was made up for each new set of PCR reactions (Table 3).

Table 3: Reagents used to make up the PCR master–mix for fluorescent PCR microsatellite analysis with labeled nucleotides (Adapted from Kocher *et al.*, 1998)

Reagent [concentration]	**Single tube volume (ml) [conc.]**	**(n+1) x single tube volume (ml)**
BioTaq buffer [10 X]	2.5 [1X]	(n+1) x 2.5
$MgCl_2$ [25mM]	1.5 [1.5mM]	(n+1) x 1.5
dNTPs [10mM of each]	0.5 [200 µM of each]	(n+1) x 0.5
FdCTP [100 µM]	0.1 [0.4µM]	(n+1) x 0.1
Forward primer [20µM]	0.4 [0.32µM]	(n+1) x 0.4
Reverse primer [20µM]	0.4 [0.32µM]	(n+1) x 0.4
BioTaq Polymerase [5units/µl]	0.2 [1 unit]	(n+1) x 0.2
Water	18.4	(n+1) x 18.4
Mix Total	24	(n+1) x 24
Template [100ng/ µl]	1	
Mix total + template	25	

The following PCR profile was used to amplify the microsatellite DNA for the fluorescent nucleotide PCR:
94°C for two minutes followed by 30 cycles of (94°C for 30 seconds, 42°C for two minutes, 72°C for two minutes) followed by 72°C for 10 minutes. Storage at 4°C indefinitely.

Fluorescent Primer polymerase Chain Reaction

A master-mix of PCR reagents was made up for each new set of PCR reactions (Table 4).

Table 4: Reagents used to make up the PCR master–mix for fluorescent PCR microsatellite analysis with labelled primers (Adapted from Kocher *et al.*, 1998)

Reagent [concentration]	**Single tube volume (µl) [conc.]**	**(n+1) x single tube volume (µl)**
BioTaq buffer [10 X]	2.5 [1X]	(n+1) x 2.5
$MgCl_2$ [25mM]	1.5 [1.5mM]	(n+1) x 1.5
dNTPs [10mM of each]	0.4 [0.16mM of each]	(n+1) x 0.4
Forward primer [20µM]	0.2 [0.16µM]	(n+1) x 0.2
Reverse primer [20µM]	0.2 [0.16µM]	(n+1) x 0.2
BioTaq Polymerase [5units/µl]	0.2 [1 unit]	(n+1) x 0.2
Water	19	(n+1) x 19
Mix Total	24	(n+1) x 24
Template [100ng/ µl]	1	
Mix total + template	25	

The following PCR profile was used to amplify the microsatellite DNA for the fluorescent primer PCR:
94°C for two minutes followed by 45 cycles of (94°C for 20 seconds, 50°C for 30 seconds, 72°C for 45 seconds) followed by 72°C for seven minutes. Storage at 4°C indefinitely.

ABI sequencer Analysis

The labelled PCR products for loci UNH104, UNH111 and UNH123 were run on an ABI377 sequencer at University of Stellenbosch, Stellenbosch. The labelled PCR for loci UNH146 and UNH192 were run on an ABI3100 at Cardiff University, Wales.

DATA PRESENTATION

Genotypes in the form of DNA fragment peak sizes were generated for each fish in a population using the ABI377 and ABI3100 fragment analysis software, Genescan and Genotyper. Data was stored as EXCEL files and later converted to ASCII file format for application in genetic analysis software.

GENETIC ANALYSES

Genetic analyses were performed on the data using the following genetic software: Tools for Population Genetic Analysis (TFPGA) (Miller, 1997), FSTAT (Goudet, 2000); RST-CALC (Goodman, 1997), Genetic Data Analysis (GDA) (Lewis and Zaykin, 2001), ARLEQUIN version 2.0 (Schneider *et al.*, 1997), and PHYLIP (Felsenstein, 1993).
The following genetic analyses were investigated: Locus Diversity, Population Diversity, Genetic Structure, Gene Flow, Genetic Distance and Phylogeography, Genetic Drift in Captivity, and Microsatellite allele Inheritance and Frequency of Null Alleles.

RESULTS:

GENETIC ANALYSES

Locus Diversity

Table 5: Locus diversity averaged over the twelve populations (TFPGA)

Locus	Sample size	Observed Heterozygosity (Ho)	Expected Heterozygosity (He)	No. of alleles
UNH104	157	0.08	0.55	7
UNH111	195	0.42	0.73	16
UNH123	237	0.53	0.84	17
UNH146	257	0.23	0.32	3
UNH192	255	0.47	0.71	14
Average	220	0.35	0.63	11.4

Population Diversity

Table 6: Population diversity averaged over the five microsatellite loci (TFPGA)

Population (N)	Type	Ho	He	No. alleles	Ave. Ho	Ave. He	Ave. alleles
M (10)	Farm	0.46	0.52	2.2		**FARM**	
NJ (10)	Farm	0.42	0.49	1.6	0.26	0.36	1.75
A (36)	Farm	0.13	0.25	1.8			
V (17)	Farm	0.03	0.17	1.4			
N (36)	Wild	0.29	0.44	5.2		**WILD**	
B (36)	Wild	0.38	0.66	8.2	0.39	0.56	5.3
E (9)	Wild	0.6	0.7	3.8			
K (36)	Wild	0.44	0.6	6.6			
O (10)	Wild	0.24	0.45	2.6			
S (36)	Wild	0.39	0.52	5.4			
VE (16)	Intro	0.22	0.37	3		**INTRO**	
L (36)	Intro	0.55	0.57	5.8	0.39	0.47	4.4
Average		0.35	0.48	4			

Genetic Structure

Monte Carlo randomisation type Exact tests (Raymond and Rousset, 1995) performed on the allelic data sets yielded significant allele frequency heterogeneity ($P < 0.001$) between the populations at all five loci. An overall F_{ST} value of 0.27 and R_{ST} of 0.29 ($P < 0.001$) were obtained, typical of a high degree of structuring, between the twelve populations (See table 7 and 8 for population pairwise F_{ST} and R_{ST} values).

Table 7: Population pairwise F_{ST} values for the twelve populations (FSTAT)

	A	B	E	K	L	M	N	NJ	O	S	V	VE
A	0.00											
B	0.29	0.00										
E	0.50	0.13	0.00									
K	0.36	0.10	0.13	0.00								
L	0.28	0.17	0.23	0.10	0.00							
M	0.46	0.25	0.27	0.23	0.27	0.00						
N	0.42	0.25	0.29	0.18	0.19	0.20	0.00					
NJ	0.61	0.25	0.30	0.28	0.28	0.14	0.14	0.00				
O	0.15	0.11	0.20	0.13	0.19	0.20	0.17	0.29	0.00			
S	0.30	0.23	0.27	0.14	0.02	0.26	0.20	0.26	0.20	0.00		
V	0.45	0.34	0.56	0.38	0.30	0.48	0.47	0.52	0.52	0.27	0.00	
VE	0.26	0.24	0.41	0.32	0.26	0.46	0.48	0.48	0.32	0.32	0.40	0.00

Table 8: Population pairwise R_{ST} values for the twelve populations (RSTCALC)

	A	B	E	K	L	M	N	NJ	S	VE
A	0.00									
B	0.22	0.00								
E	0.25	0.16	0.00							
K	0.14	0.13	0.25	0.00						
L	0.16	0.14	0.26	-0.01	0.00					
M	0.30	0.30	0.21	0.16	0.13	0.00				
N	0.18	0.27	0.17	0.15	0.19	0.22	0.00			
NJ	0.01	0.15	0.08	0.05	0.06	0.16	0.09	0.00		
S	0.23	0.19	0.25	0.05	0.03	0.20	0.14	0.16	0.00	
VE	0.16	0.10	0.28	0.24	0.20	0.40	0.36	0.23	0.29	0.00

Gene Flow

Different rates of gene flow exist between the six wild populations as indicated in Table 9.

Table 9: Number of migrants per generation between the six wild populations (RSTCALC)

	S	K	O	E	N	B
S	0.00					
K	5.15	0.00				
O	1.04	2.98	0.00			
E	0.56	0.67	1.94	0.00		
N	0.89	1.15	5.78	0.64	0.00	
B	1.09	1.82	1.57	1.03	0.74	0.00

Genetic Distance and Phylogeography

Delta-mu Squared $\{(\delta\mu)^2\}$ genetic distances were calculated for the twelve populations using four of the five loci (Table 10). The UNH104 locus was excluded from the analysis as no alleles were present at this locus for the Valley and Nick James red populations. The Neighbour Joining tree building method was used to construct a phylogenetic tree of the $(\delta\mu)^2$ distances (Figure 2). A UPGMA tree did not depict the correct clustering topology for the populations as certain conditions in the building of UPGMA trees were violated. The UPGMA trees were therefore excluded from the analysis.

Table 10: $(\delta\mu)^2$ distances between the twelve populations (RSTCALC)

	A	B	E	K	L	M	N	NJ	O	S	V	VE
A	0.00											
B	8.47	0.00										
E	38.27	30.92	0.00									
K	10.52	0.74	27.26	0.00								
L	17.57	2.83	35.69	1.33	0.00							
M	40.86	15.32	27.11	10.88	8.03	0.00						
N	2.29	10.99	27.36	11.02	18.59	35.88	0.00					
NJ	28.44	17.39	79.16	24.55	26.22	49.06	41.31	0.00				
O	8.93	8.47	11.09	8.63	16.42	24.75	6.22	37.50	0.00			
S	18.49	7.00	36.82	3.67	2.10	9.90	16.72	39.80	19.30	0.00		
V	51.12	33.06	112.26	31.53	22.31	42.36	58.67	45.63	71.46	21.41	0.00	
VE	13.04	6.74	65.57	9.01	8.47	31.70	20.81	13.19	27.34	12.25	16.09	0.00

Nei's unbiased minimum genetic distances {D_m} were calculated for the twelve pop ulations using all five loci (Table 11). The Neighbour Joining tree building method was used to construct a phylogenetic tree of the D_m distances (Figure 3). The UPGMA tree was again excluded due to discrepancies in tree topology.

Table 11: D_m distances between the twelve populations (TFPGA)

	A	B	E	K	L	M	N	NJ	O	S	V	VE
A	0.00											
B	0.22	0.00										
E	0.37	0.15	0.00									
K	0.26	0.10	0.16	0.00								
L	0.17	0.14	0.20	0.08	0.00							
M	0.27	0.25	0.27	0.22	0.23	0.00						
N	0.23	0.20	0.23	0.13	0.13	0.14	0.00					
NJ	0.35	0.25	0.29	0.28	0.27	0.13	0.13	0.00				
O	0.08	0.13	0.23	0.16	0.16	0.18	0.13	0.25	0.00			
S	0.17	0.19	0.23	0.11	0.02	0.21	0.13	0.25	0.17	0.00		
V	0.14	0.30	0.45	0.34	0.25	0.30	0.40	0.38	0.27	0.21	0.00	
VE	0.13	0.21	0.37	0.29	0.20	0.38	0.39	0.40	0.22	0.24	0.19	0.00

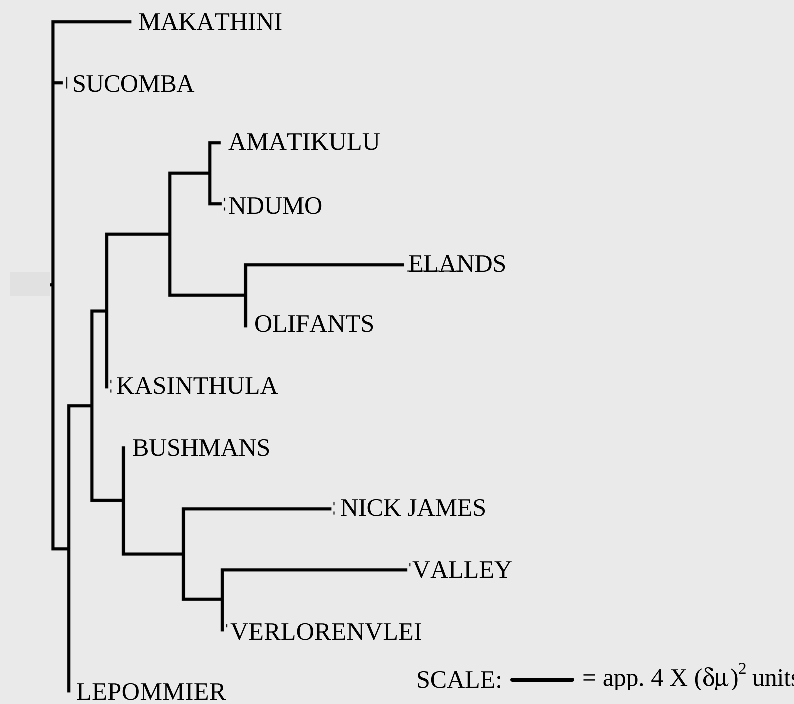

Figure 2: Neighbour joining tree of $(\delta\mu)^2$ genetic distances between the twelve populations

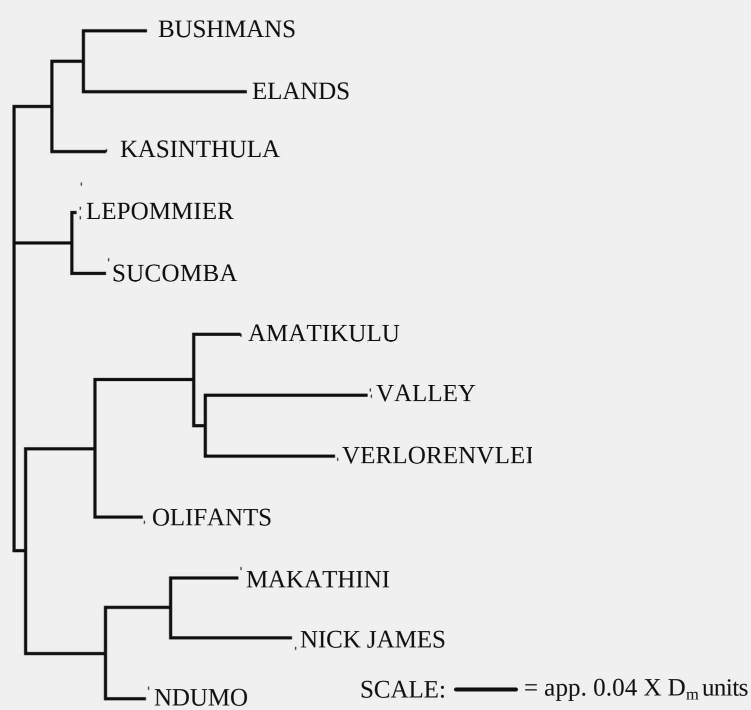

Figure 3: Neighbour joining tree of (D_m) genetic distances between the twelve populations

DISCUSSION:

GENETIC ANALYSES

Locus Diversity

The number of alleles present at the loci varied from three at UNH146 to seventeen at UNH123 (Table 5). Freshwater fish are thought to have lower levels of allelic diversity than anadromous and marine fish due to smaller effective population sizes and a less stable environment (de Woody and Avise, 2000). The average number of alleles (11.4) obtained for *O. mossambicus* (Table 5) is higher than the mean per locus for freshwater fishes (7.5) reported by de Woody and Avise (2000), but similar to Agustin's average of 15.4 alleles found at five microsatellite loci for five *O. mossambicus* populations of wild and feral origin (Agustin, 1999). Wild *O. mossambicus* may have a larger effective population size than most freshwater fishes due to the ability to enter seawater and migrate to neighbouring river systems (personal observation). In a sense *O. mossambicus* may be more similar to anadromous fishes, which have an average of 11.3 alleles per locus as reported by de Woody and Avise (2000).

Average observed heterozygosity at the loci (Table 5) was low (0.35), compared to an average of (0.46) obtained in a review of genetic variation at microsatellite loci in freshwater fish (de Woody and Avise, 2000). Average expected heterozygosity, on the other hand, was higher (0.61) to that documented in the literature (0.54) (de Woody and Avise, 2000) (Table 5). The large reduction in observed heterozygosity compared to expected heterozygosity may be indicative of null alleles at the loci leading to an apparent excess of homozygotes, or may result from inbreeding after generations of captivity that did not attempt to rigorously control inbreeding levels.

Population Diversity

The amount of genetic variation within a population can be influenced by selection, migration, mutation and genetic drift. In this study, the effects of selection can be only speculative, since the microsatellite loci are necessarily presumed to be neutral, since this is the most parsimonious explanation of microsatellite genetic variation. Of the other three factors, genetic drift across generations is responsible for losses in genetic variation while immigration and mutation increase genetic variation. The extent of genetic drift is dependent on effective population size changes for past and present generations. If a population experiences a bottleneck with subsequent population number expansion the level of genetic variation present in the resultant population will be determined by the narrowest part of the bottleneck. This phenomenon is demonstrated by the calculation of the effective population size (N_e) over many generations as the harmonic mean over those generations (Maynard-Smith, 1989). The harmonic mean will be closest to the N_e of the smallest population over the time period. The level of variation reflects the genetic drift that occurred during the bottleneck. Table 6 contains a summary of the general levels of genetic variation present in the twelve *O. mossambicus* populations. The red farm populations (e.g. Amatikulu, Nick James and Valley) may have suffered reduced effective population size in the past, evident in their reduced observed heterozygosities compared to the wild and introduced populations. Farm populations, which are not managed to maintain genetic variation, will have high rates of genetic drift compared to wild populations.

Observed heterozygosities were lower than expected heterozygosities for all twelve populations (Table 6). Possible reasons for an increased number of homozygotes include the presence of null alleles, inbreeding or population substructure (i.e. the Wahlund effect, - see Hartl and Clark (1997)). The presence of inbreeding was difficult to determine without the knowledge of effective population sizes for the populations. A suitable means of calculating effective population size was not found due to the nature of *O. mossambicus* breeding leading to the presence of overlapping generations within the populations. Overlapping generations and population substructure may have also resulted due to the supplementation of populations with low numbers using progeny from family crosses.

Genetic Structure

In aquaculture, genetic structure information serves as a guide to which populations should be kept separately for evaluation. Space permitting, however, all populations should be kept separately until their biological potential is assessed since some populations can have phenotypic advantages (Tave, 1993). This is currently the situation employed for the twelve populations. They have been maintained as separate stocks during growth trials to ascertain their potential for strain development. Molecular genetic data detected genetic structure between the populations and provided additional evidence for maintaining them separately.

Overall, a significant genetic structure was present between the twelve populations of *O. mossambicus* evident in the various structure indices that were calculated (Tables 7 and 8).

Gene Flow

The high number of migrants (5.78) between the Ndumo and Olifants populations (Table 9) may have indications of a geographic nature. The Ndumo source population occurs in the Usutu river, a tributary of the Maputo river, which drains into the Maputo Bay in Mozambique. The Olifants source population occurs in the Olifants river, a tributary of the Limpopo river, that drains into the sea north of Maputo bay near Inhambaan (Mozambique). The predominant sea currents on the coast of Mozambique move southward. They possibly provide the Olifants population with a direct migration route from the Limpopo estuary to the Maputo estuary and ultimately the Ndumo population in the Usutu river. The geographically close Sucomba and Kasinthula populations from Malawi had the next highest number of migrants (5.15) (Table 9). This was expected as the Kasinthula population at the Kasinthula research station was initiated from the Shire river, in which the Sucomba population naturally occurs.

Genetic Distance and Phylogeography

The calculation of genetic distances between populations made use of two biological evolutionary models namely the Infinite Alleles Model (IAM) and the Stepwise Mutation Model (SMM). Goldstein' s $(\delta\mu)^2$ distances were sp ecifically developed for microsatellites based an underlying assumption that a stepwise mutation model (SMM) operates at microsatelillite loci (Goldstein *et al*., 1995). Microsatellite allele frequency distribution under a strict stepwise mutation model follows a normal distribution. This model does not, however, perform well for populations with small sizes that are prone to allele frequency disruption due to genetic drift in addition to mutation. Genetic distances calculated from microsatellite data for populations of small size may be more realistic if based on the Infinite Allele Model (IAM). The IAM operates under the premise that changes in allele frequency are independent of the character of preceding alleles. Therefore Goldstein' s $(\delta\mu)^2$ distance (SMM assumption) and Nei's unbiased minimum genetic (D_m) (IAM assumption) distance were calculated for the twelve populations to facilitate construction of phylogenetic trees. Two phylogenetic tree-building methods were employed, the Unweighted Pair Group Method using Arithmetic Averages (UPGMA) and the Neighbour Joining method. The topology of UPGMA trees take on the form of population clusters with the populations being orientated according to the root of the tree. In the Neighbour Joining technique the top ology is based on distances between the populations however there is no root to the tree.

Both IAM based (D_m) and SMM based $(\delta\mu)^2$ genetic distances were calculated to compare the utility of each genetic distance measure in divulging the extent of differentiation and phylogeographic relationship between the populations (Tables 10 and 11). The population topology obtained using the UPGMA method was inaccurate due to the violation of the ultrameric data rule in which an equal rate of mutation along all the branches, as the model of evolution, is assumed. UPGMA trees were therefore excluded from the final analysis. The Neighbour Joining trees (Figures 2 and 3) depicted population clusters related to but not exclusively linked with the geographic location of the source populations. Genetic drift during time spent in captivity may have obscured some of the original genetic relationships between the populations.

A useful application of phylogenetics is in divulging the possible origins of the introduced or farmed population of *O. mossambicus* based on wild populations. One can only make inferences of population origin from the phylogenetic trees obtained for the *O. mossambicus* populations in this study (Figures 2 and 3). For example, the origins of the red coloured *O. mossambicus* populations in South Africa are unclear. A possible source includes the original introduction of red (*O. mossambicus* x *O. niloticus*) hybrids from Taiwan in 1981 to the Umgeni Hatchery in Kwazulu Natal (reviewed by de Moor and Bruton, 1988). The Amatikulu population clusters with the Ndumo which also occurs in the Northern Kwazulu Natal region. The Amatikulu red coloured fish may have originated from a mutation that occurred in a wild grey population in the Northern Kwazulu Natal region or through breeding with the red hybrids from Taiwan. The Valley population clustered with the Amatikulu population in the tree constructed from D_m (Figure 3) however it clustered with the Nick James red population in the $(\delta\mu)^2$ tree (Figure 2). This is possibly indicative of a genetic link between these three populations arising from the Amatikulu red population. Alternatively, the Nick James and Valley red strains may not have originated from the Amatikulu population. Instead, they may have possible roots with the introduced Taiwanese hybrids.

The introduced population, LePommier clustered with the Sucomba population in the $(\delta\mu)^2$ tree. This is interesting as these two populations are the most distant in terms of geographic distance. The LePommier population (most southern latitude) may have links with the *O. mossambicus* individuals introduced into Malmesbury in 1936, the Malmesbury populations in turn having links with populations originating in the Transvaal (northern latitudes, closer to the Sucomba population). The Verlorenvlei population clustered with the red populations Amatikulu and Valley in the D_m tree and with the Nick James and Valley populations in the $(\delta\mu)^2$ tree. The Verlorenvlei population is not a red coloured population though. It may have come from a farm population that was interbred with a red population in the past, the red colour having subsequently been lost. The current level of tree interpretation is only speculative. More data on the demographic history of farm and introduced populations is required in order to clarify the phylogeographic relationship between the populations. Another useful application of genetic structure and phylogeographic data is in identification of populations with conservation importance. The conservation of fish populations has often been neglected based on an 'out of sight out of mind' premise. Consequently, data concerning vulnerability to extinction of aquatic species and populations is sparse compared to that of terrestrial species (Ryman *et al*., 1995). To obtain a true reflection of a specie's or population's conservation status requires data on genetic structure, genetic variation and associated demographic history (Avise, 1994; Zhang and Hewitt, 1998; Goldstein and Schlotterer, 1999). From this information, identification and nominations of populations with evolutionary significance can be made (Avise, 1994; Bowen, 1998). Populations are referred to as management units (MUs) if they contain sufficient genetic differentiation from other populations and evolutionary significant units (ESUs) if they are believed to be important to the present and future survival of a species (Bowen, 1998; Parker *et al.*, 1999). The current level of evaluation for the twelve *O. mossambicus* populations is not sufficient to accurately classify ESUs or MU's as there is a general lack of demographic history for the populations, however, the fact that structure exists encourages the protection of habitats and river systems in which the wild source populations of *O. mossambicus* occur.

CONCLUSION:

The analysis of genetic structure and diversity provided evidence for high levels of variation within and between the twelve *O. mossambicus* populations. The populations therefore represent genetic resources from a conservation perspective and a sustainable aquaculture standpoint. Genetic diversity within the populations varied according to origin. Farm populations had reduced levels of variation compared to populations from wild and introduced sources. Introduced populations had intermediate levels of variation. The reduced heterozygosity observed for most of the populations may merely be a consequence of a high prevalence of null alleles although it is recommended that the populations be monitored occasionally to detect possible reduction in heterozygosity through inbreeding.

Genetic structure indices and phylogenetic methods divulged interesting facts on the genetic relatedness between the populations and the possible origins of some of the farm and introduced populations. Insight into the evolutionary significance of the populations will require further research on the demographic history of *O. mossambicus* populations.

A more applied approach to preventing effective population size reductions caused through infrastructure failure or through unequal family contributions when supplementing populations is advised to minimise the rate of inbreeding and genetic drift in the populations.

The development of aquaculture strains from the twelve *O. mossambicus* populations for aquaculture in South Africa is actively underway. The data presented in this study provides a basis for conservation and exploitation issues pertaining to wild and captive populations of *Oreochromis mossambicus*. Anticipated areas of future research include the continued monitoring of genetic variation in Mozambique tilapia strains and molecular characterisation of additional populations from the wild Mozambique tilapia distribution.

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Appendix 9 A report on the sustainability of small-scale aquaculture in the Western Cape carried out by the Division of Aquaculture and the Graduate School of Busines of the Universit of Stellenbosch.

(Due to the length of the report only the tables of contents are presented here. A full copy of the report is available on request.)

AN INVESTIGATION INTO THE LONG-TERM SUSTAINIBILITY OF SMALL SCALE AQUACULTURE SYSTEMS IN THE WESTERN CAPE OF SOUTH AFRICA

A project for the Division of Aquaculture
and the Graduate School of Business of
the University of Stellenbosch

in support of
DFID Fish Genetics program,
Project R 7284

by

JACOBUS HOON OBERHOLSTER

August 2001

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Draft Report

Preliminary Investigation into the Potential of Tilapia (*Oreochromis mossambicus*) as a Food Source for Lower-Income Communities

Rob Hartnell and Khalid Salie

Small Farmer Development Program

Division of Aquaculture, University of Stellenbosch

with support of

DFID Fish Genetics program, Project R 7284

"Improvement and Utilisation of Indigenous Tilapia Genetic Resources in Southern Africa"

April 2002

Preliminary Investigation into the Potential of Tilapia (*Oreochromis mossambicus*) as a Food Source for Lower-Income Communities

1. Introduction

Tilapia is indigenous to Africa and the Middle East and has for centuries been caught in the wild. Today it is one of the most important domesticated fish species and has been introduced to most continents of the world (China, East Asia, Europe, South & North America). Most of these countries cultivate tilapia mainly for domestic consumption, although international trade and export markets are fast developing. Various species of tilapia are cultivated, including: *Oreochromis aureus, O. niloticus, O. mossambicus, T. rendalli and T. zilli.* The species of relevance to South Africa is the indigenous species, *O. mossambicus.* Tilapia is known as a hardy fish, disease resistant, reproduce with ease, has the ability to digest a variety of foods and can survive in poor quality water with relatively low oxygen levels. These characteristics make tilapia a suitable candidate species for aquaculture development in developing countries.

It is rather difficult to find international trade data on tilapia because most of the stock that is produced is used for local consumption in most of the producing countries. The USA has been recorded to be the biggest importer of tilapia and markets do exist in Europe, Japan, and the Middle East. Taiwan is the largest exporter of tilapia. Other exporting countries include Thailand, Indonesia, Singapore, Costa Rica, Colombia, Jaimaca, Venezuela and Ecuador. Tilapia is reported to be a good substitute for other white marine fish species. In the USA, the retail and food service industry has over the years experimented with a variety of tilapia products. It was found that tilapia is versatile in that it can be prepared in a variety of ways and is compatible with a wide variety of ingredients and sauces. Tilapia can be poached, broiled, grilled baked and micro-waved, steamed and stir fried with relative ease. Tilapia has been characteristics in the US market as white meat, easily to filleted, boneless, odourless with a mild fishy flavour.

2. Role of Aquaculture in Development in South Africa

The development of small-scale aquaculture farming in South Africa, where the needs of local communities are met, has the potential to be the answer to some of the socio-economic problems experienced by the previously disadvantaged. This has the ability to create new employment, better standards of living by increasing household income, developing the industrial sector (both the formal and informal sectors) and develop human resources through health and education.

Health

South Africa has a major problem with protein energy malnutrition (PEM) both in the rural areas and to a lesser extent in the urban areas. This is due to social and political factors as well as the economic climate in South Africa. Available data indicate that PEM is greater amongst blacks and coloureds and is more prevalent within the rural areas. Per capita fish consumption in South Africa amounts to 3.4kg of fish per person per annum.

Fish has been proven to be an essential part of human diets. It is an excellent source of animal protein, minerals such as calcium, phosphorous, iron, vitamins, iodine and fatty acids that is essential in human brain development. Improved availability of fish through aquaculture development, can make a valuable contribution towards improvement of the nutritional status of especially rural communities

Nutritional intervention programs are usually used to alleviate nutritional inadequacies, but over-reliance on these programs can also cause a different set of problems. One of these is the inability of communities to be self-sufficient. One of the goals of any successful intervention program should be the setting short-term dietary goals as stepping stones for long term dietary improvement. All programs should have long-term goals despite the use of supplementation at the beginning of the program. Over time supplementation must be phased out and other sources should be used.

Household Food security

Household food security can be defined as access by all people at all times to the food needed for a healthy life. This entails constant stable supplies of food year after year that can be accessed equally by households or on a national level. Food insecurity therefore results from households having a lack of resources. A study conducted in 1998 indicates that many rural areas of South Africa and even the urban areas are food insecure. Economic development has the potential to increase income, which can add to the resources of households.

3. Tilapia in South Africa

Tilapia farming in South Africa is a new venture and with the result a market locally is not established. As mentioned earlier in international countries it is developing fast in the commercial arena. Research with regards to tilapia in this country has been more geared to the technical and biological aspects of farming this fish. There is a need for an intensive market research nationwide in order to determined if there is a local market for this fish in South Africa. The Small-scale Aquaculture Programme managers have taken the initiative of conducting a preliminary market search of the domestic market in order to get an idea of the consumer acceptance of this fish as a possible substitute for marine species.

3.1 Marketing Survey

3.1.1 Methodology

Semi-structured questionnaires, individual interviews and informal group discussions were used for data collection. A few of the questionnaires were completed with the assistance of a researcher, while the majority was handed to farming households for completion.. In the presence of a researcher, open-ended questions were asked to elicit or inspire respondents to freely raise their opinions. The questionnaires was also structured in such a way that respondents could relate easily without being intimidated by the science of information gathering.

The areas in which the market search was conducted were as follows:

1. Informal fish market in Grabouw, Western Cape
2. Thembalethu black township in George, Southern Cape
3. Local community in Oudtshoorn, Southern Cape
4. Farming community households at Nietvoorbij, Stellenbosch, Western Cape
5. Local community in Zwelathemba township in Worcester, Western Cape
6. Local community at Leliefontein, Namakwaland, Northern Cape

The approach in each of the 6 areas differed according to the local socio-economic environment, and therefore the presentation of the results differ. In each of the research ares, emphasis was placed on the following aspects:

- general consumer purchasing habits with regards to fish (marine and fresh water)
- importance of fish in their diets and frequency of household consumption
- methods and short recipes of preparation
- consumer attitude towards this new species of fish
- the general perceptions of fresh water fish as a food source, oppose to marine fish

3.2 Results of survey

3.2.1 Grabouw, Western Cape

Classification:	Monthly income:		Low:	R1200 – R1500 per month
	Consumption:		6.8 kg per capita	
	Awareness:	Fish:	High	
		Tilapia:	Low	

Six households from the farming community were supplied with fish and interviewed thereafter. Three of them received the fish at no cost while the fish were sold in a bunch of 4 fish for R10 (±R9t to R10/kg) to the three remaining households. The average weight of the fish ranged from 250g – 300g. It was conceived that the tilapia as supplied will have to compete with other available protein sources (chicken, sea fish, etc.) in the area. The community indicated no objection to the price of 4 for R10 (±R9t to R10/kg).

Some consumers (two households) find the fish slightly difficult to clean, as they were not accustomed to the particular species. It was however not perceived to be of a long-term nature in the event of regular supplies. It was however suggested that it should be considered to supply the fish in a fresh, gutted format. The taste and texture was found to acceptable in comparison to other available marine species.

All the respondents indicated a willingness to purchase the fish, if available, on a regular basis at the informal market. The informal traders felt positive about trading tilapia given the right price and consistency of supplies.

3.2.2 Thembalethu, George, Southern Cape

Classification:	Monthly income:		Low:	R800 – R1000 per month
	Consumption:		4.7 kg per capita	
	Awareness:	Fish:	Medium	
		Tilapia:	Low	

Eight households in Thembalethu were given fresh samples of tilapia as well as a questionnaire to complete. All questionnaires were completed. The size of the families varied from 4-8 members.
The respondents indicated fish was purchased on a weekly basis or at least twice a month. Marine fish species frequently bought included frozen hake and maasbanker. Most of the families purchase fish from fisheries and the rest purchase processed fish from Shoprite and other similar retail shops. Prices paid for fish varied from R8 - R20 per kg. Generally the respondents indicated that fresh fish was preferred to the frozen fish. Most of the respondents found tilapia tastier than the frozen marine fish that they

normally purchase. Six households grilled the fish on an open fire whilst the two other households used frying as method. All households agreed that tilapia present an except able alternative for other fish and protein source in the area, both in terms of appearance, taste and affordability, and indicated a willingness to purchase it on a regular basis as and when available. Seven respondents indicated that they would prefer buying from hawkers and 1 indicated from fisheries. Some respondents (three out of eight) felt that the average size of the fish was a bit small and would prefer buying bigger fish of about ±500g.

3.2.3 Oudtshoorn, Southern Cape

Classification:	Monthly income:		Low:	R800 – R1300 per month
	Consumption:		4.2 kg per capita	
	Awareness:	Fish:	Low	
		Tilapia:	Low	

The tilapia marketing survey was conducted in Matjiesrivier. Eleven households were included as respondents. The sample was for this area was divided into high-income groups (HI = >R2000/month) and low-income (LI = <R1000/month) groups within the community. Respondents indicated that their principle fish purchases are fresh or frozen marine fish such as hake, snoek and processed products i.e. canned sardines. Fish was purchased at 3.7 times per month for the LI and 2.5 for the HI. The main retail source is fish shops (83% for HI and 80 for LI). Other retailers are the supermarkets (50%/HI and 40%/LI) and the informal traders. No purchases from informal traders were recorded for the high income group. The lower income respondents recorded 17% purchases from informal traders. Informal traders are mainly seasonal retailers and the availability of fish is inconsistent. They usually buy fresh fish from daily catches from artisenal boats. When weather conditions are adverse, weeks may pass before these fishermen can go out to sea. Many informal traders are addressing the inconsistency of fresh fish supply by buying frozen fish from wholesalers such as I&J and Sea Harvest. These prices are higher than fresh fish and ultimately the consumer has to pay more, making it more expensive than other protein sources such as chicken.

Fresh tilapia of sixes 256g to 450 g were distributed. Ninety percent of the LI group liked the taste compared to 78% of the HI. In terms of texture 92% of the LI group indicated that they like the texture compared to 78% in the HI income group. All the LI respondents indicated that they prefer tilapia to marine fish and 40% of the HI felt the same. All the LI respondents indicated a willingness to purchase the tilapia compared to 80% of the HI income group. The LI are prepared to pay between R10 to R12 per kg fish, whilst the HI was prepared to pay between R10 to R14 per kg fish fish. If the fish are packaged in bunches the LI will pay R22.80 for 2 large and 3 medium fish, and the HI will pay R23.50 for the same bunch of fish.

3.2.4 Nietvoorbij, Stellenbosch, Western Cape

Classification:	Monthly income:		Low:	R100 – R1800 per month
	Consumption:		7.2 kg per capita	
	Awareness:	Fish:	High	
		Tilapia:	Low	

Thirty-five families on the Nietvoorbij farm were given samples of the tilapia fish as well as a questionnaire. The families had to prepare the fish and complete the questionnaire. Only 18 questionnaires were returned. The size of the families varied from 2 to 6 individuals.

Fifty percent of the families indicate that fish is bought at least once a month, 22% purchase fish at least twice a month, 5.5% buy fish at least three time a month and 22.2% indicated that they buy fish on a weekly basis. The fish species most commonly purchased range from snoek, which is most popular, to hake/stokvis, maasbanker, mackerel and silverfish. The price of these fish range from R12 to R20 per kg of fish.

With regards to tilapia, 85 % of the respondents found the fish expectable in terms of taste and appearance. 83% indicated that the fish was very tasteful. A mere 0.5% indicated that the taste was not appealing. There was also some indication that the fish was difficult to clean. Seventy-seventy percent of the respondents preferred to fry the fish as a method of preparation, using local spices. 94% of the respondents that if the fish were placed on the market, they would be more than willing to buy the fish.

3.2.5 Zwelathemba, Worcester, Western Cape

Classification:	Monthly income:		Low:	R600 – R1200 per month
	Consumption:		3.2 kg per capita	
	Awareness:	Fish:	Low	
		Tilapia:	Low	

The Worcester small-scale farmers distributed fish of different sizes amongst the township dwellers, Institute for the Physically Handicapped and to lower and middle income households. The fish stock was obtained from an experimental cage culture unit. In this study no formal questionnaires were used and the two entrepreneurs recorded responses through informal word-by-mouth sessions. The Institute incorporated the tilapia in their daily ration for the kids and the overall response from the kids were very positive. None of the individuals that consumed tilapia showed any dislike. The township had the better appreciation of the tilapia as a food source. The distributors were literally swamped during the second round of deliveries. The result provided positive feedback in terms of tilapia as an alleviate protein source amongst disadvantaged communities. The lower and middle class respondents indicated willingness to purchase tilapia if bigger fish could be supplies. Both classes like the taste.

3.2.6 Leliefontein, Namakwaland, Northern Cape

Classification:	Monthly income:		Low:	R600 – R1000 per month
	Consumption:		1.2 kg per capita	
	Awareness:	Fish:	Low	
		Tilapia:	Low	

Two researchers visited the area and prepared tilapia in different ways and present it to about 40 respondents for comments. More than 80% showed a liking in the taste and would definitely used it as a food source if it can be made available. The preferred method of preparation is fried with salt and pepper and curry stews.

3. Value Adding

The study conducted concentrates on tilapia in the product form fresh and whole. The value adding market is the alternative market that need to be investigated. This market may be easier to penetrate. Reasons for this are that is difficult to change the eating behaviour of communities that have become accustomed to a certain type of fish, especially snoek. Product trends in the US indicate that value added products have become increasingly popular. These include fillets fresh and frozen, marinade, crumbed, vacuumed packed fillets etc. With value adding the marketing may be more geared to the higher income groups as the price per kilogram will be affected. The characteristics of the meat of *O. mossambicus* make it versatile enough to experiment with different types of processing options.

Three Streams Smokehouse in Franschoek was given 4-5 samples of tilapia. These were filleted and vacuum packed. This sample was not representative enough to experiment with different processing options and the cost per kilogram for each processing option need to be determined. This will depend on the following and is recommended for further research:

a. Production cost
b. Yield after gill and gutting
c. Yield after flecking
d. Yield after filleting
e. Yield after smoking (whole of filleted)

4. Summary of Results

A summary of the results includes:

- Generally all the respondents in the survey showed a linking to the taste, texture and appearance of tilapia. A few respondents felt it was difficult to clean.
- Preferred fish sizes were not recorded for all the areas, but the Grabouw results indicate that the respondents found fish of 250g –300g to be small compared to sea species, while Matjiesrivier in Oudsthoorn indicated the willingness to buy fish of sizes 256-450g. Matjiesrivier prefer the mixing of sizes in bunches. Worcester preferred larger fish (>500g).
- Most the respondents (>85%) felt that tilapia has a pleasant taste after it had been prepared and cooked.
- The main marine fish competitors are hake (fillets and whole), snoek, maasbanker and processed products (canned fish). The indications were that tilapia would be able to compete with these species on local markets in terms price, quality and taste.
- From this survey, it can be extrapolated that tilapia has a greater market potential within the LI group compared to the HI group if it is sold in the whole fresh form.
- Proposed prices by respondents were in the region of R8.00-R12.00 per kg of fish.

5. Conclusion

The overall response with regards to consumer acceptance of tilapia was good. More research are needed to be conducted into promotion and awareness of fresh water fish amongst these communities.

A detailed cost analysis needs to be undertaken in order to determine production costs and profitable retail prices. The outcome of such a study will determine whether tilapia production will follow the high road of providing value added food commodities to consumers in the higher income brackets, or to follow the low road of providing an affordable fresh protein source to communities in the lower income brackets.

Further trails have to be conducted into the optimal fish size as some areas are willing to buy small fish, while others prefer bigger fish. A need exist to address peoples attitude to smaller fish through correct promotion or different products, preparation, cooking methods.

Fish of 600-800g is required for filleting. Important factors in determining the price per kilogram is quality, availability and size. The price per kilogram of whole fish is directly proportional to size of fish because a greater fillet yield is obtained from larger sized fish. The Western Cape may have a problem with year round supply of tilapia due to cold winters, particularly in open farming systems.

It should be determined whether the production of tilapia in South Africa should be for the domestic market, the international market or both. In the world market tilapia is well-known but in the South African market it is a relatively unknown. Development of the local market will require marketing campaigns to increase public awareness of tilapia. If production is geared at the international market then the US and Europe are the most viable markets to target. Due to the large number of countries that do export to the US and the increasing importance of US aquaculture there is increasing competition in the US market for tilapia. However the increase in demand for tilapia products in the US indicates that there is room for expansion. For South Africa to be able to compete with the other exporting countries it must be able to produce consistent year long supplies of value added tilapia products that meet all international quality and health standards.

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Appendix 11 Collaborative Links and Interactions Developed During the Project

The DFID-FGP has contributed to the establishment of collaboration with various local and international institutions since the introduction of the project in 1999. These include:

Local Institutions

- Department of Agriculture: Western Cape Province. A good system of co-operation has been reach between the UoS (DFID-AFGRP) and the DA with regard to joint objectives, planning, management and sharing of facilities with regard to aquaculture and rural development in particular. During the course of the project the Department underwent a major change in viewpoint considering aquaculture and toward the project end had adopted a policy in which aquaculture was could be considered as a major component of its rural development activities. Unfortunately the momentum of this paradigm shift was very much lost by the departure from their posts of Paul King, Jacob Swart and Robert Hartnell (VSO) towards the end of the project.
- The Aquaculture Unit of the University of the North, Pietersburg, Northern Province. Collaboration includes joint research on the quantitative selection of tilapia (*O. mossambicus*) as well as aspects of the YY-male breeding programme. The DFID-FGP at the UoS was visited by Prof. Eddie Meyer from the University of the North during November 2000 and GCM visited the University of the North in early September 2001.

- Department of Biology, University of Venda, KwaZulu-Natal.
  Collaboration includes joint research on the molecular characterisation of strains of tilapia (*O. mossambicus*). The university through Prof. Ben van de Waal is assisting with sampling of wild population in the region of Northern Kwa-Zulu-Natal and the Limpopo river system, as well as morphological analysis of wild specimens. The DFID-FGP at the UoS was visited by Prof. Ben van de Waal from the University of Venda during January 2001. The DFID-FGP at the UoS will also receive as postgraduate student from the University of Venda for training in molecular techniques during the course of 2001.

- Institute for Agricultural Engineering, Agricultural Research Council, Pretoria. The Institute is assisting with the technical design of aquaculture infrastructure (cages, ponds, biofiltration, heating, etc.), including those utilised in the DFID-FGP, as well as the design and development of least cost cage structure for community based aquaculture projects. Mr. Regard Viljoen, Project Engineer: Aquaculture, visited the DFID-FGP during October 2000 and February 2001 and hosted a tour of aquaculture operations in the North of the country for GCM in September/October 2000

- Department of Zoology, University of Pretoria. Dr. Paulette Blommer is collaborating with regard to molecular genetic investigation of indigenous aquatic species, including current work on Tilapia, *O. mossambicus*.

International collaboration

- Voluntary Services Overseas: GCM and subsequently RIL approached VSO early on in the project with the view to requesting a volunteer to work on aquaculture and project related activities with the Department of Agriculture (DA) of the

Western Cape. The application was successful and a volunteer, Mr. Robert Harntell worked closely with the DA (where he was assigned), in collaboration with UoS and with regard to project objectives for 18 months from July 1999 to December 2001.

- The Division of Aquaculture, UoS, submitted a successful project proposal as part of the Bilateral **UK/RSA S&T Research Fund**, with the **University of Wales, Swansea** as the UK partner institution. The objective of the proposal is complementary to some of the DFID-FGP objectives with regard to the molecular characterisation of the genetic structure of the Tilapia, *O. mossambicus*, in Southern Africa. The duration of the project is for the period 2001/2002 and as part of this project Mr. Edward Hall was able to travel to the U.K. for 3 months in early 2002.

- The Division of Aquaculture, UoS, was successful in submitting a project proposal as part of the **Bilateral Flemish/RSA S&T Research Fund**, with the **University of Leuven, Belgium** as the Flemish partner institution. The objective of the proposal is complementary to some of the DFID-FGP objectives with regard to the use of molecular techniques for the integrated management of aquatic resources in Southern Africa. The duration of the project is for the period 2001/2003. The UoS and DFID-FGP has received a visit by Prof. Filip Volckaert, from the University of Leuven during January 2001 and September 2002.

- Two members of staff from the UoS Division of Aquaculture, that were working in the DFID-FGP on a fulltime basis, namely Me. M. du Plessis and Mr. K. Swart were able to attend the training course on "Selective Breeding of Technology: Gift – Genetically Improved Farmed Tilapia" to be held at the **GIFT Foundation**, Munoz, Nueva Ecija, in the **Phillippines** from the 12 to the 24 of March 2001. GCM was instrumental in facilitating their participation in the ICLARM training course. This has made an important contribution as both these staff member which were assigned to the quantitative component of the DFID-FGP. Ms. Du Plessis shortly afterwards received a full time appointment in the Department of Genetics effective July 2001 but resigned from this post in December 2001 to emigrate to Europe. Mr. Swart was also subsequently appointed in the Aquaculture Section, of the Division Animal Production of the Department of Agriculture, at Elsenburg, Western Cape. The Aquaculture Section is working in close conjunction with the University of Stellenbosch, and Mr. Swart has remained involved in both the YY-breeding programme as well as quantitative genetic aspects of the tilapia research programme.

- A member of staff from the UoS Division of Aquaculture, Mr. H. Stander underwent training in technical aspects of tilapia hatchery management and fry rearing at the Freshwater Aquaculture Center in the Phillipines, working with staff trained under previous DFID funded projects there. Mr. Stander is responsible for technical management of facilities and staff related to the DFID-FGP, as well as the management of the tilapia hatchery facility providing seed material to the SFDP. GCM was again instrumental in facilitating his participation in the practical training programme in the Philippines.

- Collaboration has also been established with the US AID, CRSP Programme on Pond Dynamics and Aquaculture, in particular with the division of Prof. Claude Boyd at Auburn University, USA. The current work include aspects of tilapia pond culture and nutrition.

- Bunda College, Malawi. A faculty from Bunda college carried out is Masters study, including thesis research, at AIT under GCM. He conducted his thesis research thesis on methodologies for comparisons of improved varieties of tilapia and it is hoped to continue collaborative research on indigenous tilapia species when he returns to Malawi in November 2002.

Appendix 12 Report on the project sponsored workshop on sustainable development of indigenous resources for aquaculture development in the Southern African Region.

Report of Workshop

on

Sustainable Development of Indigenous Resources, for Aquaculture Development in the Southern African Region

presented by

Division of Aquaculture, Department of Genetics,

University of Stellenbosch,

in collaboration with

DFID Fish Genetics Programme (R7284), United Kingdom

as part of the

Sixth Conference of the Aquaculture Association of Southern Africa

AASA

10th – 13th September 2002, Stellenbosch, South Africa

Programme

Workshop on Sustainable Development of Indigenous Resources, for Aquaculture Development in the Southern African Region

Venue: 10th September 2002 Seminar Room, Department of Genetics, J.C. Smuts Building, University of Stellenbosch, Stellenbosch, R.S.A

08:00 **Registration**

08:15 **Opening**

08:30 **Presentations by invited delegates & Discussions:**

08:30 Evolutionary development and genetic structure of the African catfish, *Clarias gariepinus.*
Prof. Filip Volckaert, Laboratory of Aquatic Ecology Chatholic University of Leuven, Belgium.

09:00 Genetic variation within and relationships between Southern African and Dutch catfish, *Clarias gariepinus*.
Prof. Herman van der Bank, Dept. of Zoology, Rand Afrikaans University.

09:20 Genetic Structure and Diversity in Twelve Populations of Mozambique Tilapia, *Oreochromis mossambicus.*
Edward Hall, Division of Aquaculture, Department of Genetics, University of Stellenbosch, South Africa.

09:40 Inter species hybridisation between indigenous (*Oreochromis mossambicus*) and introduced (*Oreochromis niloticus*) species of tilapia in river systems of Southern Africa.
Prof. Ben van der Waal, University of Venda, South Africa

10:00 **Tea**

10:15 The use of molecular markers to assess the genetic status of Tilapia populations in terms of diversity and interspecies hybridisation.
Marna Esterhuyse, Division of Aquaculture, University of Stellenbosch, S A

10:30 Bioprospecting for Tilapias
Adrian Piers, African Fish Ltd., Lusaka, Zambia

10:45 Issues in dissemination of domesticated and improved fish breeds.
Graham C. Mair AIT, Thailand

11:10 **Discussion of proposed topics:**

11:10 1. The potential indigenous aquatic resources, for aquaculture development in the Southern Africa.

11:30 2. Need for conserving wild stocks, indigenous aquatic resources.

11:50 3. Potential impact of commercial species/stocks on indigenous aquatic resources and genetic diversity in the Southern Africa.

12:30 4. Dissemination of domesticated and improved fish breeds/strains.

13:00 **Lunch**

14:00 Presentation and discussion of the **Nairobi Declaration**

14:30 **Group discussions**
Group discussions on amendments and additions to the Nairobi Declaration as a framework for a **strategy/policy** for the sustainable development of indigenous aquatic resources, for aquaculture development in the Southern African region.

15:15 **Tea**

15:30 Report back & consolidation of inputs. Decision on adoption and course of action

16:30 **Closure**

Workshop on Sustainable Development of Indigenous Resources, for Aquaculture Development in the Southern African Region

Objective

The **objective** of the workshop was:

To initiate discussion that could contribute towards a strategy/policy for the sustainable development of indigenous aquatic resources, for use in aquaculture in the Southern African region.

The Workshop was attended by a total of 47 delegates (Appendix B), including delegates from 9 African countries, representatives of government, conservation, research and commercial institutions.

Outputs

The expected **outputs** of the workshop were:

- An enhanced **awareness** among potential beneficiaries of the potential of indigenous aquatic resources, for aquaculture development in the Southern Africa.
- An enhanced **awareness** among stakeholders of the potential impact of commercial species/stocks on indigenous aquatic resources and genetic diversity in the Southern African region.
- A **framework** for a strategy/policy for the sustainable development of indigenous aquatic resources, for aquaculture development in the Southern African region, with representation from government, conservation, research and commercial institutions.
- A report that could form the basis for future proposals/policies on the utilisation of indigenous species for aquaculture development in Southern Africa.

Attendance

The objective of the workshop in terms of attendance was to achieve regional representation from all countries in the Southern African Region (SADC-countries) as well as equitable representation from the various sectors including government, conservation, regulatory, research, development and commercial institutions.

Presentations by invited delegates and discussions:

Invited delegates delivered the following sequence of presentations, which were followed by general discussions.

- Evolutionary development and genetic structure of the African catfish, *Clarias gariepinus.* **Prof. Filip Volckaert**, Laboratory of Aquatic Ecology Chattholic University of Leuven, Belgium.
- Genetic variation within and relationships between Southern African and Dutch catfish, *Clarias gariepinus*. **Prof. Herman van der Bank**, Dept. of Zoology, Rand Afrikaans University, South Africa.
- Genetic Structure and Diversity in Twelve Populations of Mozambique Tilapia, *Oreochromis mossambicus.* **Edward Hall**, Division of Aquaculture, Department of Genetics, University of Stellenbosch, South Africa.
- Inter species hybridisation between indigenous (*Oreochromis mossambicus*) and introduced (*Oreochromis niloticus*) species of tilapia in river systems of Southern Africa. **Prof. Ben van der Waal**, Dept. of Zoology, University of Venda, South Africa.
- The use of molecular markers to assess the genetic status of Tilapia populations in terms of diversity and interspecies hybridisation. **Marna Esterhuyse**, Division of Aquaculture, Department of Genetics, University of Stellenbosch, South Africa.
- Bioprospecting for Tilapias **Adrian Piers**, African Fish Ltd., Lusaka, Zambia
- Issues in dissemination of domesticated and improved fish breeds. **Dr. Graham Mair**[1], School of Biological Sciences[25], University of Wales Swansea, Swansea SA2 8PP, Wales, U.K.

Abstracts of these presentations appear in Appendix A to this report.

[25] Currently based at the Asian Institute of Technology, PO Box 4, Klong Luang, Pathumthani 12120, Thailand to where correspondence should be addressed.

Discussion of proposed topics

The formal presentations were followed by an open discussion of a series of proposed topics that included:

1. The potential of indigenous aquatic resources for aquaculture development in the Southern Africa.
2. Need for conserving wild stocks, indigenous aquatic resources.
3. Potential impact of commercial species/stocks on indigenous aquatic resources and genetic diversity in the Southern Africa.
4. Dissemination of domesticated and improved fish breeds/strains.

The results of discussions are presented in a summarised format:

A. The potential of indigenous aquatic resources for aquaculture development in the Southern Africa

A discussion was held on the need for promotion of the use of indigenous species in the development of aquaculture in the region. It was felt that the use of indigenous species could make an important contribution towards sustainable aquaculture development in the region, as opposed to the historical and even current practices of introduction and transfer of exotic species which are more established in aquaculture.

Candidate species with potential to contribute towards the development of indigenous aquaculture in the region were discussed and listed in Table 1. It was however noted that in the case of many of these species further research and development is still required in order to ensure their sustainable utilisation. Areas of particular interest include cultural, economic, legal and regulatory aspects, production technology, natural and human resources. It was also felt that indigenous and traditional aquaculture practices of the region should be acknowledged and promoted. The use of alien species should not be discounted in areas where they have become established, particularly where no suitable indigenous alternative exists and where environmental conditions permit (ideally determined through appropriate environmental impact assessment).

Table 1 A list of candidate species of Southern Africa with potential to contribute to the development of indigenous aquaculture in the region.

Species	Categories of Application				
	Food	Commercial	Recreational	High Volume	High Value
Tilapias: *O. mossambicus* & other	*	*		*	
Catfishes: *C. gariepinus* & other	*	*	*	*	
Eels: *Anguillid sp*. Mullets: *Mugulidae sp.*	 *	* 		 *	*
Freshwater invertebrates: *Machrobranchium*		*			*
Freshwater Finfish: Various species	*	*	*	*	*
Marine invertebrates: Prawns: *Pinaeus indicus, P. monodon* Crayfish: *Jasis Landii* Abalone*: Haliotis midae* Mud crab: *Scylla serrata* Swimming crab		 * * * * *			 * * * * *
Marine Finfish: Sole: *S. marginata,* Spotted grunter: *P. commersonni,* Disky Cob: *Argyrosomus japonicus*		*		*	*
Sea weeds: *Ulva* and *Gracilaria*		*			*
Shellfish: Clam *Mactra glabrata* Mussels*: Mytilus galoprovincialis*		* *			* *

B. Need for conserving wild stocks, indigenous aquatic resources

The needs for the conservation of wild stocks and indigenous aquatic resources were discussed, particularly with a view to obtaining clarity or consensus among a diverse group of delegates, about the need, objectives, benefits, problems and solutions. Delegates identified the following areas where indigenous aquatic resources can make a beneficial contribution towards aquaculture development:

- Eco-tourism – various indigenous species have proven or potential eco-tourism appeal that should be utilized and exploited.
- Biodiversity – conservation of indigenous aquatic resources is of importance in both the ecological sense as well as to commercial aspects, with regard to its potential contribution towards genetic development programs.
- Bio-security – the use of indigenous species will reduce, although not eliminate, risk factors associated with bio-security, in comparison to exotic species.
- Adaptive advantages – indigenous species could have adaptive advantages to local environmental conditions, which could be beneficial in terms of their application in aquaculture, as compared to exotic species.
- Marketing – the use of indigenous species may provide beneficial opportunities in terms of "green labeling" in marketing campaigns, as well as with regard to cultural/consumer acceptance.
- Indigenous knowledge systems – the use of indigenous species will accommodate the use of indigenous knowledge systems in development programs.

The need was also expressed that all countries in the region must accept accountability in this regard. The need and responsibility for the mobilization of indigenous resources (natural, human, economic, etc.) in order to ensure the sustainable utilization of indigenous aquatic resources was also expressed. The need for contribution of resources (financial, technological, human, etc.) from outside the region for the sustainable utilization of indigenous aquatic resources was also acknowledged.

C. Potential impact of commercial species/stocks on indigenous aquatic resources and genetic diversity in Southern Africa

The potential impact of commercial species/stocks on indigenous aquatic resources and genetic diversity in Southern Africa were discussed. Delegates identified the following areas where commercial species/stocks may impact upon indigenous aquatic resources and genetic diversity:

- Habitat – impact upon and competition for habitat, impacting upon numbers, distribution, population structure, etc.
- Disease – introduction and distribution of exotic diseases.
- Genetic contamination – contamination of indigenous genetic resources through hybridization, genetic and phenotypic introgression and selective disadvantage in terms of adaptation to environmental conditions.

It was also felt that due consideration should be given to the "scale-effect" when possible impact are considered. It was felt that impact is often assumed without due consideration of the scale of impact in terms of genetic variation, numbers, time effect, etc. The approach of impact assessment should also take into consideration the "Biological Footprint" both in terms of upstream and downstream affects and benefits (human resource development, food security, socio-economics, and economic growth) that can be accrued from aquaculture development.

The contribution of aquaculture and related technologies towards the conservation of indigenous aquatic resources was also acknowledged. It was felt that aquaculture technologies could be utilised for:

- Conservation – artificial propagation and seeding of endangered species
- Bio-security – breeding of monosex or sterile varieties of aquatic species for use in aquaculture systems with a reduced bio-risk profile.

D. Dissemination of domesticated and improved fish breeds/strains

Various indigenous species have the potential to be utilized in aquaculture development within the region. It was acknowledged that domestication and genetic improvement would be required in order to ensure that sustainable development is achieved. Virtually all of the indigenous species identified as candidates for use in aquaculture lacks competitiveness in terms of growth, yield, FCR, etc., when compared to other established commercial species and strains, most of them exotic to Southern Africa. It was felt that dissemination of domesticated and improved fish species/strains should be done in accordance with:

- Impact assessment – standard national/international EIA procedures should be followed.
- Verification of genetic status – the genetic status and origin of the stocks should be verified/validated.
- Biological containment – procedures for biological containment should be implemented.
- Defining of populations – the genetic status of the stocks in the broader context of biodiversity and specific context of population structure and relationships should be considered.
- International protocol/policy/code of conduct – dissemination should be done in accordance with set international protocol/policy/code of conduct, etc. for the region.

It was requested that the convenors of the workshop should in due course give indication with references to existing protocol/policy/code of conduct in this regard, and to facilitate agreement/adherence of participating parties. It was proposed to facilitate adherence to existing policies/protocols, rather than to generate a further set of regulations..

Presentation and discussion of the Nairobi Declaration

Background of the Nairobi Declaration

An Expert Consultation on Biosafety and Environmental Impact of Genetic Enhancement and Introduction of Improved Tilapia strains/ Alien species in Africa, was convened in Nairobi, Kenya from 20-23 February 2002 under the sponsorship of ICLARM (The World Fish Center), the Technical Centre for Agricultural and Rural Cooperation (CTA), the Food and Agriculture Organization of the United Nations (FAO), the World Conservation Union (IUCN), the United Nations Environment Programme (UNEP), and the Convention on Biological Diversity (CBD), to discuss and develop guidelines that will foster the development of aquaculture while maintaining biodiversity. The meeting was attended by aquaculturists, geneticists and conservation specialists from Africa and from international organizations. Following four days of discussions the participants endorsed the Nairobi Declaration on Conservation of Aquatic Biodiversity and use of Genetically Improved and Alien Species for Aquaculture in Africa.

Delegates at the Workshop on “Sustainable Development of Indigenous Resources for Aquaculture Development in the Southern African Region” held the opinion, that the Nairobi Declaration presents an ideal background and structure for consideration as a policy framework for Southern Africa. The Nairobi Declaration was hence presented and discusses, after which the following amendments was suggested for it to be use as a policy framework for sustainable development of **indigenous** resources for aquaculture development in the Southern African Region.

Nairobi Declaration

http://www.iclarm.org/news/Nairobi%20Declaration.pdf

Nairobi Declaration

Fish are a critical source of animal protein to the people of Africa, and aquatic resources play a central role in sustaining rural and urban livelihoods across much of the region. Yet for the continent as a whole per capita supply of fish is declining and current projections of supply and demand indicate that this gap will continue to grow in the coming decades.

If this gap is to be bridged capture fisheries need to be sustained and the potential of aquaculture realised. In doing so attention needs to be given to protecting the rich aquatic biodiversity of Africa, especially the rich diversity of freshwater fish and its role in sustaining capture fisheries and providing species for aquaculture.

At present fish production from aquaculture in Africa is low. However as population increases, together with demand for fish, the aquaculture sector is projected to grow. For this to happen, a wide range of constraints need to be addressed and a greater range of management practices considered. Pond and broodstock management will need to be improved, a wider range of feeds developed, and market access improved.

In addition, there is considerable potential for improving performance of the fish species and strains used. At present many of the fish used in aquaculture in Africa are derived from undomesticated stocks. This contrasts with crops, livestock and poultry where large increases in production have been achieved through application of breeding programs and other genetic improvement procedures. However, while improved strains and introduced species have potential to increase production there is clear risk of escape into the wild, and possible negative impacts on biodiversity. If the full potential for sustainable aquaculture in Africa is to be realised these concerns need to be addressed.

Recommendations

1. Quality seed

Given that the spectrum of aquaculture from small-scale, low-input systems to large-scale intensive systems can achieve potential benefits from genetic enhancement, quality seed should be made available from the public and private sector agencies and to be used in conjunction with proper broodstock and farm management.

2. Genetics in broodstock management

Since genetic resources in cultured populations can be degraded as a result of captive breeding, best practice genetic aspects of broodstock management need to be a basic element within all aquaculture and stock enhancement programs.

3. Responsible introductions

Introductions of fishaquatic species, including genetically improved strains and alien species, may have a role in the development of aquaculture. Any movement of fish between natural ecological boundaries (e.g. watersheds) may involve risk to biodiversity and there is need for refinement and wider application of protocols, risk assessment methods, and monitoring programs for introductions of fish, including genetically improved strains and alien species. States have an important responsibility in the development and implementation of such protocols and associated regulations, the establishment of clear roles and responsibilities, and capacity building. Such efforts should be linked to obligations pursuant to the Code of Conduct for Responsible Fisheries, the Convention on Biological Diversity, and other relevant international agreements .

4. Conserving wild stocks

Unique wild stocks of important tilapia indigenous species still exist in many parts of Africa. Priority areas catchments should be identified and managed as conservation areas in which introductions of alien species and genetically improved strainsspecies and strains should be prevented.

5. Management of Transboundary problems in fish transfer

The majority of issues and problems associated with movement of fish and the use of genetically improved strains are common to most African countries. Countries are encouraged toshould: (a) look beyond borders for examples of workable policies, and legislation and regulatory and management activities, adopt them where appropriate to fill national policy gaps, and harmonize them where necessary; and (b) use existing regional/national/provincial bodies or form new bodies to assist in coordinating management activities taking into account ecological realities, in particular transboundary watersheds, and (c) monitor such transfers.

6. Strengthening access to information

Baseline information on fish genetic diversity, environmental integrity and aquaculture practices exists, but it is neither comprehensive nor easily accessible. The existing mechanisms for collection and dissemination of information need to be strengthened.

7. Controlling pathogen movement

Internationally accepted codes and protocols for reducing the risk of transboundary movement of pathogens1 through movement of fish including alien species do exist, but they do not address any specific needs regarding genetically improved species. States and other relevant bodies should evaluate the existing codes and protocols for reducing the risk of transboundary movement of pathogens through movement of fish including alien species and genetically improved strains, and adapt them for African conditions.

8. Raising awareness of risks of fish introduction

Policy makers, enforcement agencies, stakeholders and the general public need to be made aware of issues related to, and the need for, policy on the movement of alien species and genetically altered improved species, and this should be high on national agenda. This should be high on national agendas and implemented by December 2003.

9. Engaging stakeholders

Some policies relevant to movement of fishresponsible and sustainable aquaculture seem difficult to implement, are unknown to users, create conflicts of interest, or are viewed as restrictive, in part because they have been developed with limited consultation and participation. Formulation of policy and legislation concerning fish movement should seek to engage all stakeholders in a participatory process. In addition, governments should establish advisory groups with links to independent and scientifically competent expert bodies such as FAO, IUCN, and ICLARM, the World Fish Center.

10. Liability for adverse environmental impacts

Although economic benefits can be derived through the use of alien and/or genetically improved species in aquaculture, in many cases, those to whom benefits accrue do not bear the costs associated with adverse environmental impacts. In view of this, there should be provision for liability, compliance (e.g., incentives) and restoration within policies and legislation concerning the movement and use of alien and genetically improved fish species in aquaculture.

11. Additional Recommendation from the workshop

The way forward should be expressed more clearly in terms of actions, targets, dates and plans and who should be responsible for their implementation.

APPENDIX: A

ABSTRACTS

W1 Evolutionary Genetics of the African Catfish, *Clarias Gariepinus.*

Prof. Filip Volckaert, Laboratory of Aquatic Ecology Chattholic University of Leuven, Belgium.

Abstract

Favourable yet changing environmental and climatic conditions during the Late Tertiary and Quaternary in Africa resulted in tremendous opportunities for range expansion, local adaptation and speciation among both terrestrial and aquatic organisms. We have used mtDNA analysis (RFLP and sequence), in order to compare past versus contemporary influences on the structuring of populations in the African Catfis h Clarias gariepinus (Burchell 1822), a freshwater species with one of the largest distributions among African freshwater fishes. Restriction fragment analysis of a 2.5 kb fragment spanning the ND5 and ND6 genes and control region sequences (867 bp) demonstrated distinct regional clusters in eastern, south/central and northern Africa (including the Middle East) corresponding to separation since the formation of the East African Rift system or Middle Pleistocene. The south/central clade is characterised by five distinct groups, all possibly relating to distinct Late Pleistocene refugia. Intraspecific haplotype divergence was high (up to 9%), but was well correlated with current biogeographical and hydrological knowledge. Similarity in haplotype frequencies in separate rivers suggests a surprising stasis in mtDNA evolution. Based on our findings, we present a hypothesis for the rise, expansion and deep phylogenetic split of this species complex originating in the proto-savannah of the Zambesi basin and following the evolution of climate and hydrography.

W2 Genetic variation within and relationships between Southern African and Dutch catfish, *Clarias gariepinus*

Herman van der Bank. Department of Zoology, Rand Afrikaans University, PO Box 524, Auckland Park, 2006, South Africa. **E-mail**: fhvdb@na.rau.ac.za

Abstract

Aquaculture research on sharptooth catfish (*Clarias gariepinus*) in SA decreased dramatically since the demise of the National Aquaculture Programme in 1989/90. However, Hecht & Britz (1993) have projected a potential increase to ca. 5 000-6 000t per annum based on established production capacity at the time. Unfortunately, drought, marketing problems and the termination of funding for research and development were responsible for the loss of interest in catfish production. This situation has now been reversed due to good rainfall since 1996, the favourable exchange rate for export, mad-cow disease caused a worldwide consumer resistance against red meat, and people are becoming more educated regarding the health status of having fish in their diets. This is especially important in many parts of southern Africa where famine is at its worst at present and malnutrition is a major concern. For example, one catfish farmer now needs to produce 40-60 million fingerlings annually and the export of fresh and smoked fillets can earn 6.00 and 8.50 USA $ respectively (Le Roux, pers. comm., 2002). A 50 million rand fish production plant was also recently set up in SA, where fingerlings are imported from Europe, grown to marketable size in SA and then exported. Previous research at RAU on wild and domesticated catfish stocks in SA and a comparative study of Dutch catfish are summarised, and future research needs are specified.

References:

Hecht, T & Britz, PJ (1993) Aquaculture '92. *Proc. Aquacult. Assoc. sthn Afr.* 1, 198 pp.

W3 Phylogenetic Interpretation of Genetic Diversity in Southern African Tilapia Populations for Sustainable Aquaculture and Conservation of *Oreochromis mossambicus*

E. G. HALL Department of Genetics, University of Stellenbosch.

Abstract

Southern African populations of *Oreochromis mossambicus* have been classified as genetic resources in aquaculture and conservation. Integration of *O. mossambicus* into aquaculture requires management to preserve strains and to prevent contamination of unique genetic material in wild populations. Twelve populations, collected for strain development evaluation, were characterised using fluorescent microsatellite DNA analysis. Genetic diversity within and between the populations indicated that the populations represent genetically structured units. Phylogeographic interpretations of genetic diversity were made to identify populations of conservation and aquaculture importance based on their potential for sustainable utilisation in southern African aquaculture regions.

W4 Inter species hybridisation between indigenous (*Oreochromis mossambicus*) and introduced (*Oreochromis niloticus*) species of tilapia in river systems of Southern Africa.

Prof. Ben van der Waal, University of Venda, South Africa

W5 The use of molecular markers to assess the genetic status of Tilapia populations in terms of diversity and interspecies hybridisation

Marna Esterhuyse, Division of Aquaculture, University of Stellenbosch

Abstract

Forming part of a conservation programme, this study was concerned with two species of Cichlid fish (*Oreochromis mossambicus* and *O. niloticus*), which were brought into contact with each other by unnatural ways. They are now hybridizing to some extent and there is also evidence that the foreign *O. niloticus* may out compete the native *O. mossambicus*. To cast light on what the current distribution is of both these species and the hybrids in Southern Africa, it is important to identify specimens very accurately. In attempting to find genetic markers to distinguish between two species of Cichlids we tested 20 microsatellite dinucleotide (CA_n) repeats during a preliminary study and found five of these promising to exhibit little intra-specific genetic diversity but large genetic variation between species. We amplified these five loci in 145 individuals from 10 populations, which included the two species and their hybrids. Exact sizes of the fragments were determined using an automated DNA sequencer.

Between the two species, allele sizes were overlapping, but when data were analyzed by statistical models, the differences could be seen for populations, however on individual level there was overlap between the species. The hybrids were found to be intermediate positioned between the two pure species. Our attempt to assign individuals to populations provided doubtful results. Thus, using this set of markers, populations can be ascribed to one of these species, but not individuals by themselves.

W6 Bioprospecting for Tilapias

Adrian Piers, African Fish Ltd. P O Box 36383, Lusaka, Zambia. Email – imagepromotions@zamnet.za

Abstract

This paper looks at the need to secure genetic material for future use by aquaculturists to ensure food security and continued growth of Tilapia culture around the world, with particular reference to endemic species under threat in Zambia and associated watersheds. Specific threats are discussed and possible strategies to save genetic variation are put forward.

W7 Issues in dissemination of domesticated and improved fish breeds

Graham C. Mair *School of Biological Sciences*[26]*, University of Wales Swansea, Swansea SA2 8PP, Wales, U.K.*

Abstract

Whilst aquaculture genetics can still be considered to be in its infancy, progress is being made and significantly improved breeds are now available in a number of important aquaculture species including salmonids, tilapias, catfish and carps. In addition to deliberate genetic changes resulting from genetic improvement programmes, there are also very many stocks that have been substantially genetically changed by domestication, often resulting in genetic deterioration of the stocks. This paper briefly discusses technical, environmental, institutional and social issues related to the management and dissemination of improved or domesticated breeds of commercially important aquaculture species.

There are a number of classical genetics-related problems that arise during domestication and long term management of stocks in hatcheries, associated with inbreeding, genetic drift, unconscious selection and hybrid introgression. There are a number of basic broodstock management practices that can be followed to minimise these problems in hatchery stocks. In the case of exotic species, the main impact of poor broodstock management is reflected in declining culture performance. However, with the domestication of indigenous species negative environmental implication can also arise, particularly if domesticated stocks are used in restocking programmes or enhanced fisheries, as well as in aquaculture. There are already several case studies, which have demonstrated that the genetic status and population structure of indigenous species has been irrecoverably disrupted by the widespread distribution of domesticated breeds. There is thus a strong need not only for responsible practices, following precautionary principles, for the introduction of exotic species but also for responsible domestication and management of indigenous stocks.

As aquaculture develops in any region there is commonly a gradual shift in responsibility for seed supply from the state sector, commonly in centralized hatchery systems, to a more decentralized system focused in the private sector. This scenario has and is being played out in many parts of the world. It is important that the roles of formal services evolve appropriately, typically from being responsible for seed supply to taking responsibility for seed quality, avoiding unnecessary competition with the private sector along the way. Ultimately the private sector will also take on a major role in disseminating domesticated and improved breeds and eventually, even in developing the improvements. During this shift in responsibility the formal agencies should retain, and indeed expand, their role in ensuring equitable dissemination of the benefits of good quality seed to ensure that small scale and poor farmers are not further marginalised from these benefits.

[26] Currently based at the Asian Institute of Technology, PO Box 4, Klong Luang, Pathumthani 12120, Thailand to where correspondence should be addressed.

Appendix B List of Overseas, African and other delegates attending the workshop on "Sustainable Development of Indigenous Resources, for Aquaculture Development in the Southern African Region" on the 10th September 2002, Stellenbosch, South Africa.

Overseas & African Delegates

Surname	Name	Country	Institution	Address	e-mail
Akande	Rotime	Lagos	Department of Fisheries	PO Box 9783, Ikeja, Lagos	rotimiakande@yahoo.com
Beardmore	John	U.K.	University of Wales, Swansea	School of Biological Sciences, University of Wales, Swansea SA2 8PP, Wales, U.K.	j.a.beardmore@swansea.ac.uk
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Appendix 13 Guide for extension services on the development of SMMEs in Aquaculture (full publication available on request).

Guide for Extention Services on the Development of SMME's in Aquaculture

Developed by the Department of Agriculture, Western Cape
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in collaboration with the
Division of Aquaculture,
University of Stellenbosch

October 2001

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Appendix 14 Training Manual

TRAINING MANUAL FOR SMALL SCALE FISH FARMING SYSTEMS

Compiled by

Division of Aquaculture, University of Stellenbosch

with support from

DFID Fish Genetics program, Project R 7284

PRODUKSIE PLAN VIR KLEINSKAAL VISBOERDERY

saamgestel deur

Divisie vir Akwakultuur, Universiteit Stellenbosch

in samewerking met

DFID Fish Genetics program, Project R 7284

Die handleiding is 'n praktiese opsomming van die belangrikste aktiwiteite van 'n kleinskaal visboerdery projek. Die volg van hierdie handleiding sal bydrae tot die lewering van goeie resultate en kwaliteit.

PRODUKSIE AKTIWITEITE

1. AANKOOP VAN VINGERLINGE

Die aankoop van goeie gehalte vingerlinge (vissies) is die bepalende faktor vir die suksesvolle produksiefase. Die volgende is riglyne daarvoor:

1.1 Bepaal koopprys vooruit en vergelyk met ander verskaffers.

1.2 Kyk na die algemene voorkoms, die aantal, algemene grootte en ouderdom van die vis wanneer dit aangekoop word.

1.3 Neem 'n lewendige monster van 100 - 200 visse om in die dam te plaas vir sowat 'n week voordat die groot groep visse ingesit word. Dit help om die toestand van die dam te evalueer en om enige risikos te verminder.

1.4 Kom ooreen oor die betaalmetode en tyd.

2. VERVOER

Die voorbereiding van toerusting en vis sowel as die toesig gedurende die vervoer van die vis is baie belangrik om die suksesvolle vervoer van lewendige vis te verseker. Die volgende riglyne moet gevolg word.

2.1 Voor vervoer moet vis vir 24 - 48 uur nie gevoer word nie.

2.2 Maak seker die toerusting is in goeie werkende toestand voordat vis vervoer word.

2.3 Hou ‘n ekstra bottel suurstof byderhand vir noodgevalle.

2.4 Laai die vis volgens die voorgestelde digtheid (1.0 - 1.2kg vis per 10 liter water)

2.5 Bepaal die suurstofvlak en kondisie van die vis nadat dit gelaai is en vóór vervoer. Maak seker dat die pype nie deur die watertenke gedruk word nie.

2.6 Hou altyd die weerstoestande in gedagte, veral in warm weer, want dan het die hele prosedure meer aandag nodig.

2.7 Kyk elke 20 minute gedurende vervoer of alles nog in orde is; let op na die suurstofvlak en water temperatuur. As die vis lusteloos lyk en voorkom asof dit lug soek (vis reik na die oppervlakte), moet ekstra suurstof gegee word. Die vis moet lewenslustig vertoon en gemaklik rondbeweeg.

2.8 Probeer die temperatuur en chemiese elemente tussen die vervoereenheid en die hok dieselfde hou deur 50% van die dam water oor ‘n tydperk van 10 - 15 minute by te voeg.

2.9 Wag omtrent 12 ure voor die eerste voeding.

2.10 Hou die vis deeglik dop vir 24 - 48 uur nadat dit in die dam gesit is.

2.11 Onthou dat vis op eie risiko vervoer word, tensy anders ooreengekom.

2.12 Die drywer moet die druk van die bande nagaan en baie versigtig bestuur. (1 tenk water met vis kan tot een ton weeg !)

2.13 ‘n PERMIT moet van die Natuurbewaring personeel verkry word 14 dae voor die beplande vervoer datum. Onderhandeling met die US kan help om die proses te bespoedig.

2.14 Vervoer-toerusting word deur die US voorsien.

3. VOEDING

Voeding is die belangrikste komponent in die produksie koste (tot 60%) en beïnvloed dus die inkomste. Hier is 'n paar riglyne om goeie voedingsbestuur te verseker.

3.1 Moenie meer as 4 - 6 weke se voer koop nie; stoor dit in 'n koel droë plek met goeie ventilasie; hou dit weg van mure om te verhoed dat klammigheid die kwaliteit van die voeding beïnvloed. Maak seker dat die stoorplek vry is van knaagdiere.

3.2 Kies die korrekte korrel-grootte en voersoort vir die groei-fase van die vis.

3.3 Volg die aanwysings in die voedingsprogram en **MOENIE OORVOED NIE**. (Voeding programme word deur die Oranje Visvoerprojek, US, voorsien.)

3.4 Hou by die korrekte voedingsmetodes byvoorbeed:- voer teen dieselfde tempo, dieselfde manier van verspreiding van die kos en voer 2 tot 3 keer per dag, min of meer dieselfde tye. Hou die visse dop en die opsigter moet sy diskresie gebruik volgens hoe die visse reageer.

3.5 Evalueer die voeromsettingsverhouding op 'n maandelikse basis - die optimale verhouding is tussen 1.4 en 1.6 d.w.s. 1.4 kg voer vir 1 kg massa toename

3.6 Hou elke dag dop hoeveel en hoe hulle eet en stel onmiddellik ondersoek in as hulle skielik minder begin eet as wat hulle normaalweg inneem.

3.7 Die byvoeging van karoteen moet betyds begin word; 8-10 weke voor bemarking of sodra die vis 50% van die verwagte bemarkingsgewig het (bv as jy die vis wil verkoop as hulle 600g weeg, moet jy karoteen insluit sodra hulle 300g weeg).

3.8 Wanneer die water troebel is veral na goeie reëns, is die voer moeilik sigbaar vir die vis. Voer moet stadiger gedien word en die aantal kere per dag verhoog. Staak die voer van vis vir die betrokke voersessie indien reaksie van vis traag is. Sodra vis beter reageer probeer om verlore sessies in te haal om die voerprogram op stryk te bring

4. GROEIFASE BEHEER

Die groeitempo van die vis moet gedurig gemonitor word regdeur die seisoen. Enige afwyking in verwagte groei is ‘n vroegtydige indikasie dat daar probleme is met betrekking tot die voeding, water-kwaliteit of siekte onder die visse. Hou ook in gedagte dat steurnis van mense of diere (bv voëls) ook kan bydrae tot slegte groei.

4.1 *Toerusting benodig*: skryfblok, trekskaal (50-100kg), drom (50-80 liter), weegraam en ‘n skepnet.

4.2 *Prosedure:*

a) Honger die vis uit vir 24 ure voor die toetsmonster geneem word.

b) Bring die vis bymekaar deur geleidelik die hok vlakker te maak, tot ‘n diepte van 1 tot 2 meter.

c) Verdeel die hok deur ‘n tou kruis te span onder die net.

d) Weeg en tel 10kg vis tien keer (omtrent 10% van totale vis per hok)

e) Hanteer die vis versigtig en voltooi die prosedure so gou as moontlik. Sak die nette en laat die res van die vis vry.

f) *Bepaal die volgende:*

1. Gemiddelde massa = totale massa deel deur die aantal vis geweeg
2. Totale gewig = gemiddelde massa x aantal vis in hokke.
3. Massa toename = huidige gewig - vorige gewig.
4. Voeding omsetting = voeding gebruik per maand deel deur die massa toename van al die vis.

‘n Voorbeeld van ‘n steekproef:

1. 100kg / 400 visse = 250g
2. 250g x 4 000 = 1 000kg (1 ton)
3. 250g - 150g = 100g
4. 600kg Voeding / 400kg massa-toename
   1.5 : 1 = (1.5kg voeding tot 1kg massa-toename)

4.3 Vergelyk die resultate met die verwagte gewig volgens die voedingsprogram en stel ondersoek in as daar enige verskil van meer as 5% is.

4.4 Vergelyk die voeding omsetting met die verwagte 1.4:1 tot 1.6:1 en ondersoek enige afwyking.

4.5 Evalueer die uiterlike kondisie/voorkoms van die vis gedurende hantering met die toets-monster en rapporteer enige opsigtiglike abnormaliteite soos verwering van die vinne, blindheid en velwonde.

4.6 Moenie **OORREAGEER** as enige abnormaliteite sigbaar is nie. Probeer die grootte van die probleem vasstel en kontak akwakultuur persone.

5. VISSIEKTES

Siektes is nie algemeen onder visse nie, maar die gesondheidstoestand van die visse moet gedurig dopgehou word. Let veral op die volgende:

5.1 Koop slegs vis van geregistreerde handelaars.

5.2 Neem die volgende algemene simptome in ag en rapporteer waarnemings

- voerinname neem af
- lusteloosheid, veral tydens voersessies
- meer visse wat doodgaan
- vel wonde en snye
- kiewe wat geswel is

Verwyder die dooie vis onmiddellik; dooie vis lê gewoonlik op die bodem vir ‘n paar dae voordat dit weer opkom. Wanneer die roetine visnet inspeksies gedoen word, moet die dooie vis verwyder word.

5.3 Maak seker dat die hokke beveilig word teen diere soos voëls en otters.

5.4 Vis wat gedurig gestres word, is meer vatbaar vir siektes.

5.5 **HANTEER VIS TE ALLE TYE VERSIGTIG.**

6. KWALITEITSKONTROLE

Kwaliteitsbeheer/kontrole is baie belangrik aangesien die mark sterk gekant is teen swak kwaliteit. Die kriteria vir kwaliteit is:

Visgrootte: minimum is 1kg (* ideaal 1.2 - 1.4kg

Uitslag %: skoongemaak met kop (* 85% van lewende massa)

Uitslag %: skoongemaak sonder kop (* 75% van lewende massa)

Vleiskleur: Roche skaal: (min 15; * ideaal 16 plus)

Uiterlike voorkoms: gesonde liggaamsvorm met geen vel wonde

Vleiskwaliteit: vermy inwendige bloeding en gapings in die vis

Smaak: swak watertoestande en sekere alge veroorsaak 'n slegte nasmaak.

Toets die kwaliteit eienskappe 4 tot 6 weke voor bemarking.

7. OES-PROSEDURE

Die korrekte oesmetode is baie belangrik vir kwaliteit vis. Die volgende riglyne moet gevolg word:

7.1 Honger vis uit vir 5 tot 7 dae voor oes sodat die maag leeg kan wees. Dit verhoed 'n slegte nasmaak en verbeter higiëne gedurende die proses.

7.2 Gradeer die vis in eweredige groottes voor die oes.

7.3 Oes so vroeg as moontlik in die oggend wanneer die temperatuur nog koel is.

7.4 Hou die vis koud - benede 16 ºC as dit binne een uur na die verwerkingsfabriek vervoer gaan word. Stoor die vis benede 5 ºC en bedek met lae dun ys as dit oor 'n langer tydperk afgelewer word.

7.5 Versmoor die vis in 'n plastiek houer of slaan dit oor die kop. Vermy onnodige stres en kneusing van die vis aangesien dit die kwaliteit van die vleis mag affekteer.

7.6 Lewer die korrekte hoeveelheid vis in skoon houers op die afgesproke tyd en datum.

Let wel: Dit is goeie bestuursprosedure om 'n klein hoeveelheid vis (50-80kg) vooraf na die verwerker te stuur. Hy moet dan besluit of die vleiskleur, smaak, grootte en algemene voorkoms goed is. Slegs dan behoort die res van die vis afgelewer te word.

8. HOKINSTANDHOUDING

Gereelde en deeglike instandhouding van die hokke speel 'n belangrike rol in die vermindering van bestuurskoste en verbetering van produksie doeltreffendheid. Doen die volgende gereeld:

8.1 Ondersoek die nette vir enige skade wat kan veroosaak dat die vis ontsnap. Doen dit op 'n weeklikse basis en ook wanneer die nette hanteer word gedurende die oestydperk en wanneer toetsmonsters geneem word.

8.2 Hou die nette skoon van enige alge wat die water sirkulasie beïnvloed deur dit uit te hang om droog te word of vee die alge af met 'n growwe besem.

9. OMGEWINGSIMPAK

Die akwakultuur sisteem werk in samewerking met die omgewing en daarvoor word boere aanbeveel om die impak daarvan op die omgewing gereeld te ondersoek.

Watermonsters behoort maandeliks geneem te word en dit met die vorige uitslae te vergelyk om te bepaal of die waterkwaliteit nadelig beïnvloed word. Dit sal help om die nodige voorkomingsstappe in die bestuursplan in te werk.

TABEL VIR DAAGLIKSE TAKE VIR SUKSESVOLLE VISBOERDERY IN DAMME.

DATUM	VOEDING	DOOIE VIS	WATER TEMP.	VIS GEDRAG	HOK NAGAAN	ANDER
1 2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15 16						
17						
18						
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25						

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Appendix 15 Procedural Manual for the UoS Small Farmer Aquaculture Programme

Procedural Manual

SMALL FARMER AQUACULTURE PROGRAMME

Procedural Manual for the Implementation of a Small Scale Aquaculture Project

compiled by

Division of Aquaculture, University of Stellenbosch

with support of

DFID Fish Genetics program, Project R 7284

”Improvement and Utilisation of Indigenous Tilapia Genetic Resources in Southern Africa”

June 2001

CONTENTS

1. Steps in the process

1.1 *Permission of the farm owner*

It is important that the potential entrepreneur obtain the permission of the owner to proceed with the implementation of the project on the farm. The owner has the right to negotiate a contractual settlement with the entrepreneur.

1.2 *Location of the farm*

In the assessment for site suitability, the proximity of the farm to the nearest town or metropole as well as to the sea would play a vital role in marketing, input provision, extension and other support services.

1.3 *Suitability of the dam for aquaculture*

A physical and chemical analysis of the water environment is necessary to determine the suitability for fish farming. Other aspects to look for are the number of water plants present, any other organisms living in the dam, the source which supplies water, depth of the dam maintained throughout the year, how often it replaces itself in volume, the size of the dam, the water temperature over winter and summer months, the length of the cold and warm seasons and accessibility for the transport of fish and suitability for cage construction.

1.4 *Selection of an entrepreneur/s*

The entrepreneur/s must show initiative to manage an aquaculture venture successfully. The criteria for selection will differ on each farm dependent on the institutional and socio-economic conditions and the prevailing farm management culture. It is the prerogative of the farm owner or manager to select an entrepreneur or to allow the employees to decide amongst themselves whom they regard as most capable of running such a project. Only a limited number of persons (preferably 1-3) can be accommodated in the initial stage of the project. If the farm has a worker's committee they can take the initiative to run the project in the interest of the community. It would be their responsibility to mobilise the participants to manage the project properly. Other potential entrepreneur/s could be pensioners, retired or disabled persons. The project can also be targeted at the women folk on the farms who are generally unemployed or seasonal employed.

1.5 *Marketing of the product*

The marketing of the tilapia, carp or fresh water mullet should be directed at the informal sector (i.e. low income consumers) on the farm and the surrounding areas. The price of the product should be competitive with poultry and marine fish. The tilapia has an advantage over marine fish in that it can be harvested and supplied fresh to the consumer whereas the marine fish is sold as frozen products in the rural areas. The production cost of trout is higher than tilapia and is aimed at up-market consumers. Tilapia's initial function would be to provide an alternative protein source to supplement the diet of the rural populace. With establishing networks, other markets can be supplied and tus increasing turnover generated by tilapia sales. The turnover of the trout production would spell a considerable profit that will benefit the entrepreneur financially. The bulk of the net farm income would thus mainly be generated by trout production initially.

1.6 *Construction of the cage structure*

As soon as the dam has been identified as suitable the building of the float can be started. After the float has been stabilised on the dam, the cages can be suspended on the frame. The standard measurement of the cages is 10 x 10 x 5m with a maximum production capacity of four tons. The nets are factory-made.

1.7 *Training of the prospective fish farmer*

With training, the skills and the educational background of the entrepreneur must always be kept in mind. The success of the aquaculture initiative rides on the application of the learned skills to ensure that management proceeds smoothly. There are certain times of production that requires more attention than other especially when environmental conditions suddenly change that can effect the fish adversely. The rest of the time would be routine work such as the daily feeding, sampling and monitoring the behaviour patterns of the fish. Support services will be provided.

1.8 *Introduction of the fingerlings*

The objective is to farm on a rotational basis with warmer water species e.g. tilapia *(Oreochromis mossambicus*), carp or mullet over the summer months and colder water species e.g. trout (*Oncorhynchus mykiss*) over the winter months. The tilapia fingerlings should be introduced into the cage in November/December and trout in April/May. The preferred mass for the fingerlings should be 5g - 10g for tilapia and 200 - 300g for trout. The number of fingerlings will be determined by the number and size of cages the dam can accommodate. At least one month without production should be allowed between the seasons to maintain a resting period and to increase the water quality for the next production phase.

1.9 *Growth period of the fingerlings to marketable size*

With the correct management and application of technology as derived from the University of Stellenbosch (US) and the Department of Agriculture (DoA), the tilapia should grow by an average size of 50-80 grams per month. Trout should grow at about 150g per month (200g April - 1.3kg November). The time allocated should be enough to ensure that the entrepreneur gets his fish to a marketable size. Certain management practises have to be applied during this period e.g. following a feed program, test sampling, checking the water quality on a regular bases, monitor the fish for any diseases, removing dead fish, etc.

1.10 *Finance*

Different financial schemes can be used for operation in concurrence with the dynamics prevalent on the farm. The US will assist the process.

2. Procedures and Responsibilities

Before anything is initiated, the farm owner should be approached by his employees to get ***permission*** to establish a Small-Farmer Aquaculture Project on his farm. After his permission has been obtained, the University of Stellenbosch will send an aquaculture specialist to ***inspect*** the dam and determine whether it is suitable for production. A detailed report containing the physical and chemical results of the water quality will be compiled and presented to the project co-ordinator. If the dam meets the necessary criteria, implementation can begin.

The next step is to ***select*** the entrepreneur/s. The US will arrange a meeting with the identified parties to discuss the project format and discuss the advantages and disadvantages. Informal questions will be asked to establish the entrepreneurial potential and the level of ambition and enthusiasm of the potential entrepreneur. If there are more than three persons interested, all of them will undergo the evaluation process and the eventual entrepreneur/s will be selected by a process of evaluation. If a suitable entrepreneur cannot be found then the responsible parties can look at the other options of selecting an entrepreneur.

The necessary funding should be sourced for the entrepreneur/s to undergo the required ***training*** to manage an aquaculture enterprise. The training will be provided by the US and DoA. The US operates an Aquaculture Research Unit where production of trout farming is maintained throughout the year. Research is being done on all the phases of production from artificial fertilisation of the eggs to the processing of the market size fish. Other research fields include genetic selection, feeding, etc. The DoA has a tunnel system at Elsenburg specifically for research on tilapia and is busy developing technology which is specifically suited for the implementation by the small farmer. The entrepreneur/s will spend at least one week at each institution to undergo intensive practical and theoretical training.

The building of the ***floating cage system*** will be assisted by the technicians at Elsenburg. It will take place on the farm preferably by the entrepreneur/s and other voluntary workers. Since most of the materials required for the system may be available on the farm, the entrepreneur is encouraged to use these materials thus effecting greater cost saving and decreasing the production cost of the system substantially. The materials referred to are drums for buoyancy and wooden poles for the walkway. The rest of the material such as the net, angle iron, rope and bolts most probably have to be purchased. As soon as the floating structure is complete, the nets can be fixed to the structure. There is also the option of buying a premanufactued production system from the Agricultural Research Council.

The ***trout fingerlings*** can be obtained from the cage production system at Kleinplaas, Jonkershoek (US). The ***tilapia fingerlings*** will be supplied by the warm water tunnel system at Elsenburg. The transport for the fish must be arranged by the entrepreneur. If the farm owner cannot make his truck available or does not possess one, alternative transport can be hired with the expense falling under the direct overhead expenses.

The routine ***feeding*** of the fish to get it to market size plays a very important role in the production. Up to 60% of the production cost goes into feeding and therefore great emphasis will be placed on this aspect in the training of the entrepreneur. Feed is supplied by WPK, Aquafeeds, Malmesbury. This company functions in collaboration with US who appointed a nutritionist who does continuous research on fish feed to improve the quality. This ensures that the fish farmer receives the best feed at all time. The company delivers the feed to the farm or the entrepreneur can fetch it at their warehouse. If the company delivers it, the transport cost is extra to the unit price and will depend on the distance covered.
For the first two trout ***harvests*** the market will be prearranged by the co-ordinators. The entrepreneur will be responsible for marketing the following season. This will create maturity in yearling with market related issues and develop trading skills. The tilapia is the sole

responsibility of the entrepreneur from the first harvest onwards. The co-ordinators will continue to do market research to assist the entrepreneur.

The ***finance*** to purchase the necessary infrastructure and cover operating costs is carried by the entrepreneur. The University of Stellenbosch will assist the entrepreneur in sourcing the necessary finance. Different equity arrangement for projects can be negotiated with US.

3. Contact Details

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Appendix 16 Presentations made by Project Associates.

Brink, D., 2001. An overview of aquaculture research and development in South Africa. Proceedings: Conference of Animal Production Association of Southern Africa, Pretoria, South Africa. October 2001.

Brink, D., 2001. The development of sustainable tilapia farming systems in Southern Africa. Aquaculture Workshop, Cape Town, South Africa. 20th September 2001.

Brink, D., Hoffman, L., Mair, G.C. (2000) Fish Genetics & Aquaculture Research Programme Workshop (DFID) Hanoi, Vietnam, Nov. 7-12, 2000. Project presentation and Discussions plus working group sessions.

Brink, D. and Lewis, R.I. (2001) Genetic improvement in candidate species for small scale fish farming in Southern Africa, with reference to rainbow trout (Oncorhynhus mykiss) and tilapia (Oreochromis mossambicus). Workshop on "Kleinboeren in Aquacultuur" (Small Farmers in Aquaculture): Flanders/RSA collaborative programme, 30th May 2001, Katholic University of Leuven, Belgium.

D'Amato, E (2002) Sixth Conference of the Aquaculture Association of Southern Africa. (AASA). University of Stellenbosch, South Africa. September 11-12, 2002. Assessment of the genetic status of broodstock and genetic management practices through the use of molecular markers.

De Wet, L.F. and Brink, D. (2001) Nutritional Improvements in fish feeds for Small Farmer Aquaculture in Southern Africa. [Workshop on "Kleinboeren in Aquacultuur" (Small Farmers in Aquaculture): Flanders/RSA collaborative programme, 30th May 2001, Katholic University of Leuven, Belgium.

Du Plessis, M. and Wallecan, S. (2000) The influence of temperature on sex differentiation on the Mozambique tilapia, O. mossambicus. "Aquaculture Africa" Fifth Conference of the Aquaculture Association of Southern Africa, 24 to 28th September 2000, Pretoria, R.S.A.

Esterhuyse, M. (2002) Workshop on Sustainable Development of Indigenous Resources, for Aquaculture Development in the Southern African Region. (DFID – UoS) University of Stellenbosch, South Africa. September 10, 2002. The use of molecular markers to assess the genetic status of tilapia populations in terms of diversity and interspecies hybridisation.

Esterhuyse, M. (2002) Conference of the Aquaculture Association of Southern Africa (AASA). University of Stellenbosch, South Africa. September 11-12, 2002. The use of molecular markers to assess the genetic status of tilapia populations in terms of diversity and interspecies hybridisation

Hall, E. (2002) Genetic Workshop on Sustainable Development of Indigenous Resources, for Aquaculture Development in the Southern African Region. (DFID – UoS) University of Stellenbosch, South Africa. September 10, 2002. Structure and Diversity in Twelve Populations of Mozambique Tilapia, *Oreochromis mossambicus*.

Hall, E. (2002) . Conference of the Aquaculture Association of Southern Africa (AASA). University of Stellenbosch, South Africa. September 11-12, 2002

Microsatellite DNA: a valuable tool in the sustainable use of the Mozambique tilapia genetic resource.

Hartnell, R. and Brink, D. (2001). Workshop on the Small Scale Aquaculture Program. Elsenburg Agricultural Institute, Stellenbosch, South Africa. 26-27th July 2001.

Lewis, R.I., Hall, E.G., Marais, P.S., Brink, D., Hoffman, L.C. Mair, G.C. and Beardmore, J.A. (2000) The Conflict Between the Conservation of Genetic Resources and Exploitation: An Example from African Populations of Oreochromis mossambicus (Peters). The 7th IAGA meeting (International Association of Genetics in Aquaculture), Townsville, Queensland, Australia, July 2000.

Lewis, R.I. (2000) The utilization of Molecular Markers in Aquaculture. "Aquaculture Africa" Fifth Conference of the Aquaculture Association of Southern Africa, 24 to 28th September 2000, Pretoria, R.S.A.

Mair, G.C. (2000) International Conference on Aquaculture in the Third Millenium (NACA and FAO), Bangkok, Thailand, Feb. 20-25, 2000. Panel Member on Genetics in Aquaculture

Mair, G.C. (2000) The Fifth International Symposium on Tilapia in Aquaculture (ISTA), Rio de Janeiro, Brazil, Sept. 3-8, 2000. Selective improvement of Genetically Male Tilapia.

Mair, G.C. (2000) Aquaculture Africa 2000 (AASA), Pretoria, South Africa Sept 26-29, 2000. Review of Tilapia Genetics.

Mair, G.C., King, P and Swart, J. (2001) Aquaculture (2001) World Aquaculture Symposium, Orlando, Florida, U.S.A, Jan. 21-25, 2001. Review of Tilapia Genetics (GCM)

Mair, G.C. (2001) Sixth Steering Committee Meeting of the International Network on Genetics in Aquaculture (INGA - ICLARM), Hanoi Vietnam, 8-10 May, 2001. Oral presentations on GMT technology plus facilitation of workshop session on dissemination of improved breeds.

Mair, G.C. (2001) Tilapia 2001 (INFOFISH, ICLARM, FAO, Ministry of Agriculture, Malaysia), Kuala Lumpur, Malaysia. May 28-30, 2001. Invited presentation on review of tilapia genetics

Mair, G.C. (2001) Sixth Symposium on Central American Aquaculture (Andah, GAA, Zamorano and PD/A CRSP). Tegucigalpa, Honduras 22-24 August, 2001. Invited review paper on tilapia genetics.

Mair, G.C. (2001). ASEAN-SEAFDEC Conference on Sustainable Fisheries for Food Security in the New Millennium "Fish For the People" Bangkok, Thailand. 19-24 November, 2001. Panel member Sustainable Aquaculture – Supply of Good Quality Seeds. (GCM, presenter and panel member)

Mair, G.C. (2002) Tilapia Forum 2002, Los Banos, Philippines. (PCMARD, SEAFDEC & BFAR). Feb 25-27, 2002. Genetics and Breeding in Tilapia.

Mair, G.C. (2002) International Technical and Trade Symposium on Tilapia (CAPPMA), Haikou, Hainan, China. April 18-20, 2002. Invited review paper on tilapia genetics

Mair, G.C. (2002) Expert Consultation on Strategies/Plans for Dissemination of Improved Fish Breeds (ICLARM-INGA). Bangkok, Thailand. June 4-7, 2002. Dissemination of Genetically Male Tilapia: Experiences from the Philippines. Also facilitation of working groups sessions on strategies for dissemination of improved breeds.

Mair, G.C. (2002) Workshop on Sustainable Development of Indigenous Resources, for Aquaculture Development in the Southern African Region. (DFID – UoS) University of Stellenbosch, South Africa. September 10, 2002. Issues in dissemination of domesticated and improved fish breeds.

Mair, G.C. Sixth Conference of the Aquaculture Association of Southern Africa. (AASA. University of Stellenbosch, South Africa. September 11-12, 2002. 1. Tilapia Aquaculture in Asia and 2. Tilapia Genetics Update including a case study of GMT in the Philippines.

Salie, K and Brink, D., 2001. Development through small scale fish farming enterprises in South Africa: Proposing a model for entrepreneurial growth. World Aquaculture Conference, Orlando, USA, 21-25 January, 2001.

Salie, K. (2002) Conference of the Aquaculture Association of Southern Africa (AASA). University of Stellenbosch, South Africa. September 11-12, 2002. Small-scale fish farming in the Western Cape Province, South Africa: An analysis of the socio-economic impact of the programme.

Posters

Esterhuyse, E., Lewis, R.I., Brink, D., Van der Waal, B. Investigation of Hybridisation in two tilapia Species using Molecular Genetic Markers. "Aquaculture Africa" Fifth Conference of the Aquaculture Association of Southern Africa, 24 to 28th September 2000, Pretoria, R.S.A.

Hall, E.G. Lewis, R.I. Marais, P.S. An Assessment of DNA Microsatellite Variation in Brood - Stock Groups of Mozambique Tilapia, *Oreochromis mossambicus*. "Aquaculture Africa" Fifth Conference of the Aquaculture Association of Southern Africa, 24 to 28th September 2000, Pretoria, R.S.A.

Lambrechts, D., Lewis, R.I., Hall. E.G.. The use of Molecular Genetic Markers for the Verification of Andro- and Gynogenesis Procedures during the development of YY-males in the Tilapia, *Oreochromis mossambicus*. "Aquaculture Africa" Fifth Conference of the Aquaculture Association of Southern Africa, 24 to 28th September 2000, Pretoria, R.S.A.

Salie, K. An extensive fish rearing system in the Northern Cape Province, South Africa: Aquaculture to rural communities. "Aquaculture 2001" International Conference and Exposition. January 21-25th, Orlando, Florida, USA.

Salie, K. and Brink, D. (2001) Impact of small scale fish farming on the water quality of irrigation dams in the Western Cape of South Africa, with reference to integrated water use. Workshop on "Kleinboeren in Aquacultuur" (Small Farmers in Aquaculture): Flanders/RSA collaborative programme, 30th May 2001, Katholic University of Leuven, Belgium.

Salie, K., Brink, D. and Hoffman, L.C. (2001) Small-scale rainbow trout producing cage systems in the Western Cape Region of South Africa. "Aquaculture 2001"

International Conference and Exposition. January 21-25th, Orlando, Florida, USA.

Salie, K and R. Hartnell. (2002) Small-scale fish farming in the Western Cape Province, South Africa: An analysis of the socio-economic impacts. World Aquaculture Conference, Beijing, 2002.

Salie, K. and Oberholster, K. (2001) The long-term sustainability of small scale aquaculture systems in the Western Cape of South Africa. Workshop on "Kleinboeren in Aquacultuur" (Small Farmers in Aquaculture): Flanders/RSA collaborative programme, 30th May 2001, Katholic University of Leuven, Belgium.

Salie, K. and Oberholster, K. (2001) An investigation into the long-term sustainability of small-scale fish farming in the Western Cape Province, South Africa. "Aquaculture 2001" International Conference and Exposition. January 21-25th, Orlando, Florida, USA.

Salie, K. and H. Wiggins. (2001) Development through small-scale fish farming enterprises in South Africa: Proposing a model for entrepreneurial growth. "Aquaculture 2001" International Conference and Exposition. January 21-25th, Orlando, Florida, USA.

In addition:

- Workshop on Sustainable Small Scale Aquaculture Systems, University of Leuven, Belgium (29st-30th May 2000: RIL, DB, Khalid Salie (KS) and Lourens de Wet (LFDW) of the UoS attended the workshop and made presentation on aspects of the SFDP, related to ecology, genetics and nutrition.

- VSO Project Asia Training Mission, 1st to 31st July 2000, AIT, Bangkok, Thailand: RH participated in a technical training programme on aquaculture development and small scale farming systems in Asia, arranged by GCM, prior to his arrival in South Africa on a two year VSO assignment with the Dept. Agriculture: Western Cape, South Africa.

- The 7th IAGA (International Association of Genetics in Aquaculture) meeting in Townsville, Queensland, Australia in July 2000: Was attended by JAB, GCM and RIL during which seven scientific contribution were presented by the group although only two related directly to the project.

- Aquaculture Africa, Fifth Conference of the Aquaculture Association of Southern Africa, 26th to 29th September 2000, Pretoria, South Africa. The conference was attended by RIL, DB, RH, KS, MdP, and four additional members of staff from the UoS, associated with the DFID-FGP, whilst a total of eight scientific contributions were presented. GCM also delivered an invited keynote address at the conference reviewing tilapia genetics. Edward Hall won best poster award for his poster resentation on the molecular characterisation of *O. mossambicus*, carried out under the project. A total of four presentations were made by project associates and relating to the project.

- The DFID FGRP-3 and ARP Workshop in Hanoi, Vietnam, from 10th to the 13th October 2000 was attended by DB, LCH, RH, and RIL During the workshop data was presented with regard to project R7284, in the context of the DFID Sustainable Livelihoods approach. DB, LCH, and RIL subsequently visited rural aquaculture sites in Vietnam and Thailand, hosted by the Vietnam RIA#1 aquaculture research institute, and by GCM/AIT in Thailand.

- DFID - Southern Africa Workshop on Sustainable Livelihoods Approach Training, Pretoria, 20-22 February 2001: The workshop was attended by RH and KS as representatives of the DFID-FGP in view of the contribution to rural development as one of the objectives of the FGP. The objectives of the workshop were (a) to establish an understanding of Sustainable Livelihoods Approach (SLA), and (b) to apply the SLA in development programmes and projects. The participants believe SLA provides a framework to be used in the Small-scale Aquaculture Projects and ultimately endeavor to achieve the goal of facilitating sustainable livelihoods to the people where it is needed most.

- “Aquaculture 2001, International Triennial Conference and Exposition" in Orlando, Florida, North America from the 21st to the 25th of January 2001. The conference was attended by KS of the UoS during which he presented one paper and three posters on matters related SFDP. The meeting was also attended by Paul King and Jakob Swart of the Western Cape Dept. of Agriculture, providing further indication of their renewed interest in the potential for aquaculture as a component of agricultural development in the region. The meeting was also attended by Graham Mair, John Beardmore and Eric Roderick from UWS, with the former giving a review presentation on tilapia genetics during a producers session, which included details of the project.