Behavioural, Reproductive and Growth Studies on *Oreochromis mossambicus* (Peters 1852)

BY

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THESIS ABSTRACT

A major obstacle facing the successful creation of an African aquaculture industry, based upon Oreochromids, is the irregular supply of good quality fish seed. There are several causative biological processes behind its irregular supply. The aim of this research was therefore to determine the basic requirements for the establishment and maintenance of a small breeding facility, for *O. mossambicus*. The goal was to make a unit that was simple and which could be easily replicated in rural, satellite aquaculture seed stations. The results obtained illustrate that a small reproduction unit can produce large quantities of healthy 90-day fry.

Asynchronous hatching of the eggs and spawning asynchrony in female *Oreochromis mossambicus* are two elements which negatively affect uniformity in the fry produced. Typical fish seed production uses large ponds partitioned into breeding allotments or a series of breeding pools. While the earthen ponds provide a substrate in which a nest can be excavated, its presence is not required for mating success in the closely related *O. niloticus* (Linneaus 1758). Female mate choice, as well as apparent fecundity, according to nest size has been clearly recorded in related cichlids but no investigations have been made as to nest size and spawning synchrony in *O. mossambicus*. The main focus of this investigation was to ascertain whether *O. mossambicus* would accept artificial nest substitutes in preference to their own constructed ones and secondly, whether different alternatives would elicit different levels of acceptance. The observed results indicate a ready acceptance for artificial nest alternatives, with nest lip height being prioritised by the fish . The implications thereof are discussed in relation to the potential for optimization of breeding arenas for *O. mossambicus* by the provision of artificial nests whose dimensions satisfy both male and female preferences.

In established communities, *Oreochromis mossambicus* display various complex and ritualised behaviours during stable and disruptive events. The aim of this research was primarily to produce a glossary of behaviours defining these interactions, particularly with reference to male-male behaviours. Three males and six females were allowed to acclimatise over one month, with various social groupings being established within the first few days. Results from this study illustrated not only a dynamic social structure, signaled via various chemosensory and visual methods, but also supported recent findings in apparent male-male courtship and the underlying

causes. Furthermore, the observed male-male activity of the nestholder malesfirmly corroborate the current practice in aquaculture whereby only one male is allocated per breeding arena.

The use of artificial incubation of *Oreochromis spp*. eggs has become widespread in high intensity fish seed production. Various types of incubator exist, and their selection is dependent on the specific attributes of the egg to be incubated. Currently available incubators are typically of a funnel (up-flow) or round bottomed (down-flow) design. Neither permits easy access to the eggs, which is particularly important when dealing with poor quality water as is typically found in rural areas. The aim of this study was to devise and test an easy-to-use incubator, applicable to rural seed production projects, which offers advantages over currently available incubator types. The final design, WETNURSE Type II, offered improved hatching rates over Type I, with a mean hatching success of 75%. While falling short of the desired 80% success rate (Rana 1986), the various other benefits provided by the design justify further optimization and testing.

Three distinct populations of *O. mossambicus*, representing populations of inbred, randomly mated and genetically unknown (wild-caught) pedigree were analysed according to their food conversion efficiency (FCE). The intra- and inter-sample crosses were done with single males in order to produce half-sib progeny batches which allowed for the assessment of sire influences on the FCE of the progeny batches. The results show that the population of unknown pedigree is comparable to that of the randomly mating population, indicating the presence of sufficient genetic variation to permit further selection; the genetic contribution of the males to their respective progeny was insignificant in relation to that made by the female.

DECLARATION

The experimental work described in this thesis was carried out in the School of Biochemistry, Genetics, Microbiology and Plant Pathology, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Prof. Mark Laing and the co-supervision of Dr. Annabel Fossey.

These studies represent original work by the author and have not otherwise been submitted in any other form to another university. Where use has been made of the work of others, it is duly acknowledged in the text.

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FOREWORD

All research presented in this thesis was conducted at the Animal House, Department of Zoology and Botany, University of KwaZulu-Natal, Pietermaritzburg, South Africa. The work presented is the culmination of seven years of research which started as an honours seminar and developed into a Master's thesis. All of the laboratory work was conducted in the first three years.

As an avid proponent for the adoption of a national aquaculture industry in South Africa based on *Oreochromis spp*., the main focus of the research was to target the well-documented problems regarding *Oreochromis spp*. seed production. Behavioural observations were to be used to identify areas which may be artificially manipulated to improve breeding synchrony. These observations would also be used to produce a glossary of behavioural actions related to reproduction which would facilitate subsequent quantification.

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CONTENTS

THESIS INTRODUCTION

Aquaculture in South Africa has been regularly proposed as an option for inclusion in variable intensity agriculture systems. This is in part due to its small requisite ‘footprint’ per unit protein produced (land, water, high rearing densities, and culturing process demands), as well as the presence of a suitable indigenous aquatic species, *Oreochromis mossambicus,* which competes favourably with globally cultured species, particularly as regards food conversion efficiency (FCE).

Two factors were identified as potential stumbling blocks toward the successful adoption of aquaculture by rural South Africa. The primary obstacle was identified as aquaculture novelty or the non-recognition of the industry. This novelty has been proposed to be due to a lack of historical contact, which is particularly endemic to South Africa owing to our lack of a lacustrine culture. This situation has ensured that imported ideas regarding the industry have remained rurally exclusive.

A further, well documented, problem which affects the African aquaculture industry in general, is the irregular and unreliable supply of the requisite fish fry needed for the maintenance of the industry.

The Pietermaritzburg campus of the University of KwaZulu-Natal had no existing Ichthyology Department or established aquaculture facility. The onus for establishing the facility including research and the reviewing of relevant literature on aquaculture facilities, to purchase or obtain suitable equipment, to set up the equipment, to maintain and evaluate the necessary systems and processes, as well as to completely design and execute the studies contained within was borne by the author. This was done with little technical assistance from the University.

At the onset of this research, the focus was primarily the quantification and comparison of the feed conversion efficiency between inbred and outcrossed populations of *O. mossambicus,* as well as inter-species comparisons, compared with the FCE values reported for *O. niloticus*. The study evolved over time, owing to substantial obstacles and a subsequent literature research, into a review and testing of various systems - proven in *O. niloticus* and *O.*

mossambicus - as well as the proposal and testing of novel enhancements by which the production of *O. mossambicus* fry could be achieved with minimal infrastructure.

Chapter 1 deals with a review of available literature concerning the global importance of *Oreochromis spp.*; the problems of aquaculture in Africa, including South Africa; the positive and negative attributes of *O. mossambicus* with respect to an aquaculture product in an aquaculture-unaware society and systems by which to circumvent these problems; the various genetic methods by which to enhance productivity, developed chiefly with *O. niloticus*, via breeding schemes; and the potential of *O. mossambicus* within rural outreach and food security schemes.

Chapter 2 covers the practical aspect of this MSc thesis, dealing with the setup and maintenance of a small breeding facility of *O. mossambicus*, including all requisite regimens and sampling protocols.

Chapter 3 is the first of three consecutive chapters dealing with strategies by which to facilitate the regular and synchronous breeding of *O. mossambicus* under basic conditions. Problems existed with the shared filtration and the colonization of the aquaria by a resistant biomat. A related issue was whether the bottom filter gravel substrates would impact on filtration efficiency, led to the inception of trials to test whether *O. mossambicus* would accept nest substitutes, thereby not only eliminating the substrate but also providing pliable arenas for observation.

In Chapter 4, research was conducted to test the current commercial techniques by which *Oreochromis spp*. seed production is achieved, in particular, focusing on the impact of multiple males on desirable social stability, and the effect of subsequent introductions of new males of different sizes.

The research covered in Chapter 5 deals with the engineering, testing and final patent design of a novel rotary, drum-based incubator for *O. mossambicus* eggs. Very poor fry survival was experienced during community establishment. Poor water quality, in particular, the bacterial and fungal inoculum levels, was deemed to be the primary causal factor. The use of available incubator types did not improve hatching success, so a new type of incubator was envisaged whereby the eggs would be immediately accessible for medication or removal. Two generations of prototypes were developed and tested. Further benefits of the design are also discussed.

Research in Chapter 6 deals with the quantification and analysis of the Food Conversion Efficiency (FCE) of three populations *of O. mossambicus,* and the genetic status of the wild population sampled. These local results are compared against reported FCE figures for *O. mossambicus* and *O. niloticus,* and a breeding program to select for enhanced growth rates is proposed.

CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION

Aquaculture is defined by the United Nations Food and Agriculture Organization (FAO), as the "farming of aquatic (freshwater, saltwater or brackish) organisms including fish, mollusks, crustaceans and aquatic plants. The term, "farming", implies some level of control in the establishment and/or rearing of the particular organism to maximise production, such as regular stocking, feeding and protection from predators. Farming also implies individual or corporate ownership of the stock being cultivated…" (FAO 2002).

With the rapid growth in the global aquaculture industry and resultant diversification at the end of the last century, an ambiguous subdivision as regards aquatic type has seen the term "aquaculture", while still referring to the subset of agriculture, being quoted as having a freshwater focus while the term "mariculture" refers to production systems in salt and brackish water (FAO 2002). This ambiguity has been recently clarified by the use of 'inland' and 'marine' aquaculture terms. The latter is referred to as "mariculture" and is the delineation seemingly adopted by the FAO in its biannual SOFIA report (the State Of World Fisheries and Aquaculture) (FAO 2004, FAO 2006).

Furthermore, it is important to note the differences between culturing the organisms as opposed to simply harvesting the aquatic organisms by various means. This is referred to as capture fisheries or artisan fishery (Diegues 2002) and does not entail any culture of the organisms harvested.

1.2 GLOBAL IMPORTANCE OF AQUACULTURE

The SOFIA report describes present trends in all fishery sectors, including production amounts, economic value, as well as a host of socio-economic components in a per nation format. The establishment of this report reflects the expansion of aquaculture within the last three decades (Naylor *et al.* 2000, Hardjamulia *et al.* 2001, Beveridge and Little 2002, Changadeya *et al*. 2003, White *et al.* 2004).

Reported aquaculture figures in the first half of the 20th Century were typified by low volume, high value product such as ornamental fish, sport fishing as well as jewelry production based on freshwater and saltwater pearls (Landman *et al.* 2001). Consequently the global figures for aquaculture from the mid-century mark, in terms of tonnage reared (Figure 1.1), was dwarfed by the capture fishery industry. Aquaculture production stagnated, relative to capture harvests, during the 1960's and 1970's due to the introduction of new techniques and technologies which boosted global capture fishing hauls (White *et al.* 2004). Toward the end of the 1970's, concerns regarding the depletion of marine and inland stocks, as well as the refinement of recirculating systems and the proven successes of various culturing schemes, particularly salmon culturing in Norway, began to drive investment into sustainable practices with the resultant increase in production (Singh - Renton 2002). This increase in aquaculture has largely been evident in East Asia where 26% of the animal protein is derived from fish alone, while globally 1 billion people (16% of the global population, United Nations Population Division 1999) rely primarily on fish as their sole protein source (FAO 2002). This importance is also reflected economically. The global trade in aquaculture products exceeded US$ 50 billion in 2000, employed over 36 million people directly, while a further 160 million people obtained indirect income via various aquaculture related avenues (Garcia and Newton 1997, FAO 2002).

From 1970 to 2005 aquaculture production of food (products for direct human consumption only) increased from less than a million tonnes to approximately 50 million tonnes. This increase equates to an increase of 8.8% per annum. The increase supersedes all other food production sectors. In contrast, capture fisheries peaked at approximately 90 million tonnes since the turn of the century, excluding anchoveta (*Engraulis ringens,* Jenyns 1842) catches that are severely affected by *el Niño* years (FAO 2006). Livestock production, while still increasing at roughly 3% p.a. has mitigating circumstances due to the significant attitude conversion of cattle owners in Africa. Traditional draught utilisation of cattle and their cultural indication of wealth is being replaced by their direct use in meat production(Naylor *et al.* 2005).

Total world fisheries, which includes culture as well as catch industries, increased by only 7% from 2000 to 2004, compared with the 9% increase in aquatic food consumption over the same time frame. This shortfall was absorbed by the markedly slower increase in fisheries product for non-food uses. As consumption increases, however, this ‘cushion’ will be quickly depleted and future demand can only be relieved by aquaculture production, which increased by 28% during the same period. During this time frame, capture fisheries and aquaculture industries together employed 41 million people worldwide who produced approximately 106 million tonnes of food fish in 2004, providing a per capita complement of 16,6 kg p.a., and a record export value of US$ 71.5 billion. The aquaculture sector alone provided 45.5 million tonnes, or 43%. This total food fish amount supplied twenty percent of the average per capita protein intake for 2,6 billion people, and this is an underestimate due to the paucity of records in terms of subsistence fishing practices (FAO 2006).

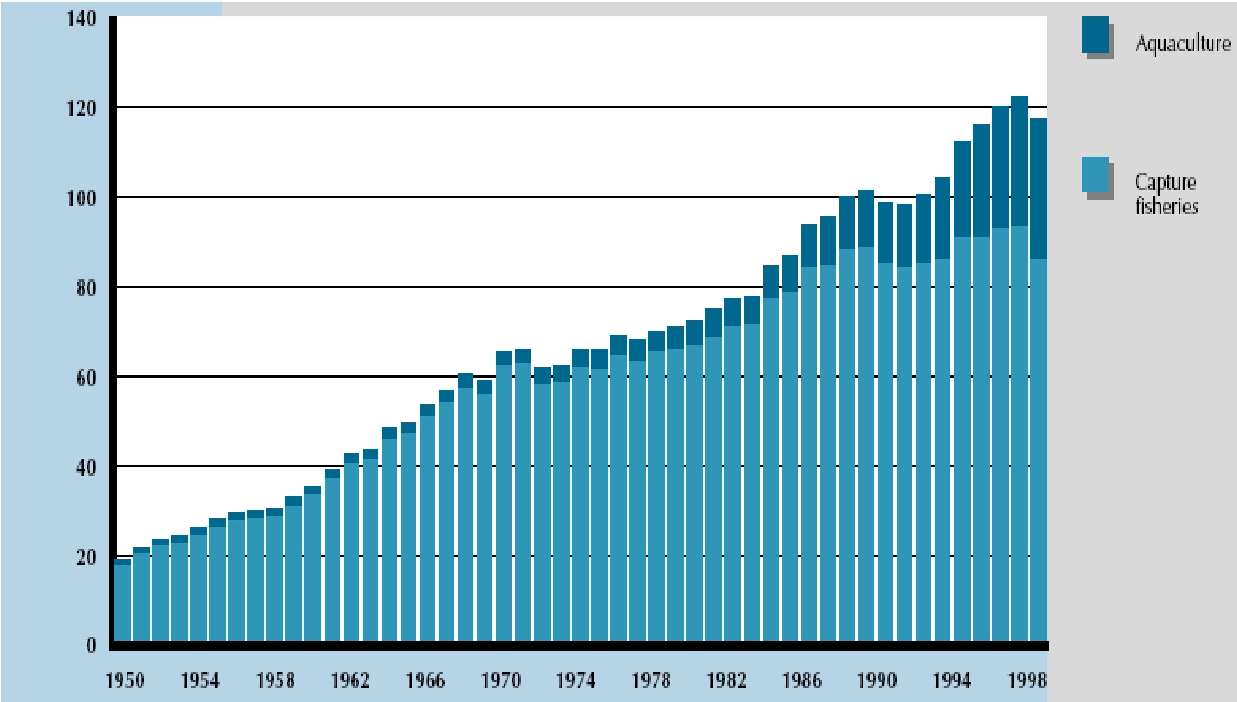


Figure 1.1: World capture fisheries and aquaculture production, 1950 - 1998 (Taken from FAO-SOFIA 2000).

Table 1.1: Overview of the relative importance of aquaculture and capture industries in 2004. Taken from FAO (2006).

2004	Tonnage (millions)	Export value (billion U.S.$)	Employment (millions)	Top species (tonnage + value)
Capture Fisheries	95	84.9	30	Anchoveta
Aquaculture*	45.5	63.3	10	Carp

* Excluding aquatic plants

Table 1.2: Regional breakdown of aquaculture production for 2004. Taken from FAO (2006).

Region	**Production Volume Percentage**	**Production Value Percentage**
China	67.3	51.2
Rest of Asia-Pacific	22.3	29.3
Europe	4.8	8.62
Americas	4.2	9.33
Near East and Africa	1.4	1.55
World Total	100	100

It is interesting to note that of the developing nations, which as a group, have shown robust growth in aquaculture employment, Africa as a continent lags behind in aquaculture industries, despite maintaining a stable quarter share in global inland capture fisheries product, which implies a substantial nutritional and economic dependence on freshwater species. Furthermore, the top global species group caught, within the inland capture fisheries sector in 2004, as well as being ranked eighth in the global aquaculture species caught (by weight) was the grouping of tilapia and other cichlids, which is dominated by indigenous African fish species of the *Oreochromis* genus (FAO 2004, 2006).

1.3 AQUACULTURE IN AFRICA

SOFIA statistics (taken from FISHSTAT PLUS, available at: http://www.fao.org/fishery/statistics/software/fishstat/en) for 2004 show aquaculture production to be minimal within the African continent. Africa supplied 1.02% of the global weight in aquaculture product and 1.55% of the global aquaculture value produced (FAO 2006). While these figures, particularly of tonnage produced (Table 1.3), is indicative rather than exact, due to the complete absence of census data in many cases, the trend implied is one of below potential industry production when compared against other developing nations. While the species listed under the African countries are ‘typical’ species farmed globally, the production quantities are insignificant. The main causes for this were identified by Changadeya *et al.* (2003) to be industry novelty and the prevalence of low input, culture based systems.

Aquaculture novelty in Africa

Aquaculture’s rapid introduction into the global market was spearheaded by agencies such as USAID (United States Agency for International Development), ICLARM (International Center for Living Aquatic Resources Management, now the World Fish Centre) and CGIAR (Consultative Group on International Agricultural Research). These agencies were established to disseminate research and development findings on agricultural practices to at-risk nations (Egna 1998). U.S. foreign policy during the height of the cold war in the 1960’s and 1970’s however, judged Asia and South America to be of a higher priority with respect to financial aid, and, coupled with regional instability in Africa, resulting in land tenure insecurity, labour shortages, and a lack of stocking material; the infrastructure development within funded nations, whose evolution would lead to the inclusion and expansion of aquaculture, superseded that of Africa (Aguilar-Manjarrez and Nath 1998, FAO 2000, Changadeya *et al*. 2003).

Table 1.3: Ranking of aquaculture producing nations within Africa. Quantities are in metric tonnes.

Ranking within Africa	Total tonnage	Top Species	Tonnage of top species	Global top five nations
1. Egypt	471 500	Nile Tilapia *Oreochromis niloticus**	200 000	China (43 271 000)
2. Nigeria	44 000	North African Catfish *Clarias gariepinus***	15 800	India (2 800 000)
3. Madagascar	8 800	Giant Tiger Prawn *Penaeus monodon****	6 200	Phillipines (1 717 000)
4. South Africa	6 000	Aquatic Plants	2 800	Indonesia (1 469 000)
5. Tanzania	6 000	*Eucheuma* seaweed	6 000	Japan (1 261 000)

* Linnaeus 1758 ** Burchell 1822 *** Fabricius 1798

Prevalence of culture based systems

Culture based aquaculture is a compromise between pure capture fishery practices and the typical culture of organisms. It is generally defined by the cyclical stocking of desirable species into low intensity, but extensive, grow-out systems. It is particularly prevalent in African regions where aquatic species contribute seasonal bounty toward dietary intake and allows for a simple transfer of seasonally and temporally desirable species between regions. This type of aquafarming has had notable success in China where the stocking of gullies and winter reservoirs in rice paddies with various freshwater species has resulted in a 7.8% increase in yield and a 41% increase in value per hectare (Xuegui *et al.* 1995). While it does supply a year round dietary component in China, yields in Africa remain low due to inherent characteristics of the species cultured and does not provide a reliable market supply of fish (Kocher 1997, Changadeya *et al.* 2003).

In summary, the general conditions that are reported to restrict growth of the aquaculture industry in Africa are as follows:

1. A lack of regional expertise among African farmers regarding species identification
2. The general non-existence of sound, aquaculture-friendly agricultural foundations.
3. The above are further fuelled by generally poor economic conditions including limited credit services. All these combine to effect poor aquaculture management (FAO 2000, Changadeya *et al.* 2003, Moehl *et al.* 2006).
4. The absence of a self-perpetuating aquaculture industry, coupled with the prevalence of low intensity culture systems, results in a lack of knowledge of baseline genetic data of the species cultured. These data are vital for the optimization of culture systems, as well as the cultured species found within. Such optimization would remove losses of strain purity and genetic contamination. Most notably, this concerns the hybridization of 'foreign' strains with endemic strains and all the consequent issues concerning genetic 'fouling'(FAO 2000, Changadeya *et al.* 2003, White *et al.* 2004, Hecht 2006, Moehl *et al.* 2006).

1.3.1 Aquaculture in the Republic of South Africa

Aquaculture production in South Africa has been described as negligible (Hoffman *et al.* 2000, Weyl *et al*. 2007). While the rest of the world's developing nations incorporated aquaculture into existing agricultural practices, the South African aquaculture industry displayed low investment, low returns and the subsequent low penetration of aquaculture knowledge into existing agricultural usage. Per capita consumption of fish in the sub-Saharan region alone has decreased from 9.9 kg in 1982 to 1.7 kg in 2003 (FAO 2006).

Contributing factors to this status were formalized in a problem statement section within the governmental policy for the development of a sustainable freshwater aquaculture sector in South Africa (RSA 2006a). A further prohibitive point is the general lack of a historical lacustrine culture within the dominant black South African peoples. Fish and their harvest are generally seen as a seasonal food supplement and thus an infrequent bounty, with a low priority in day-to-day dietary intake (Andrew 2001). This lack is primarily due to the environmental conditions which describe South Africa as a semi-arid region with frequent droughts and the subsequent absence of large natural lakes which may have generated a historic reliance upon fish. This point is reflected by current subsistence fishing seen in the iSimangaliso Wetland Park and other minor estuary systems on the Kwa-Zulu Natal (KZN) north coast, where intricate fish trapping systems are established by local communities and frequently harvested (Sunde and Isaacs 2008). Past governmental policies also had a role to play because the apartheid regime, in power during the surge in global aquaculture industry, criminalized subsistence fishing from dams (Weyl *et al.* 2007) and was in complete control of land use determination. This further limited the potential reliance on aquatic harvests.

Limited aquaculture successes within South Africa can be attributed to high value species such as the Nile crocodile (*Crocodylus niloticus* Laurenti 1786), Rainbow Trout (*Oncorhynchus mykiss* Walbaum 1792), Shrimp (*Penaeus indicus* H. Milne Edwards 1837), and Abalone (*Haliotis midae* Linnaeus 1758). These species and the resultant amounts harvested (Table 1.4) are indicative of a private sector-only approach to the industry. Further concerns are published discrepancies regarding aquaculture production values. This fragmentation of the industry is evident in the discrepancies between two independently published articles (Botes 2006 and DWAF 2006) both reporting on production volumes for 2003 with widely varying values (Table 4).

This situation does seem to be changing as the South African government re-evaluates the potential role of aquaculture, not only in alleviating food security issues, but also in its use as a vehicle by which to foster entrepreneurial and small industry development. This re-evaluation is evidenced by the government's publication of several domestic policies: the Marine Living Resources Act, the Integrated Coastal Management Act, and the Marine Aquaculture Development Plan (MADP), as well as being a signatory to regional and international committees (SADC, NEPAD), which support aquaculture and mariculture practices (FAO 2006, RSA 2007).

Table 1.4: South African aquaculture production for 2003. Taken from DWAF 2006.

Freshwater Species	**Common Name**	**Quantity (metric tonnes) / Botes 2006**	**Value (million R)**
Aponogeton distachyos Linn.f.	Hawthorne	170	0.280
Carasius auratus Linnaeus 1758	Goldfish	930 000* / -	3.319
Cherax tenuimanus Smith 1912	Crawfish	13	0.699
Clarias gariepinus Burchell 1822	Catfish	240	3.202
Crocodylus niloticus Laurenti 1786	Crocodile	8550*	11.500
Cyprinus carpio Linnaeus 1758	Carp	80	1.106
Koi carp (*C. carpio*)	Koi carp	110 000*	1.563
Micropterus salmoides Lacepède 1802	Bass	8	0.072
Mugulidae	Mullet	17	0.231
Onchorhynchus mykiss Walbaum 1782	Trout	1750 / 353.79	44.011
Oreochromis mossambicus Peters 1852	Tilapia	210	3.098
Oreochromis spp.	Tilapia spp.	52	0.739
Ornamental fish spp.	Ornamental	7	0.520
Penaeus indicus H. Milne Edwards 1837	Shrimp	130	11.830
Subtotal		**2677**	**82.170**
Marine Species			
Crassostrea gigas Lamarck 1818	Oysters	250 / 194.6	1.600
Gracilaria spp.	Seaweed	48	0.265
Haliotis midae Linnaeus 1758	Abalone	515 / 344.41	134.000
Mytilus galloprovincialis Lamarck 1819	Mussels	900 / 600	5.135
Subtotal		**1713**	**141.000**
TOTAL		**4 390**	**223.170**

* Units of species, not tonnes

Administratively, the amalgamation of previously fragmented authorities responsible for aquaculture such as the Marine Coastal Management, the Department of Environmental Affairs and Tourism, and the National Department of Agriculture (RSA 2006b) into the newly formed Department of Agriculture, Fisheries and Forestry (DAFF) further reflects the national government's attempts to streamline the process whereby the potential for the industry can be fully realised in South Africa.

The DAFF strategic plan for 2010/11 states the following as opportunities available to a South African aquaculture industry: "good natural resources and infrastructure; a demand for affordable alternative protein sources; a high potential for agricultural diversification; the potential for export opportunities and a growing economy with a good economic climate" (DAFF 2010). While the strategic plan also stated that a current lack of species choice was a threat to such an industry, this threat needs to be viewed in light of our historical marine capture industry and our infancy in freshwater aquaculture. Current research and development into a freshwater fish species capable of intensive and extensive culture, while adhering to environmental legislation regarding alien fish introduction into low management systems, needs to focus on an indigenous species with attributes applicable to rural realities. These attributes should include: general hardiness, ease of reproduction, good taste and market acceptability. One such species is the Mozambique tilapia, *Oreochromis mossambicus* (Peters 1852).

1.4 TILAPIA

1.4.1 Evolutionary Origins & Biogeography

Tilapia (Thlape - Tswana word for fish) is the generic term used to primarily describe members of the Tilapiini tribe of the Cichlidae family of fishes. Believed to have had a maritime ancestor, the proto-Cichlids' retention of a generalized physiological design, allowing for both freshwater and saltwater colonization, permitted the cichlids to speciate successfully throughout Africa from western and eastern foci following the retreat of the ancient Tethys Sea (Philippart and Ruwet 1982), resulting in a present day total of some 900 native species in Africa alone and an almost worldwide distribution in the tropics, including Central and South America, Africa, Madagascar, the Levant, and parts of Arabia and India (McAndrew 2000, Skelton 2001). This basic, adaptable physiology permitted the cichlids to speciate successfully and thus colonise varied water bodies from typical river systems to large bodies of water, where speciation is apparent at different depths and feeding zones, and at the extreme, in hot springs (McAndrew 2000).

The broad term ‘tilapia’ is due to the historic grouping of three genera of the Tilapiini tribe under the genus *Tilapia,* a grouping which has since been reevaluated, resulting in the genus divisions of *Sarotherodon, Tilapia* and *Oreochromis*. This subdivision was promoted by Trewavas (1982) who described the Tilapiini as having an African and Levantine assemblage. This classification has been widely accepted by the scientific community. Due to this reassessment taking place in the early 1980’s, older classifications still persist in literature. The determining factors involved in the subdivision between the genera is the parental brooding and nesting behaviours (Trewavas 1983, McAndrew 2000).

The *Tilapia* genus is currently believed to be the most ancient of the three genera and displays a biparental concern for the fry and territory maintenance; and while young are gathered into the mouths of either parents during times of danger, they do not mouth brood *per se* but are substrate spawners and do not construct nests (Trewavas 1982). The *Sarotherodon* genus occupies the transitional evolutionary stage with various species using either biparental or paternal mouthbrooding to care for the fry. Nests or spawn pits are constructed by the pair within the male’s territory (Trewavas 1983).

The *Oreochromis* genus is the most advanced of these three tribe members and has ‘lost’ the biparental attitude toward fry care; this has been replaced with maternal mouthbrooding, whereby the fertilized eggs are kept in the mother’s mouth until hatching and maternal concern continues for a substantial time thereafter, offering sanctuary to the young. The male practices polygyny while constructing a number of deep nests within his territory, which he alone defends vigorously (McAndrew 2000). The *Oreochromis* genus which houses the three most important aquaculture species, namely: *O. niloticus* (Linneaus 1758), *O. aureus* (Steindachner 1864) and *O. mossambicus*.

The variations in parental behaviour as well as its predominantly herbivorous nature saw the initial introduction of the tilapia into Java in the 1930’s as an ornamental fish (Stickney 2000). Furthermore, its general body design, aggressive behaviour, and opportunistic diet have allowed it, upon escape, to successfully establish itself in feral populations worldwide. This ability to thrive under varying environmental conditions is one of the principal reasons behind the promotion of tilapia as the “most important aquaculture product of the 21st Century” (Fitzsimmons 2000).

O. niloticus and *O. aureus* are both under widespread cultivation following from their introduction into various areas of the globe. Their resulting pest status from mismanaged aquaculture programmes has led to a number of nations, including South Africa, restricting their use in rural aquaculture ventures (RSA 2009). *O. mossambicus* is similarly labeled an aggressive invader but, owing to its indigenous nature, it is therefore exempt from the restrictions.

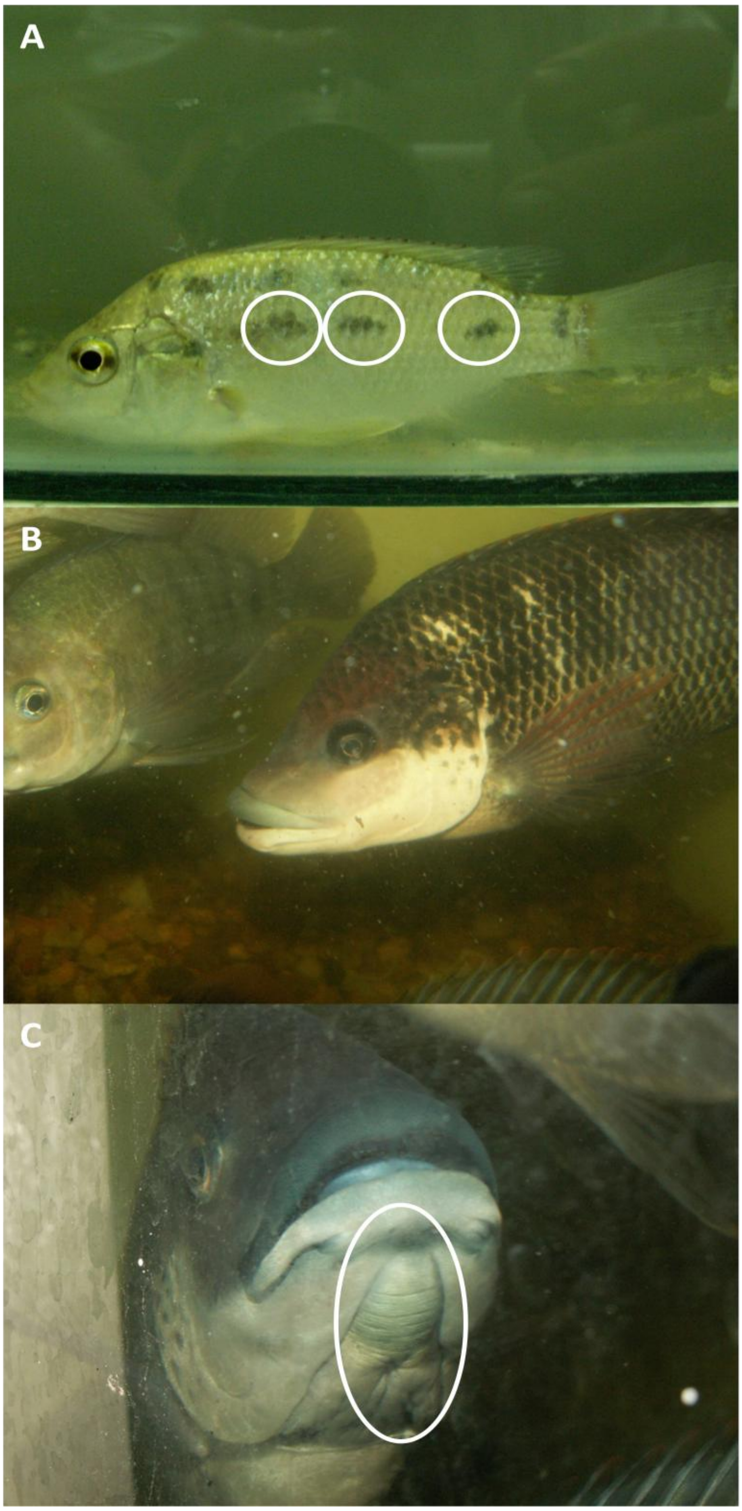
1.5 Oreochromis mossambicus

1.5.1 Description

Oreochromis mossambicus (Greek *oreos*, "of the mountains" and *chroma*, "colour"; *mossambicus* geographical range) follows the general physical appearance of the genus, as well as of the tribe, by being laterally compressed, with a deep body cavity. The head is large and usually extends approximately ¼ of the total body length (TL) Males generally grow larger than females, with average lengths of 400 mm (SL - standard length) (Skelton 2001). Paired fins lie adjacent to the gill operculum as well as singular dorsal, anal and caudal fins. Mozambique tilapia have 28-31 vertebrae and 14-20 lower gill rakers. The spine / ray count is: Dorsal = XV-XVII + 26-29; Anal = III=IV + 9 - 10 (Skelton 2001).

A definitive characteristic for the species is the presence of three dark spots of highly variable form which lie along the lateral line, anterior to the caudal peduncle (Figure 1.3A)(Skelton 2001). Sexual dimorphism is difficult to ascertain in non-breeding colouration because both sexes display the silvery olive colouration, which is furthermore shared by juveniles, although lighter in hue. Older males which have previously bred may show concavity of the upper jaw line compared to the more convex shape of females and young males (Figure 1.3B). With the onset of the breeding season the male is easily distinguished by his breeding colours. The males become almost completely black along the entire body except for a prominent white patch below the lower jaw and the edges of the dorsal, pectoral and caudal fins which are red (Figure 1.3B). This colouration is under direct control and stress will cause a sudden loss of the colouring.

The females maintain their silvery olive colouration throughout the year and the only two physical characters that may indicate breeding status are (1) the increase in contrast of the horizontal bars across the bridge of the upper jaw, and (2) the distension of the sac between the two lateral lower jawbones (Figure 1.3C). This sac is evident on both sexes and while its use by the females is primarily for the oral incubation of eggs (Ahmed *et al*. 2007), its use in males is not documented.



A. Lateral view of *O. mossambicus* indicating the 3 spots lying on the lateral line.

B. Male *O. mossambicus* displaying breeding colouration. Note the concavity on the upper jaw.

C. Frontal view of male *O. mossambicus* indicating the buccal sac.

Figure 1.3: Phenotypic characteristics of *O. mossambicus*

1.5.2 Biology

O. mossambicus may be generally defined as a filter feeding cichlid with opportunistic habits (Fryer and Illes 1972). Filter feeding in this species is not achieved by fine gill rakers but rather by the secretion of mucus which traps the plankton into a bolus which is then ingested. Its opportunistic diet is largely dependent on its environment but records show insects and their larvae, detritus, and even small fish will all be taken (Fryer and Illes 1972, Robins *et al*. 1991, Allen *et al*. 2002).

A further reflection of its adaptability is the various types of waters in which it thrives. *O. mossambicus* will inhabit most types of waters that have an average temperature of greater than 13°C (Philippart and Ruwet 1982). Preferred habitats are extensive bodies of water with no or minimal water movement such as all types of dams. Rivers are also successfully inhabited given that they include areas which provide the required refuge necessary for territory establishment, mating and fry incubation (Allen *et al*. 2002). *O. mossambicus* are also known to successfully inhabit various estuary systems worldwide, owing to their euryhaline nature (Chervinski 1982, Philippart and Ruwet 1982, van der Audenaerde 1988).

A defining behavioral characteristic of the *Oreochromis* genus is the maternal care given to the young as opposed to biparental care (*Tilapia spp*.) and paternal care (*Sarotheradon* spp.) of other tribe members (Fryer and Illes 1972, Trewavas 1983, Turner and Robinson 2000). Furthermore, *O. mossambicus* has been described as a lek breeder whereby males congregate in a particular area, or lek, where courtship displays are enacted (Nelson 1995, Oliveira and Almada 1996). Breeding in *O. mossambicus* is typically seasonal, with the elevated water temperatures being regarded as the pivotal trigger for the onset of mating related behaviours (Turner and Robinson 2000).

Typical of the genus, male *O. mossambicus* construct saucer shaped nests, which are then used as focal points of the courtship displays, as well as the mating act (Trewavas 1983). A distinct hierarchy exists within this male aggregation and the more dominant males may construct multiple nests, depending on their territory size (Oliveira and Almada 1998). Once the female has chosen her mate she follows him back to his nest site whereupon she lays her eggs in discrete batches which the male fertilizes. Once the last batch has been fertilised the female takes the eggs into her mouth and leaves the lek. The female will then orally incubate the eggs for approximately one week before they hatch. Her care for the fry then continues well into their second or third week by providing sanctuary should danger threaten. *O. mossambicus* are further described as precocious breeders because sexual maturity has been recorded in individuals as young as three months old (Bell-Cross and Minshull 1988, Turner and Robinson 2000).

1.5.3 Economically Important Traits of *O. mossambicus*

While *O. niloticus* is currently the world leader in terms of tonnage produced, value obtained and worldwide distribution, *O. mossambicus* possesses comparable economic traits which have catapulted the Tilapiine to the forefront of aquaculture research.

Growth rate.

Baseline food conversion efficiencies (FCE) of 1:2 have been recorded for *O. mossambicus* which are able to gain 1g in weight for 2 g of feed supplied. Under intensive culture conditions, with selective breeding having taken place, GIFT strains (genetically improved farmed tilapia) of *O. niloticus'* FCE approaches 1: 1.4 where 1.4 g of feed results in 1 g of weight gain. A linked attribute is the omnivorous diet of *O. mossambicus.* One of the largest economic constraints facing aquaculture of finfish is the need to supply fishmeal diets in order to effect optimal growth (Millamena 2002). This is not the case with *O. mossambicus* because they occupy a place low on the trophic level with 'filter' feeding as their mode of feeding. This technique implies that low intensity culture systems which support nutrients such as algal blooms or duckweed (*Lemna* spp.) provide efficient methods by which to feed *O. mossambicus*. In practical terms, water is fertilised and subsequent natural pathways allowed to develop. Even in intensive systems, the rearing of tilapia in green water is a viable option in that *ad libitum* feeding is completely satisfied without the costs of high quality feed, approximately 60% of running aquaculture costs (Gabriel *et al.* 2007), and concomitant water treatments to keep the water clear. The disadvantage in green water systems is the difficulty in disease diagnosis due to potential pathogen introduction via the fertilizing agent as well as the resultant difficulties in observation.

Sexual plasticity.

This term describes the ability of *O. mossambicus* fry to have their phenotypic sex altered before 21 days of age. The result does not affect gametogenesis but simply the organ structure which produces the gametes, i.e., a sex reversed male (neo-female) will produce eggs in roughly equal X and Y proportions. This ability, easily achieved, has large ramifications for a cultured organism, allowing for even greater selection potentials to be effected on future potential broodstocks (Mair *et al.* 1997).

Hardiness.

Tilapia species are often described as robust fish due to their ability to thrive under various environmental conditions. This hardiness is believed to be related to the same basic physiology that they have maintained throughout their evolution, from a maritime ancestor, which has allowed them to exploit different niches (Trewavas 1982). The two crucial metabolic modifiers which have a large impact on aquaculture species are salinity and temperature.

Salinity tolerance by the Tilapiine is one such well documented environmental parameter which definitively excludes the culture of the majority of freshwater fish and, conversely increases the potential water sources available to tilapia aquaculture systems. Typically an increase in salinity negatively affects osmoregulation of freshwater species, resulting in a reduction in ionic ($Na+$) gradients across cellular membranes, culminating in cellular dysfunction. Suresh and Kwei Lin (1992) studied the effect of salinity tolerance of a large number of freshwater fish. They found that the most commonly cultured Tilapiine all show a wide range of salinity tolerance. Furthermore, fry become euryhaline after the age of 45 days; prior to that, eggs and fry alike have a 96 hour median lethal salinity of 18.9 gl^{-1} (Ross 2000).

Temperature is a vital environmental factor, due to the fact that fish are thermal conformers, i.e., ambient temperatures directly affect their metabolic processes (Brett 1979). Thus temperatures at which fish species grow optimally are therefore of crucial interest to aquaculturists. Chervinski (1982) studied the thermal tolerance of several members of the Tilapiine and recorded a range from 7°C to above 38°C. This tolerance has been suggested to be the result of selection pressures on the lacustrine proto-tilapia where large diel ranges had to be assimilated in order to survive. While *O. mossambicus* growth is greatly reduced at low temperatures; the genetic modifications that could be potentially utilized to affect such a eurythermic tilapia, capable of good growth in both a wide range of temperatures and salinities, would be greatly optimised by the use of closely related sister taxa.

In combination, salinity has been shown to provide an exacerbating effect on transfers between waters of differing temperatures. Al-Amoudi *et al.* (1996) reported that *O.*

mossambicus, which underwent a 10°C transfer in freshwater showed no significant increase in mortality. An equal temperature change in saline water, however, did produce a significant increase in mortality

In addition to these two parameters, temperature and salinity, tilapias also display good aquaculture attributes as regards other aquatic parameters such as pH, dissolved oxygen concentration (DO) as well as stocking densities.

pH is the standard measure of free hydrogen ions (i.e., acidity vs. alkalinity) in a water body. While tilapias typically thrive under neutral or near neutral conditions (van Ginneken *et al.* 1997), tolerance for alkaline waters is greater than that of acidic waters, and is exemplified by *O. alcalicus grahami* (Boulenger 1912) which occurs in environments with pH values between 9.6 and 10.0 (Johnston *et al.* 1983).

Dissolved oxygen is the level of oxygen available in the water column. Oxygen is far less freely available to aquatic organisms than to terrestrial based organisms, typically a few milligrams per litre in water compared with 20% of air (Ross 2000). As oxygen is a vital component of efficient metabolic activity, saturation of the water column with dissolved oxygen is a desirable goal toward realizing any economically profitable stocking densities. While Tilapia will gulp at the water's surface if the DO falls too low, this is a short term behavioural adaptation and survival, let alone optimal growth, will be compromised. Typically a DO between 6 and 8 mgl^{-1} will provide a suitable oxygen concentration for tilapia (Boyd 1982, Suresh 2003, Gonzales *et al.* 2007). Stocking densities typically recorded from commercial operations indicate a density at harvest of 25 to 40 $kg\ m^{-3}$ with a cost benefit ceiling of 100 $kg\ m^{-3}$ due to oxygen depletion (Muir *et al.* 2000). Due to the decrease in dissolved oxygen content in warmer waters, these densities compare favourably against cold water species such as Rainbow Trout (*Oncorhynchus mykiss*) (North *et al.* 2006).

In summary, tilapia and particularly those of the *Oreochromis* genus, display positive aquaculture attributes regarding typically difficult or exclusive environmental parameters for traditional freshwater fish. While selective breeding and hybridization has shown merit, the inevitable future incorporation of molecular techniques, such as gene transfer, will be greatly assisted by the variation inherent within this single genus.

1.6 GENETIC MANIPULATIONS OF *OREOCHROMIS SPP.*

1.6.1 Overview of Tilapiine sex determination.

A typical tilapia genome consists of 44 chromosomes, with one large chromosome pair, a second smaller pair and then twenty chromosome pairs of a small size which to date have not been identified nor characterised according to centromeric position (Martins *et al*. 2004). The ancestral teleost fish, from which the Tilapiine are derived, had a karyotype of 2n=48. FISH (Fluorescent In Situ Hybridisation) examinations by Chew *et al*. (2002) supported the hypothesis that the existing chromosome 1 was the result of fusion between three ancestral chromosomes.. While current research is pointing toward the two largest chromosomes being involved in sex determination, numerous studies have revealed that they are not the only components, genetic or otherwise, of the final sexual phenotype (Mair *et al.* 1997, Campos-Ramos *et al.* 2003).

Several different sex determination mechanisms are known to exist in fish, namely hermaphroditism (Price 1984, Chourrout 1988); polygenic systems (Kallman 1984); monofactorial systems (Chourrout 1988) and environmental systems (Conover and Heins 1987). Within the *Oreochromis* genus, both male homogamety (ZZ & ZW) and female homogamety (XX & XY) systems have been proposed. Initial experimental results in *O. mossambicus* (Clemens and Inslee 1968) as well as *O. niloticus* (Tave 1988) showed that male heterogamety (= female homogamety) was indeed the case, i.e., males = XY and females = XX (Campos-Ramos *et al.* 2003). Subsequent scientific endeavours have been structured to elucidate whether this sex determination system was monofactorial (as with humans) or multifactorial. The multifactorial hypothesis was confirmed by the use of successive pseudofemale lines (Desprez *et al*. 2003). A pseudofemale, or neo-female, is a phenotypic female with a masculine genotype. In tilapia this is achieved by immersing the fry in gynogenetic hormones before sexual differentiation has taken place, usually within thirty days after fertilization (AF). In the case of the heterogametic male *O. mossambicus*, a neo-male is produced by masculinising a typical XX female. This neo-male will then produce homogametic eggs and the resulting cross with a typical female should produce monosex progeny if a monofactorial system is in effect, i.e.,

XX♀ X XX '♂' (sex reversed) ⟶ 1♂(XX): 0♀

Any deviation from this expected 100% ratio would indicate that some other factor, environmental or genetic or a combination of both, was playing some role in the determination of sex. Several authors have recorded just such deviations when dealing with the Tilapiine, and hypotheses put forward to explain the deviations include a polygenic system of sex determination; epistatic autosomal genes; or a purely environmental influence (Wohlfarth and Wedekind 1991, Baroiller and Toguyeni 1996). Mair *et al*. (1991) proposed a polygenic system of sex determination based on the existence of autosomal recessive genes (F/f), which are epistatic to the main sex chromosomes. In other words, in *O. aureus* the combination of a homozygous recessive autosomal allele (ff) with either pair of 'male' sex chromosomes ZZ or ZW would produce a phenotypic female (ZZff or ZWff). This hypothesis was supported by Wohlfarth and Wedekind's (1991) experiments with *O. niloticus,* which indicated that an autosomal factor(s) was/were affecting the monofactorial system of sex determination. Further evidence toward the polygenic hypothesis in *O. mossambicus* was revealed by investigations into *O. niloticus* that proved the presence of a temperature dependent gene, which is directly involved in androgen production that results in all male progenies at elevated temperatures (D'Cotta *et al.* 2001). In an attempt to manipulate the dominance relationships between the two systems in order to produce monosex progeny, it was shown that pairings between female *O. mossambicus* (XX) and male *O. hornorum* (ZZ) resulted in all male broods (ZX), indicating a dominance of the Z allele over the X allele (Scott *et al*. 1989, Mair *et al*. 1991, Beardmore *et al*. 2001).

Thus the sexual phenotype of *Oreochromis spp.* can be deduced to be under multifactorial control with a dominance of sex-chromosomes augmented or adjusted by discrete autosomal genes and / or environmental conditions (Campos-Ramos *et al.* 2003). This system, coupled with the relatedness of the Oreochromis genus, permits not only typical selective breeding and direct hormonal manipulation, but also intra-genus hybridisation as well as various molecular techniques in order to further manipulate the parental broodstocks to produce progeny with desirable attributes. The main targets of these manipulations, within the aquaculture industry, has been either to induce all male progeny or to genetically sterilise specimens that pose a feral establishment risk.

1.6.2 Selective Breeding

In 1988, ICLARM, in conjunction with several Asian aquaculture agencies, initiated a breeding project of *O. niloticus* to determine potential genetic gains under selection for growth. Eight strains were collected: four from the wild in four North African countries and four existing strains from Israel, Singapore, Taiwan, and Thailand respectively. These strains were then crossed in an 8 X 8 diallele cross to determine the level of genotype by environment interaction. This was found to be negligible and so a pure breeding system based on selection for growth to maturity was undertaken. After five generations the resulting average genetic gain in growth per generation lay between 12 and 17%, while the cumulative genetic gain overall compared to that of the base population was 85%. This final population came to be known as the GIFT (genetically improved farmed tilapia) strain (Eknath *et al.* 1993). It is important to note that no transfer of genetic material took place in the development of this strain and therefore GIFT is a non-GMO (Aquafish CRSP 2007). The gains made were due solely to family selection, a selection tool which is based upon family merit and not individual merit, which, while showing slower specific gains in desirable traits, does produce a generally more fit organism due to a greater degree of heterosis, which is a necessary characteristic in a culture species with worldwide application in varying environments (Tave 1988, Eknath *et al.* 1993, Ross 2000).

The GIFT strain has not been transferred to Africa, as the World Fish Center (previously ICLARM) has strict policies regarding the introduction of selectively bred organisms into Centers of Origin, where the potential for escape and establishment is sufficient enough to seriously affect natural genetic reservoirs. Furthermore, in South Africa, current legislation (RSA 2009) forbids the culture of *O. niloticus* due to its invasive potential within our borders. This invasive potential is not merely due to direct competition with indigenous fish species but also its ability to hybridise with indigenous *O. mossambicus*. This potential may already have been realized with findings of *O. niloticus* within the Limpopo and Shashe rivers in 1996 and 1998 and concomitant records of hybrids between these two species (Moralee *et al.* 2000, van der Waal and Bills 2000).

1.6.3 Hybrids

O. niloticus has been the target of the majority of hybridisation attempts, due to its faster growth. It is therefore dominant in aquaculture production, relative to other *Oreochromis spp*. Some of the genetic factors successfully targeted for induced and captive hybridisation include: increased salinity tolerance (*O. niloticus* X *O. spirulus*, Ridha 2010) (*O. niloticus* X *O. mossambicus*, Hena *et al.* 2005); increased growth rate with cold tolerance (*O. mossambicus* X *O. urolepsis hornorum* X *O. niloticus* X *O. aureus*, Desprez *et al*. 2006); and the production of all-male progeny (Table 1.6).

Table 1.6: A listing of reported inter-species hybrids which produce monosex progeny. Taken from Beardmore *et al*. (2001).

Female Parent	Male Parent
O. niloticus	*O. aureus*
O. niloticus	*O. u. hornorum* (Trewavas 1966)
O. niloticus	*O. variabilis* (Boulenger 1906)
O. mossambicus	*O. aureus*
O. mossambicus	*O. u. hornorum*
O. spirulus niger (Günther 1894)	*O. macrochir* (Boulenger 1912)
O. aureus	*O. u. hornorum*

1.6.4 Karyotype manipulations

1.6.4.1 Polyploidy Inductions

The ploidy of an organism is the number of sets of each distinct chromosome that is represented in the genome. Diploidy is the most common form of ploidy in animals, indicating a pair of each chromosome is present. More than a pair of each chromosomes is referred to as polyploidy and in a number of plants, natural polyploidy is evident, while a number of breeding strategies, namely in the cereals, has resulted in permanent polyploidy. This has resulted in more robust crops with measurable heterosis. The ability to alter chromosomal sets in fish also has a number of positive aspects (Ambali and Malekano 2004, Piferrer *et al.* 2009):

1. With functionally sterile triploids it reduces the risk of the establishment of escaped genetically modified organisms (GMO's) in wild populations due to the unbalanced meiosis which results in abnormal gamete production.
2. Polyploidy does not maintain itself naturally and thus puts a direct management control onto the GMO.

3. Functional sterility would lead to an increase in cost efficiency, as the energy from converted feed would not be spent on reproduction.

The induction of polyploidy requires that the meiotic division be interrupted in such a way as to depolymerise the microtubules, which are essential for the formation of the spindle apparatus (Razak *et al*. 1999). Factors that affect the spindle apparatus forming are environmental shocks such as heat (Mair 1993); cold (Don and Avtalion 1990); pressure, and pressure in combination with ether (Hörstgen-Schwark 1993). These shocks are usually applied within 30 minutes of fertilization as in the induction of tetraploidy in tilapia, which results after immersion into a 41°C bath for two minutes, twenty minutes after fertilization (Mair 1993).

Triploidy

This is the presence of three of each chromosome in the genome and, in induced triploidy, normally implies a functional sterility due to the unbalanced meiosis, which leads to abnormal gamete production. Triploidy in tilapia can be achieved by shocking the oocyte, resulting in the retention of the polar body (Razak *et al*. 1999). This diploid egg is then fertilised by the spermatozoa, resulting in a 3n zygote (Razak et al. 1999). As male tilapias are the heterogametic sex, triploidy induction must be coupled with hormone treatments to masculinise the zygote (Razak et al. 1999). Triploidy, however, is very labour intensive, costly and has a low success rate (Ambali and Malekano 2004).

Tetraploidy

This is induced by the interruption of the first mitotic division, resulting in a 4n cell, which is able to produce viable diploid gametes which, with a haploid gamete from the normal diploid, will produce a triploid zygote . Matings between tetraploids and normal diploids, therefore, would result in 100% triploid progeny being produced (Mair 1993). Numerous studies have shown the occurrence of triploidy, as well as tetraploidy, within a single spawn. It has been suggested that this phenomenon is due to asynchronous development of the eggs laid (Myers 1986).

1.6.4.2 Euploidy inductions

These inductions are used to produce either an all maternal or all paternal zygote.

Androgenesis

This is the development of a zygote (termed an androgenote) with an all-paternal genotype and it can be achieved in two ways. Both methods require the neutralisation of the egg genome, which can be achieved by irradiating the egg with gamma rays, X-rays or UV radiation to neutralise the maternal genome (Karayucel and Karayucel 2003). UV radiation is preferred for safety and simplicity concerns but also because it dimerises the DNA rather than disintegrating it (Shelton 1989).

Method 1.The neutralised egg is fertilised with a normal haploid spermatozoa. The first mitotic division must then be arrested to produce a diploid zygote, which will contain a double paternal genome (Shelton 1989).

Method 2. The fertilisation of the neutralised egg is done by the diploid spermatozoa of tetraploid males.

Both of these procedures will result in a completely homozygous genome, which may have deleterious combinations of disease causing alleles. However, it is the simplest manner by which to induce a YY male, without hormones, and also provides a rapid method by which to produce inbred lines. Furthermore, androgenesis permits the production of nucleocytoplasmic hybrids between different species (Shelton 1989).

Gynogenesis

This is the development of a zygote with an all-maternal genotype and can be divided into two types, namely gynogenesis by suppression of either mitotic or meiotic events:

Method 1. Suppression of meiosis produces a meiogynote and occurs when the polar body is retained resulting in a 2n all-maternal egg, which is then activated by a neutralized spermatozoon (Stickney 2000). Unlike androgenesis, the resulting diploids will not be homologous at all loci due to some recombination having taken place.

Method 2. Suppression of mitosis results in a mitogynote, which results from the activation of a normal haploid egg by a neutralised spermatozoon. In other words, the haploid (n) egg begins to replicate (mitotically) and division is then halted resulting in a 2n all-maternal genome (Stickney 2000). This genome will be homologous at all loci.

However, these processes are far too time consuming and labour intensive to be economically feasible for same-generation monosex induction. Rather they are used as precursors for subsequent monosex induction (Stickney 2000).

1.6.5 Monosex Induction

Since the introduction of tilapia into intensive aquaculture systems and their undesirable, precocious breeding habits became apparent, methods have been investigated to produce single sex progeny batches. The preferred sex is male due to their superior growth (Beardmore *et al.* 2001). An immediate 'fix' to the situation is the use of androgens, which are a class of hormones able to masculinise the sexually plastic fry (Baroiller and D'Cotta 2001). Another approach sees the manipulation of the ploidy level so as to effect the same masculinising result, with the added benefit of severely limiting potential genetic fouling by escapees due to the concomitant infertility. A third option, which removes the continuous cost and stigmas regarding hormone treatment, while furthermore not requiring the expensive apparatus of polyploidy induction and providing a higher success rate, is the production of a YY or super male tilapia (Stickney 2000, Beardmore *et al.* 2001). It uses SRT (Sex Reversed Tilapia) technology with subsequent backcrossing to determine the YY carriers.

1.6.5.1 Sex Reversed Tilapia (SRT)

SRT is a well established aquaculture technology because the process is simple and produces a high percentage of male fry per batch. The androgen, 17-α-methyltestosterone (MT), is simply added to the water of the fry to affect a certain concentration. This additive is replenished in the water supply for the first month of growth of the fry, after which sexual organ differentiation has been set and further exposure will have no effect (Stickney 2000).Disadvantages regarding this process are the obvious potential for uptake of the hormone by the consumer and the less than 100% success rate (Stickney 2000). Recent findings into the longevity of the hormones have disproved potential health risks but concerns surrounding associated derivatives remain (Beardmore *et al*. 2001).

1.6.5.2 YY Male Technology and Genetically Male Tilapia®(GMT)

GMT® is a registered trademark of Fishgen Ltd and refers to the development of a YY male tilapia, which in pairing with a typical XX tilapia female will produce a 95% or better monosex male progeny batch. The 5% uncertainty is due to epistatic genetic interaction, which may result in female production, depending on the heritable genetic co-factors within the parental lines.

The development of the GMT, also known as the super male or YY male, was initiated by the desire to provide a non-hormone treated product which would remove concerns regarding the potential transfer of hormones from the product to the consumer. To produce GMT the use of hormones is only used to affect the parental (P1) broodstock and the product to be harvested is two generations distant (Stickney 2000, Beardmore *et al.* 2001) (Figure 1.4). However, GMT utilisation ignores the maternal genetic line and thus cannot ensure a 100% all male line. To achieve this, the maternal pedigree must be fully known, particularly in relation to those autosomal factors which affect the final sexual phenotype.

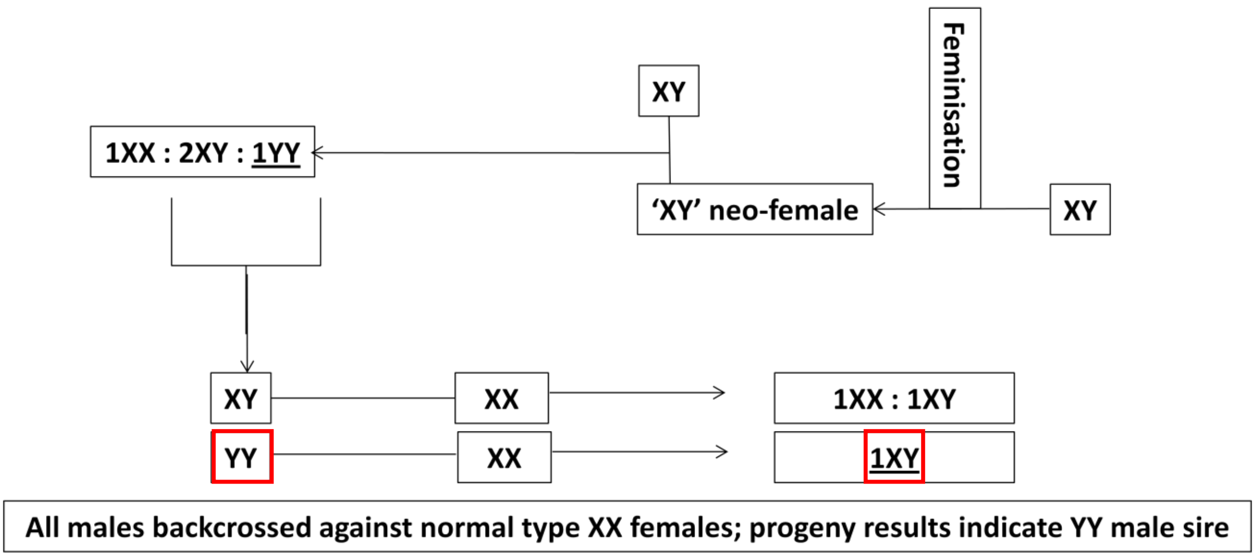


Figure 1.4: A schematic representation of the creation and identification of a YY male.

While the above process does have constraints, such as the extensive record-keeping that YY male technology requires, the ability to produce a YY male reliably and cost-effectively will allow smaller aquaculture endeavours, such as rural outreach programmes, the opportunity to purchase high percentage male fry in a per-season basis, as is done with various cereals to date. Furthermore, YY male production has already been assisted by the complimentary processes of polyploidy and / or euploidy alterations (Beardmore *et al.* 2001)such that YY males are now available for purchase as broodstock (http://www.fishgen.com). These genetic options, while maintaining the *Oreochromis* environmental hardiness, will provide greater profit margins, regardless of the culture system used.

1.7 AQUACULTURE SYSTEMS

Within aquaculture systems, there are three main infrastructure types that are used for a majority of the species cultured, namely: pond, cage and recirculating systems. These three types may be seen as an evolution of farming practices, improving from outdoor, minimal-control pond systems to indoor, fully controlled recirculating aquaculture systems (RAS's). These three types of culture can also reflect the level of capital investment, increasing substantially from pond through cage to recirculating systems. Globally, East Asia leads the industry with the amount of water under culture, particularly in reference to semi-intensive aquaculture practices. Comparisons between East Asian and African practices indicate the potential growth possible of semi-intensive aquaculture systems in Africa.

1.7.1 Pond Culture

This may be viewed as the most ancient of aquaculture system types and is still in widespread use in developing countries, where minimal financial backing, necessary for more intensive operations, predisposes the establishment of low maintenance systems (Changadeya *et al.* 2003, El-Sayed 2006). Culture based pond systems comprise simply of a constructed earthen pond which is stocked directly with the species desired and harvesting is done on an *ad hoc* basis. Conversion of metabolites within the water body is achieved via natural processes found in the substrate, plants and resident microorganisms. Levee or dike ponds are more intensive variations whereby the pond is constructed in such a manner as to facilitate rapid draining at harvest time. Biological processes are re-initiated by fertilization after each harvest. The use of this system in a commercial environment is dependent on site choice and water resource availability (Muir 2005, El-Sayed 2006).

Pond culture of tilapia in East Asia is almost exclusively freshwater based and is often used in integrated culture systems such as rice-fish (tilapia are cultured in irrigation canals), livestock-fish (livestock excreta fertilises the water body), and pen-cum-pond systems where complimentary fish species, such as catfish (*Clarias spp.*) are housed alongside tilapia in separate enclosures within the same water source. Intensive pond farming of tilapia in Taiwan has the highest recorded figures of 12 - 17 $Mt.ha^{-1}$ (Dey 2001). While small scale and medium intensity systems are both reported in Africa (Jamu 2001), Egypt is the largest producer on the continent (Table 1.3) where the vast majority of tilapia production takes place in monoculture, brackish water, systems.

1.7.2 Cage Culture

As the name implies, this system involves the complete culture of the product within the confines of a cage, which greatly increases the efficiency of harvesting. The cage, or hapa, is kept afloat by floatation devices and moored to fixed objects such as jetties or the substrate (El-Sayed 2006). Due to biofouling, age cohorts are cultured in cages of progressively larger mesh sizes. This system is chiefly used within large water bodies such as dams, lakes and the open oceans. Within aquaculture, it is the only available system used for the culturing of open ocean species where its capital investment is considerable (Muir 2005).

Its use in Asia is chiefly due to complimentary weather conditions, the prevalence of suitable water bodies and moderate management requirements. While data on the actual production amounts are scarce, a peak of 2700 Mt was recorded in Thailand (1991) from this system alone (El-Sayed 2006). In Africa, Zimbabwe produces the most amount of tilapia in this manner, with 2000 Mt having been recorded from the Lake Harvest operation on Lake Kariba (El-Sayed 2006).

1.7.3 Recirculating Aquaculture Systems (RAS)

RAS's are the preferred, usually indoor, setup types for intensive grow out operations. A RAS is defined by continuous recycling of the water through mechanical and biological filters and finally into a settling tank before returning to the grow-out structure, thus removing the need for large water replacements (El-Sayed 2006). A number of variations on the general concept exist, depending on the species under culture, and the surrounding environment. Two main variations are raceway and circular systems, and their use is dependent on the species cultured. Trout (*O. mykiss*) are often cultured in raceways as they prefer a moderate current, whereas tilapias prefer a far gentler flow of water such as found within circular or rounded square, tank systems. A RAS system provides complete control over all aspects of the products environment lifecycle, particularly temperature, and while more expensive in setup, culture density is greatly enhanced to ensure a greater return on investment (ROI) (Muir 2005).

East Asian utilisation of RAS' for the grow-out of tilapia is low. This is chiefly due to the low value attributed to tilapia, which limits the initial capital investment available to ensure profitability (Dey 2001, El-Sayed 2006). Its primary use in tilapia production is therefore currently confined to environmentally unsuitable countries such as Israel (water scarce) and Canada (low temperatures) where complete environmental control is a prerequisite (El-Sayed 2006). A further use for RAS' within tilapia production is that of fish seed production. This incorporation would greatly alleviate the industry-wide shortfall of high quality, regularly available, fish seed. This alleviation would greatly benefit the establishment of more profitable aquaculture ventures in Africa.

These three general systems cover all possible forms of operation and choice is therefore made on location, the species in question, and the capital investment available. Of all the species currently under global culture, the Tilapiine *Oreochromis* genus surpasses all others in global utilization. This feat is largely due to its members' robust nature as well as their opportunistic feeding strategies. This synergy allows them to be farmed within any system and at varying degrees of intensity.

While low production values for Africa have been attributed to many factors, it is this authors belief that fish seed production, of both high volume and quality is the single biggest hurdle for African aquaculture to overcome. With reference to aquaculture in South Africa, accepting the role that *O. mossambicus* has to play is the first step. Analysing behavioural and biological aspects of this species, particularly with respect to breeding and wild genetic reservoirs, would be the next logical step. The aim of this research was therefore:

1. To establish and maintain a small breeding facility for the production of *O. mossambicus* fry.
2. To observe social and breeding behaviours of *O. mossambicus* in order to highlight possible enhancements for improved breeding synchrony.
3. To quantify FCE values of wild-caught *O. mossambicus* within said small breeding facility and thereby produce a suitable breeding program for the improvement of weight gain.

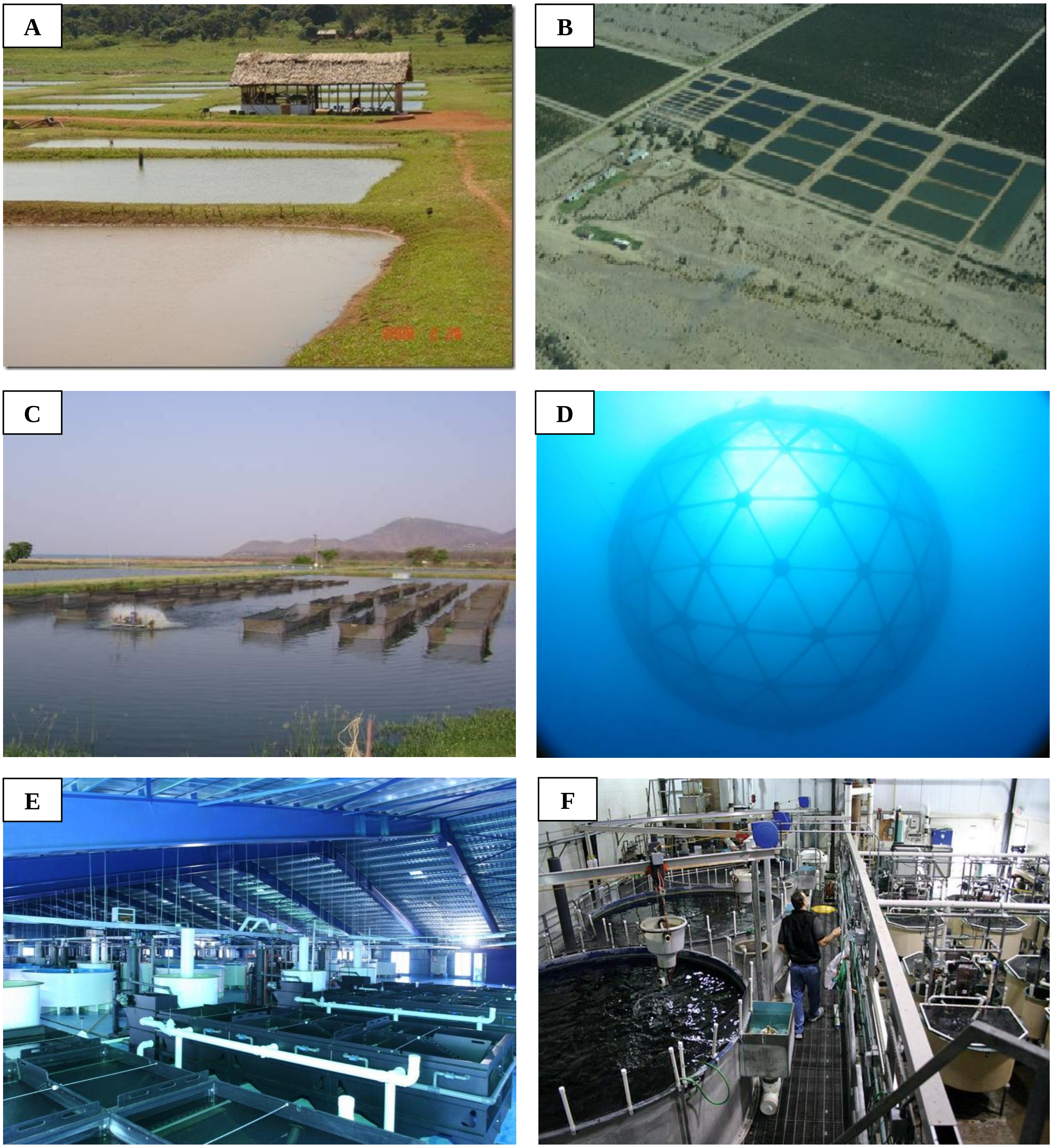


Figure 1.5: Pond, cage and recirculating systems in use.
A. Tilapia culture in earthen ponds in Uganda. Taken from http://www.sterenbergsalinas.nl/?p=38
B. Levee style pond used for finfish in Arizona. Taken from http://ag.arizona.edu/azaqua/extension/Classroom/AquaStruc.htm
C. Tilapia culture using Hapa system in Ghana. Taken from http://www.cde.int/documents/20094226E7TIOR6L4.htm
D. Aquapod® in use off Puerto Rico for the culture of Cobia (*Rachycentron canadum*, Linnaeus 1766). Taken from http://technicalstudies.youngester.com/2008/09/self-propelled-aquaculture-cage-debuts.html
E and F. Taken from http://www.csmonitor.com/Environment/2010/0224/Recirculating-aquaculture-systems-The-future-of-fish-farming

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CHAPTER 2

THE ESTABLISHMENT AND MAINTENANCE OF A SMALL BREEDING FACILITY FOR *Oreochromis mossambicus* (Peters 1852)

ABSTRACT

A major obstacle facing the successful creation of an African aquaculture industry, based upon *Oreochromis spp.*, is the irregular supply of good quality fish seed. There are several causative biological processes behind its irregular supply. The aim of this research was therefore to determine the basic requirements for the establishment and maintenance of a small breeding facility, for *O. mossambicus*. The goal was to make a unit that was simple and which could be easily replicated in rural, satellite aquaculture seed stations. Protocols regarding collection, habituation, medication, and feeding throughout the various life stages were established. The results obtained illustrate that a small reproduction unit, contained chiefly within the dimensions of a standardised shipping container of $40m^3$, can produce large quantities of healthy 90-day old fry. The implications for the unit's potential role in rural fish seed production systems are discussed.

2.1 INTRODUCTION

The potential for a South African aquaculture industry to alleviate food insecurity in rural areas, while assisting in job creation and skills development will, in large part, be dependent upon the selective use of successful international practices (Hecht 2006). This use of international aquaculture operations must accurately reflect the expected realities of our rural locales. These realities include disadvantages, such as that most of South Africa is defined as being water-scarce (Prinsloo *et al.* 2000); and advantages, such as the availability of various indigenous fish species, such as the sharp toothed catfish (*Clarias gariepinus*, Burchell 1822) and the Mozambique tilapia (*Oreochromis mossambicus*, Peters 1852) which are cultured globally (Hecht 2006). The use of an indigenous species, such as *O. mossambicus*, should be prioritised in local aquaculture projects owing to their regional recognition, ease of production, low trophic level, good food conversion efficiency, and mild taste (Muir *et al.* 2000). Furthermore, *O. mossambicus* is already a globally accepted aquaculture product whose adoption would greatly increase the impact, and speed of acceptance, of an aquaculture industry within rural communities whose fish consumption is declining (Bruton and Merron 1985, Hecht 2006, RSA 2006).

Appropriate aquaculture sites for the cultivation of *O. mossambicus* within South Africa do exist in the forms of various dams, and estuaries, as well as numerous abandoned aquaculture project sites that could be successfully utilised as grow-out arenas (Hoffman *et al.* 2000). A large problem facing aquaculture in sub-Saharan Africa in general, however, is the reliable supply of large quantities of healthy fish fry with which to stock these waters (Hecht 2006, RSA 2006). *O. mossambicus*' precocious nature eliminates the typical need for expensive pre-conditioning of brood pairs, such as with *C. gariepinus* (Viveiros *et al.* 2002, Adebayo 2006). A single female is able to produce eggs throughout the year, dependent on water temperatures being kept at appropriate breeding levels of greater than 25 °C (Bruton and Boltt 1975, Muir *et al.* 2000).
The aim of this research was therefore to detail the basic requirements, both structural and process-wise, for the reliable, systematic production of large quantities of *O. mossambicus* fry.

2.2 MATERIALS AND METHODS

In order to create a fully functioning breeding facility, the process was deconstructed into constituent components deemed required for fry production.

Table 2.1: A listing of preliminary and multiplication components necessary for the establishment and multiplication of a trial *O. mossambicus* population.

PRELIMINARY	MULTIPLICATION
Physical structure	Sexing and sex ratios
Sampling - sourcing and methodology	Nest building
Habituation and quarantine	Courtship
Water temperature	Mouthbrooding and post hatching care
Water quality	Incubation system
Feed	

2.2.1 Preliminary Components

These were defined as the physical infrastructures required for the establishment, maintenance and multiplication of the tilapia population.

2.2.1.1 Physical Structure Component

The animal house within the Zoology Department at the University of KwaZulu-Natal Pietermaritzburg (UKZN-P) was chosen as the trial population site because it contained a room which had three large inbuilt aquaria (Figure 2.1) and a dedicated filtration system. Further structural criteria that needed to be fulfilled were:

- Multiple additional aquaria of 50 l and 400 l capacities for breeding and grow-out stages, to allow for an increase in fry production.
- Requisite equipment for the aquaria. These included submersible filters and heaters (thermostat controlled), lids and aquaria floor cleaning tools, nets, buckets and lengths of airhose.
- Ambient temperature control to reduce heat loss from the glass aquaria
- Light / Dark cycle control to ensure uniform environmental conditions
- Light and dissecting microscopes for minute observations of eggs and early larval stages
- A digital scale capable of 0,01 g increments

The three extant built-in aquaria (BIA1,2,3) were concrete tanks with 20 mm front glass panels with no lids. They were all supplied with water from a 5000 l reservoir which acted as a dechlorination stage for municipal water. The volumes of the three BIA were 350, 700 and 350 litres, respectively (Figure 2.1). Air was supplied to the three BIA via a compressor, controlled via industrial screw valves. Introduction of air into the water column was achieved by airstones suspended approximately 50 mm above the respective aquaria floor to maximise water column contact and thereby oxygen diffusion. Filtration of the three BIA was achieved via undergravel filters which fed a standard swimming pool type sand filter. The use of a single filter for all three BIA ensured equal aquatic parameters across the three aquaria, but it also permitted the cross contamination of the three water bodies with a particularly resilient biofilm, containing a range of organisms including various bacteria and nematodes, as well as aquatic invertebrates.

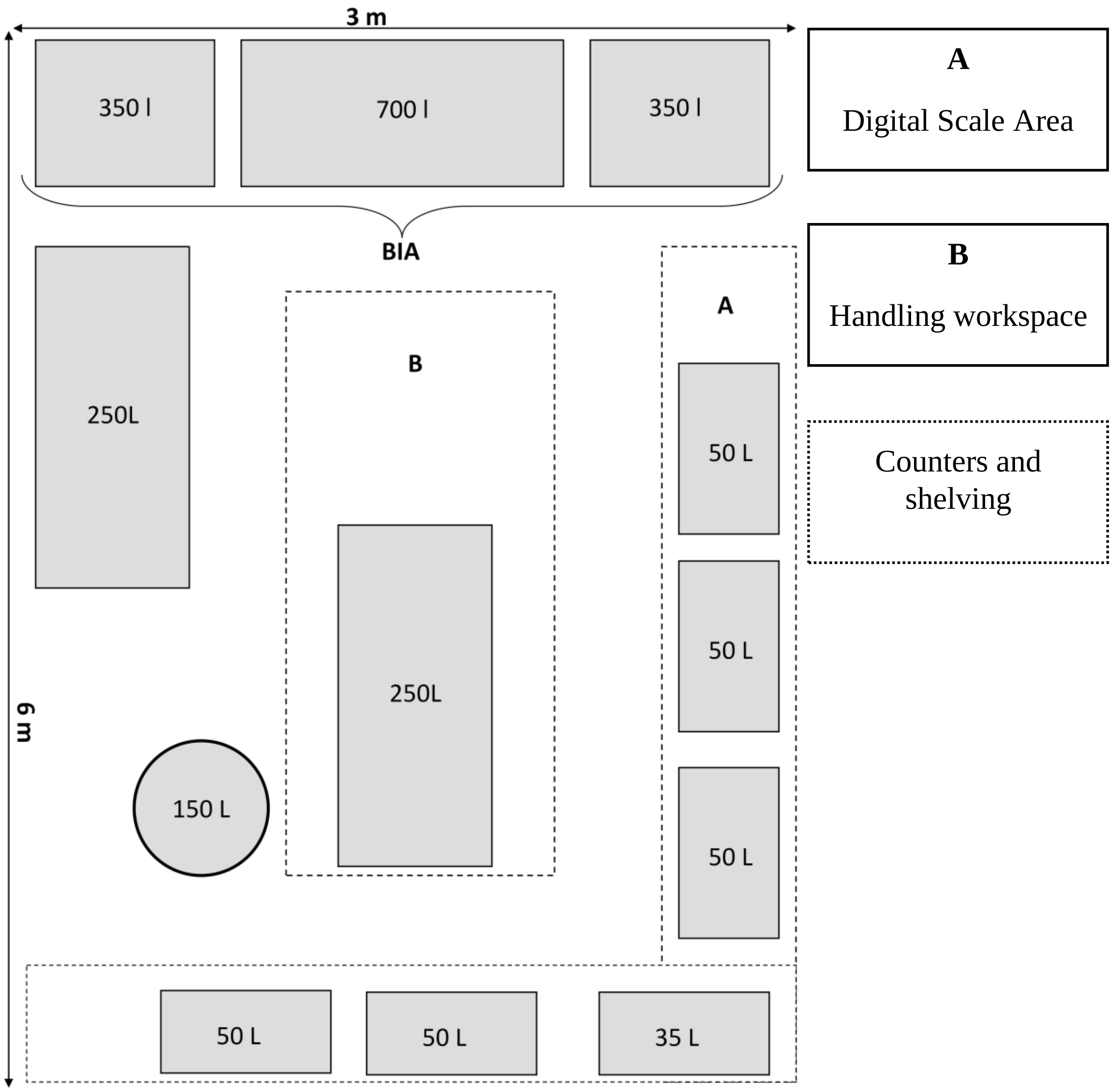


Figure 2.1: Lab dimensions and layout with various aquaria and workspaces

Other problems included:

- The presence of only three grow-out arenas, of which two (BIA1, 3) had equal dimensions while the dimensions of BIA2 were twice as large. No further aquaria of similar volumes could be added due to space constraints;
- The lighting ran on an inbuilt circuit with no timer to regulate the light / dark cycle; Due to the construction design of the room and the embedded filtration network, it was not possible to completely clean the filtration system.

2.2.1.2 Sample Sites - Sourcing and Sampling Methodology

Sourcing of fish

With the abundance of small rivers proximal to UKZN-P, Umgeni Water (Pty) Ltd was approached to supply local river survey records. Three rivers were identified according to the known occurrence of *O. mossambicus* and proximity to the UKZN-P campus. (Map 1: Msunduze R below the Earnie Pearce weir; Blackborough Spruit River at the N3 highway; Foxhillspruit River between Jesmond and Lindup Roads). Foxhillspruit River (29° 37′ 28″S; 30° 23′ 25.5″E) between Jesmond and Lindup Roads was finally chosen as the Pietermaritzburg site due to its accordance with desirable sampling attributes, namely: a slow flowing, small volume stream with discrete large ponds which would facilitate the use of electro-shocking and trapping processes for the sampling of fish.

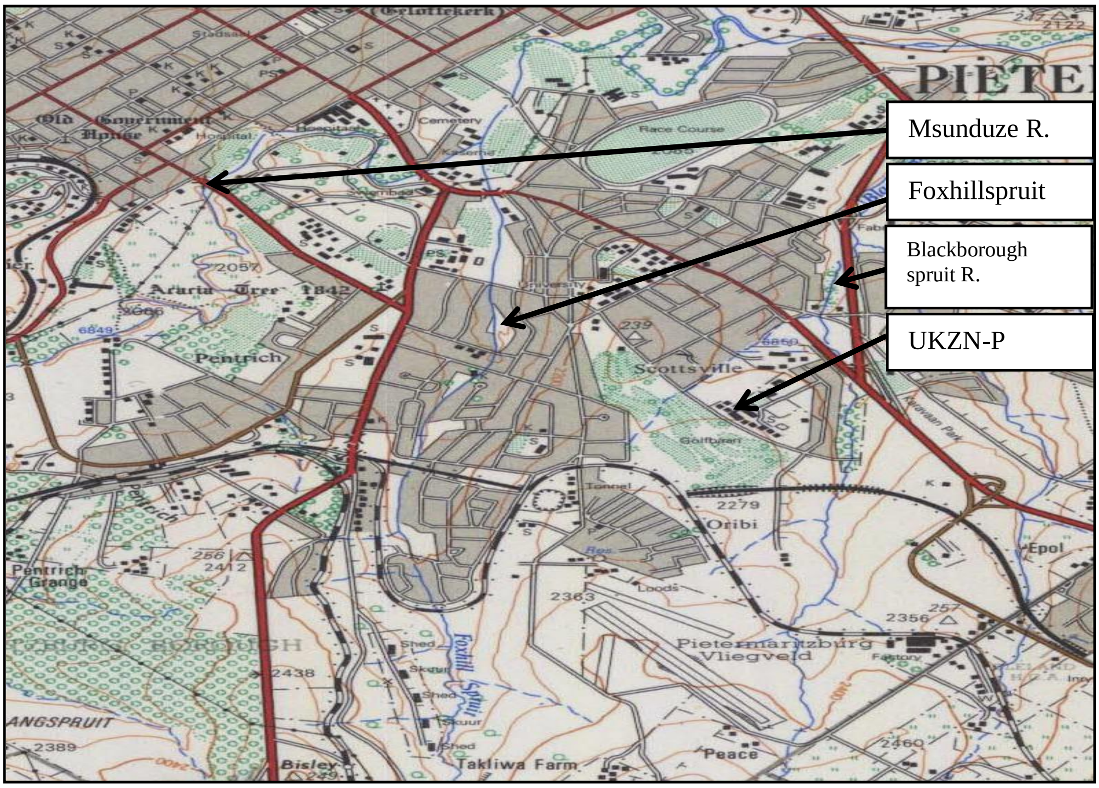
Sampling Methodology

Electro-shocking is a recommended method (A. Sundram pers. comm.) and was incorporated into subsequent sampling techniques. Plastic bottle traps were also used based on previous personal experience.

Pre Sampling

- The relevant permits for the capture of *O. mossambicus* from the wild were obtained from Ezemvelo Wildlife at Queen Elizabeth Park;
- Five 20 l plastic lidded buckets were prepared for the temporary holding of samples by filling them with 15 l of water from the sample site;

- Three small nets of various sizes were also used for the transfer of fish and the removal of non-target species;
- Dungaree type plastic waders were utilised to insulate the sampler against the discharged current. This permitted the sampling of central areas of water bodies.



Map 1: Location of sampling sites

Sampling Method 1 - Electro-shocking (ES)

This was the primary sampling method employed, due to its ease of use and the large mesh size used, which filtered out small, immature specimens.

Construction: Designed and built by Umgeni Water (Pty) Ltd, the ES comprised of a battery pack, step-up transformer with variable voltage and Hertz dials, copper cable (anode) and a wooden, long-handled metal net that acted as the cathode. Shock durations were regulated by the trigger mounted on the wooden handle (Figure 2.2).

Application: Test shocking, less than a second in duration, was done in a side-to-side manner from the exit side of the pond toward the entrance point to effect a 'herding' of the aquatic fauna. Once a shallow area had been reached, lengthier shocks were applied to produce immobilized fish which were then collected in the net.

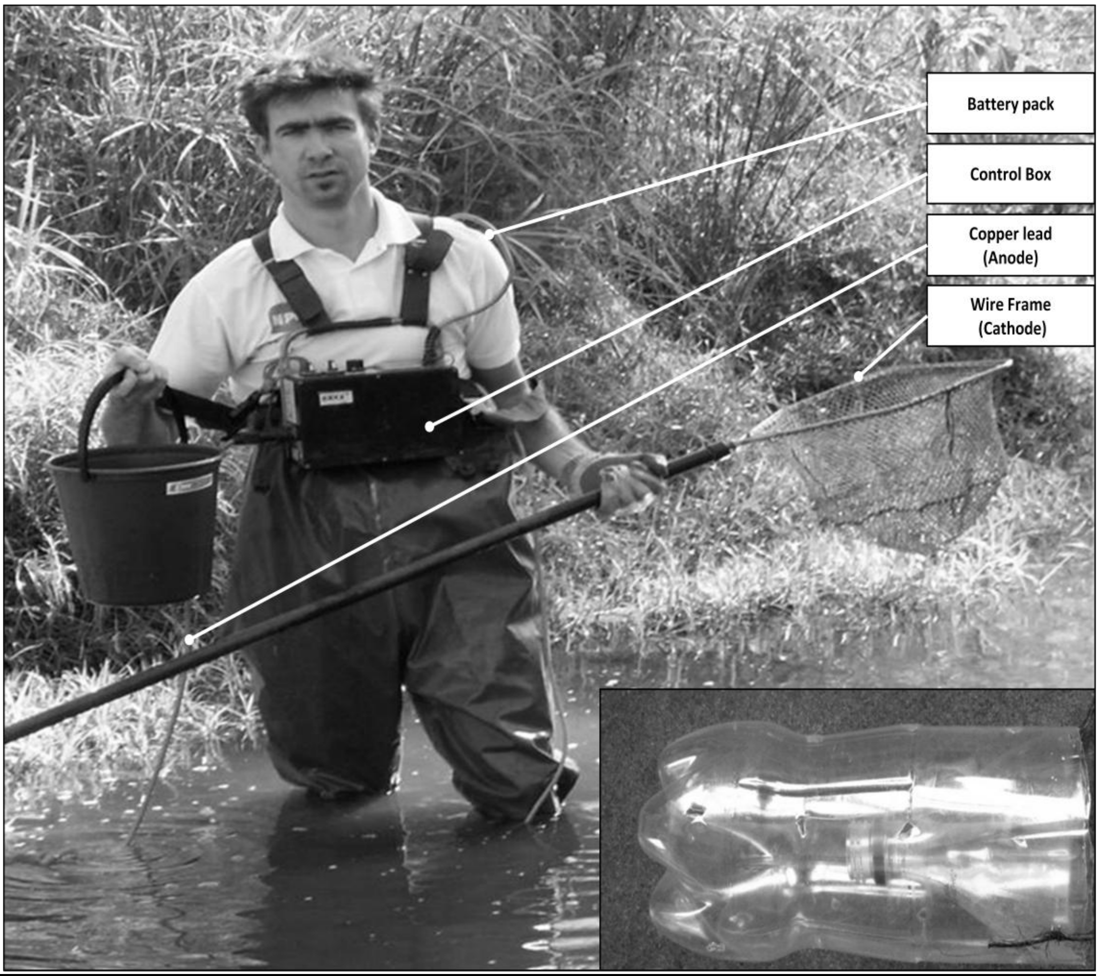


Figure 2.2: Author with ES apparatus on site at Foxhillspruit River. Inset: Bottle trap.

Sampling Method 2 – Bottle Trapping (BT)

Construction: A 2 litre plastic soda bottle was cut, the neck was removed, reversed and stapled back into place. Small holes were introduced into the sides and base of the BT to allow for dispersal of the baits' scent as well as to allow fresh water entry for the trapped fauna (Figure 2.2, inset). Numerous BT entrance sizes were tested, with an aperture size of no more than 30 mm being suitable for river bottom and riverbank application due to the prevalence of platanna's (*Xaenopis laevis,* Daudin 1802) and freshwater crabs (*Potomonautes sp*.).

Application: Canned vegetable bait (KOO™ Tomato and onion mix, 2004/5) was put into the base of the trap. The trap was submerged and secured with string to the river bank, and left for two hours before being checked. Upon observation of trapped species, the head section of the trap was removed and the species poured into a shallow sorting basin. Non-target species were recorded before being returned to the river, while *O. mossambicus* fish were then transferred to the temporary holding buckets.

2.2.1.3 Quarantine and Habituation

Caught fish were kept in 20 l lidded plastic buckets with water obtained from the sample site. Upon arrival at the animal house, UKZN-P Zoology Department, the fish were acclimatized to new water parameters over 30 minutes. This was done by slowly introducing the target aquarium water into the sample buckets at a rate of 25% of the buckets volume (5 l) every 10 minutes to allow the fish to adapt to pH, ammonia, dissolved oxygen (DO) and temperature discrepancies. The fish were then introduced into BIA1 (subsequent habituations and quarantines took place *ex situ* in a 500 l aquarium) and monitored daily. During this quarantine stage the top of the tank was partially covered while the front glass panel was covered in brown paper. This was done in order to minimise fish escaping the tank and to decrease disruptive visual stimuli respectively. The fish were not fed for the first two days during quarantine. Abnormal actions of the fish, such as flicking and scraping against objects, were diagnosed as being symptomatic of external gill flukes. This condition was treated with Parasite Clear Tank Buddies (Jungle™)[1] because it targeted both external and internal parasites present. Diagnoses and recommendations were obtained after consultation with various commercial aquarists as well as aquaculture consultants (L. ter Morshuizen and H. Holder pers. comm.). The lighting was maintained at 12L:12D. Sections of the brown paper were then removed over the course of a week to acclimatize the fish to daily observations. Temperature was kept at 20°C until after the ectoparasite medication was complete, whereupon it was increased to 28°C. Ich / Ick (*Ichthyophthirius multifilis*, Fouquet 1876) outbreaks were only observed after this increase in water temperature, which is consistent with optimal temperatures for *I. multifilis* (Popma and Lovshin 1996) and were successfully treated with Ick Clear Tank Buddies (Jungle™). Habituation to feeding was then initiated by the use of sinking groundfeeder tablets. The use of this type of feed was favoured over the

[1] Jungle Labs ™ www.junglelabs.com

floating type to assuage the observed aversion, of the fish, against coming to the surface to feed.
Habituation was defined as complete upon the observation of ritualised greeting behaviour at feeding times. This typically involved the complete suspension of social hierarchies and the gathering of the fish near the water surface in the anticipation of feed. Quarantine was likewise deemed concluded upon the lack of any signs of distress displayed by the fish. Once all fish displayed good health, they were hand sexed (Figure 2.3) and grouped according to suitable sex ratios per breeding aquarium.

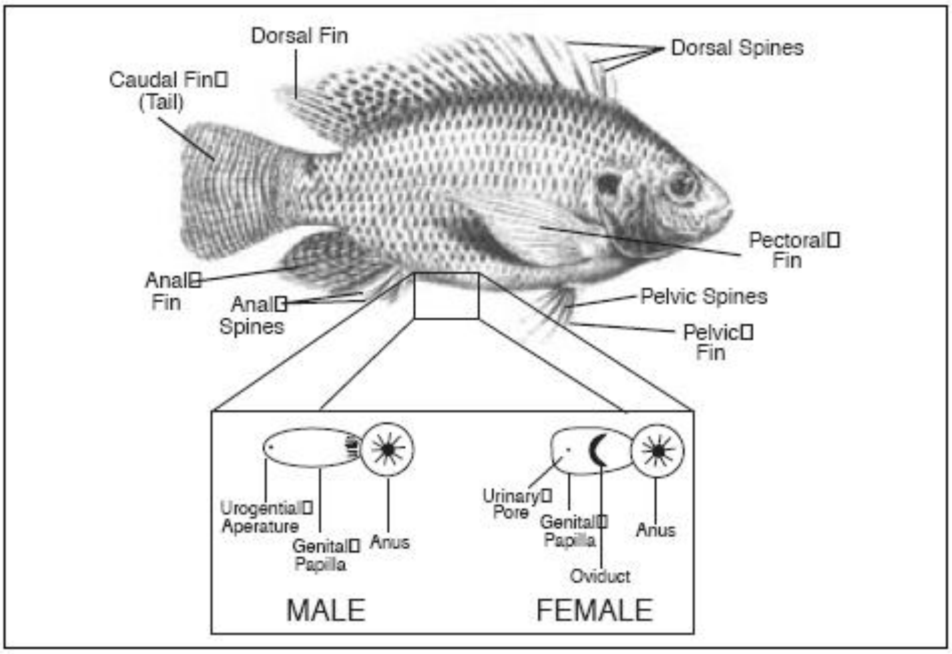


Figure 2.3: Sexual dimorphism in genitals of *Oreochromis spp*. used for hand sexing. Taken from Popma and Masser (1999).

2.2.1.4 Water Temperature

Optimal growth for *O. mossambicus* is typically achieved at 28°C. Water temperature for all aquaria was thus maintained at 28 °C via thermostat-controlled, submersible heaters (Bruton and Boltt 1975, Muir *et al.* 2000) (Table 2.2).

2.2.1.5 Water Quality

Management of water quality is crucial for any aquatic breeding venture. Although *O. mossambicus* is often termed hardy, this refers to its survival and growth. Breeding success however, is greatly impacted by water quality due to its effect on overall broodstock health, with better water qualities resulting in improved fecundity and egg integrity (Meyer 1991). Under the SBF mandate which stipulates that rural waters are to be utilised and treated in-house, water management was solely concerned with the crucial parameters, namely: pH, ammonia, temperature, and DO levels, as well as the maintenance of the biological filters.

- The series of BIA's were not tested due to its large volume of recirculating water (>5000 l). Ammonia levels were assumed below toxic levels due to the non-stressed disposition of the fish and lack of mortalities. All other aquaria had a 10% addition of dechlorinated water per week.
- Evaporative dechlorination was achieved by setting up a large 200 l plastic tub which acted as a reservoir during the removal of chlorine. A standing time of 48 hours was deemed sufficient (L. ter Morshuizen, H. Holder pers. comm.).
- The temperature of all the aquaria was maintained via the use of thermostat-controlled heaters, which were equilibrated to aquatic thermometers, set to 28 °C.
- Efficient filtration was assumed but regular backwashing of the swimming pool type filter (BIA) was carried out. Bi-weekly cleaning of the 250 l and 50 l tanks' submersible filters was undertaken to ensure water flow through the filtration media.
- Optimal DO levels in all aquaria were assumed, owing to low fish densities and the continuous, high volume supply of air to the aquaria via airstones.

Table 2.2: Aquaria dimensions, corresponding volumes and heating supplied

Aquaria Exterior Dimensions l X b X h in mm (glass panel width)	Aquaria Volumes Maintained (l)	Uses	Heating supplied
450 X 320 X 305 (5 mm)	35	Brine shrimp hatchery	50 W
610 X 330 X 250 (5 mm)	50	Swim up fry (<30 d)	50 W
1220 X 440 X 445 (6 mm)	250 **	Juvenile (<60 d)	200 W
BIA 1 & 3: 740 X 540 X 880 (20 mm)	350 **	Grow out	1 X 300 W
BIA 2: 1500 X 540 X 880 (20 mm)	700 **	Mating, grow out	2 X 200 W
2030 X 600 X 500 (10 mm)	600 **	Habituation	2 X 300 W

* *Ex situ*
** Excludes volumes within external filtration system

Table 2.3: Dimensions, volumes, and dedicated filtration utilised per aquarium type

Aquaria Volumes Maintained (l)	Filtration Utilised
50	Magi - 200 (Magic - Jet Filter, by Resun™)
220	Jebao 918 (Zhongshan Jeboa Electrical Appliances Co., Ltd.)
350,700,350*	Swimming pool sand filter 750 W, 4.8 A, 250 V
700	Bowini 915

*excludes 5000 l reservoir and piping volume

2.2.1.6 Feed

Various feed types (protein/carbohydrate ratio, floating or sinking, pellet or flake) are commercially available for cichlids. Previous experience, as well as advice from commercial fish breeders, led to the development of Table 2.4. The amount of feed supplied to each tank was an *ad libitum* quantity until feed was left over for 2 minutes. Excess pellets were then removed, counted, and discarded. Weight of excess feed was then calculated according to

Table 2.4. The amount consumed by the fish was termed the meal feed amount (MFA). Doubling of this equaled the daily feed amount (DFA) and multiplication by a factor of 14 was termed the weekly feed amount (WFA). Weekly feed amounts were determined at the beginning of each week.

Table 2.4: Devised feeding regime according to life stage recruitment

Life Stage Component	Feed Type	Protein %	Carb. %	Mean Weight (g)
Habituation	Groundfeeder tablet GFT	43	8	0.214g / tablet
	floating pellet 2 FP2	42	6	0.02g / 70
Conditioning	bloodworm BW	6.5	0.57	2.86g / tab
	Groundfeeder tablet	43	8	0.214g / tablet
	floating pellet 2 FP2	42	6	0.02g / 70
Fry (<21d)	brine shrimp BS	65	19	n/a
Fingerling 1 (22 – 29d)	Groundfeeder tablet	43	8	0.214g / tablet
Fingerling 2 (30 – 60d)	floating pellet 1 FP1	42	6	0.01g / 150 pellets
Juvenile (61 – 90d)	floating pellet 2 FP2	42	6	0.04g / 150 pellets

Habituation. Nutrafin Basix[2] Groundfeeder tablets (GFT) were used during the habituation stage to supply a high protein food.

Conditioning. This stage was primarily aimed at the females to ensure an optimal supply of nutritional requirements for oogenesis. During conditioning, GFTs were supplied in conjunction with Type 2 floating pellets (Aquafin Colour Enhancing Pellets for Cichlids, large size, FP2)[3] as well as bloodworm (BW). Bloodworm (*Chironomus spp.*) is used as a feed treat by aquarists and was immediately recognised by the fish. It is supplied in frozen tabs of 100 mm^2 which, when thawed, release a few hundred dead bloodworms. Providing less than 10% in protein (Bogut *et al.* 2007), its inclusion in the conditioning phase was to provide natural prey items.

[2] Nutrafin, www.Hagen.com
[3] *www.aquafin.net*

Fry <21 d. Once roughly 80% of the fry batch had completely absorbed their yolk sacs, the first feed type was introduced, namely brine shrimp (*Artemia spp.*). Brine shrimp (BS) are utilised in the aquaculture industry for the rearing of fry due to their high lipid and unsaturated fat content (Sorgeloos *et al.* 2001). Commercial brine shrimp are supplied in cyst form (the embryonic life stage of *Artemia*), which then need to be hatched out. *Artemia* cysts need to be hatched out because the cyst shells are inedible, and may lead to gut blockage of the fish fry. The hatching protocol (Appendix 2.1) was adapted from various sources to produce a 7 day WFA (weekly feed amount) of brine shrimp in solution which was administered via a syringe into the nursery aquaria (Sorgeloos 1980, Sorgeloos *et al.* 2001).

Fingerling Stage 1 (21 – 29 d). This weeks' feeding via GFT was implemented to give a sustained supply of food over an entire day as well as to familiarize the fish with artificial feed types which were to follow. Each nursery tank received one tablet broken into four pieces and dispersed around the base of the tank, with each quarter supporting approximately 5 feeding fry at any one time. This was done in increasing increments culminating in one full tablet broken down twice a day. Breaking and dispersal of the tablets was done so as to reduce the bullying behaviour of the larger individuals over smaller ones.

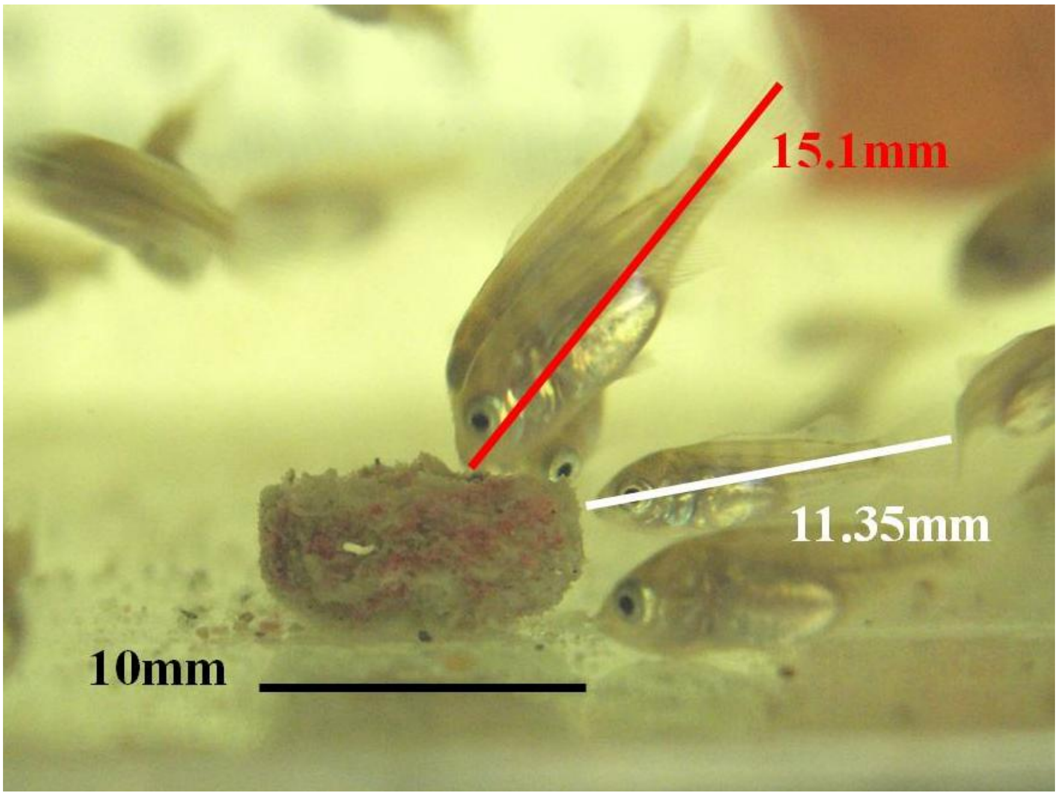


Figure 2.4: Size estimation of young fry (22 d) feeding on GFT. Note the size variations.

Fingerling Stage 2 (30 - 60 d). This growth stage utilised the final feed type, namely Aquafin's Colour Enhancing Cichlid Pellet[4]. This brand comes in two sizes: a mini, and a maxi, which are prescribed according to the buccal size of the fish. Fingerlings in Stage Two were fed the Mini version (FP1). The fingerlings in Stage Two were fed twice a day.

Juvenile (61 – 90 d). Fry in the final stage were then fed the largest pellet size of feed: Aquafin's Colour Enhancing Cichlid Pellet Maxi (FP2) [5]. Juveniles were fed twice a day.

2.2.2 Multiplication Components

These were defined as the biological aspects of *O. mossambicus* concerned with reproduction as well as the necessary protocols, regimes and technologies required for the management of the reproduction on a continuous basis. Due to the scarcity of information pertaining to artificial enhancements to promote regular matings, daily observations of the relevant fish behaviours were made. One set of observations commenced prior to each feeding and were terminated one hour after feeding. The pre-feeding observations of the fish were ultimately discontinued as their social hierarchies dissolved upon their anticipation of food. A further 60 minute observational period was also initiated at a random time throughout the day where my presence was judged not to cause an interruption of ongoing behaviours. Some of the observations made allowed for the engineering of certain enhancements to provide a system for the reliable mating, ease of stripping and subsequent rearing of the fry.

2.2.2.1 Sexing and Sexual Ratios

Sexual dimorphism in *O. mossambicus* can be problematic because the masculine breeding colours are under direct control and can thus be quickly lost under stress (Amorim and Almada 2005). Hand sexing techniques were employed whereby each fish of a sample was examined as to the structure of the urinogenital and anal tracts. Males possess a combined urinogenital tract and thus have two external openings in relation to the females' three

[4] www.Aquafin.net
[5] www.Aquafin.net

openings (Figure 2.3). The three BIAs had been designated as breeding tanks. Typical breeding ratios used in commercial hatcheries propose a 1: 3 male to female ratio (Oliveira and Almada 1998). This ratio was used for all subsequent trials with male numbers per tank being based on 1 dominant male for BIA1 and 3, with a doubling to two territorial males for BIA2. This difference in male numbers was due to the smaller internal floor areas of BIA1 and 3, which dictated a single territory, whereas BIA2 with a larger floor area could accommodate two territories.

2.2.2.2 Nest Building

The methodology and findings are reported in Chapter 3.

2.2.2.3 Courtship

The methodology and findings are reported in Chapter 4.

2.2.2.4 Mouthbrooding and Post Hatching Care

The methodology and findings are reported in Chapter 5.

2.3 RESULTS AND DISCUSSION

2.3.1 Physical structure

The floor space of the laboratory equated to 18 m^2, excluding the pool sand filter, the 700 l *ex situ* habituation aquarium and the 5000 l water reservoir. Should this structure have been purpose-built, only the large water reservoir would need to be housed externally because the actual physical space available would have allowed for the inclusion of the other two components. These dimensions compare favourably with a standardised shipping container of dimensions 6.1 m X 2.4 m X 2.6 m which approximates to 40 m^3 in volume (Export 911 2009).

Despite the small size, some 3000 fish were hatched and grown to swim-up stage within the confines of this structure over a period of three years, all with a staff of one. This admittedly lengthy amount of time, however, is justified in terms of:

1. The lengthy initiation and setup stages as well as the simultaneous testing of different systems, protocols and enhancements included in Chapters 3 to 6.
2. The fact that the trials included in Chapter 6 required that progeny of a single cross had to be housed independently of each other till 90 days of age. This together with the restricted number of grow-out aquaria further lengthened the time required and reduced the stocking rate of the various aquaria.

This compact size is therefore worthy of further investigation, in light of the previously proposed scheme for satellite fish seed stations.

2.3.2 Sampling

Seasonal variation in temperatures and precipitation had a significant impact on species caught, as well as size and abundance of those caught. A further factor on fish abundance was the frequently observed mounds of dumped waste materials, ranging from paint and solvents through to rotting food, corroded metal and human waste, lying along the river bank which polluted the ponds to a greater extent in winter than in summer when regular rainfall events helped flush the system.

Nevertheless, the two methods utilised were successful for the capture of *O. mossambicus* specimens throughout the year, with the electro-shocking (ES) method being more effective due to its greater speed of capture. This difference in speed was exemplified by the typical capture (ES method) of more than 30 fish specimens (pre-identification) within 30 minutes, whereas the Bottle Trap (BT) method required lengthy setup periods of approximately 10 minutes per trap.
Physical removal of the Bottle Trap from the water, for checking at 30 minute intervals, was also needed to be done due to the poor water visibility. This check would further lengthen capture times due to the disturbance caused. Successful capture within a single BT would range from one to five unidentified fish specimens per 30 minute wait period. Due to this lengthy process, five traps would be laid at once which again negatively impacted setup time. Comparative catch per unit time for the two methods could not be done however, as the two methods were never tested against each other, as the BT method was only utilised on occasions where the ES apparatus was unavailable.
A further negative aspect of the BT method was that while the capture of non-target species occurred with both methods, the sedentary positioning of the Bottle Traps did capture

freshwater crabs (*Potomonautes spp*.) while the ES method did not. The capture of crabs, on occasion, led to visible injuries being inflicted on the fish by the crabs' chelae, while furthermore impeding the quick emptying of a BT due to their tenacious grip within the bottle.

Table 2.5: A list of non-target fauna caught using the electro-shocking and bottle trap methods

Electro-shocking	Bottle Trapping
Platanna (*Xaenopis laevis,* Daudin 1802)	Platanna (*Xaenopis laevis*)
Common River Frog (*Rana angolensis,* Bocage 1866) ad. and juvenile	Southern Mouthbrooder (*Pseudocrenilabrus philander*, Weber 1897)
Banded Tilapia (*Tilapia sparrmanii,* Smith 1840)	Banded Tilapia (*Tilapia sparrmanii*)
Barbel (*Clarias gariepinus,* Burchell 1822)	Red breasted Tilapia (*Tilapia rendalli,* Boulenger 1897)
	Freshwater crabs (*Potomonautes spp.*)
	Bow Stripe Barb (*Barbus viviparous,* Weber 1897)

2.3.3 Habituation and quarantine

Habituation and quarantine were achieved simultaneously within two weeks of capture, with specimens caught in summer having lengthier (5 to 10 days) necessary quarantine events than those caught in winter (3 to 6 days). This discrepancy may indicate a simple causal effect that due to colder water temperatures, ectodermic parasites produced less of a parasite load on the host; and furthermore, young weak fish and old compromised fish would have already been overcome by biotic stresses, as well as the abiotic fouling factors mentioned previously. This is supported by the literature whereby thermal tolerance of *O. mossambicus* is usually exhausted at 11° C (Popma and Lovshin 1996) and thus the recorded temperature, at capture, of 11°C (July 2005) would have permitted the survival of only the hardiest specimens.

2.3.4 Water temperature

The only temperature issues faced were during the numerous electricity supply load shedding events experienced in Scottsville, Pietermaritzburg during 2005 and 2006. Temperatures did recede from the optimal temperature range of 27°C - 29°C, but went no lower than 25°C and then only for 36 hours at the most.

2.3.5 Water quality

DO and filtration were the two vital parameters that were most severely affected by the electricity load shedding events. This lack of electricity led to the use of very inefficient battery operated bubblers (to sustain DO) because the room was not connected to the established backup generator circuit. The lack of filtration capabilities was the greatest concern, however, because the metabolites from the fish waste would not be broken down efficiently into nitrite and then nitrate but rather permitted to remain in the highly toxic ammonia form. To combat the potential for lethal ammonia spikes, feeding was completely suspended during the load shedding events and regular cleaning of the tank floor was undertaken. Despite the efforts undertaken, optimum water quality was not maintained and an impact on overall growth was to be expected.

It is worth mentioning that this extreme dependence on electricity for the running of aquaculture ventures must be seen as a double edged sword in that while the dependence is vital, particularly in high density schemes, it also means that the venture can be placed literally anywhere provided that sufficient, reliable power from a central grid or from a generator is available.

2.3.6 Feed

The devised feeding regime, having been based upon behavioural observations, proved reliable in the habituation of the wild-caught specimens, as well as acclimatising the fry to artificial feeds.

2.4 CONCLUSION

Despite the small confines of the laboratory and the related space issues, 3000 fry were hatched and grown to the swim-up stage of development over a three year period. While the majority of the fry produced were not weighed, 300 fry were assessed according to weight attained per unit feed over 90 days growth (Chapter 6).

These first three months in culture of *O. mossambicus* are widely regarded as crucial to attaining a good harvest weight, particularly when one considers that these 90 days comprise 25% of the typical goal of one year of growth till harvest. Furthermore, survival rate of fry sharply increases once the swim-up stage of development has been achieved due to various physiological maturations. As such, it is typical in industry that first handling of fry takes place only upon swim-up stage, with the stocking of outdoor ponds and water bodies taking place at 90 days Stickney 2000, El-Sayed 2006). The results from this mini hatchery therefore, have a number of relevant attributes in relation to rural outreach programs aimed at creating subsistence and/or commercial fisheries

As mentioned above, the dimensions of the workspace fit within a standard shipping container of $40m^3$ in volume. This size opens up various logistic opportunities to cater for the setting up of satellite aquaculture stations or mobile hatcheries via road or rail transfer. Tanks and aquaria need not be made of glass but can be replaced with tougher plastics which would handle transportation as well as providing a reduction in weight and therefore fuel costs. This is typified by commercial operations where very little glass is used, save for quarantine stations, due to their observational requirements. Further benefits afforded by the tested SBF model are the simple and easy to maintain regimes regarding filtration, lighting and heating; as well as the required components which are widely available and competitively priced. With all these factors taken into consideration, it is this author’s belief that such a simplified, and potentially mobile, SBF format would auger well for the uptake of simple aquaculture basics so necessary for a successful, bottom-up approach to skills uptake and implementation in an agricultural field whose minimal environmental footprint and low-fat protein production possibilities can no longer be ignored.

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APPENDIX 2.1: Brine shrimp hatching protocol (from various sources)

1. Aquaria filled to 35 L level, heater set to 25 °C, and allowed to dechlorinate (48hr).
2. Mass of cysts required calculated:
    a. To produce a 3 gl^{-1} concentration of feed within a 250 ml glass storage container, hatched cysts weight must approximate to 0,75 g.
    b. Cysts hatch success = 80%
    c. Therefore CDM (total cyst dry mass) X 0.8 = 0.75 g = 1,88 g of dry cysts to be hatched.
3. Dry cysts added to freshwater (25 °C) hatch apparatus and saturated under aeration for 2hrs.
4. Entire content of hatch apparatus poured through stocking (hosiery) within small net, allowed to drain for 2 minutes.
5. Stocking placed in pure bleach for approximately 10 minutes. A colorimetric judgment as the cysts turn orange when sufficiently scarified. This scarification process is used to speed up the process as well as ensure synchronicity of the hatching.
6. Stocking with bleached cysts then flushed with 8 L freshwater (dechlorinated) and drained.
7. 4,5 g of non iodated sea salt was added to the hatch cylinder and dissolved with agitation.
8. Water in hatch cylinder made up to 750 ml to produce 6 g/l salt concentration. Water taken from surrounding aquaria.
9. Stocking inverted and placed within hatch cylinder, cysts flushed off stocking with syringe. Water for syringe taken from hatch cylinder.
10. Aeration set so as to ensure continuous bubbling of water surface and allowed to run at 25 °C for 36 hours.
11. Aeration and heating removed from hatch system.
12. Hatch cylinder removed from 35 L aquaria and covered within a developed shroud. Light source placed at base of shroud to attract phototrophic Brine shrimp. Hatched Brine shrimp migrate down leaving unhatched cysts and cyst shells floating. Shrouded for 15 minutes.
13. Shroud removed and air hose inserted to base of hatch cylinder, Brine shrimp solution siphoned into 500 ml glass A. The solution was allowed to settle under shroud with base light again for 7 minutes. Remainder of hatch cylinder solution (no Brine shrimp) discarded.
14. Shroud removed; siphoned again into final 250 ml glass storage container B. Excess water containing no Brine shrimp was discarded.
15. At every feeding, Brine shrimp solution was agitated to ensure homogeneity before siphoning off the required amount with a syringe and applied to the nursery aquaria. Brine shrimp MFA's were determined by the observation of visible gut distension of the fry.
16. A Brine shrimp solution was calculated for 5 days and refrigerated between meals.

CHAPTER 3

NEST PREFERENCES IN *Oreochromis mossambicus* (Peters 1852): DETERMINED NEST BUILDER OR OPPORTUNISTIC SQUATTER?

ABSTRACT

Asynchronous hatching of the eggs and spawning asynchrony in female *Oreochromis mossambicus* are two confounding elements which affect uniformity in the fry produced. Typical fish seed production uses large ponds partitioned into breeding allotments or a series of breeding pools. While the earthen ponds provide a substrate in which a nest can be excavated, its presence is not required for mating success in the closely related *O. niloticus* (Linneaus 1758). Female mate choice, as well as apparent fecundity, according to nest size has been clearly recorded in related cichlids but no investigations have been made as to nest size and spawning synchrony in *O. mossambicus*. The focus of this investigation was to ascertain whether *O. mossambicus* would accept artificial nest substitutes in preference to their own constructed ones and whether different alternatives would elicit different levels of acceptance. The observed results indicate a ready acceptance for artificial nest alternatives, with nest lip height being prioritised. The implications are discussed in relation to the potential for optimization of breeding arenas for *O. mossambicus* by the provision of artificial nests whose dimensions satisfy both male and female preferences.

3.1 INTRODUCTION

One goal included in all culturing practices is to provide the optimum environment for the species in question to mate successfully, and as often as possible, without impacting negatively upon long-term productivity. In aquaculture, *O. mossambicus* are referred to as precocious breeders, on the basis of their ability to breed from a very young age of approximately 4 months (Medonça and Gonçalves-de-Freitas 2008, de Lapeyre *et al.* 2009).

This has a large impact on farm productivity if allowed to proceed unchecked because it produces small, non-marketable fish (Kuwaye *et al.* 1993, Bell-Cross and Minshull 1988, Coward and Bromage 2000, Edwards *et al.* 2000, Lowe-McConnell 2000, Turner and Robinson 2000). In order to combat this inherent ability, culture systems for the production of tilapia fry have various protocols in place to ensure frequent removal of fry from predefined mating arenas. Low to medium intensity fry production systems typically take the form of outside earthen ponds which are sectioned off into $1m^3$ pens where one male is stocked with three to five females (Little *et al.* 1993, Little and Hulata 2000). Higher intensity production sees the use of numerous plastic or concrete pools, typically indoors, which replace the outdoor earthen pond setup and allows for greater control of environmental parameters. Another benefit to the constructed tanks is the control of potential pathogen outbreaks that is minimised by the absence of any form of substrate within the tank (Muir *et al.* 2000). Regardless of the operation, the females are then routinely checked for signs of mouthbrooding which is typically indicated by an engorged buccal sac which houses the fertilised eggs (Little and Hulata 2000).

In nature, *O. mossambicus* males construct saucer shaped nests by excavating the substrate with their mouths and then ejecting the material over the rim of the nest thereby creating a sharply defined rim, while deepening the cavity and increasing the nest lip height (Bruton and Boltt 1975). The nest is used as a mating focal point, analogous in some respects to a bird's bower, by which the nest dimensions reflect the male's 'fitness', while also luring the female toward it for the mating act (Oliveira *et al.* 1996, Taylor *et al.* 1998, Tweddle *et al.* 1998, Popma and Masser 1999, Amorim *et al.* 2003, Njiru *et al.* 2006). While it has been reported that the lack of substrate, in high intensity systems, has no impact on mating success in the closely related *Oreochromis niloticus* (Linneaus 1758) (Medonça and Gonçalves-de-Freitas 2008), other reports have indicated that not only the amount of eggs laid by other cichlids are affected by certain nest dimensions, notably nest height, but also that basic welfare of cichlids is affected negatively by the absence of suitable nesting substrate (McKaye *et al.* 1990, Galhardo *et al.* 2008).

In commercial operations therefore, courtship processes are largely dependent on the intensity of the operation because earthen pond mating systems do allow for instinctive nest building to take place, with only a few weeks given for the females to incubate the young from hatching till independent locomotion or swim-up fry stage (Little and Hulata 2000). *O. mossambicus'* well documented spawning asynchrony may be due to numerous random, negative, retarding events which affect the quality of the mating processes (Little *et al.* 1993, Tsadik and Bart 2007). Such events, excluding environmental, can be sub-optimal nest construction / placement by the male or mobbing of the mouthbrooding females by non-brooding females i.e. kleptoparasitism (Dominey and Snyder 1988). These events may result in the following impacts: the male requiring longer to mate with all the females; eggs or young may be ingested by the mouthbrooding females due to stress or be consumed by pen-mates; the loss of condition of the mouthbrooding dam due to the typical non-feeding during incubation, as well as stress due to pen-mate avoidance and / or harassment (Schreck *et al.* 2001, Galhardo and Oliveira 2009). These errors, multiplied over a farm-wide system of such pens and, excluding the well documented and confounding asynchronous fertilisation / spawning issue of *Oreochromis spp.*, usually results in the production of batches of fish young of various ages. These multi-aged 'cohorts' are then grouped together due to the finite number of grow-out ponds available. However, this forced grouping of mismatched ages however invariably leads to bullying and cannibalism, and a decrease in productivity of the operation, not just due to fatalities but also due to the subsequent need for the reconditioning of the dams, which are stripped of fry and not eggs, in order to regain their pre-incubation weight, a parameter that is directly proportional to fecundity (Brandtmann *et al.* 1999, Lorenzen 2000, Hazeltine and Bull 2003, Hatikakoty and Biswas 2004).

The hypothesis behind this study therefore, is that greater synchrony may be possible by the dictating of both nest placement and nest dimension. If aquaculturists were able to dictate both nest placement and nest dimensions within a breeding arena; that this would aid in greater synchronisation between mating pairs by providing greater control over the stimuli required by the fish. The aim of this study was therefore to firstly quantify the relationship between the male's size and the dimensions of his constructed nest and then to determine which factors potentially affect nest placement within a nesting arena. Secondly, this male : nest ratio would then be used to select a commonly available, inexpensive item as a nest substitute. Common availability of the nest substitute, it is hoped, would ensure its uptake in rural aquaculture fish seed stations. Future investigations into whether nest dimensions may affect hormonal response are also discussed.

3.2 MATERIALS AND METHODS

Thirty five *O. mossambicus* specimens were obtained from the Foxhillspruit River in Pietermaritzburg and, following quarantine and habituation to captivity, were sexed and placed in three breeding aquaria (BIA 1,2,3) in sexual ratios of one male to three females per aquarium. Due to the larger floor area of BIA2 ~ 2 m^2, two males and six females were stocked. Thus, 1 male and three females in both BIA 1 and 3, and 2 males and 6 females in BIA2. BIA2 would therefore constitute two arenas of equal size to BIA1 And 3, in effect producing four arenas of comparable size. All of the males used in the following trials ranged in size from 120 mm to 160 mm while the females' size range lay from 75 mm to 130 mm. Feeding was done twice a day till satiation. Temperature of the water was maintained at 28°C.

3.2.1 Trial 1: BIA1, 2, 3. Self built nests

The aim of this trial was to provide basic measurements regarding the nest diameter versus male total length (TL). Corollary observations were also made regarding nest placement choices made by the males in BIA2, owing to the larger floor area, which permitted two territories to be set up. These observations would be used in subsequent investigations into substitute nest preference and placement (Trials 2 and 3).

- Washed river gravel with a minimum size of 10 mm^3 was supplied to all three BIA's to a depth of 30 mm. River gravel was chosen as the medium because it would lessen the impact on the undergravel filtration system, which may have become blocked if a finer material was used.
- With the establishment of a territory by a male and his construction of nests, observations were made as to the size of the nests in relation to the male's size. Each male produced more than one nest. Sizes of the nests were measured with a 5m tape measure extended down and run across the nest diameter. The tape measure had white adhesive tape stuck to it at 20 mm intervals to aid in length determination through the glass and water media.
- The tape measure was then affixed in place and the male was allowed to return. His length was then likewise determined. The size difference between the male and his nest, as well as the ratio of nest to body size of the male fish was then calculated.
- A generalised floor plan was made of the placement of nests constructed by the male *O. mossambicus.*

3.2.2 Trial 2: BIA2, Alternative Nests, Territorial Males Only

Subsequent to the findings of Trial 1, additional trials were then run to ascertain a nest substitute which elicited the strongest response from the males within BIA2. The male-centric preferences were prioritised owing to the sex specific roles in nest construction and defense. Two choices in alternative nest type were made according to the recorded nest diameter in Trial 1 (Table 3.1), while an additional third alternative was also offered which, while of a natural material, was of a substantially smaller diameter. This was done to determine if male *O. mossambicus* would favour natural nest media over an artificial nest of a larger diameter.

- All females were removed from BIA2 to isolate male preferences, and the two remaining males (male 'A' and male 'B') were allowed to acclimatise to the 'empty' tank for two days.
- To ascertain the willingness of the territorial males to accept a ready-made structure as a nest substitute, three different containers were chosen which, while they all provided variations on a nest theme, each alternative also provided singular attributes which would help determine parameter priority according to male *O. mossambicus* (Table 3.1). All substitutes were cured in water for three days prior to use in the trials.
- The gravel from Trial 1 was not removed and the self-built gravel nests were not destroyed. Their placement by the male was assumed to convey that particular males prime choice in that given environment.
- At the onset of each sub-trial I both males' territories received one of the substitute nest types, and the substitute nests were placed adjacent to the existing self-built gravel nests (Figure 3.1). This was done in order to determine the males ranking of his self-built nest (size and placement) versus an adjacent artificial one.
- Time Taken for Acceptance (TTA) was recorded in seconds. This was defined as the time taken for the male to hover over the nest for at least 3 seconds (Oliveira and Almada 1998).
- Nests were replaced after fifteen minutes. Positions of replacements were not altered. Upon change of nest type, two minutes were awarded for acclimatisation due to the disturbance.

Table 3.1: Substitute nest types and corresponding parameters

Type	Description	Lip Height	Nest Diameter	Nest Colour
A	Clay Pot Holders	30 mm	200 mm	Brown
B	Plastic Dogs Bowls*	150 mm	400 mm	Green
C	Coconut Halves*	60 mm	150 mm	Brown

* Items were glued to roof tile fragments to combat buoyant tendencies. Glue was silicone sealant and each nest item was cured for three days in dechlorinated water.

Table 3.2: Order of Replacement of Nest Types in BIA2 during Trial 2

Sub-Trial	Nest Types	Position in BIA2
I	A then B then C*	1
II	A,B,C**	2
III	A, B	1 / 2
IV	A, B*	2 / 1

* Each nest alternative was removed after 15 minutes
** Type C was discarded after Sub-Trial II. Position Numbering Refers to those stipulated in Figure 3.1.

3.2.3 Trial 3: BIA2, Mixed Community with Nest Substitutes

Following from the results from Trials 1 and 2, a similar trial was run with the inclusion of female *O. mossambicus*. This was done to determine the acceptance / preference of the females for the nest substitutes and the effect of the females' presence on male acceptance of the nest substitutes. Nest type C was discarded during Trial 2 as it was seen as a food source.

- BIA2 community was allowed to acclimatise socially for two days prior to the trial. During this time territorial males rebuilt / repaired previous gravel nests. Gravel nests (self built) were not destroyed.
- BIA2 received the two nest substitutes from Trial 2 (A - clay underpot and B - dog bowl) Nest substitutes were placed according to Table 3.3.
- Each male received the same nest type at the same time.
- Two minutes were awarded for the disturbances between nest changes.

- TTA was again deemed an accurate parameter of nest choice because ‘settling’ in this case included the proximity of a female. TTA was recorded in seconds.

Table 3.3: Order of Nest Type Substitution in BIA2 during Trial 3

Sub-Trial	Nest Type	Position
I	A	1
II	B	1
III	A	2
IV	B	2

3.3 RESULTS

3.3.1 Trial 1: BIA1, 2, 3, Self Built Nests

Table 3.4: Dimensions of males and respective self-built nests

Parameters recorded	BIA1	BIA2*		BIA3	Mean ± SD
Number of Territorial Males	1	2		1	
Number of Nests Built	1	3	2	2	
Mean Nest ø (mm)	240	202	182	155	193 (24)
Mean Nest Lip Height (mm)	70	70	70	70	70 (0)
Male length (mm)	150	140	120	130	135 (12.9)
Nest diameter / Male TL	1.6	1.4	1.5	1.2	1.4 (0.2)

*Two territorial males in BIA2

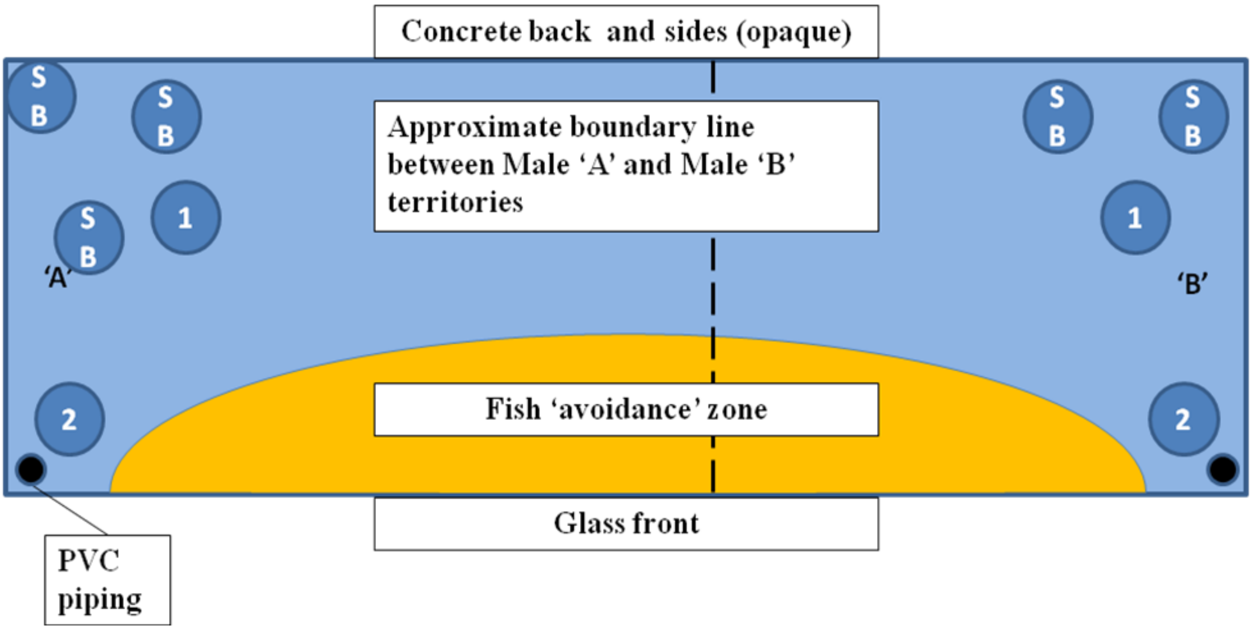

Figure 3.1: Generalised layout of self built (SB) nests by territorial males 'A' and 'B' in BIA2. Positions 1 and 2 indicate substitute nest placement points for Trials 2 and 3.

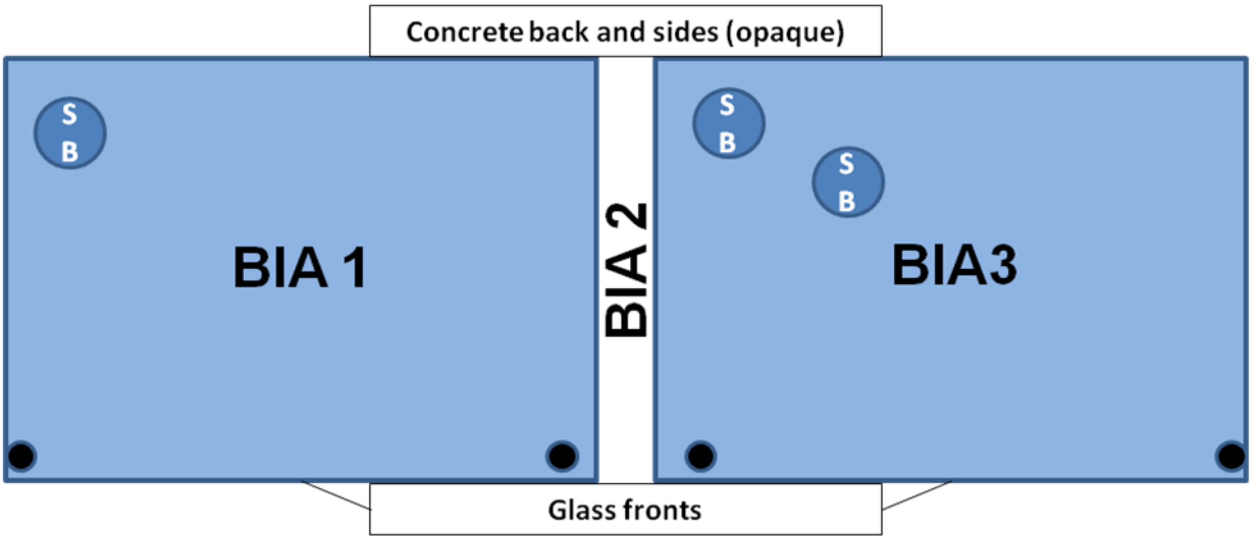

Figure 3.2: Generalised layout of self-built (SB) nests by territorial males in BIA 1 and 3.

3.3.2 Trial 2: BIA2, Territorial Males Only, Nest Substitutes

Table 3.5: Time taken for acceptance (TTA) of nest substitutes by male *O. mossambicus*, recorded in seconds

Trial	Nest Type Offered	Position in BIA2*	TTA in seconds for males A and B per nest type		
I	A then B then C	1	308	273	-
II	A,B,C**	2	230	175	-
III	A*** B	1 2	183	170	
IV	A B	2 1	-	200	

* Position refers to Figure 3.1.
** Nest Substitute Type C was removed from testing after Sub-Trial II
*** Nest type A at position 1 followed by nest type B at position 2
**** Sub-trials were run once due to the learning behaviour of the fish

3.3.3 Trial 3: BIA2, mixed community with nest substitutes

Table 3.6: Time taken for acceptance (TTA) of nest substitute types in BIA2 in seconds.

Sub-Trial	Nest Type	TTA Male A	TTA Male B
I	A - Clay Pot	442	367
II	B - Dog Bowl	485	450
III	A - Clay Pot	-	115
IV	B - Dog Bowl	930	915

*Sub-trials were run once only due to the learning behaviour of the fish

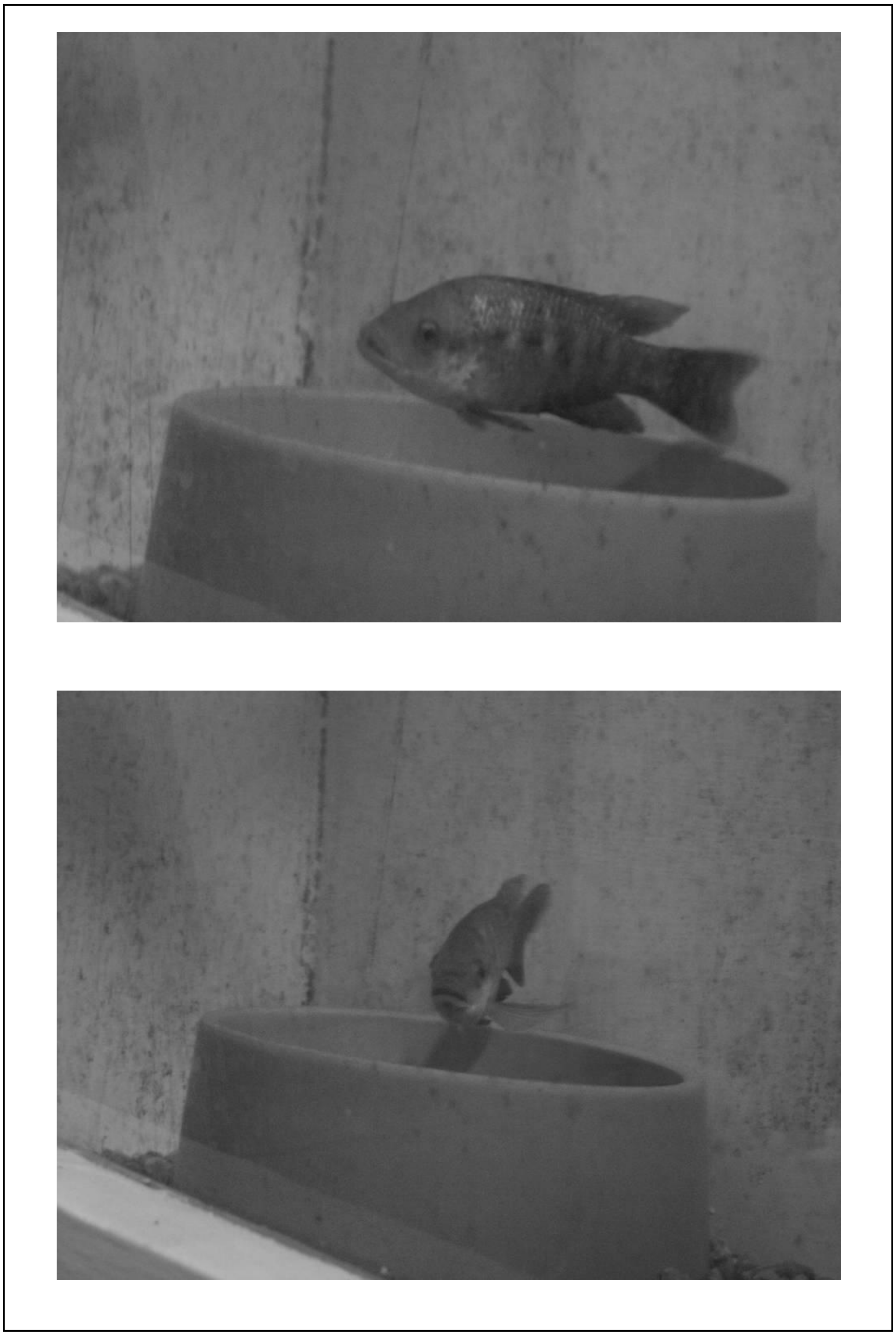

Figure 3.3: Typical “hover” display of nest acceptance by Male ‘A’ over nest Type B.

3.4 DISCUSSION

In general, the results indicate a ready acceptance of artificial nest structures by both *O. mossambicus* males and females, which supports previous investigations in the closely related *O. niloticus* whereby nest (self-built) deprivation had no impact on mating success (Medonça and Gonçalves-de-Freitas 2008). An earlier investigation into a criterion for sexual selection by the female as regards nest dimensions was also supported by these findings in that nest height of the nest structure seems to be the governing factor as regards structure preference by the males and the females in the cichlid *Cyrtocara eucinostomus* (Regan 1922) (McKaye *et al.* 1990).

The male *O. mossambicus'* varied preference for the different nests did indicate differences, but the lack of replication excludes statistical analysis. The apparent preference, however, may indicate an instinctive scale-of-fit to which the artificial nests were compared and then scored; the highest scoring being the structure most favoured. One such factor of the nest structure which seems to be scored highly by male *O. mossambicus* is the lip height as was proposed elsewhere (Miranda *et al.* 2005). A similar template for the mating arena is proposed here to occur in female *O. mossambicus* which have been shown to be able to 'improve' their fecundity based purely on nest size (McKaye *et al.* 1990).

From Trial 1 it can be reasonably assumed that the self-built gravel nests, specifically the sizes and positions within the tank, indicate the optimal compromise between attraction potential for the females, and security and energy expenditure by the male builder. In experiments examining *O. niloticus'* mating success without substrate, it was suggested that territory defense and aggression displayed by the territorial males without any substrate was due to vacuum behaviour, i.e., the behaviour manifests itself without the prescribed stimulus. It is this author's belief however, that the substrate is not the stimulus worthy of defense but rather that the optimal nest / mating sites available are the stimulus, which in this case, would be the corners, furthest from the glass and related visual disturbances (Mendonça and Gonçalves-de-Freitas 2008, Galhardo and Oliveira 2009). This is further supported by the observations in BIA 1 and 3, where the territorial males likewise built their nests against the far wall corners (Figure 3.2).

In Trial 2, without any females present, nest Type B or plastic dogs bowls were seen to be the preferred choice of nest as its acceptance was faster than that of Type A or C. The reasons for its acceptance are supported in part by the results from Trial 1, whereby the largest male produced the highest nest lip. The provision then of a nest (alternative) whose dimensions provided an acceptable and perhaps superior nest structure worthy of defense may be the factor which assured its preferential selection by the males. Time spent over nest Type B far exceeded time spent over other artificial or self-built nest types. The provision of coconut halves (Type C) was not accepted as a viable nest because the males treated them solely as a potential food source, which resulted in them being pushed and pulled randomly and in some cases even being pulled into a nest, destroying a portion of the nest lip which led to the coconut half being removed from the nest to allow for nest repair to take place.

In Trial 3, an apparent paradox was noted because Male A, the TDM, took longer to accept the new nest structures compared to Male B, the subservient male. This paradox, noticeable in Trial 2 as well, may be explained by the social hierarchies evident during these trials. While Male 'A' was the tank's dominant male and exerted greater influence over Male 'B', the majority of his time was spent in recruiting the females (Trial 3) and patrolling the boundary line between his territory and that of Male 'B' (Trial 2 and 3). Male B was thus most often seen to be guarding his nests, and was thereby quicker to hover over a new nest offering.

In summary, the trials indicate a generalist acceptance of nest structures by both male and female *O. mossambicus*. This acceptance however, was observed to be substantially lower in importance than the proximity of any female, because approaching females were always courted toward the nearest available nest - be it self-built or artificial. This fact was supported by the females' acceptance of the male's lead, i.e., females never went to empty nests when being courted. This may indicate two complimentary sex-specific strategies for optimizing genetic investment in that specific mating process: Firstly, if female fecundity is directly affected by visual and olfactory stimuli in *O. mossambicus*, then the above observations would indicate a female strategy whereby evaluations of mate choice by the female results in her laying egg numbers proportionate to the nest size to which she was being led, as well as the strength of the chemical signals being used by the courting male (Oliveira *et al.* 1996). The second strategy (male) is that, owing to the male-directed courtship, his selfish interest may lie heavily skewed toward mating success with as many different females as possible. This promiscuity with multiple partners improves, genetically, both the chances for heterozygosity and fry survival (more maternal care). A further factor may also be the effective ‘swamping’ of females with his progeny which, although the batch may be short of maximum amounts (i.e. continuing the argument that non-optimal nest dimensions would produce non-optimal egg batch sizes) their oral incubation would effectively exclude other males from producing progeny with that female during that spawning event.

These proposed relationships need to be further investigated whereby nest sizes offered, female mass and resultant batch sizes are examined concomitantly, and quantitatively, to further prove the existence of a direct-visual response in *O. mossambicus*, as in other cichlids. An additional proposal worth investigating is the sexual specificity of the observed nesting behaviour. Should this well documented and rapidly employed ‘hover-over-nest’ behaviour be conclusively proved to be a pure male behaviour, then the artificial nests could be utilised in systems to assist in the sexing of fish. Results from such investigations would provide great insight into the sexual processes involved in *O. mossambicus* and provide tools for their manipulation in order to maximise productivity.

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CHAPTER 4

A GLOSSARY OF BEHAVIOURS (SOCIAL INTERACTIONS AND DOMINANCE) AMONG A MIXED-SEX CAPTIVE GROUP OF *Oreochromis mossambicus* (Peters 1852) DURING STABLE AND ENFORCED DISRUPTIVE EVENTS

ABSTRACT

Oreochromis mossambicus displays various complex and ritualised behaviours during stable and disruptive relations with other community members. The aim of this research was to establish a definitive glossary of behaviours that qualitatively captured the major behaviour patterns of *O. mossambicus*. These results reflect not only a dynamic social structure, signaled via various chemosensory and visual methods, but also supported recent findings in apparent male-male courtship and the underlying causes. Observed activities of two territorial males, which shared a common aquarium, included numerous antagonistic behaviours absent in other aquaria where only a single dominant male was present. This corroborates the current practice in aquaculture whereby only one male is allocated per breeding arena. This glossary will permit further quantification of the observed behaviours in order to determine levels of energy investment which has a direct impact on fertility and fecundity.

4.1 INTRODUCTION

Oreochromis mossambicus, as a maternal mouth-brooding member of the species-rich cichlid family of teleost fishes, displays significant plasticity in mating behaviours. One important factor is the establishment of dominance, upon which all subsequent mating behaviours and social interactions are based (Oliveira and Almada 1996a, 1998a, 1998b, Taborsky 2001, Chase *et al.* 2002, Oliveira *et al.* 2005, Barata *et al.* 2007).

In nature, male *O. mossambicus* males aggregate in arenas, termed leks, and excavate pits or nests in the substrate, which are then vigorously defended. Female *O. mossambicus* which are ready to breed travel to these leks and courtship of the females by the males commences. It is at this point that inter male-dominance hierarchies come to the fore. *O. mossambicus* dominance hierarchies have been described as linear (Oliveira and Almada 1996a, Oliveira and Almada 1996b), while displaying reversible plasticity (Oliveira *et al.* 2005). Linear dominance implies that dominance of Male A over Male B and B over C, equates to Male A being 'automatically' dominant over Male C. Reversible plasticity refers to the ability of *O. mossambicus* males to utilise both bourgeois, direct, primary access to females due to nest construction, defense, and marketing thereof, and parasitic, secondary, shared access to spawning females via sneaking and kleptogamic fertilisation modes. Both achieve mating success, regardless of social rank (Taborsky 1994, Oliveira and Almada 1996a, Taborsky 2001).

Because of these various complex and energy expensive male-specific strategies, as well as the well documented asynchronous hatching and spawning asynchrony of the females, typical aquaculture operations dealing with fry production of *Oreochromis spp.* rely on breeding tanks with 1 male to 2 - 5 females per partition (Bentsen *et al.* 1998, Bhujel 2000, Little and Hulata 2000, Bolivar and Newkirk 2002). Outdoor earthen ponds are most often utilised in fry production systems because they are able to produce large numbers of fry and consist of more natural arenas for courtship and mating by providing a nesting substrate which, while not essential for mating success (Medonça and Gonçalves-de-Freitas 2008), has been shown to affect mating quality through its impact on welfare of the male *O. mossambicus* (Galhardo *et al.* 2008).

The aims of this study were firstly, to establish a stable community with multiple males in order to map aquarium partitioning and to note the behaviours used by various social groups while furthermore evaluating observational evidence for the commercially accepted single male breeding system. A second aim was to make note of any social changes within the community resulting from the introduction of differing sized new males into the community. Results from these trials would firstly provide a reference table of behaviours which would permit subsequent quantification, while responses to enforced disruptions may also indicate further areas of research toward the management of commercial seed production of *O. mossambicus*.

4.2 MATERIALS AND METHODS

4.2.1 Sample Sourcing and Environmental Regimens

- The specimens of tilapia (*O. mossambicus*) were obtained from the Foxhillspruit River in Pietermaritzburg (29° 37′ 28″S; 30° 23′ 25.5″E). Sampling protocol and habituation is covered in Chapter 2.
- One community aquarium (BIA2, 700 L: 1500 X 540 X 880 mm) was stocked with a sex ratio of 1 male: 3 females. Previous observations during habituation indicated that two territories were the norm for this aquariums available floor area. An additional male was added however, to fulfill the role of 'floater' (Oliveira and Almada 1998a, Oliveira *et al.* 2005) thus totaling the aquarium occupants to 3 males and nine females.
- Water temperature was maintained at 28°C to simulate breeding conditions.
- The fish were fed twice a day till satiation with Aquafin Colour Enhancing Cichlid Pellet.
- The light: dark cycle was kept at 12L: 12D.
- The fish were grouped according to size, with BIA1 containing the largest, BIA2 the average and BIA3, the smallest of the fish sample.
- The two adjacent tanks BIA 1 and 3 (350 L: 740 X 540 X 880 mm) were equally stocked with 2 males and 6 females each. These were to provide the males for Trials 2 and 3.

4.2.2 Trial 1: stable community

The BIA 2 community was allowed to acclimatise over one month. No intervention was made on brooding females. Following this, observations of social interactions were made during the 60 minutes prior and post feeding. The prior observations were subsequently discarded due to the conditioning of the fish which resulted in the abandonment of social interactions in their anticipation of food. The males (A, B, C) of BIA2 were all between 120 to 160 mm long and known to be sexually mature from previous observations of breeding colours. The females' lengths ranged from 75 mm to 130 mm. Cleaned river gravel, > 20 mm^3, was supplied to a depth of approximately 50 mm.

Post feeding, observations were made of aquarium partitioning according to social group, as well as the causative sex specific interactions resulting in the partitioning.

Males: Observations were made of male behaviours (active or passive) and classed according to male - male or male-female interactions. Behaviours were monitored until termination of said behaviour. Termination of behaviour was defined by the male either:

1. Coming to rest,
2. Initiating a different behaviour,
3. Targeting a different individual.

The initiation and resultant impact of these behaviours on other community members was used to infer the behaviour's social role. Aquarium utilisation by males was also recorded in order to determine their sex-specific component in aquarium partitioning.

Females: Observations were similarly made of female behaviours (active or passive) as well as the breeding status of the monitored female. Aquarium utilisation by females was also recorded.

4.2.3 Trial 2: addition of similar sized new male

A new male (D) was introduced into BIA2 from BIA3. Male D was of a similar length (140 mm) to the two territorial males already present. The BIA2 non-territorial Male C was not removed. The new male had been judged to be a floater / sneaker male as he did not build a nest nor defend a territory but did, on occasion, display breeding colours while following females (Oliveira *et al*. 2001). This new male was tagged by clipping the ventral fin. Observations were made for one hour after each of the two daily feedings as the social interactions resumed post-feeding. The new male was removed after fourteen days and returned to BIA3.

4.2.4 Trial 3: addition of significantly larger new male

A larger male (E, 180 mm) was introduced into BIA2 from BIA1. The BIA2 non-territorial Male C was not removed. The new male had been judged to be the BIA1 floater / sneaker male according to Oliveira *et al*. (2001). Observations were made for one hour after each of the two daily feedings. The new male was removed after fourteen days and returned to BIA1.

4.3 RESULTS and DISCUSSION

4.3.1 Trial 1: stable community

Social partitioning and tank possession.

The tilapia all displayed previously reported behaviour typical of group formation and social dominance in *O. mossambicus* (Oliveira and Almada 1998a, Amorim *et al.* 2003). In BIA2, two males set up territories at opposite ends of the aquaria (A and B, Figure 4.1). Both were termed territorial males or nestholder males (Oliveira and Almada 1996a) but A was termed the Tank Dominant Male (TDM) as he exerted visible dominance over Male B. The remaining ten fish, including the non-territorial Male C, occupied the higher shaded area of approximately the upper third of the tank volume. This was termed the 'female' stratum (Figure 4.1). Both Male A and B excavated two prominent nests, each with other smaller, indistinct depressions also being visible. The area which saw responsive antagonistic behaviours between male A and B was inferred to be the common boundary between male A and B.

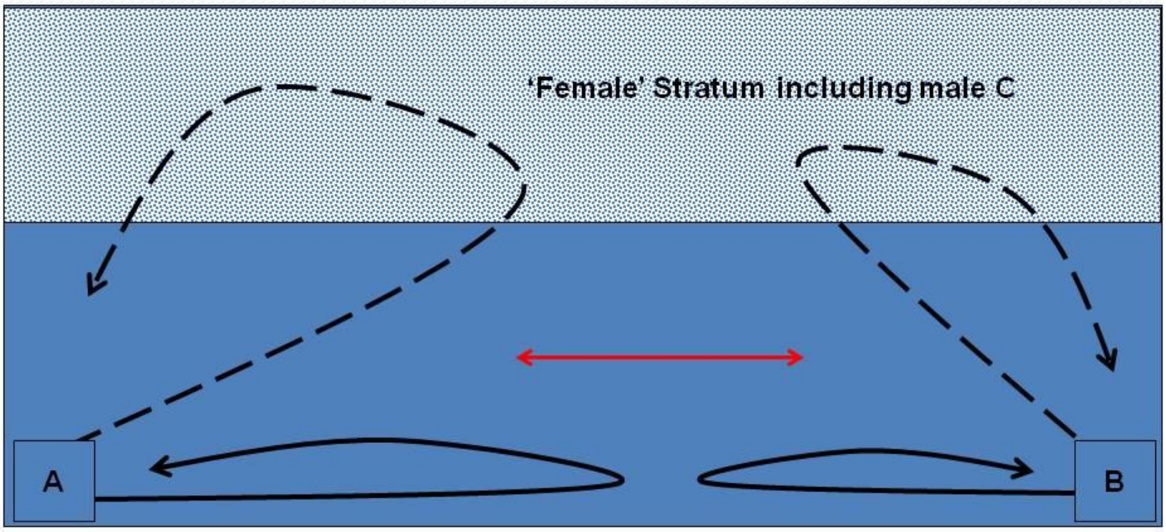

Figure 4.1: Generalised lateral representation of relative aquarium possession and actions by territorial Males A and B as well as the 'female' stratum. Red denotes where responsive agonistic behaviours were witnessed. Boxes denote general nest(s) area.

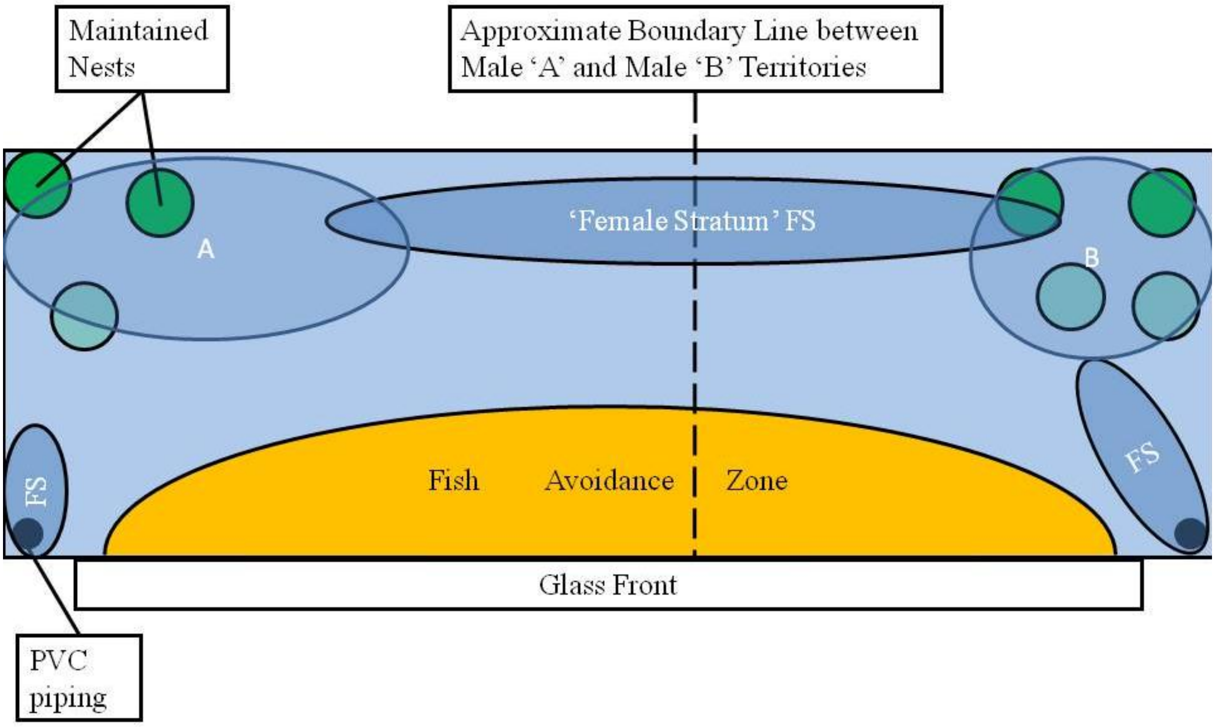

Figure 4.2: Top down, generalised view of tank partitioning, nest placement, and high and low prevalence areas for community members.

Owing to their recent introduction to captive conditions, the tilapia tended to shy away from the glass front of the aquarium. The largest community segment, the female stratum (FS) tended to school continuously in the upper third of the water column (Figure 4.1), with the majority of their time spent at the rear wall, with individual fish intermittently using the vertical PVC pipes in the front corners as refuge sites (Figure 4.2). These smaller sites were most regularly used by mouthbrooding females, as the space between pipe and aquarium wall provided a darker recess in relation to the rest of the aquarium. Brooding *O. mossambicus* females tend to occupy areas on the fringe of a female grouping in order to avoid harassment. The utilisation of these refuges by the fish during these trials does corroborate a need for such sites, particularly within breeding arenas where aggression is more commonplace (Oliveira and Almada 1998c).

The two territorial males were never observed to swap or alternate their respective nests and with the dominance of Male A over Male B and his heightened aggression and resultant instigation in both male - male and male - female encounters. These actions resulted in the FS gathering directly over Male B's nesting area. This further escalated agonistic behaviour between the two males and led to repeated dashes by Male A in an attempt to dislodge their 'encampment' over the subordinate male's territory. "Dashing" is a term proposed here, due to its prevalence during all trials, as well as its observed role in both male - female and male - male courtship encounters. The movement appears very similar to the upward charge to retrieve floating food. Here a modified version was observed where the object was to scatter the FS into smaller, manageable pieces (broken line, Figure 4.1). This action was also taken by Male B, although to a far lesser extent in both duration and intensity, which is most likely explained by the relative proximity of the FS as well as the subsequent aggressive response from Male A.

Male - Female Interactions

While charging *per se* has been reported as a typical antagonistic behavioural pattern in *O. mossambicus* (Oliveira and Almada 1996a), observations made here indicated a modified strategy whereby the male-male charge had been altered to serve the nestholder males in a courtship manner within this captive community. Typical courtship in *O. mossambicus* requires a single female to be the target of the male's attention, which the close-knit 'female' school did not permit. An inference, as to the role of the behaviour, was then made in that this grouping of females had to be broken up prior to the typical rear charging which was always focused on a single target. These modified charges, or dashes, would always commence from near the nest, i.e., low in the water column, and then proceeded rapidly into the ‘female’ stratum and back to the starting point (dashed lines, Figure 4.1). These rapid upward paths would be repeated continuously until one of two things happened:

1) The two territorial males would approach the “no-man’s land” whereupon a border rivalry would ensue between the territorial males with repetitive mock charges against one another, the gap between the two males slowly widening until a ‘truce’ had been reestablished (solid lines, Figure 4.1). The males would then return to their nests to hover for a short period (< 3 minutes) before resuming either the upward dashes or general exploratory behaviour.

2) A female target was successfully separated from the ‘female’ group and in their avoidance of the male had actually neared his nest.

The ‘promotional’ looping dashes, exemplified by Male A, having resulted in the breaking up of the ‘female’ community into fragments, allowed the nestholder male to target single fish for subsequent courtship. At this point, one of two strategies would become apparent, dependent on the position of the female target in relation to his nest. These strategies were descriptively termed ‘pushy’ and ‘coy’ strategies. If the target female was still high up in the water column, or too close to Male B’s nest, Male A would continue with the dashing raids (pushy) in an attempt to drive the target downward and toward his nest. The target female’s vertical position in the water column was observed to be of greater concern to the male than its horizontal distance from the nest; and all the males observed would first force the target down before luring them along the horizontal plane. These aggressive measures would only lessen upon firstly, the female target dropping to a satisfactory level and secondly, the target becoming motionless.

Subsequently, the suitor male would then adopt the ‘coy’ strategy whereby his aggressive physical attacks would lessen in intensity and he would instead attempt to lead the target from the front. This ‘leading’ was done by coaxing body positioning with repeated dashes to the rear of the female (’pushing’) and various suggestive actions such as swimming to the nest and then back to the female (‘leading’).

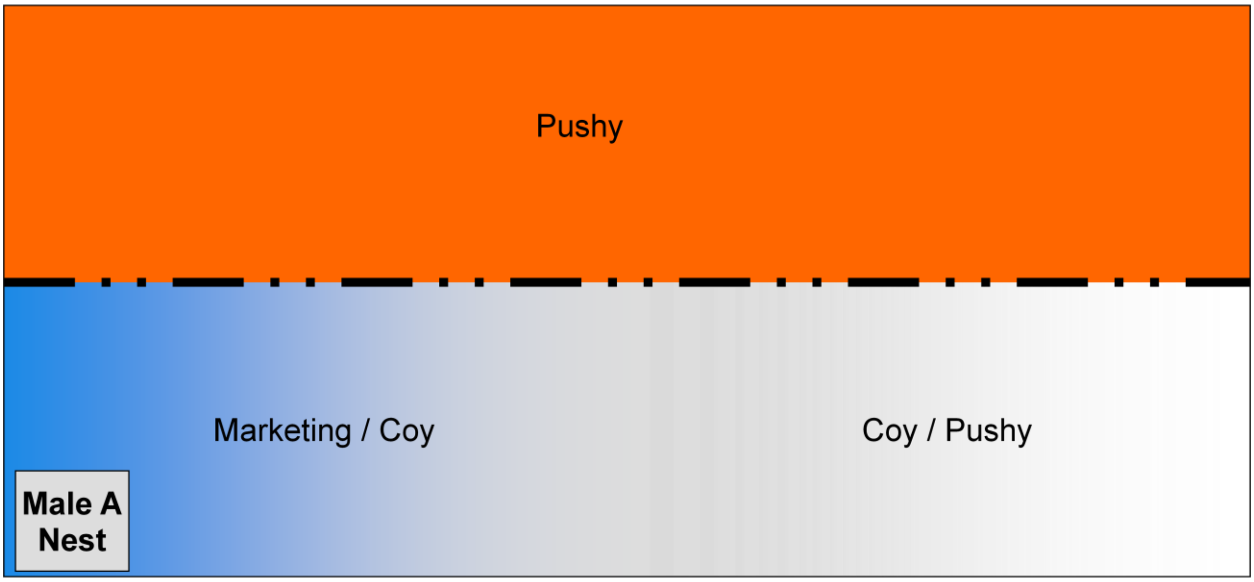

Figure 4.3: Generalised zoning of BIA2 according to the observed pushy / coy and nest marketing strategies employed by tank dominant Male A.

One such action that was often used was that the male positioned himself ahead and just above the target. Territorial males were never observed to position themselves in a direct, obstructive line between target and nest. The male would then rapidly thrash his posterior in a side to side manner which would physically thrust the female downwards. Tail beating / wagging has been reported before, as an agonistic interaction between competing males (Oliveira and Almada 1996a, Amorim *et al.* 2003). The observations here, witnessed to occur against both females and males, was termed 'body washing' and was observed to occur in an increasing intensity upon the cessation of movement of the target within the 'coy' zone. This action was never witnessed taking place above that approximate halfway mark denoted in Figure 4.2. Other than producing the desired downward movement, literature does provide another motivation for this energetically demanding action. Oliveira *et al.* 1996c, Oliveira *et al.* 2005, Barata *et al.* 2007, Almeida *et al.* 2005 all reported that this action may facilitate the simultaneous release of pheromones via urine, which would then be washed over the female in an effort to elicit a complimentary hormonal response from the potential mate.. All movements and actions of the male would increase in speed and intensity as the female approached the nest, whereupon 'marketing' of the actual nest would commence.

The ‘nest marketing’ display by the male involved the male repeatedly swimming from behind the female and then entering the nest cavity. The male would settle on to the base of the nest and begin twitching his body in an attempt to lure the female down to him. It has been reported that it is at this stage that sound production and some milt excretion by the male *O. mossambicus* takes place (Amorim *et al.* 2003, Amorim and Almada 2005, Oliveira *et al.* 2005). These actions were often repeated consecutively while the female would observe motionless from the rim edge of the nest. Her ultimate movement over the central regions of the nest directly above him would result in the male lying motionless at the bottom of the nest. The mating act, however, was never witnessed during these trials which may be due to their recently captured status, and insufficient habituation, as well as insufficient observation, in both length and frequency.

Male - Male Interactions

Literature review of the male-male interactions observed indicated typical agonistic behaviours between competing *O. mossambicus* males (Oliveira and Almada 1996a, 1996b, 1998a, Amorim *et al.* 2003, Oliveira *et al.* 2005, Barata *et al.* 2007). However, numerous observations of male - male courtship were also observed. Male A, upon successfully fragmenting the ‘female’ stratum began to ‘court’ one of the other two males (B and C). His actions were identical to those when the target was a female and has been reported above. Earlier investigations into such male - male, courtship action had been initially proposed to be due to the targeted male ‘allowing’ himself to be courted so as to minimise aggression from the territorial male, while also allowing the ‘sneaker’ male closer proximity to established nests during spawning events (Turner 1986, Oliveira and Almada 1998b, Oliveira *et al.* 2005). This was in part evident in the observed targeted males’ assumption of female colouring. However, a more recent report into male - male signaling via urine has shown that the urine’s complement of androgens has a modulating effect on other subservient males, further establishing the territorial male’s dominance (Barata *et al.* 2007). Observational support for this was deduced as it was always at this action when a targeted male (B or C) would flee from Male A.

4.3.2 Trial 2: new male, similar size.

The new male (D) did not alter the dominance hierarchy already established in BIA2 during the two weeks that the trial was run and it assumed membership of the 'female' stratum. The new male was not observed to attain male breeding colours within the female stratum. These observations support the literature, in which it is reported that fighting experience does affect subsequent hierarchy interactions (Hsu *et al.* 2006, Barata *et al.* 2007).

4.3.3 Trial 3: new male, larger size.

Following approximately three hours of acclimatisation (no observation), the new male (E) was observed to take up a position near the aquarium bottom, with resultant hostile responses from Males A and B. Following the second feed of Day Two after introduction (<48hrs), territory B was in obvious dispute between resident Male B and the new Male E. The following morning, prior to feeding, the replacement of the resident Male B by the new larger Male E had already taken place. During the remaining 12 days of the trial, the initial social system (Figure 4.1) was reversed with the conflict zone now inside Male A's half with a respective increase in territory E, Male E had become the new TDM (Figure 4.3). The ousted Male B would briefly and intermittently regain his breeding colours while in the female stratum but the trigger for this could not be determined. The length of each, seemingly sporadic, re-colouring event by Male B decreased over the remaining days but was still witnessed once on the ultimate day of the trial.

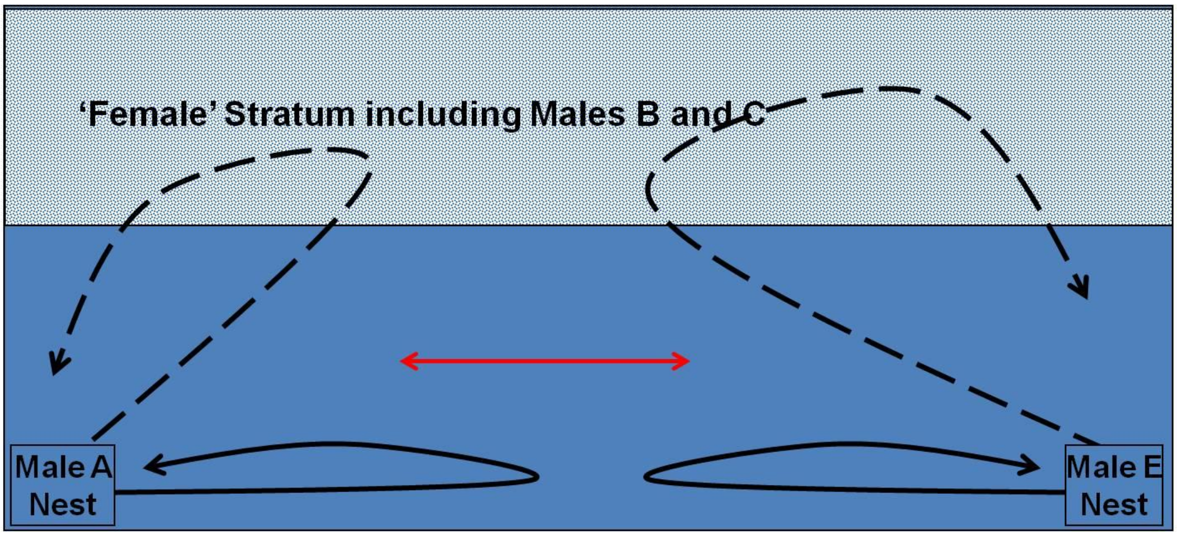

Figure 4.4: Representation of final relative aquaria possession and actions by territorial Males E (new) and A (previously tank dominant).

After the removal of the new Male E, ousted Male B regained his previous territory within a single day, with negligible interactions from either Male A or non-territorial Male C. It took a further week for Male A to reassert his previous territory size. Size discrepancy between the new Male E and the resident females was also of interest because the females suffered far greater observable stress upon the inclusion of the larger male, and resulted in a shift of the FS toward Male A's territory. Lengthy confinement with such a size discrepancy in favour of a large male would result in chronic stress of the females, impacting upon commercially important attributes such as fecundity, which directly affects body size and general health of the broodstock (Schreck *et al*. 2001, Hatikakoty and Biswas 2004).

Table 4.1: A glossary of observed instigative / responsive behaviours of *O. mossambicus* in a male-shared aquarium. Note: commas separate alternative responses, which may also have followed chronologically

Orientation of behaviour	Instigative Behaviour	Response
Male - Male		
Tank dominant male (TDM)	Nest hover and explore (not approach boundary	Nest hover, explore
	Mock charge (including up to approximate boundary)	Hover, mock charge, return to nest
	Active charge (past boundary)	Hover, confront*
	Mock charge (post confrontation), explore	Mock charge, return to nest, explore
	Return to nest (post confrontation)	Return to nest
	Body wash	Hover, follow, return to nest
Subordinate male	Nest hover, explore	Explore, test dash, dash
	Mock charge	Mock charge, Active charge, confront, ignore (explore)
Male - Female		
Either male	Dash	Scatter, school away, hide
	Body wash	Hover and follow, hover and return to female stratum
	Lead	Follow, return to female stratum
	Push	Approach nest and hover, return to female stratum
	Enter nest and twitch	Hover at nest rim, return to female stratum

4.4 CONCLUSION

The commercially accepted technique of only utilising one male per breeding arena was well supported by these three trials due to the presence of solely male-male behaviours. These behaviours, while not recorded as regards energy expenditure, did imply energy expenditure not directed at breeding. This single aspect of energy utilisation, as viewed from a fry production standpoint, has potential follow-on effects.

Male broodstock quality

Utilisation of energy away from purely breeding behaviours has the potential for a concomitant impact on spermatogenesis and immune response on the subservient males resulting in the potential for pathogen establishment and subsequent spread (Turner 1986, Binuramesh *et al.* 2005). In addition, the fact that the two males displayed agonistic behaviours toward one another implied a stress which was inferred to be a sub-optimal allotment of space. This competition would have compromised their establishment of optimum nests and territories, resulting in two territories of sub-optimal resource value. This decrease in resource (space, nest sites) value, which is suggested to have been the discriminating factor during these trials, may then have had a consequent negative effect on female fecundity - and thereby progeny production - as female mate choice in lek-breeding cichlids is largely dependent on territory quality and size (McKaye *et al.* 1990, Dijkstra and van der Zee 2008).

For example: Male A is dominant over Male B. He must 'choose' the level of energy he is willing to expend to either:

(i) Defend the entire tank floor as his own and ensure total dominance and optimal nesting sites, or

(ii) Mate with as many females as possible.

Behaviour in social animals such as *O. mossambicus* is obviously not an either-or-situation but a continuum of compromises or evolutionary stable strategies. Subjugating other males totally would leave him with less energy with which to undertake successful mating. Conversely, mating as often as possible would leave his territory exposed to sneaker / floater males encroaching on his territory. Male A therefore could not do both, and the evolutionary stable strategy dictates the maximisation of gene flow from Male A to his progeny, requiring a balance between (i) and (ii).

Female Broodstock Quality

The aggression of the TDM toward the other nestholder male, and the resultant displaced attacks on females, particularly brooding females who showed no response to courtship, were visible components of the negative impacts of a male-shared mating arena. Harassment of brooding females by persistent males and non-receptive females, intent on either egg cannibalism or the use of the brooding female as a dither factor against persistent males, in addition to the enforced starvation during incubation, all amount to stress situations (Galhardo and Oliveira 2009). Although female *O. mossambicus* have been reported to handle stress more effectively than males, the optimization of fry production should focus on the minimisation of all stress events.

In summary, current legislation in South Africa is such that *O. mossambicus* is the only viable aquaculture species available for culture within the cichlid family. In addition, stress initiation and regulation in cichlids has been extensively researched with respect to physiological processes and metabolism, under laboratory conditions. However, research into practical, field-based systems by which to minimise these stress factors on commercial productivity, would be advantageous to future seed production in systems for this indigenous species in South Africa.

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CHAPTER 5

THE DESIGN OF A NOVEL ROTARY INCUBATOR FOR THE INCUBATION OF *Oreochromis mossambicus* (Peters 1852) EGGS

ABSTRACT

The use of artificial incubation of *Oreochromis spp*. eggs has become widespread in high intensity fish seed production. Various types of incubator exist, and their selection is dependent on the specific attributes of the egg to be incubated. Currently available incubators are typically of a funnel (up-flow) or round bottomed (down-flow) design. Neither permits easy access to the eggs, which is particularly important when dealing with poor quality water as is typically found in rural areas. The aim of this study was to devise and test an easy-to-use incubator, applicable to rural seed production projects, which offers advantages over currently available incubator types. The final design, WETNURSE Type II, offered improved hatching rates over Type I, with a mean hatching success of 75%. While falling short of the desired 80% success rate, the various other benefits provided by the design justify further optimization and testing.

5.1 INTRODUCTION

A commercially important aspect of *Oreochromis spp.* culture is the production of large synchronous batches of healthy fry. Size and health of the batches produced can be negatively affected by the presence of bacterial (*Aeromonas hydrophila* and *Pseudomonas fluorescens*) and fungal pathogens such as *Saprolegnia spp.* that are omnipresent in most culture waters (Subasinghe and Sommerville 1985, Meyer 1991, Bolivar 2004). This relationship is true for *Oreochromis mossambicus* whose adult and egg stages are susceptible to attack from the *Saprolegnia spp*. of fungus (Subasinghe and Sommerville 1985, Khoo 2000, Osman *et al.*

2008). While adult afflictions can be dealt with symptomatically and effectively, pathogenic infection of eggs may result in total batch failure. Pathogenic attacks puncture the egg shell (chorion), destablising the osmotic safeguards, resulting in egg death via yolk sac rupture, or bleb formation, and the further proliferation of pathogens (Subasinghe and Sommerville 1985).

In nature, the non-breeding season results in a drastic reduction in bacterial and fungal spore density in the water by the removal of potential targets / reservoirs, such as fish eggs. During the warmer breeding season, however, when eggs are plentiful, a further limit, not evident in the literature, on bacterial and fungal pathogen growth is believed to occur in that the brooding dam is able to discriminate between unfertilised, broken or infected eggs and subsequently ingests them.

The hatching rate of *O. mossambicus* eggs, as with *O. niloticus*, is temperature dependent, with warmer temperatures (>25°C) significantly reducing the incubation times required (Rana 1990, Subasinghe and Sommerville 1992, Popma and Lovshin 1995). In the aquaculture industry therefore, water temperatures are maintained at these levels in order to (1) ensure year round reproductive cycles by the brood fish; and (2) to reduce the hatching times of the fertilised eggs or seed. The continuous production of *O. mossambicus* eggs is thus easily achieved. However, with eggs being continuously, especially in hapa-based systems which share water, egg-specific fungal and bacterial pathogens may proliferate and result in pond-wide reproductive failures if not controlled early enough.

Fortunately, in most fish seed production systems, the aquaculturist leaves the eggs with the female until they attain independent exogenous feeding stages, allowing the dam to remove any infected eggs during incubation. This typical system requires numerous pens, which are used to house 2 to 5 incubating dams each (Popma and Lovshin 1995). Furthermore, each dam may incubate for up to 18 days (temperature dependency) during which time she does not feed, resulting in a weakened broodfish. The dam is then typically reconditioned so she returns her to her previous weight, a factor which directly affects her fecundity (Hatikakoty and Biswas 2004). Reconditioning includes a higher protein (more expensive) feed, as well as her continued isolation from males to minimise fights which, in their weakened condition, may negatively affect future broodstock quality (Fessehaye *et al.* 2006).

An alternative approach, gaining in acceptance, is to strip the fertilised eggs from the dam's buccal sac two days post-fertilization. This independent hatching of the eggs allows the aquaculturist complete control over the environment of the eggs, a desirable attribute if the

water supply is not optimal, as well as providing a tool by which to accurately age the eggs/fry in order to ensure homogenous aging within a grow-out cohort (Rana 1986).
In addition, the eggs can be treated symptomatically without any fear of affecting the dam. Furthermore she will have only undergone a minimal enforced starvation, and will recover far quicker, with a corresponding decrease in the inter-spawning interval or ISI (Rana 1986).

Many types of incubators or tumblers exist, depending on the type of eggs to be hatched. *O. mossambicus* eggs are typical of the mouthbrooding type: round, smooth, denser than water and non-adhesive. The incubator type is therefore typically either: an enclosed cylinder with water entering from the bottom at the correct velocity to keep the eggs suspended, and rolling but not 'boiling'; or a down-flow system in which the water current is reflected back up off a curved base and in so doing, keep the eggs suspended (Kristanto *et al.* 1998, Rana 1985). However, these incubator systems are closed units and thus not applicable to poor water quality systems. The aim of this study was therefore to design a simple incubator system which would satisfy the following requirements:

- The incubator must be open ended to allow easy access to the eggs, whether for the medication of the eggs or for their addition or removal.
- The incubator must be made of inert materials, presenting smooth surfaces that can be easily cleaned.
- The incubator must provide sufficient impetus to the eggs, in effect replicating the tumbling experienced within the mouthbrooding parent's buccal cavity.
- The incubator must have variable control of tumbling speeds to deal with small or large batches of eggs.
- The incubator system must incorporate all necessary heating, filtration and aeration requirements.

Utilising hatching rates from Rana (1986) and El-Naggar *et al.* (2000), the successful hatching of 80% of the eggs over 3 batches (maintained at28 °C and 12L: 12D) was set as a goal.

5.2 MATERIALS AND METHODS

Broodstock and fertilisation management

1. Broodstock choice was made on previous observations of the successful production of at least 75 swim-up fry under ‘natural’ mating systems i.e. no intervention as regards fertilisation and incubation.
2. Broodstock were all maintained on a 42% protein diet (Conditioning diet, Chapter 2) with a light/ dark regime of 12L: 12D at a ratio of 1 male to 3 females.
3. Fertilisation of the eggs was achieved naturally.

To replicate the movement of the eggs within the dam’s buccal cavity, a rotary incubator was envisaged whereby the eggs would be continuously rolling over one another. Typical tilapia tumblers utilise upwelling and/or down-welling and are thus funnel-shaped (Rana 1986). However this shape hinders the efficient removal of diseased eggs. The incubator was designed to function within a 50 L glass aquarium, termed an incubator aquarium (IA).

5.2.1 WetNurse Type I (WN-1) construction

1. A plastic 2 litre soda bottle was cut and a stocking diaphragm was introduced. The upper and middle segments were reattached with marine seal ™ with a 3M adhesive tape spine. The base was discarded.
2. The incubator drum (base and wall join) was cured in water for two days.
3. The Incubator Drum was placed (free) onto the spray bar of a filter pump (Magi-Jet 200).
4. The Incubator Drum was angled at 45° to retain eggs while providing sufficient slope to initiate rolling.
5. The spray bar of the Magi-Jet 200 filter was angled to direct its force in such a manner as to produce rotation of the drum from within the neck of the base.

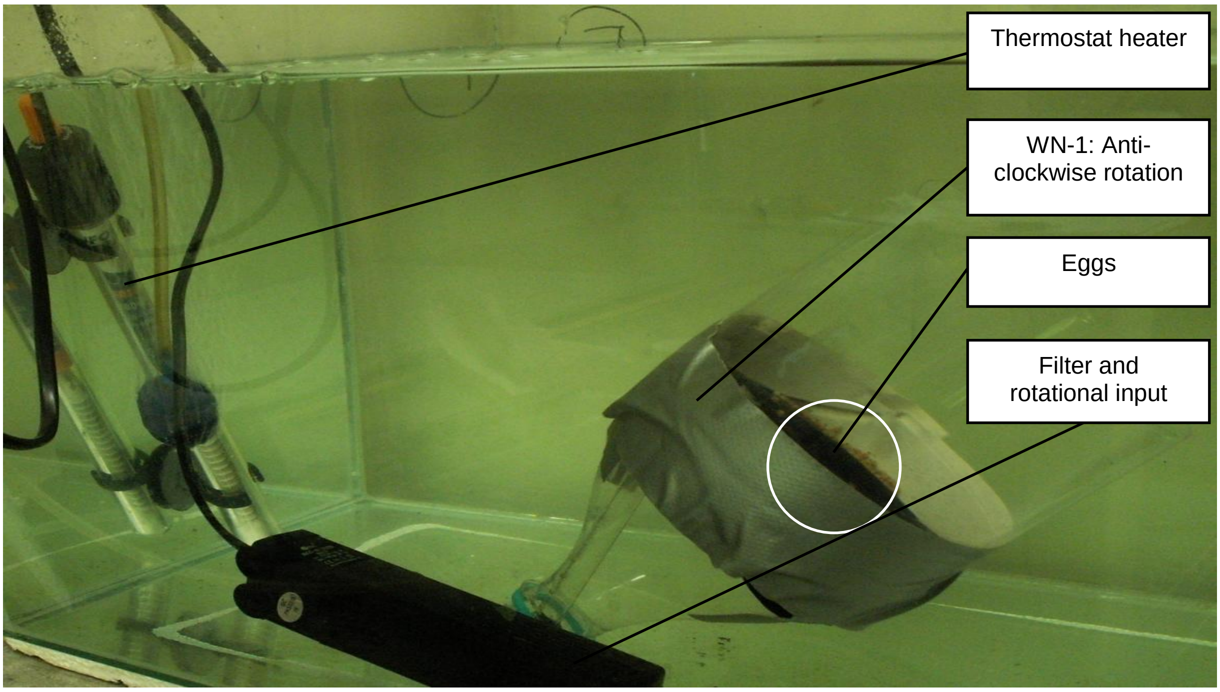

Figure 5.1: Incubator Aquarium (IA) including Wet Nurse I (WN-1) incubator, filter and thermostat heater

Problems with WN-I

- The eggs only rolled along the stocking / wall join, increasing the chances for eggs to become stuck.
- The stocking (60 dernier) stretched after two separate batch loads, resulting in folds and fine threads which both resulted in the entrapment of the eggs, leading to egg death and fungal proliferation.
- Discontinuous and haphazard rotation of the incubator drum was also experienced, particularly during power outages (ESKOM load shedding events) and substitution of the filter by the provision of airflow from a battery operated compressor or ‘bubbler’ to generate turn, lacked the required airflow volume.

Due to these problems, WN-I was rejected and a modified version, WN-Type II, was constructed.

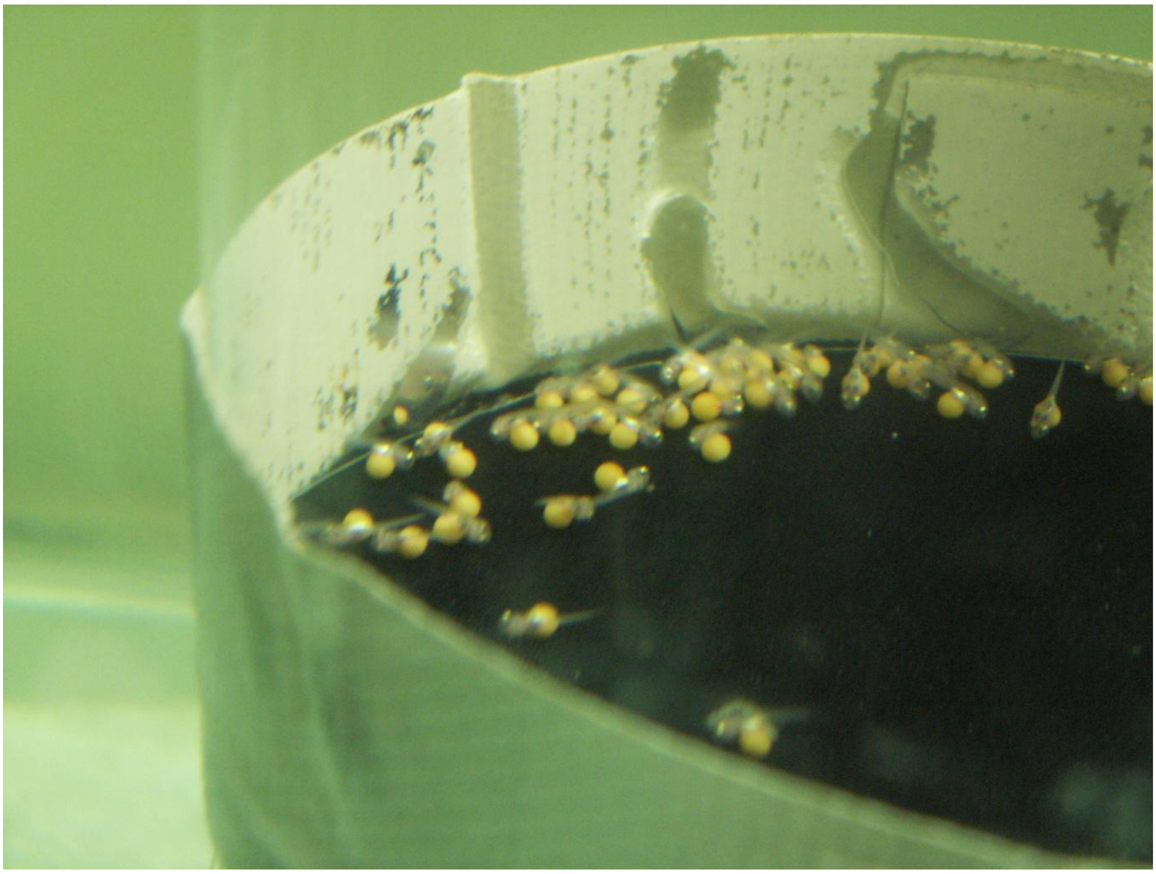

Figure 5.2: View of hatched *O. mossambicus* fry within WN-I.

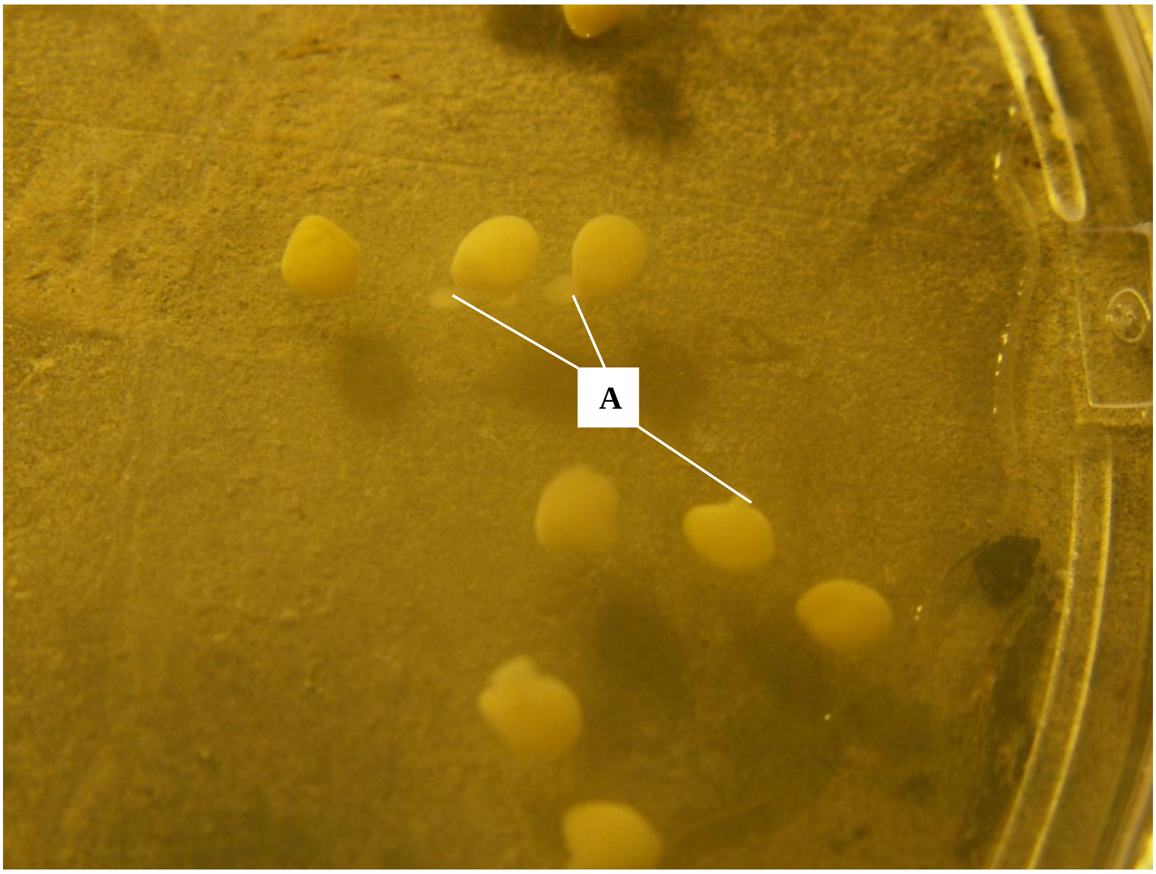

Figure 5.3: Egg rupture due to pathogenic attack. Bleb formation is visible as well as the subsequent fungal colonization (floccous protrusions from the eggs - A).

5.2.2 WetNurse Type II (WN-II) construction

1. The stocking diaphragm was replaced with a plastic cradle type diaphragm. The cradle was cut from the base of a plastic 2 L soda bottle.
2. Due to the increase in weight, rotation via the spray bar was augmented by the addition of mill-type paddles (hydrofins) which would assist rotation as well as allowing for a variable rotation dependent on airflow. Hydrofins (4) were cut from same 2 L plastic soda bottle.
3. Due to the tight fit of the cradle and base, adhesive was only required for the hydrofins attachment (3M Marine Seal™)[6].

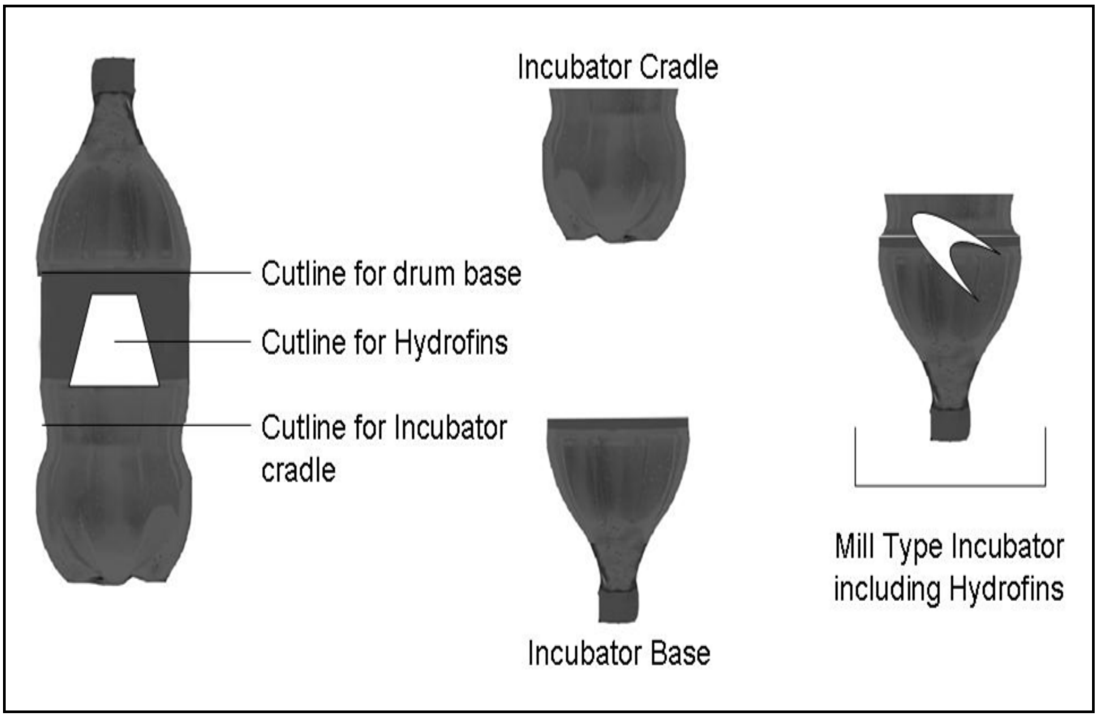

Figure 5.4: Construction of WN-II. All components from a single plastic 2 L soda bottle.

[6] 3M (Pty) Ltd South Africa: 146A Kelvin Rd, Woodmead, Sandton, Private Bag X926, Rivonia, 2128

Figure 5.5: WN-II in operation with hatched fry.

5.2.3 Trial Design

Two incubator aquaria (IAa and IAb) were set up to house the incubators. Three trials were run in IAa utilising WN-I once and WN-II twice. This was repeated concurrently in IAb. Batches of 70 eggs were added to each incubator per trial and resultant egg hatching rate was recorded. Subsequent fry survival to the swim-up stage of development was also recorded to confirm that the incubator did not adversely affect survival rate by inflicting unobserved injury on the yolk sac which would have manifested later.

Aquarium setup

1. Municipal water was dechlorinated over three days prior to any use in any of the trials
2. Incubators were cured for two days in municipal water prior to use in the trials.
3. Water temperature in the IA’s was maintained via thermostat controlled submersible heaters (50W) at 28°C.
4. Upon attainment of swim-up fry stage, the trial was terminated.

Egg selection

1. Each batch of 70 eggs came from a randomly selected single female. All females shared the same breeding aquarium and male.
2. Stripping and egg selection was done as per a stripping protocol (Appendix 5.1: Stripping Protocol) at 2 days post fertilisation. Successfully fertilised eggs were judged according to colour (yellow tint).
3. Eggs were photographed and counted on the digital image and recorded (Konica-Minolta Dimage Z5).
4. Eggs were then introduced into an operating incubator along with a dose of methylene blue as per Bolivar *et al.* (2004) (3 mgl^{-1}).The inclusion of methylene blue was done due to low hatching rates experienced after the colonization of the breeding aquaria by a biomat.

Rotational velocity

Rotational velocity was adjusted to provide 20 rpm at the onset of each trial, to be slowed to 10 rpm upon visual estimation of 50% of the batch having hatched.

Due to the lack of necessary replication, statistical analysis of the data was not done. Data presented are indicative of potential production values utilising this system

5.3 RESULTS

Table 5.1: Hatching success and survival to swim-up (SU) stage using WN-I and WN-II. Results from conical and round-bottomed incubators, taken from Rana (1986), are included for comparison.

Incubator	Number of trials	Mean hatching rate % (SE)	Mean swim-up survival rate %
WN-I	**2**	**53 (9)**	**39**
WN-II	**4**	**75 (3.13)**	**70**
Conical (Rana 1986)	**62**	**74.6 (1.61)**	**59.7**
Round-bottomed (Rana 1986)	**105**	**91.6 (1.16)**	**84.5**

5.4 DISCUSSION

WN-I and especially WN-II provide a viable alternative to current incubator designs. While 80% mean hatching was not achieved, the positive attributes of the WN-II design merits further refinement and optimization. The WN-II design successfully incorporated all the desirable attributes of an independent incubator for *O. mossambicus* eggs with unknown water qualities. The control of airflow to the airstone allowed for control of rotational speed while ensuring optimal DO (dissolved oxygen) for the developing eggs. Optimal DO (> 8 $mg.l^{-1}$, Boyd (1982), Suresh (2003), Gonzales *et al.* (2007)) was assumed due to the small volume of water in the aquaria, the continuous supply of air via the airstones and the small size of the fish.

The ability to easily remove the incubator from the pivot allowed for ease of sampling of the young fish, as well as the removal of dead eggs. The form of the plastic, as well as its transparent nature, also permitted the detailed observations of impacts between eggs and incubator. Impacts were always tangential, with the force being dissipated into a desirable forward movement of the egg.

The open end of the incubator permitted easy access to the eggs, and provided two additional advantages:
Firstly, the hatched fry were able to escape the incubator once sufficient swimming ability had developed. This desire to escape was observed in all incubator trials, and should reduce any chance of damage to new appendages by either the unhatched eggs or the incubator surface. This characteristic opens up various possibilities for the investigation into the assumption of motor control by the young fish, but also the establishment or refinement of either visual acuity or environment awareness, because the fry always swam directly out of the opening, i.e., their escape was not due to accident or current. Observations of the swimming potential required to escape was approximated at 3 seconds of continuous beating of the tail.

Secondly, the incubation, hatching and rearing of the fry to the swim-up stage could all be accomplished within one aquarium, removing the need for damaging transfers via nets. The potential for this system's adoption in aquaculture processes is chiefly due to the reduction in footprint, which is particularly relevant in high intensity culture systems where space, volume of water and water heating are priorities.
Another aspect relevant to both commercial and research projects is that due to its non-fixed nature, the incubator drum is not affixed to the spray bar and thus when eggs are to be (re)moved, the cradle is simply lifted and either moved to a new aquarium or allowed to rest on the water surface, where detailed examinations and manipulations can take place. Due to the internal ridging, the egg batches are easily sorted into 5 manageable portions which assist in enumeration, as well as the detection of dead eggs.

5.5 FUTURE PROSPECTS

Two inherent attributes of *O. mossambicus* reproduction which conflict with the desired continuous system of any culturing practice are: asynchronous hatching, due to prolonged spawning events and resultant staggered developmental stages within a single female's batch of eggs, as well as asynchrony between females with respect to their reproductive cycle. While the use of artificial incubators to hatch eggs has had a marked effect on reproductive asynchrony, due to the reduction in ISI (Rana 1986), the WN-II incubator is able to couple that aspect, along with releasing the fry at distinct developmental stages, such as the observed escape response. While this may be an artifact of some unknown laboratory attribute, its analysis in further research may well prove useful in the grading of, and subsequent grouping of, fry into developmentally appropriate cohorts, thereby permitting the establishment of more uniform harvests of juvenile tilapia for aquaculture stocking purposes.

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APPENDIX 5.1: Stripping protocol for fertilised eggs

1. The stripping basin was prepared with water taken from the breeding aquarium from which the brooding dam was caught. Methylene blue was added at a concentration of 3 mgl^{-1} 10 minutes prior to stripping.
2. The brooding dam was caught in a small meshed net and transferred into the stripping basin.
3. The brooding dam and all ejected eggs were emptied into the stripping basin.
4. The female was held firmly in one hand and forced backward and forward to flush all remaining eggs from her buccal cavity.
5. Once visual inspection of the dam's mouth indicated that no more eggs were present, the dam was returned to her BIA.
6. Dechlorinated water was added to the stripping basin till full. Aeration was then supplied as well as gentle agitation to ensure that the eggs remained in motion.
7. Eggs were visually sorted as to integrity; unfertilised, diseased, broken and malformed eggs were removed with a turkey baster and discarded.
8. All the remaining eggs were siphoned off into another glass container (750 ml) with only fresh, dechlorinated water and methylene blue at a concentration of 3 mgl^{-1}. The container was agitated for five minutes to allow for oxidative action by the sterilising agent.
9. The eggs were then introduced into the incubator drum by gently pouring the entire contents of the container into the IA via a funnel.

CHAPTER 6

QUANTITATIVE ANALYSIS OF THREE POPULATIONS OF *Oreochromis mossambicus* (Peters 1852) FOR THEIR FOOD CONVERSION EFFICIENCY (FCE)

ABSTRACT

Three distinct populations of *O. mossambicus*, representing populations of inbred, randomly mated and unknown (wild-caught) pedigree were analysed according to their food conversion efficiency (FCE). Comparisons between the three resulting batches permitted the ranking of the unknown population with a subsequent evaluation of the population as a potential genetic reservoir for further selection. The intra- and inter-sample crosses were done with single males in order to produce half-sib progeny batches which allowed for the assessment of sire influences on the FCE of the progeny batches. The results show that the population of unknown pedigree is comparable to that of the randomly mating population, indicating the presence of sufficient genetic variation to permit further selection; the genetic contribution of the males to their respective progeny was insignificant in relation to that made by the female.

6.1 INTRODUCTION

The feed conversion efficiency (FCE) (or ratio, FCR) of a particular species, or strain, can be defined as the amount X of saleable weight gain, given a particular feed of weight Y (Doupe and Lymbery 2003). Various notations to describe the ratio are evident in the literature and in this study the X:Y notation will be used for simplicity, where X g of gross weight gain results from Y g of feed given. Furthermore, the FCE of a particular species or strain must be viewed in light of the culture environment. Temperature, size and age of the stock, feeding level, nutritional composition, body weight and composition, physical exercise, as well as density dependent social factors such as competition, antagonism and stress have all been shown to impact gross FCE values (Doupe and Lymbery 2003). Therefore, the FCE must be viewed as a summation of the physiology of the species under culture as well as the culture environment.

A low FCE is one of the economically important attributes of the *Oreochromis spp*. in aquaculture, relative to other farmed species. Domesticated fowl (broiler) and swine (weaner age) strains, which have undergone numerous generations of selective breeding, are the nearest with ratios of approximately 1:2 and 1:3 respectively (Ferguson *et al.* 2002, Króliczewska *et al.* 2005). In comparison, wild-type *Oreochromis niloticus* have recorded FCE ranges of 1:1.4 - 1:8 (Abdel-Tawwab 2004, Khattab *et al.* 2004, Mamun *et al.* 2004). Genetically improved farmed tilapia, the GIFT strain of *O. niloticus*, has had varying reviews in relation to FCE, with both improved (Ridha 2004) and unimproved ratios reported (Mamun *et al.* 2004). Another factor which stresses the importance of a low FCE in finfish culture is the cost of aquaculture feed. Cited as the single largest expense in semi-intensive and intensive production systems (Mbahinzireki *et al.* 2001, Ahmad and Diab 2008), the efficient use of feed will improve the return on investment (ROI).

The development of the GIFT strain of *O. niloticus* was primarily aimed at achieving such an improvement in growth rate, in essence, its FCE. The base population for the GIFT strain was created from the parents of best growers in an 8X8 full diallele cross which utilised four north African and four cultured Asian strains. Subsequently, Genomar have improved on the findings of the GIFT strain to produce Genetically Male Tilapia, or GMT, with improved growth rates compared with mixed sex and sex-reversed tilapia (Beardmore *et al.* 2001, Eknath 2007). Current legislation in South Africa, however, forbids the cultivation of *O. niloticus* in South Africa due to its invasive and hybridisation potential.

With the establishment of an aquaculture industry in South Africa as a focus, four issues need to be realised:

1. *O. mossambicus* with its comparable attributes is the only realistic candidate for rural aquaculture schemes due to its ease of growth, general hardiness, indigenous nature, low trophic level, euryhaline ability, market recognition and cross-cultural acceptance.
2. The much-criticised precocious nature of *O. mossambicus* can be managed, as it is with *O. niloticus*, to produce monosex progeny whose impact, on potential escape, would be negligible.

3. The numerous technologies researched in *O. niloticus*-based aquaculture can be adapted to local realities, particularly in view of the close intra-genus relationship which exists between *O. niloticus* and *O. mossambicus*.
4. The creation of a selectively bred line of *O. mossambicus*, aimed at growth optimisation, would greatly benefit its uptake in the intensive aquaculture industry.

Therefore, if an aquaculture industry in South Africa were to be successfully based upon the use of indigenous *O. mossambicus*, an evaluation of wild genetic stock would be the first logical step. The aim of this study was therefore to determine the FCE's of a sample of wild-caught specimens (reflecting a potential wild genetic reservoir); an inbred population of known pedigree and a synthetic population (reflecting maximum outcrossing) created by crosses between the former two samples. Comparisons between the three sets of FCE values were used to rank the existing feed conversion efficiency available in the wild-caught sample and thereby evaluate its potential role in a growth selection program.

6.2 MATERIALS AND METHODS

Three populations were to be analysed according to their FCE. One population, which implied successive generations of inbreeding, was sourced from Yellowwood Park, Durban. The second population, wild-caught and of unknown heterogeneity, was sourced from the Foxhillspruit River in Pelham, Pietermaritzburg. The third population was to be a synthetic one, made up of crosses between the former two populations. Their geographic relation and partial history were used to judge these two populations as unrelated. Their cross breeding would therefore produce progeny which would describe an outcrossing or randomly mating population.

6.2.1 Experimental Design

1. Each of the sampled populations (Yellowwood Park, Foxhillspruit) was to undergo one round of intra-sample mating to produce three progeny sets of 30 fry each.
2. The third, synthetic, population was created from crosses between the above two populations to produce 4 sets of 30 fry each.
3. All progeny were to be grown to 90 days of age with individual weighings being done at 56, 70 and 90 days

4. Due to space constraints, no replicates were possible. Pseudoreplication due to single aquarium environmental effects was minimised by the use of only two aquaria from swim-up stage onwards. The progeny from crosses were randomly assigned to the two 250 L and then again to the two 350 L final grow-out aquaria.

6.2.2 Population Choice

The inbred population (Y) was obtained from a private residence in Yellowwood Park, Durban (29° 55′ 8.5″S; 30° 56′ 33″E). The *O. mossambicus* inhabited a pond feature which had been stocked with seven *O. mossambicus* individuals in 1993 with no further additions till the date of capture (2006). No records were available on the sexual ratio at the time of stocking, but three males and nine females were collected for this study.

The Foxhillspruit River (R) population (29° 37′ 28″S; 30° 23′ 25.5″E) was utilised as the population of unknown inbreeding. Seasonal inbreeding was suggested due to the river's fragmentation into small rocky breeding pools, interspersed with long shallow stretches of river which are devoid of *O. mossambicus*. Assuming minimal new introductions of *O. mossambicus* into the system, the only genetic transfer between the breeding pools would be during large summer storms whereby gene flow would only be downstream owing to the weak swimming ability of *O. mossambicus.* This therefore, excludes the potential for any gene flow upstream from the large, assumed to be randomly mating, population in the Umsunduse River. The Foxhillspruit River population was sampled at the end of September, prior to the summer rains. Three males and nine females were collected

The Synthetic population (S) was created from an equal cross between both sampled populations (Table 6.1).

6.2.3 F1 Generation

Within each Y and R sample 1 male and 3 females were randomly chosen from the sample collected. The male was mated against each female once to produce 3 half-sib groups. The S population F_1 had two sires and four dams, split equally between F and R samples (Shaded section, Table 6.1).

Table 6.1: F_1 creation and nomenclature system used. Shaded sections refer to synthetic (S) population crosses

	r♀	**r♀**	**r♀**	**y♀**	**y♀**	**y♀**
Y♂	Yr_1	Yr_2	-------	Yy_1	Yy_2	Yy_3
R♂	Rr_1	Rr_2	Rr_3	Ry_1	Ry_2	-------

Table 6.2: A listing of the various half-sib groups produced

MALE	Y	R
Half Sib Progeny	Yy1, Yy2, Yy3, Yr1, Yr2	Rr1, Rr2, Rr3, Ry1, Ry2
FEMALE	Y	r
	Yy1, Ry1	Yr1, Rr1

6.2.4 Fry Selection and Housing

Each fry batch was initially housed in their respective IA's (Incubator Aquaria, see Chapter 5). Post hatching and yolk sac absorption, 40 swim-up fry per cross were randomly selected prior to the supply of any artificial feed. Thirty fry were then randomly selected from an initial 40 and transferred to 250l tanks and ultimately transferred into large, grow-out tanks of 350l capacities at 50 days of age. The remaining 10 fry were maintained in separate 250l tanks under equal conditions to the trial group, to act as reliable replacements in the event of trial batch mortalities. Due to space constraints, the trials had to be staggered at two batches per growth stage and the trial replacements were removed once the trial group attained 60 days of age.

6.2.5 Feed Type and Feeding Regime

All batches were fed twice a day (10h00 and 16h00) and light was maintained at 12L: 12D starting at 08h00. The amount of feed fed per meal was termed the MFA (meal feed amount) and was averaged between the two *ad libitum* feedings given at the start of each week. Feed type per growth stage was provided according to Table 6.2 with constituent components indicated in Table 6.4 (for feed type choice, please see Chapter 2: 2.2.1.6 Feed).

Table 6.2: Feeding regime indicating total amounts per feed type. Amount per growth stage is mean of feed given per stage over all trials. Feed amount given per batch, prior to the removal of trial replacements has been adjusted to reflect trial participants only.

Feed type	**Age in Days**			
	0-21d	**22 - 28d**	**29 - 56d**	**57 - 90d**
Brine Shrimp	6g			
Ground Feeder Tablet		6.5g		
Aquafin mini			90g	
Aquafin maxi				160g

Table 6.3: Feed type and constituent components given at various life stages.

Life Stage	**Feed Type**	**Protein %**	**Carb. %**	**Weight (µ)**
Fry (<21d)	brine shrimp (BS)	65	19	1.1g / 100
Fingerling 1 (22 – 29d)	Groundfeeder tablet (GFT)	43	8	0.214g / tablet
Fingerling 2 (30 – 60d)	floating pellet 1 FP1	42	6	0.5g / 100
Juvenile (61 – 90d)	floating pellet 2 FP2	42	6	1.5g / 100

6.2.6 Weighing Protocol

At 56, 70 and 90 days, the 30 individuals were individually weighed according to the following protocol:

1. The batch to be weighed was transferred to a separate basin
2. A container of water (1l) placed on the digital scale which was then zeroed.
3. Ten fry would be added singly to the container with weights between additions recorded. Individual weights were then determined via subtraction.
4. The ten recorded individuals were then returned to their aquarium, and the next ten were weighed as above.

6.2.7 Data Analysis

1. The weights achieved, over the time taken (56d, 70d, 90d), were plotted on weight vs. time graphs (Figure 6.1):
    a. Each population was treated separately (Y, R, and S).
    b. Comparisons were made between the three populations.
2. Comparisons were made between the Y and R males half-sib groups produced (Figure 6.2).
3. The weights achieved, per unit feed given, were then used to calculate the FCE (Table 6.6).
4. One way ANOVA's were done of the three samples' weights obtained at 90 days using cross, male, female, and population as variates. (Table 6.7).

6.3 RESULTS

The results are presented below, in Figures 6.1 and 6.2, and Tables 6.6 and 6.7.

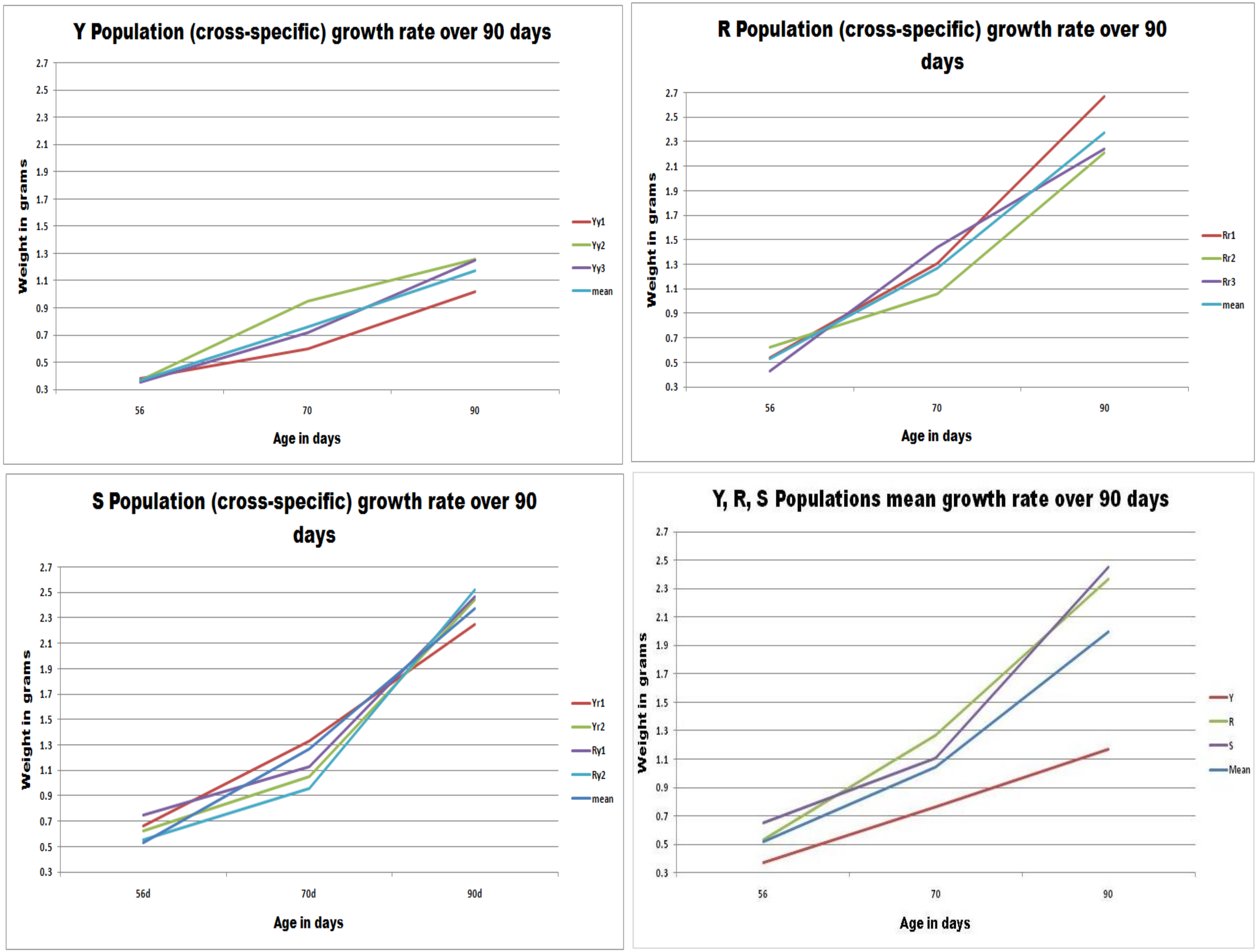

Figure 6.1: Graphical representation of the various weight gains over 90 days per population (Y, R, and S) and overall

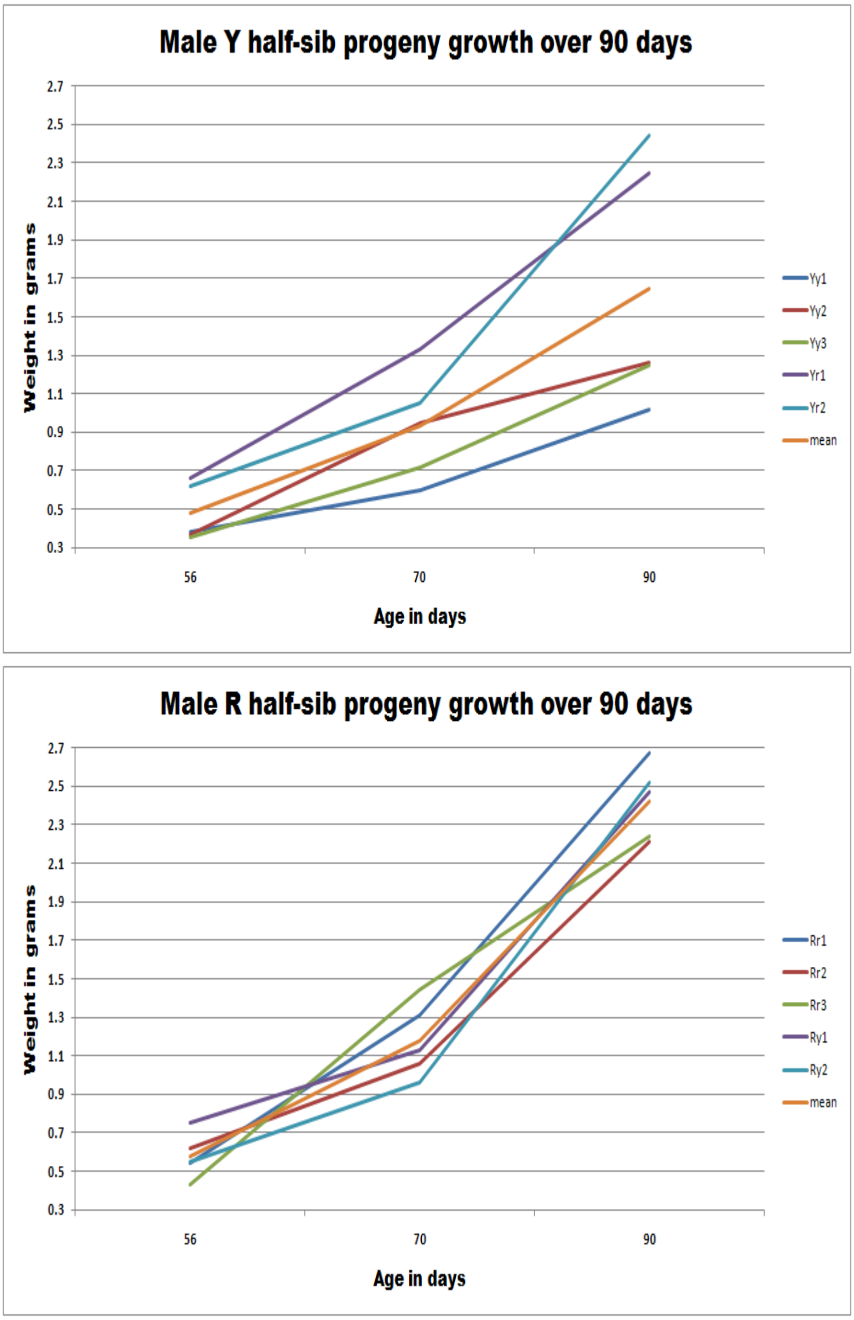

Figure 6.2: Graphs of Y and R males' half-sib progeny performance over 90 days

Table 6.6: FCE calculated for the various crosses at 90d

	Cross									
	Yy1	**Yy2**	**Yy3**	**Rr1**	**Rr2**	**Rr3**	**Yr1**	**Yr2**	**Ry1**	**Ry2**
Feed supplied (g)	259	262	264	265	268.5	267.5	261	262	265	262.5
Total cross weight (g)	30.68	37.66	37.36	80.21	66.28	67.19	67.46	73.07	73.98	75.74
Cross FCE	1:8.44	1:6.96	1:7.07	1:3.3	1:4.1	1:3.98	1:3.87	1:3.59	1:3.58	1:3.47
Total pop weight	105.69			213.73			290.25			
Pop FCE	1:7.49			1:3.78			1:3.63			

Table 6.7: one-way ANOVA utilising Cross, Male, Female and Population as variates

Source of variation	**D.F.**	**S.S.**	**M.S.**	**V.R.**	**F pr.**
Cross	9	101.1398	11.2378	34.54	<.001
Residual	290	94.33983	0.3253		
Total	299	195.4796			
Male	1	41.7312	41.7312	80.88	<.001
Residual	298	153.7484	0.5159		
Total	299	195.4796			
Female	5	44.7513	8.9503	17.46	<.001
Residual	294	150.7283	0.5127		
Total	299	165.4796			
Population	2	94.7270	47.3635	139.62	<.001
Residual	297	100.7526	0.3392		
Total	299	195.4796			

6.4 DISCUSSION

Considering the multifactorial nature of feed conversion efficiency, the three populations of tilapia subscribe to the expected performances. The inbred population (Y) showed significantly depressed feed conversion efficiencies, while the synthetic population (S), exemplifying a random mating population, displayed increased efficiency in feed conversion. Utilising these two results as the two extremes, the river population (R) of unknown pedigree was determined to display variation similar in scale to that of the synthetic population and can therefore be seen as a potential genetic reservoir for FCE-targeted breeding programmes.

However, two contradictory factors need to be assessed:

1. The high degree of significance between the intra-sample crosses of the inbred, Y, population. Assuming the owner's correct recollection of the year of stocking and no subsequent additions, the Y sample should not have shown significant differences between the group means (Yy1, Yy2, and Yy3). Successive inbreeding within an initial stock of seven adults should have shown greater homogeneity among the results, particularly as one sampled male was also common to all three matings. Three factors may account for some form of mitigation:
    a. The large number of genes involved with metabolism, protein deposition, waste production, etc., may require further inbreeding to display homogeneity.
    b. No aging of the captured fish was done. There is a probability that the selected individuals were not results of successive inbreeding but may have been several years of age and thus still heterogeneous at FCE-related loci. The depressed form of their growth curves can then only be ascribed to the homogeneity of several loci whose products deal directly with growth.
    c. The outdoor pond from which they were sampled still provided selective pressures, in the form of biotic (predation and competition) and abiotic (temperature) factors, by which the successively inbred, and less fit, progeny may have been selected against reducing the numbers of inbred individuals.
2. The spread of weights visible at 90 days for the Y population, with an opposite clustering of weights for the outcrossed samples (Figure 6.1):
    a. The spread of weights for Y can be attributed to the female (y1, y2, y3) influence. This is corroborated by the weights found in the Y half-sib grouping (Figure 6.2), where the introduction of females r1, r2 narrowed the 90 day weight grouping as with the R intra-sample cross. This influence may be due to

the y females possessing differing, inbred, FCE-related loci resulting in depressed but variable weights.

b. The tight grouping of the R and S population samples is more than likely attributable to the small size of the cross and not due to inbreeding. This is supported by the elevated feed conversion, with respect to the inbred Y population in both the intra and inter-sample crosses (Figure 6.1 and 6.2). A further factor worth considering is the meal frequency which, at twice a day, may have obscured finer genetic variations by providing a glut of food with an increase in waste metabolites. Commercial aquaculture operations typically supply feed 4 - 6 times a day. Further weighings at 120 and 150 days may have provided more accurate results based on twice-a-day feeding.

The wild-caught population, while hypothesised to display significant inbreeding depression in FCE due to a predicted seasonal bottle necking, displayed robust feed conversion ability, comparable to that of the synthetic population. This result, obtained from a small population, surviving in a sometimes heavily polluted environment, substantiates the widely recognised hardiness of *O. mossambicus* and provides support for its use in rural aquaculture projects. Furthermore, the low FCE values obtained and their similarity to those of the synthetic population indicate that this natural stock would be a valid genetic reservoir for a growth selection program.

6.5 FUTURE PROSPECTS

Choice of a growth selection program for *O. mossambicus* for rural aquaculture schemes however, needs to take into account the following three factors: (1) environmental variations at grow-out sites and their ‘blurring’ genotype X environment effect on phenotypic variation (Mair 2007); (2) a low (assumed) heritability for weight gain of 0.15 (Bolivar and Newkirk 2002); and (3) the asynchronous breeding of *O. mossambicus* (Bhujel 2000). Combining these, a within-family, cohort-organised, selection breeding programme is proposed here:

1. Sourcing of the founder parents (FP_0) should be from as far afield as possible so as to maximise genetic heterogeneity, as was done with the GIFT strain of *O. niloticus*. The breeding project should also utilise similar culture systems and management regimes as would be expected at the grow-out sites, to minimise any potential environmental influence (Eknath 1993, Bentsen and Olesen 2002).

2. The founder population (FP_1) should be derived from a full diallele cross between at least 25 males and 50 females, with each female mated to only one male. (F_1= 1250 families of 40 progeny) (Eknath 1993).
3. Due to the asynchronous breeding of *O. mossambicus,* the breeding strategy will have to be organized according to age cohorts. While this is typically a management intensive procedure, the utilisation of the WetNurse incubator, which provides a chronological discrimination, would permit simple allocation of escapee fry into a particular age cohort.
4. Grouping of different family cohorts into large grow-out ponds could be done, provided they were of similar ages (within 48hrs) and that identity marking, or DNA typing, of the various families was done (Tave 1995, El-Sayed 2006).
5. Selection procedures typically consist of either a single selection event, typically at harvest, or a dual selection, where selection is done at fingerling stage and then again at harvest. Considering the type of rural grow-out scheme envisaged, a single selection event at harvest is proposed, with those 'culled' being eaten (Tave 1995, Ponzoni 2009).
6. Selection intensity for single event systems should be of the order of 25%. Typical phenotypic characters selected for are length, and length with body conformation as weight is too difficult to obtain on such a large scale. This selection needs to de done equally across all cohorts (Bentesen and Olesen 2002).
7. The 25% selected would then comprise the broodfish for the subsequent (F_2) generation. Random mating between the selected is recommended because rotational mating requires intensive record keeping (Bentsen and Olesen 2002, Ponzoni 2009).
8. The top 25% progeny of the F_2 generation will produce the following (F3) generation, etc.

Utilising such a breeding program, increases in saleable weight gain per generation would be expected. The magnitude of such increases however, would be dependent on any environment X genotype interactions, realised heritabilities, as well as stringent record-keeping. Utilising a low per-generation-gain of 5%, this would result in a significant increase in growth after five years of selection, i.e., within 5 generations. Such a program needs to be an integral part of any future aquaculture research which is aimed at rural aquaculture and food security.

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APPENDIX 6.1: Y, R and S Populations mean weights ± Std Dev per cross over time.

Cross	**56d**		**70d**		**90d**	
Yy_1	0.382933	0.093607741	0.602226	0.099957239	1.022667	0.252039041
Yy_2	0.365741	0.111001127	0.953778	0.202582223	1.255187	0.202306669
Yy_3	0.346867	0.056781938	0.720667	0.242557257	1.245167	0.3731609
Cross	**56d**		**70d**		**90d**	
Rr_1	0.547833	0.073750317	1.308	0.508624921	2.6735	0.835284538
Rr_2	0.622333	0.169434828	1.061344	0.354470579	2.209273	0.467749876
Rr_3	0.4285	0.117430464	1.439338	1.380689059	2.239567	0.497121103
Cross	**56d**		**70d**		**90d**	
Ry_1	0.74906	0.384800565	1.131068	0.386422911	2.465947	0.672341001
Ry_2	0.622333	0.169434828	1.04552	0.284650511	2.435667	0.649661362
Yr_1	0.657333	0.149064516	1.326348	0.407676863	2.248667	0.688785705
Yr_2	0.553693	0.153404603	0.955734	0.311721366	2.52463	0.702857425