

**Quantifying the Effect of Inbreeding
On the Growth Performance and Yield of Mozambique Tilapia,
Oreochromis mossambicus (Peters, 1852)
Over Three Generations of Repeated Full-Sib Mating**

BY

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Science in Agriculture at the Faculty of Agricultural Sciences, Stellenbosch University.*



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DECLARATION

To the best of my knowledge I do hereby declare that this thesis represent my own work. It has not been submitted in any form for another degree or diploma to any other University or other Institution of education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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ABSTRACT

The effects of acute inbreeding on growth performance, yield and occurrence of deformity traits were studied in experimental full-sibling inbred populations of Mozambique tilapia *Oreochromis mossambicus* at three levels of inbreeding coefficients, namely $F = 0.000$, $F = 0.250$ and $F = 0.375$. The base population with $F = 0.000$ was established through the crossing of two geographically separated and genetically unrelated farm stocks. At each generation, the inbreeding depression indicated by body weight (BW), standard length (SL), specific growth rate (SGR) and yield were highly significant, but no linear relationship was found between level of inbreeding and inbreeding depression. Both condition factor (K) and the number of observed deformities appears not to be significantly affected by inbreeding at all three levels.

Over all, the average inbreeding depression at $F = 0.250$ and $F = 0.375$ respectively was found to be 46.5 percent and 46.6 percent for body weight (BW); 18.2 percent and 18.0 percent for standard length (SL); 21.8 percent and 20.3 percent for specific growth rate (SGR) and 5.752 percent and 8.940 percent for flesh yield. The outbred Control group differed significantly ($P < 0.05$) from the six inbred family groups in terms of body weight (BW), standard length (SL), specific growth rate (SGR) and yield at all levels of inbreeding studied ($F = 0.000$, $F = 0.250$ and $F = 0.375$).

Average inbreeding depression for body weight (BW) amongst the six inbred families ranged from 39.6 to 54.2 percent at $F = 0.250$ (in Gen 2) and 45.6 to 47.3 percent at $F = 0.375$ (in Gen 3). The inbreeding depression coefficient for body weight (BW) per 10% increase in F , amongst the six inbred families, ranged from 15.9 to 21.7 percent at $F = 0.250$ and from 12.2 to 12.6 percent at $F = 0.375$.

Average inbreeding depression for standard length (SL) amongst the six inbred families ranged from 14.0 to 22.3 percent at $F = 0.250$ and from 17.2 to 18.4 percent at $F = 0.375$.

The inbreeding depression coefficient for standard length (SL) amongst the six inbred families ranged from 5.6 to 8.9 percent at $F = 0.250$ and from 4.6 to 4.9 percent at $F = 0.375$.

Average inbreeding depression for specific growth rate (SGR) amongst the six inbred families ranged from 17.9 to 27.9 percent at $F = 0.250$ and from 16.7 to 27.2 percent at $F = 0.375$. The inbreeding depression coefficient amongst the six inbred families ranged from 7.2 to 11.2 percent at $F = 0.250$ and from 4.5 to 7.3 percent at $F = 0.375$.

Average inbreeding depression for yield amongst the six inbred families ranged from 0.4 to 7.7 percent at $F = 0.250$ and from 8.5 to 10.2 percent at $F = 0.375$. The inbreeding depression coefficient for yield amongst the six inbred families ranged between 0.2 and 3.1 percent at $F = 0.250$ and from 2.3 to 2.7 percent at $F = 0.375$.

The condition factor (K) of the six inbred families showed no significant differences to the Control ($P > 0.05$) at all levels of inbreeding with K-values ranging from 1.42 to 2.85.

The occurrence of morphological deformities in all seven family groups including the Control showed no noticeable trend, with a random, nonlinear occurrence of fluctuating asymmetry observed at different inbreeding levels in *O. mossambicus*.

This study demonstrates that inbreeding has a significant negative effect on the production traits of *Oreochromis mossambicus*, especially growth. Results from this study emphasize the need to create awareness amongst small scale farmers of the importance of preventing uncontrolled inbreeding in production systems, as well as to monitor inbreeding levels during the process of dissemination of improved fish strains to small scale fish growers in developing countries, including Africa.

OPSOMMING

Die gevolge van akute inteling op die groei prestasie, opbrengs en voorkoms van misvorming eienskappe bestudeer in eksperimentele full-broer ingeteelde bevolkings van Mosambiek tilapia *Oreochromis mossambicus* op drie vlakke van inteling koëffisiënte, naamlik $F = 0,000$, $F = 0,250$ en $F = 0,375$. Die basis bevolking $F = 0,000$ gestig deur die kruising van twee geografies geskei en geneties onverwant plaas aandeel. Op elke generasie, die inteling depressie aangedui deur die liggaam gewig (BW), standaard lengte (SL), spesifieke groeitempo (SGR) en opbrengs was hoogs betekenisvol, maar geen lineêre verband is gevind tussen vlak van inteling en inteling depressie. Beide toestand faktor (K) en die aantal waargeneem deformiteite verskyn om nie beduidend beïnvloed deur inteling op al drie vlakke.

Oor alles is, die gemiddelde inteling depressie by $F = 0,250$ en $F = 0,375$ onderskeidelik gevind om 46,5 persent en 46,6 persent vir die liggaam gewig (BW) wees; 18,2 persent en 18,0 persent vir standaard lengte (SL); 21,8 persent en 20,3 persent vir spesifieke groeitempo (SGR) en 5,752 persent en 8,940 persent vir vlees opbrengs. Die outbred beheer betekenisvol verskil ($P < 0,05$) van die ses ingeteelde familie groepe in terme van die liggaam gewig (BW), standaard lengte (SL), spesifieke groeitempo (SGR) en opbrengs op alle vlakke van inteling bestudeer ($F = 0,000$, $F = 0,250$ en $F = 0,375$).

Gemiddeld inteling depressie vir die liggaam gewig (BW) onder die ses ingeteelde families gewissel 39,6-54,2 persent by $F = 0,250$ (in Gen 2) en 45,6-47,3 persent by $F = 0,375$ (in Gen 3). Die inteling depressie koëffisiënt vir die liggaam gewig (BW) per 10% toename in F , onder die ses ingeteelde families, het gewissel 15,9-21,7 persent by $F = 0,250$ en 12,2-12,6 persent by $F = 0,375$.

Gemiddeld inteling depressie vir standaard lengte (SL) onder die ses ingeteelde families gewissel 14,0-22,3 persent by $F = 0,250$ en 17,2-18,4 persent by $F = 0,375$. Die inteling

depressie koëffisiënt vir standaard lengte (SL) onder die ses ingeteelde families gewissel 5,6-8,9 persent by $F = 0,250$ en 4,6-4,9 persent by $F = 0,375$.

Gemiddeld inteling depressie vir spesifieke groeitempo (SGR) onder die ses ingeteelde families gewissel 17,9-27,9 persent by $F = 0,250$ en 16,7-27,2 persent by $F = 0,375$. Die inteling depressie koëffisiënt onder die ses ingeteelde families gewissel 7,2-11,2 persent by $F = 0,250$ en 4,5-7,3 persent by $F = 0,375$.

Gemiddeld inteling depressie vir opbrengs onder die ses ingeteelde families het gewissel van 0,4-7,7 persent by $F = 0,250$ en 8,5-10,2 persent by $F = 0,375$. Die inteling depressie koëffisiënt vir opbrengs onder die ses ingeteelde families gewissel tussen 0,2 en 3,1 persent by $F = 0,250$ en 2,3-2,7 persent by $F = 0,375$.

Die toestand faktor (K) van die ses ingeteelde families het geen betekenisvolle verskille op die Beheer ($P > 0,05$) op alle vlakke van inteling met K-waardes wissel 1,42-2,85.

Die voorkoms van morfologiese afwykings in al sewe familie groepe, insluitend die beheer het geen merkbare neiging, met 'n ewekansige, nie-lineêre voorkoms van wisselende asimmetrie waargeneem op verskillende vlakke in inteling *O. mossambicus*.

Hierdie studie toon dat inteling het 'n beduidende negatiewe uitwerking op die produksie-eienskappe van *Oreochromis mossambicus*, veral groei. Resultate van hierdie studie beklemtoon die noodsaaklikheid om bewustheid onder kleinboere van die belangrikheid van die voorkoming van onbeheerde inteling in die produksie stelsels, sowel as om te monitor inteling vlakke tydens die proses van verspreiding van verbeterde vis stamme klein skaal vis produsente in ontwikkelende lande te skep, insluitend Afrika.

DEDICATION

I dedicate this Thesis to God Almighty for His favors and mercies. A special feeling of gratitude to my loving parents, Alhaji and Alhaja Kolawole Akinoshun, whose words of encouragement and push for tenacity ring in my ears; and to my sisters, Tolamise, Temitope and my little brother Abiodun Akinoshun, who have never left my side and are very special.

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GLOSSARY

Allele Frequency: The relative proportion of all alleles of a gene that are of a designated type.

Allozyme: Any of the alternative electrophoretic forms of a protein coded by different alleles of a single gene.

Androgen: An individual whose nuclear DNA is all paternally inherited. Also a term used for hormones that stimulate activity of accessory sex organs and sexual characteristics in males. Testosterone is one of these hormones.

Autosomes: Chromosome pairs which are alike in both sexes; all chromosomes other than the sex chromosomes.

Backcross: Cross of an F1 heterozygote with a partner that has the same genotype as one of its parents.

Breed: Group of animals having a common origin and identifying characters that distinguish them as belonging to a breed group.

Breeding value: Genetic worth of an animal's genotype for a specific trait.

Carrier: Heterozygote for a recessive allele.

Dam: Female parent, the mother of an animal.

Deleterious gene: Gene which in either homozygous or heterozygous state has an undesirable effect on an individual's viability and usefulness.

Effective population size: The number of adult individuals in a population who contributes offspring to the next generation.

Environmental variance: The variance, in absolute terms, of any character in a population which is due to environmental influences.

Epistasis: Genetic effect due to interaction among two or more pairs (or series) of non-allelic genes (genes at different loci).

F₁: First filial generation from a given mating; the first generation of descent from a given mating.

F₂, F₃ ... F_n: Subsequent filial generations of descents from a given mating, produced by inter-crossing or self-fertilizing individuals from previous generation.

Family: The denotation of a line of descent representing a group of animals having a genetic relationship.

Fitness: A measure of the average ability of individuals with a given genotype to survive and reproduce.

Full-sib: Progeny or offspring from the mating of a specific male and female.

Gene expression: The multistep process by which a gene is regulated and its product synthesized, thus making a contribution to the phenotype.

Gene frequency: The proportion in a population of the loci of a given allelic series occupied by a particular gene or the frequency of a gene at a locus for a population.

Gene pool: The totality of genetic information in a population of organisms.

Genetic drift: Changes in gene or allele frequency in a population due to chance variations in proportions of gametes that are formed carrying specific genes or that succeed in accomplishing fertilization. Also called "random genetic drift" and is caused by small reproducing population numbers.

Genetic marker: Any pair of alleles or DNA sequence whose inheritance can be traced through a mating or a pedigree.

Genetics: Basically the study of heredity and the variation of inherited characteristics.

Genotype frequency: The proportion of members of a population that are of a particular prescribed genotype.

Genotype: The complete genetic make-up of an individual.

Genotype-environment (GxE) interaction: When the rank or value of a genotype changes relative to other genotype when the environment changes.

Genotypic variance: The part of the phenotypic variance that is attributed to difference in genotype.

Half-sib: One of a pair of animals having one common parent; half-brother or sister.

Heterosis: The superiority of hybrids or offspring over either parent in respect of one or more traits.

Heterozygote: An individual in which a given locus in a chromosome pair carries dissimilar alleles of one or more genes; an individual with different alleles at some particular locus.

Homozygote: An individual in which both members of a chromosome pair carry the same gene at a specific locus.

Inbreeding coefficient, F : A measure of the probability that two genes at any locus in an individual are identical by descent from the common ancestor(s) of the two parents. (Inbreeding values range from 0 - 1); 0 indicates no inbreeding. Under normal assumptions of panmixia the fixation index and the inbreeding coefficient are synonymous ($f = F$).

Inbreeding coefficient, f : refers to the inbreeding coefficient based on pedigree relationships, which under specific conditions (e.g. recent population bottlenecks) are not synonymous to the fixation index ($f \neq F$).

Inbreeding depression: Decrease in performance of traits due to inbreeding.

Inbreeding: A system of mating in which mates are more closely related than average individuals of the population to which they belong; mating between relatives.

Line breeding: A form of inbreeding in which effort is made to maintain high relationships in subsequent generations with a favored ancestor.

Line: A population or genetic group developed by direct breeding usually involving some type of inbreeding, line-breeding or selection.

Parent generation: The parent used in a cross or the original parents in a series of generations.

Pedigree: A record of the animals from which a given individual has descended; a diagram representing the familial relationships among relatives.

Phenotype: The external appearance, performance or some other observable or measurable characteristics of an individual.

Population: A group of organisms of the same species inhabiting a single locality and forming a single unit.

Progeny: Young or offspring of a given individual.

Random genetic drift: Fluctuations in allele frequency from generation to generation resulting from restricted population size.

Random mating: A breeding situation in which any male or female has an equal probability of mating with any other individual of opposite sex in the population regardless of similarity or dissimilarity of appearance, measurable characteristics or parentage.

Recessive allele: Genes that have no observable effect unless present in both members of a chromosome pair, expressed only in homozygous state.

Siblings: A brother or sister; each having the same parent.

Sire: Male parent, the father of an animal.

Species: A group of animals possessing common (one or more) distinctive characteristics and which are fully fertile when inter-mated. They are kept genetically distinct through various forms of reproductive isolation from other species.

Strain: A breeding unit within a species with the same origin and history that possess at least one unique trait different from other strains.

Trait: Any aspect of the appearance, behaviour, development, biochemistry, or other features of an organism.

1 INTRODUCTION

1.1 BACKGROUND

The world population is expected to increase from the current 7.5 billion to 9.6 billion by 2050, with malnutrition to affect over 800 million people on a global scale (FAO, 2012). The challenge remains to provide for the growing demand for food and animal protein in particular, while sustaining the natural resources and the environment for future generations. Fish is an important food source providing 16 percent of the total human consumption of animal protein (FAO, 2014), whilst global per capita consumption of fish increased from 9.9kg in the 1960s to about 19.2kg in 2012 (FAO, 2014). Aquaculture is recognised as the fastest growing food producing sector in the world with an average annual growth rate of 8.6 percent over the past two decades, reaching a total of 70.5 million tonnes in 2013, and contributing close to 50 percent of global fish consumption (FAO, 2014). The significance of China as the leading contributor of 65 percent of global aquaculture production (43.5 million tonnes in 2013) deserves specific mention in this regard (FAO, 2014).

A stagnant supply from capture fisheries, together with increased per capita consumption, global population growth and rising income levels is expected to provide further impetus for the rapid expansion of aquaculture production in order to meet this rising demand for a sustainable and affordable source of animal protein (Bentsen & Olesen, 2002; FAO, 2014).

1.2 AQUACULTURE IN AFRICA

Unlike in Asia and Europe, aquaculture in Africa is slow in responding to advances in production with a total contribution of only 2.23 percent (1.49 million tonnes, US\$3 billion) to global aquaculture production in 2012 (FAO, 2014). The slower than expected growth is attributed to various limiting factors such as lack of focus on the entire fish value chain (low quality feeds, genetically unimproved seeds, lack of improved techniques of culturing, processing and marketing of fish and fish products). The novelty of aquaculture on the continent, the general poor economic condition of many countries in Africa and the relative

lack of entrepreneurial skill and credit facilities coupled with a weak government interest also hinders its development (FAO, 1997). Africa, nevertheless, has good potential for aquaculture development and expansion in terms of the availability of land and water resources (Changadeya *et al.* 2003).

Aquaculture production in Africa is predominantly composed of species that feed low in the food chain, such as cyprinids and tilapia, as well as catfish. Tilapia is a major aquaculture species in Africa, and accounts for 40 percent of aquaculture production in Africa (FAO, 2014). Production mainly comes from small and medium scale farmers in rural areas that practice fish farming as a part-time activity or secondary form of agriculture, using extensive to semi-intensive fresh water culture systems.

1.3 GENETIC IMPROVEMENT STRATEGIES IN AQUACULTURE

In the case of terrestrial farm animals and crops, genetic improvements have made a significant contribution to increased productivity over centuries of on-farm selection and application of modern breeding and selection techniques (Bentsen & Olesen, 2002).

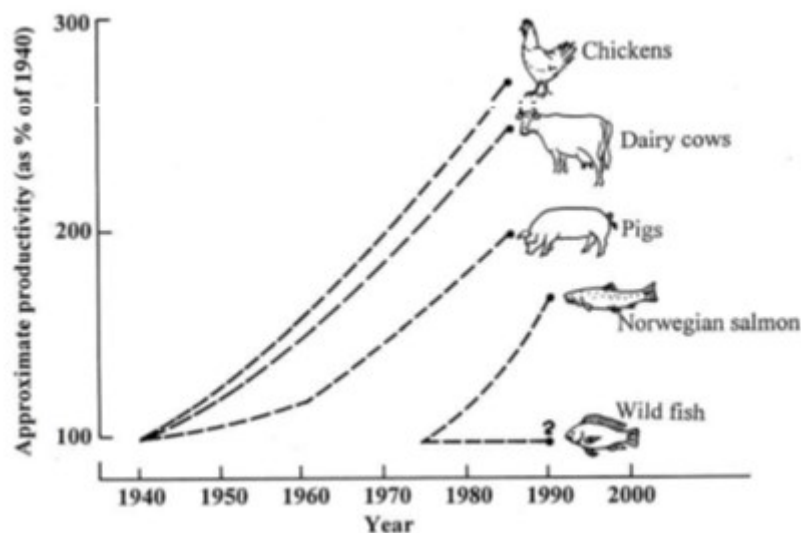


Figure 1.1: Trend of genetic improvement in terrestrial farm animals and fish (Gjedrem, 2005)

On the contrary, less than 10 percent of the current world aquaculture production is based on improved stocks (Gjedrem *et al.* 2012). Production of many species still depends on wild caught fry and broodstock which have been described often to have a poor yield and low

genetic variance due to non-randomness or limited sample size (McAndrew & Napier, 2011). In developing countries and Africa in particular, fish species used in aquaculture are mainly undomesticated and often even genetically inferior to their wild and undomesticated genotypes. This has been described to be as a result of small founder populations, poor broodstock management, irrational mating systems and poor understanding of basic genetics among fish farmers, which leads to genetic drift, inbreeding depression and retrogressive hybridization in most culture populations (Pullin & Capili, 1988; Eknath *et al.*, 1991; Brummett *et al.* 2004).

The adoption and application of genetic improvement techniques including individual and family selection, hybridization, sex reversal, chromosome set and gene manipulations have contributed to the development of genetically superior strains and improved performance in aquaculture species such as Atlantic salmon (Gjedrem, 1997), Rainbow trout (Donaldson & Olson, 1955), Pacific oyster (Evans *et al.*, 2004), Nile tilapia (Eknath, 1995; Eknath & Acosta, 1998; Dey & Gupta, 2000; ICLARM, 2001) and Common carp (Wohlfarth *et al.*, 1983; ICLARM, 2001). The development of the genetically improved farmed tilapia (GIFT) strain of *Oreochromis niloticus* at the World Fish Centre in the Philippines is a distinct example of genetic improvement with up to 85 percent increase in growth rate (Eknath & Acosta, 1998). Similar protocols are being adopted in Egypt, Cote d' Ivoire, Ghana and Malawi to develop local strains of *Oreochromis niloticus* and *Oreochromis shiranus* (Gupta *et al.*, 2001). These genetic improvement programs are designed to maximize farming efficiency, reduce cost of production, and bring socio-economic benefits, especially to poor rural small and medium farmers in the developing countries and in Africa by improving growth, survival, feed conversion ratio and resistance to diseases (Bentsen & Olesen, 2002).

The contribution of such genetic improvements to increase production in small and medium scale aquaculture farming in developing countries, is however compromised by poor genetic maintenance of these strains within small breeding population, poor understanding of basic principles of genetics and non-random mating that result in reduced effective population

size, random genetic drift, increased rate of inbreeding and inbreeding depression of production traits.

1.4 INBREEDING AND INBREEDING DEPRESSION

Inbreeding is a phenomenon which could undermine genetic gains and production performance when not controlled and monitored in production systems and breeding programs (De Donato *et al.*, 2005). Inbreeding refers to mating between close relatives in a population that leads to a reduction in heterozygosity of genes and an increase in homozygosity on the allelic level, which may expose deleterious or lethal recessive alleles (Lande, 1994; Lynch & Walsh, 1995) and their expression in the homozygote state (Barrett & Kohn, 1991; Brock & White, 1992; Pray *et al.*, 1994; Vrijenhoek, 1994). This negatively impacts life history traits and productive fitness causing reduced growth, low survival rate during the eggs, fry and adult stages, low reproductive performance with a higher proportion of biochemical disorders and deformities in fish populations (Gjedrem, 2005). The loss of fitness and productive performance in inbred individuals is referred to as inbreeding depression (Wright, 1977; Shields, 1987), quantified in terms of the inbreeding coefficient, F .

In developing countries *per se*, inbreeding has been a major problem, yet poorly understood both with regard to the establishment and management of farmed stock (Lacy *et al.*, 1993; Crnokrak & Roff, 1999). The main contributing factors are the small effective population size and the high fecundity of the breeders that lead to an increased probability of mating among relatives with the inevitable increases in inbreeding (Bentsen & Olesen, 2002).

1.5 VULNERABILITY OF SMALL SCALE FARMERS TO INBREEDING

Small scale farming systems, making up the bulk of aquaculture production in Africa and other developing regions, are particularly exposed to the deleterious effects of inbreeding. The high relative fecundity of the major cultured species such as catfish, carp and tilapia implies that only a small number (10–30 individuals) of broodstock is required to produce sufficient quantities of seed to sustain annual production. The use of a limited number of

broodstock and non-random mating contributes to a reduction in the effective population size, followed by accelerated inbreeding and cumulative levels of inbreeding depression in production traits (Eknath & Doyle, 1990).

Even when improved genetic materials are made available to these small scale farmers, the poor understanding of causes and effects of inbreeding, together with a lack of basic knowledge of genetic maintenance of such improved strains, rapidly erodes the genetic integrity of such strains, undermining the objectives of such genetic improvement program and the expected improvement in production performance (Gjedrem, 2005).

There is therefore a need for the establishment of effective strategies for the dissemination and maintenance of genetic materials amongst rural small scale farmers in developing regions including Africa, together with creation of an awareness of the deleterious effects and extent of inbreeding in closed aquaculture environments.

1.6 AIMS OF STUDY

Although inbreeding has been shown to depress fitness and production performance in a number of aquatic species such as salmonids (Kincaid, 1983), carps (Eknath, 1991), tilapia (Ch'ang, 1971b), shrimps (Crococ *et al.*, 2002; De Donato *et al.*, 2005), there is relatively little literature on the effects of inbreeding in the Mozambique tilapia (*O. mossambicus*). The aim of this study therefore, is to quantify the effects of inbreeding on the growth performance and yield of Mozambique tilapia (*O. mossambicus*), thereby creating an awareness of the deleterious effects on production performance and the need to promote effective guidelines for monitoring and control of inbreeding among small and medium scale producers in developing countries, including Africa.

1.7 SPECIFIC OBJECTIVES OF THE STUDY

The specific objectives of this study are:

- To estimate the level of inbreeding depression in relation to growth and yield traits in Mozambique tilapia (*O. mossambicus*), over three generations of repeated full-sib mating.

- To describe the occurrence of morphological deformities associated with increased inbreeding in Mozambique tilapia (*O. mossambicus*).

1.8 SUMMARY

Aquaculture is seen as an important contributor to future global food supply (Pullin *et al.*, 1994; Williams & Bimbao, 1998; Dey & Bimbao, 1998; FAO, 2012); whilst genetic improvement is expected to contribute towards improved productivity through the domestication of wild stocks and the development of genetically improved strains (Changadaya *et al.*, 2003). Productivity of small scale farming systems in Africa and other developing regions however is undermined by the occurrence of uncontrolled inbreeding and subsequent inbreeding depression in production traits (Brummett *et al.*, 2004; Brummett & Ponzoni, 2009).

This study aims to quantify the effect of inbreeding on growth traits in *O. mossambicus* and propose specific measures and protocols to protect the genetic quality of farmed stocks, with specific reference to rural small and medium farming systems.

2 LITERATURE REVIEW

2.1 BACKGROUND

The development and application of the theory of genetic improvement in relation to fish farming has been well documented by Dunham *et al.* (2000), Dunham (2004) and Gjedrem (2005). Although widely applied to animal breeding since the 1900s, little progress was made in relation to fish due to the limited scale of aquaculture at the time. Since the 1970s, genetic improvement of aquatic organisms gained momentum and the first long term breeding programs were established for salmon in Norway and catfish in the U.S.A. (Hulata, 2001). Multinational programs such as the Genetically Improved Farmed Tilapia (GIFT) in the Philippines on *Oreochromis niloticus* was established in the 1990s, already exceeding 21 generations (Dey *et al.*, 2000; Dunham, 2011).

Currently genetic improvement of cultured fish species focus on a variety of production traits aimed at reducing total production costs by increasing growth rate, improving feed conversion efficiency (FCE), enhancing fish disease resistance, and improving yield and product quality (Gjedrem, 2000). Examples of such improvement was documented with the Nile tilapia in the Philippines with reports of up to 85 percent improvement in growth (Eknath & Acosta, 1998, Eknath *et al.*, 1998; ICLARM, 2001), 46 percent in survival and yield improvement of 25–78 percent depending on local conditions (Eknath *et al.*, 1998; ICLARM, 1998) in the GIFT strain (Hulata, 2001). Other fish species that have been developed as such includes the Channel catfish, Rainbow trout, Coho salmon, Atlantic salmon, Pacific oyster and Common carp through the use of selective breeding and biotechnology techniques. However, aquaculture is still impeded by the lack of genetic improvement in various species where producers are still dependent on the use of wild undomesticated stock; especially in developing countries including Africa, where aquaculture still lacks development and the technology is not readily available (e.g. Shrimp, Tilapia and African catfish). Despite the genetic gains that have been reported for aquatic species, it is

estimated that less than 10 percent of global aquaculture production originates from genetically improved stocks (Gjedrem, 2005).

2.2 GENETIC IMPROVEMENT IN AQUACULTURE

Compared to plants and terrestrial livestock industries, aquaculture still trails behind in utilizing genetic improvement and selective breeding techniques as tools for improving production efficiency of fish stock (Delgado *et al.*, 2003). More so even in relation to developing countries including Africa where productions are mostly dependent on the use of genetically inferior undomesticated wild stocks (Eknath, 1991; Bentsen & Olesen, 2002; Brummett *et al.*, 2004; McAndrew & Napier, 2011). Several large scale experiments and breeding programs have shown 10–15 percent genetic gains for various traits per generation, which is much higher than that reported for other farm animals (Gjedrem, 2000; Hulata, 2001).

The improvement of knowledge in relation to reproductive biology of fish, artificial reproduction and early larvae rearing, including genetic developments of economically important aquatic species, have recently allowed fish breeders to genetically improve fish species through domestication and artificial selection (Bentsen & Olesen, 2002). Significant improvements have been achieved in the last few decades in farmed fish species such as in Rainbow trout, *Oncorhynchus mykiss* W. (Donaldson & Olson, 1957; Gjerde, 1986); Atlantic salmon, *Salmo salar* L. (Gjerde, 1986; Gjerde & Korsvoll, 1999; Gjedrem, 2000); Coho salmon, *Oncorhynchus kisutch* W. (Hershberger *et al.*, 1990; Myers *et al.*, 2001); Channel catfish, *Ictalurus punctatus* R. (Smitherman & Dunham, 1985; Dunham & Brummett, 1999) and Nile tilapia *Oreochromis niloticus* L. (Eknath *et al.*, 1993; Eknath *et al.*, 1998; Bentsen *et al.*, 1998; Basiao & Doyle, 1999; Rye & Eknath, 1999) through genetic manipulation, biotechnology and selection for valued traits.

Genetic improvements of growth in fish have also been linked to improved feed conversion efficiency and survival rate (De Donato, 2005). Improvement of growth has been the main

focus of selective breeding in fish, compared to other traits, because of the overall improvement in utilization of limited resources such as feed, labour, water and land which are generally experienced by farmers in the developing world, including Africa.

Poor genetic practices such as inbreeding can lead to deterioration in performance of cultured stock compared to wild populations (Gjerde *et al.*, 1996; Komen *et al.*, 2006). Inbreeding is caused mainly by small effective population size and subsequent loss of genetic variation (Bentsen & Olesen, 2002). Examples have been reported in Nile tilapia, *Oreochromis niloticus*, where uneven contributions amongst broodstock have resulted in significant reduction in effective population size, resulting in significant inbreeding depression, genetic drift and reduced genetic variation in farmed stock (Fassehaye, 2006; Fassehaye *et al.*, 2007; Fassehaye *et al.*, 2009; Blonk *et al.*, 2009).

In addition, the high fecundity of the major aquatic species that are cultured in Africa, such as catfish and tilapia, requires the farmers to use only a limited number of broodstock of about ten to thirty to produce adequate number of seed (Bentsen & Gjerde, 1994). Thus, this shows that even where selective breeding is applied, the high fertility and small number of broodstock allow for very high selection intensities within the population, resulting into genetic degradation of stock due to the rapid accumulation of inbreeding and inbreeding depression (Ekmath & Doyle, 1990; Evans *et al.*, 2004; Pante *et al.*, 2001a; Rye & Mao, 1998). This is especially relevant in the case of small and medium scale African farmers who often lack basic knowledge in relation to good genetic management systems.

2.3 APPROACHES TO GENETIC IMPROVEMENT

Genetic modification methods such as selection, gene transfer, chromosome set manipulations, crossbreeding and hybridization etc. hold tremendous potential for improving the quality and quantity of fish reared in aquaculture, though not without significant consumer reactions and risks (Delgado *et al.*, 2003). Genetic improvement programs have contributed significantly towards more efficient, productive and profitable aquaculture

systems in the developed countries. Such programs involve selecting superior broodstock, using optimum breeding techniques, and minimizing environmental impacts in relation to conservation and biodiversity. The key methodologies applied with regards to genetic improvement of aquaculture species are summarised in [Table 2.1](#).

Table 2.1: Genetic improvement methods and their application in aquaculture

Improvement Methods	Applications (Species)	References
<p>1. Selective breeding</p> <p>a) Mass selection</p> <p>b) Family selection</p> <p>c) Combined or index selection</p> <p>d) Marker Assisted Selection (MAS), including Quantitative Traits Loci (QTL's)</p>	<p>Kuruma shrimp <i>Penaeus japonicus</i> in Australia; Rainbow Trout in USA; Nile Tilapia in Philippines; Brown tiger prawn <i>P. esculentus</i> in Australia; Coho salmon in Chile and Iceland; Rainbow trout in Chile, France and U.S.A., <i>Oreochromis shiranus</i> in Malawi; Blunt snout bream <i>Megalobrama amblycephala</i> in China; Pacific oyster <i>Crassostrea gigas</i> in Australia and Oregon, USA; Sydney rock oyster <i>Saccostrea glomerata</i> in Australia; Channel catfish, <i>Ictalurus punctatus</i>; Rainbow trout; Atlantic salmon, <i>Salmo salar</i>; Coho salmon, <i>Oncorhynchus kisutch</i>; Nile tilapia, <i>Oreochromis niloticus</i>.</p>	<p>Hetzel <i>et al.</i> (2000); Donaldson & Olson (1957); Eknath <i>et al.</i> (1998); Preston <i>et al.</i> (2001); Jónasson (1996), Jónasson, Gjerde & Gjedrem (1997); Hulata <i>et al.</i>, 1999; Li & Yang (1996), Li & Cai (2000); Ward <i>et al.</i> (2000), Hedgecock <i>et al.</i> (1996); Nell, Smith & McPhee (2000); Smitherman & Dunham (1985), Dunham & Brummet (1999); Gjerde (1986); Gjerde & Korsvoll (1999), Gjedrem (2000); Hershberger <i>et al.</i> (1990), Myers <i>et al.</i> (2001); Eknath <i>et al.</i> (1993), Bentsen <i>et al.</i> (1998), Basiao & Doyle (1999), Longalong <i>et al.</i>, 1999, Rye & Eknath (1999).</p>
<p>2. Hybridization and Crossbreeding</p> <p>a) Species (Hybridization)</p> <p>b) Strains (Crossbreeding)</p>	<p>Common carp hybrid in Israel, Vietnam, China and Hungary; Pacific Oyster; The sunshine bass hybrid between female white bass <i>Morone chrysops</i> and male striped bass <i>M. saxatilis</i>; Reciprocal crosses between silver carp <i>Hypophthalmichthys molitrix</i> and bighead carp <i>Aristichthys nobilis</i>; Cyprinid hybrid of grass carp <i>Ctenopharyngodon idella</i> x bighead carp <i>Aristichthys nobilis</i> in Hungary; Hybrid of the channel catfish <i>I. punctatus</i> female x the blue catfish <i>I.</i></p>	<p>Wohlfarth (1993), Bakos & Gorda (1995), Hulata (1995); Hedgecock, McGoldrick & Bayne (1995), Hedgecock <i>et al.</i> (1996); Harrell (1997, 1998); Issa <i>et al.</i> (1986); Bakos, Krasznai & Marian (1978); Smitherman & Dunham (1985); Wohlfarth & Hulata (1983), Penman & McAndrew (2000); Dorson, Chevassus & Torhy (1991); Galbreath & Thorgaard (1996).</p>

	<p><i>furcatus</i> male; Hybridization between Nile tilapia <i>O. niloticus</i> x the blue tilapia <i>O. aureus</i>; <i>O. niloticus</i> x <i>O. urolepis hornorum</i>/<i>O. macrochir</i>, <i>O. mossambicus</i> x <i>O. urolepis hornorum</i>; Triploid hybrids of rainbow trout <i>Oncorhynchus mykiss</i> x char <i>Salvelinus</i> spp.; Triploidy of Atlantic salmon <i>Salmo salar</i> x brown trout <i>S. trutta</i> hybrids.</p>	
<p>3. Genetic manipulation and Biotechnology</p> <p>a) Sex manipulation</p> <p>b) Chromosome set manipulation</p> <p>c) Genetically modified organisms (GMOs)</p>	<p>Monosex female salmonids; Monosex female rainbow trout in the UK, France, Canada and Japan; Cloned hirame (Japanese flounder, <i>Paralichthys olivaceus</i>); Triploid grass carp in U.S.A.; YY male genotypes of Nile tilapia; Transgenic Atlantic salmon <i>Salmo salar</i>, Rainbow trout <i>Oncorhynchus mykiss</i>, Tilapia <i>Oreochromis niloticus</i>, Common Carp <i>Cyprinus carpio</i>, Channel catfish <i>Ictalurus punctatus</i>, African catfish <i>Clarias gariepinus</i> and northern pike <i>Esox Luciu</i>; Gold fish <i>Carassius auratus</i>.</p>	<p>Donaldson & Devlin (1996); Chourrout <i>et al.</i> (1986), Quillet <i>et al.</i> (1991), Arai (2001), Devlin <i>et al.</i> (1991, 1994); Yamamoto (1999); Cassani (1996); Mair <i>et al.</i> (1995, 1997), Tuan, Little & Mair (1998, 1999), Abucay <i>et al.</i> (1999), Beardmore, Mair & Lewis (2001). Rahman, Iyengar & Maclean (1997), Venugopal <i>et al.</i> (1997), Rahman <i>et al.</i> (1998), Hinitz & Moav (1999), Kang <i>et al.</i> (1999), Rahman & Maclean (1999), Li & Tsai (2000), Sin <i>et al.</i> (2000), Tsai (2000); FAO (2000a).</p>

2.3.1 SELECTIVE BREEDING

Selective breeding is a process used to exploit heritable phenotypic variance to identify and select as parents for the next generation individuals that have the highest additive genetic merit for the selected trait(s). Selection is a proven method of domestication and obtaining continuous long-term genetic improvement and has yielded positive results in relation to a significant number of aquaculture fish species (Gjedrem, 1997, 1998). Some of the species that have been improved via individual, family or combined selection techniques include:

Table 2.2: Selective breeding methods and application in aquaculture species

Species	Trait	Gain	Reference
Atlantic salmon	Growth rate per gen	10.6-14.4%	Gjerde (1986)
Coho salmon	Growth rate per gen	10.1%	Hershberger <i>et al.</i> (1990)
Rainbow trout	Growth rate per gen	13%	Gjerde (1986)
Channel catfish	Growth rate per gen	12-20%	Bondary (1983); Dunham (1987)
Tilapia	Growth rate per gen	15%	Eknath & Acosta (1998)
Shrimp	Growth rate; FCR	13%, 14.5%; 21%; 35%	Luo <i>et al.</i> (2014); De Donato <i>et al.</i> (2005); Goyard <i>et al.</i> (2002a,b)

2.3.1.1 MASS SELECTION

Mass selection or individual selection is the most commonly used method of artificial selection in aquaculture due to its simplicity and cost efficiency (Bentsen & Olesen, 2002; Doyle, 2002). This selection method involves selecting parents based on individual phenotypic values. Family relationships are totally ignored and individuals are selected or culled based on merit for the selected trait(s) (Tave, 1999).

One of the major constraints of mass selection though is inbreeding resulting from poor genetic management and non-random mating in the population, which may affect fitness related traits in particular and counter genetic gains (Moav & Wohlfarth, 1976; Hulata *et al.*, 1986; Teichert-Coddington & Smitherman, 1988; Doyle, 2002; De Donato *et al.*, 2005).

2.3.1.2 FAMILY SELECTION

This selection method involves the ranking of family groups according to the mean performance of each family relative to the trait(s) of interest. At the end of evaluation, each family group is either discarded or selected for that particular trait as breeders for the next generation (Lush, 1947). This type of selection is more efficient when the heritability for the selected trait is low, a large number of families are involved and environmental factors constitutes significantly to the phenotypic variance of the selected trait(s).

There is a distinction with regard to:

- Between family selection – commonly referred to as family selection i.e. selection of the top performing family (-ies) as a whole.
- Within family selection – selection of the top performing individuals from each of the families involved.

2.3.1.3 COMBINED OR INDEX SELECTION

This method is the most efficient method of selection as it combines information from the individuals, full and half-sib families and progeny groups, as well as pedigree information altogether in a breeding plan. It can also incorporate performance in relation to several traits into an index or breeding value, taking into account the relative economic value of the traits under selection.

A suitable example where this method of selection has been exclusively used to improve genetic quality of fish is the “Genetic Improvement of Farmed Tilapias” (GIFT) Project in the Philippines, Asia initiated by the International Centre for Living Aquatic Resources Management (ICLARM) in 1988. The primary objective of the project was to develop improved breeds of Nile tilapia for low-cost sustainable aquaculture. The project adopted a combined family and within family selection strategy whereby test fish were ranked based on their breeding value. The selected fish had a faster growth rate of 20 percent than the base population and was about 60 percent heavier at harvest than the most commonly farmed

strain in the Philippines (Ekanth, 1995; Dey & Gupta, 2000). Further selection through generations resulted into an average genetic gain in growth rate of 12-17 percent and a cumulative genetic gain of 85 percent compared to the base population (Eknath & Acosta, 1998).

2.3.1.4 MARKER ASSISTED SELECTION

Complementary approaches such as hybridization, chromosome manipulation, sex reversal and applications of molecular technology always start with the best stock available from selection, in order to maximize the expected genetic output of these other breeding tools. These selection process can be made more reliable with genetic markers, used to identify loci that controls quantitative traits (QTL) which could be used to develop superior strains. It involves the use of molecular markers such as Randomly Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), and microsatellite markers to generate genetic data to access genetic variability and population structure in fish stock.

A quick example would be a reported reduction in genetic variation in the F7 generation of domesticated populations of *P. vannamei* from Ecuador, compared to wild stocks, using microsatellites markers to characterize both populations (Wolfus *et al.*, 1997). While the genetic makers are faster and more reliable in identifying quantitative trait loci, the only downside to this technique is the cost implication of developing linkage maps.

2.3.2 SPECIES HYBRIDIZATION AND CROSSBREEDING

The occurrence of external fertilization in aquaculture species and the subsequent ease of gamete collection have facilitated numerous examples of inter-specific and inter-generic hybridization and the potentials of using such hybrids in aquaculture (Bartley *et al.*, 2001). Crossbreeding involves the mating of fish from different populations to produce hybrids which are phenotypically superior to both parents (Tave, 1999). Crossbreeding has the advantage of simplicity and its impact is often immediate on animal performance (Hulata, 2001). However, if crossbreeding is the only tool for breeding, further genetic improvement is close to zero beyond that achieved in the first generation (Gjedrem, 1985). Recent studies

have shown that crossbreeding together with pedigree based selection can produce tremendous improvements in aquaculture species (Vandeputte, 2009). An example of crossbreeding in the industry will include the use of *O. niloticus* × *O. aureus* hybrids displaying beneficial all male oriented sex ratios and improved cold tolerance (Hulata *et al.*, 1993), the Szarvas hybrid carps with average growth and survival rate of about 15.4 percent and 28.7 percent respectively (Bakos & Gorda, 1995) and also the *Morone chrysops* × *Morone saxatilis* hybrids, referred to as sunshine bass that grows faster and has better overall culture characteristics than either parental species under commercial culture conditions (Smith, 1988). However, despite the large numbers of reported hybrids few have been successfully cultured for extended periods because of the added complexity of production and the introgression of the hybrids back to the parental species (Penman, 1999) including loss of beneficial characteristics due to inbreeding.

2.3.3 GENETIC MANIPULATION AND BIOTECHNOLOGY

The external fertilization characteristics of most finfish and shellfish species have made possible the manipulation of sex and chromosome numbers of an individual or group of individuals from artificial production exercises.

2.3.3.1 GENETIC SEX MANIPULATION

This genetic improvement technique is employed to produce individuals with only the paternal chromosomes (Androgenesis) or individuals carrying only the genetic materials of the mother (Gynogenesis). Production of mono sex animals is of particular interest in species where one sex grows faster than the other. In some species male grow faster than females like in tilapia and some salmonids while it is the opposite in other species such as rainbow trout *Oncorhynchus mykiss* and Atlantic halibut *Hippoglossus hippoglossus* therefore, it might be of commercial interest to produce all-female or all-male population (Quillet *et al.*, 1991).

This technique has been used to develop various species in aquaculture including the rainbow trout *Oncorhynchus mykiss* and Atlantic salmon *Salmo salar* where females are the

homogametic sex (Gjedrem, 2005). It involves producing phenotypic males from genetic females by sex-reversing with 17 α -methyl-testosterone during first feeding period. The sperm from these sex-reversed females is further used to fertilize eggs from normal females before triploidization to produce all-female triploids.

Another application of this technique would be the birth of the Genetically Modified Tilapia (GMT) in the Philippines which under culture conditions has increasing yields of up to 58 percent compared to the mixed sex tilapia of the same strain (Mair *et al.*, 1995). In Nile tilapia, males are the heterogametic sex and all-male population is more advantageous in commercial production. All-male population has been achieved by producing the YY males (also known as super males) through feminized genetic males (Neo females) crossed with normal males (XY) to give 25 percent females (XX), 50 percent males (XY) and 25 percent super males (YY) (Mair *et al.*, 1997). The YY super males were identified through progeny testing and mated with normal females to produce overall sex ratio of 95 percent viable and fertile males to the majority being males (Scott *et al.*, 1989; Varadaraj & Pandiana, 1989; Mair *et al.*, 1991; Hussain *et al.*, 1994).

2.3.3.2 CHROMOSOME SET MANIPULATION

A wide range of genetic and environmental manipulations have been applied to the chromosome sets of aquatic fish species. For instance, when normally fertilized eggs are heated or pressure shocked at the 2nd meiotic division, a triploid (3n) embryo, containing three complete chromosome set is produced. Triploidy is associated with effective sterility and is applied in relation to species in which maturation slows down growth and/or affects quality, such as rainbow trout (Bye & Lincoln, 1986), channel catfish (Wolters *et al.*, 1991), Clams (Allen *et al.*, 1982), Oysters (Stanly *et al.*, 1984), and common carp (Basavaraju *et al.*, 2002) to mention a few. This technique was also used to improve growth rate and flesh quality in Pacific oysters (Guo *et al.*, 1996).

Bencze (1988) reported a faster growth in triploid rainbow trout compared to diploid reared in seawater cages with no significant difference in chemical content, shape and flesh colour.

Triploid Sydney rock oyster showed an average growth rate of 41 percent compared to its diploid family with higher dry meat weight and higher condition index values after a growth period of 2.5 years (Nell *et al.*, 1994). Similar result was reported for Pacific oysters (Guo *et al.*, 1996) however, triploid tilapia were not significantly different from diploid females in growth rate and proximate compositions (Hussain *et al.*, 1995). Triploid induced sterility is also applied to reduce the risk of stocked or escaped farmed strains interbreeding with native populations (Kozfkay *et al.*, 2006; Mair *et al.*, 2007).

In addition, shock treatments during the first mitotic division will produce a tetraploid embryo with four sets of chromosomes (4n). Male tetraploids produce diploid sperm that can be used to fertilise normal diploid (2n) females to produce triploid (3n) individuals directly, avoiding the need to treat large numbers of fertilized eggs to produce a triploid fish. This has been demonstrated in rainbow trout (Myers *et al.*, 1986). These protocols have now been reported to be available for over 30 different fish and shellfish species in aquaculture (Dunham, 2004).

2.3.3.3 GENETICALLY MODIFIED ORGANISMS (GMOs)

Various molecular technologies have been applied to effect gene manipulations and to produce transgenic fish in an effort to improve production traits. This technique involves the transfer of genes that regulates growth hormones, disease resistance, osmoregulation and general metabolism into aquatic species. To date, at least 10 species including salmon, carps, catfish, tilapia and rainbow trout have been modified for enhanced growth, although none has yet been approved for commercialization (FAO, 1997; FAO, 2000a; Aerni, 2001).

In tilapia, transgenics that contain the exogenous growth hormone (GH) gene construct derived from Chinook salmon have demonstrated growth enhancement (Rahman & Maclean 1997). Studies in salmonids also indicates that the spectacular improvements in growth seen by incorporating growth hormone gene constructs into slow-growing wild strains were not repeated when the same constructs were incorporated into fast-growing domesticated stocks (Devlin *et al.*, 2009). Concerns have been raised by various consumer groups and

conservation agencies on moral, ethical and safety grounds about the use of genetic modifications, which have limited applications in commercial production.

Even though, trans-genesis offers several advantages to tilapia culture, the rate of genetic changes in transgenic tilapia are such that their phenotypic and behavioural properties cannot be easily predicted (Mair, 2002). Apart from the issue of perceived health risks and acceptability, a range of practical constraints such as cost, expertise and environmental risks (Delgado *et al.* 2003) provide indications that transgenic fish are unlikely to become a commercial practice in the immediate future (Mair *et al.*, 2007). Functional genomic work is nevertheless continuing with identifying candidate transgenic genes in the area of improving disease resistance.

In conclusion, application of techniques such as species hybridization, chromosome set manipulations and sex reversal strategies provide opportunities for improvements within one or two generations, though not cumulative in nature as in the case of long term improvement strategies through selective breeding. These improvement techniques should however be used in combination with conventional breeding methods to provide targeted genetic improvements.

2.4 INBREEDING

Understanding the effects of inbreeding is important for the success of aquaculture breeding programs, most importantly in relation to hatchery operations of small and medium scale producers in developing countries with small effective breeding population size. Even though, the development of aquaculture production in Africa is dependent on making genetic gains through applied breeding programs, so is the maintenance of these genetic improvements within the various culture environments (De Donato *et al.*, 2005), by ensuring good broodstock management, keeping appropriate pedigree records and optimum mating strategies to control the effect of inbreeding and inbreeding depression in domesticated and improved fish population.

Inbreeding is a key phenomenon which could undermine genetic gains and genetic variability in fish stock if not monitored and controlled in a breeding program or production systems (Falconer & Mackay, 1996). Although, a small degree of inbreeding in a breeding program or a closed culture population is often inevitable if maximum gains are to be derived from a large number of potential parents, it should be minimized and controlled to avoid inbreeding depression offsetting genetic progress (Falconer & Mackay, 1996).

Inbreeding is widely reported in literatures to be detrimental to aquaculture stocks, although the genetic bases of its effects often remain poorly understood (Husband & Schemske, 1996; Charlesworth & Charlesworth, 1999). Biologically, inbreeding is the mating of individuals more closely related to one another than the average pair in a population, the consequence of which is a reduced variation in life history traits within and among offspring of such populations. Genetically, inbreeding is known to increase homozygosity of genes and reduce the frequency of heterozygous genotypes in a population (Falconer, 1989; Barrett *et al.*, 1991; Brock & White, 1992; Pray *et al.*, 1994; Vrijenhoek, 1994).

Related individuals share common alleles through one or more common parents because each inherited some portion of its genes from the common ancestor(s). When related individuals mate, the alleles they share as a result of their common ancestor(s) may be paired and this produces progeny that are homozygous at one or more loci and are referred to as inbred. For this reason, an individual could become homozygous for deleterious, semi-lethal or lethal phenotypes, which could result into a considerable loss of heterosis (Charlesworth & Charlesworth, 1987), reduce productive performance in relation to growth rate, viability and survival, and increase biochemical disorders and deformities.

Mating between unrelated individuals could also produce offspring that are homozygous at one or more loci as the inbred individuals; however, the difference can only be explained by how these alleles are inherited. Inbred individuals are homozygous because they have alleles that are common by descent whereas non-inbred individuals are homozygous due to alleles that are alike in kind within the population. An experiment by Launey & Hedgecock

(2001), showed that a large load of recessive lethal alleles were responsible for mass mortality of inbred by descent (IBD) F_2 families of the Pacific oyster *Crassostrea gigas*, examined with microsatellite DNA markers.

Nonetheless, inbreeding does not change the gene frequencies in a population, but alters the genotypic frequencies in that population resulting into higher than expected levels of homozygosity in the genotype of the entire population and consequent reduction of heterozygosity in individual genotypes.

The rate of inbreeding in fish population is quantified in terms of the inbreeding coefficient (F) which could be estimated as a function of its effective population size “ N_e ” (Falconer & MacKay, 1996, Tave, 1999) as;

$$F = \frac{1}{2N_e} \quad \text{----- (1)}$$

$$N_e = \frac{4N_m N_f}{N_m + N_f} \quad \text{----- (2)}$$

$$F = \frac{1}{8N_m} + \frac{1}{8N_f} \quad \text{----- (3)}$$

$$F = 1 - (1 - \Delta F)^t \quad \text{----- (4)}$$

$$F_x = \sum \left[\left(\frac{1}{2} \right)^N (1 + F_A) \right] \quad \text{----- (5)}$$

Equations (2) and (3) are most applicable in the case of uneven sex ratios where N_m is the number of males and N_f is the number of females involved in the breeding exercise.

It was reported by Agustin (1999) that the founder stock of Mozambique tilapia (*O. mossambicus*) introduced into Indonesia in the 1950s consisted of only five individuals comprising of three males and two females, even though Atz (1954) reported differently that Mozambique tilapia were actually introduced into Indonesia as far back before 1939. This small parent stock gave rise to progenies for tilapia culture and feral stock of *O. mossambicus* in most part of Asia, inevitably leading to the accumulation of significant levels

of inbreeding within the stock and associated stunting, early sexual maturation, body deformities, low survival rate and poor growth. This has invariably resulted in Mozambique tilapia being disregarded as a potential species for culture in most Asian countries and it has subsequently been replaced with the introduction of Nile tilapia and various other tilapia hybrids.

2.5 INBREEDING DEPRESSION

The associated loss of fitness and productive performance observed in inbred individuals is referred to as inbreeding depression, which is usually expressed as per a 10 percent change in the coefficient of inbreeding (Weaver & Hedrick, 1995; Falconer & Mackay, 1996). Inbreeding depression primarily results to the reduction of productive strength shown by traits connected with reproduction (fecundity, egg size and hatchability) or physiological traits such as growth rate, survival and biological disorders (Gjedrem, 2005).

Inbreeding depression was calculated by Falconer & MacKay (1996) as;

$$ID = \frac{W_{control} - W_{inbred}}{W_{control}} \times 100 \quad \text{----- (6)}$$

$$IDC = \frac{1 - \frac{W_{inbred}}{W_{control}}}{F_{control} - F_{inbred}} \times 10 \quad \text{----- (7)}$$

Where:

ID = Inbreeding depression of individual

IDC = Inbreeding depression co-efficient

$W_{control}$ = Mean weight of control

W_{inbred} = Mean weight of inbred individuals

$F_{control}$ = Inbreeding level in control group

F_{inbred} = Inbreeding level in inbred group

Where pedigree information are unavailable, as in the case of wild fish and for most cultured population in developing countries, data from molecular markers could be used to estimate F for individuals. However, one can also estimate the rate of inbreeding occurring in the population by making a key simplifying assumption that - assuming each individual contribute equally to the propagation of the stock, a mean “ F ” for the population can be estimated. Likewise, if the number of breeders of each sex that were used to propagate a stock is known, the inbreeding coefficient of that stock can be estimated (Tave 1990, Tave 1993).

2.6 MAIN CAUSES AND CONSEQUENCES OF INBREEDING

The main causes of inbreeding in aquaculture populations are:

- Small effective population size (*due to high fecundity of species and small scale of operations*)
- Non-random mating (*genetic drift due to small population size*)
- Unequal sex ratio (*preferred managerial practices*)

The general consequence of inbreeding is the reduction of genetic variance. Every individual carries deleterious recessive alleles that are not expressed because they are masked in the heterozygous state. In situations where these recessive alleles are paired and expressed in their homozygote state, they negatively impact the fitness of the carrier by decreasing larval viability, reducing survival, decreasing growth rate, lowering fecundity and reproductive ability of the fish and may increase the frequency of abnormalities in the population (De Donato *et al.*, 2005). The high fecundity associated with species cultured in Africa, including catfish and tilapia, requires a relative small number of broodstock to propagate a large number of stocks, sufficient of what the entire aquaculture facility needs for its annual production.

Besides the reduction of productive performance, morphological traits have also been reported to suffer the consequences of inbreeding. Morphological traits such as bilateral

asymmetry which could be defined as the unbalanced numbers for meristic traits on the right and left halves of the body have been observed to be an indirect indication of homozygosity of recessive genes in fish (Leary *et al.*, 1983, 1984, 1985a, b, c; Alibert *et al.*, 1994). It was reported by Doyle (2003) that random asymmetry can be interpreted as failure of the genotype to adequately regulate the development of the phenotype since the two halves of the embryo should be genetically identical. Similarly, Lerner (1954) considered fluctuating asymmetry as a measure of developmental stability to reflect the inability of an organism to develop precisely along determined pathways. Although bilateral asymmetry is usually undesirable in stock, it may not always be a reliable indicator of reduced fitness and inbreeding depression (Bourget, 2000).

2.7 EXTENT OF INBREEDING IN AQUACULTURE

Inbreeding can occur in both cultured and natural populations and harmful effects have been reported in numerous animals and plant species. Levels of inbreeding in natural populations are highly variable (Keller & Waller, 2002; Marshall *et al.*, 2002) and could be due to random factors such as population size, limited dispersal, or active mate choice for relatives (Shields, 1993). In relation to aquaculture species, the magnitude also appears highly variable depending either on the genetic constitution of the founding population, the particular trait or character under observation (Bentsen & Olesen, 2002), together with the interaction of genotypes with the environment (Hedrick & Kalinowski, 2000).

Inbreeding depression is mostly evident in traits associated with fitness (Lynch & Walsh, 1998; Falconer & Mackay, 1996) and could result in a decrease of up to 30 percent or greater in life history traits such as survival (Camara *et al.*, 2007), egg viability and reproduction (Bondari & Dunham, 1986; Falconer & Mackay, 1996; DeRose & Roff, 1999), but could also affect production traits such as growth (Bondari & Dunham, 1986; Camara *et al.*, 2007), flesh yield and food conversion efficiency of fish (Kincaid, 1976a, b, 1983a; Dunham, 1996). While body size/weight is considered a morphometric trait in endotherms, it is more closely associated with fitness in ectotherms, including finfish, due to the strong

relationship between body size and fecundity (Mousseau & Roff, 1987; Rawson & Hilbish, 1990).

In the aquaculture environment, records of the deleterious effects of inbreeding are quite limited but well documented for species such as rainbow trout (Calaprice 1969; Aulstad & Kittelsen, 1971; Bridges 1973; Kincaid, 1976a, b, 1983b; Gjerde *et al.*, 1983; Su *et al.*, 1996; Rye & Mao, 1998; Pante *et al.*, 2001a,b; Arkush *et al.*, 2002) and salmon (Gallardo *et al.*, 2004). In cold water fishes, inbreeding depression has been estimated at 3 – 6 percent per 10 percent increase in inbreeding for different traits (Kincaid, 1976; Gjerde *et al.*, 1983). In Coho salmon, significant inbreeding depression has been detected for the gonadosomatic index and body length at spawning in two independent populations (Gallardo *et al.*, 2004). However, there was no evidence of significant impact of inbreeding levels on other traits such as body and gonad weight, number and survival of eggs and fecundity in relation to these populations (Gallardo *et al.*, 2004; Neira *et al.*, 2006). In other salmon species except Coho salmon, significant inbreeding depression has been reported for growth in Atlantic salmon with inbreeding depression range of -0.6 to -2.6% (Rye & Mao, 1998) and rainbow trout, with inbreeding depression range of -1.6 to -5.0% (Pante *et al.*, 2001a) for each 10% increment of inbreeding. In this regard, it has been proposed that a rapid increase of inbreeding has a greater negative effect than slower accumulation in close culture systems (Bentsen & Olesen, 2002; Evans *et al.*, 2004; Pante *et al.*, 2001a; Rye & Mao, 1998).

Other studies include the Brook trout (Cooper 1961; Davis 1976), Brown trout (Davis, 1976), Common carp (Moav & Wohlfarth 1968), Mozambique Tilapia (Ch'ang 1971a, b), and Channel catfish (Bondari 1984b; Bondari & Durham 1987). Only with few exceptions, all these studies showed similar trends that inbreeding depressed most production traits and phenotypes such as growth, fecundity, survival rate and perhaps increased the number of abnormalities in fish.

The average inbreeding depression of individuals of different breeding populations have been reported at the same average level of inbreeding, e.g. for survival to alevin, inbreeding depression was 11.3 or 4.8% per ten percent inbreeding in Pacific salmon (Hard & Herschberger, 2002). Populations of rainbow trout with low rates of inbreeding (Pante *et al.*, 2001a) had lower inbreeding depression in body weight than populations with high rates of inbreeding (Gjerde *et al.*, 1983).

Kincaid carried out detailed studies on inbreeding using rainbow trout in the 70s and 80s, and his results suggests that the concept of inbreeding maybe true in rainbow trout. Kincaid (1977) estimated that the critical level of inbreeding in rainbow trout is about 18 percent, a level at which productivity was depressed significantly, but below which inbreeding produced few problems. Gjerde *et al.* (1983) studied the effect of three levels of inbreeding ($F = 0.25$, 0.375 , and 0.5) on survival and growth rate in rainbow trout. The average inbreeding depression was 10 percent for eyed eggs, 5.3 percent for alevins and 11.1 percent for fry. No linear relationship between inbreeding and inbreeding depression was found. Growth of fingerlings did not show significant inbreeding depression, while the growth of adults showed an increasing growth depression with increasing inbreeding. Per ten percent increase in inbreeding coefficient, inbreeding depression was found to be 4.5 percent, 5.3 percent, and 6.1 percent at inbreeding levels of 0.25, 0.375 and 0.5, respectively.

Su *et al.* (1996) found also in rainbow trout that the spawning age of females was delayed by 0.53 percent per ten percent increase of the inbreeding coefficient; and egg number decreased by 6.1 percent. However, Inbreeding did not significantly affect egg size or spawning age of males. Inbreeding depression for body weight per ten percent increase in inbreeding varied from 2.26 percent to 5.77 percent, with the tendency that inbreeding depression increased with increasing weight.

Inbreeding experiment by Bondari & Dunham (1986) on the Tifton strain catfish, *Ictalurus punctatus*, shows significant depression in the first generation of full-sib mating, with up to 17 percent reduction in average egg weight. In the second generation, body weight was

significantly depressed (about 19 percent), but did not influence survival rates to various ages. Experiment with channel catfish by Bondari & Dunham (1987) reported that inbreeding level of 0.25 increased the number of days required for eggs to hatch, but did not significantly influence spawning weight or hatchability. Two inbreeding studies on channel catfish gave contrasting results which could be due to the differences in the starting heterozygosity of genes in each population that gave one family a buffering effect over the other before the expression of inbreeding depression. One population was initiated by crossing six strains together with presumably more heterozygosity than the second population originating from a single strain. At $F = 0.25$, the first population, presumed a more heterozygous population exhibited no inbreeding depression, while the second population from just one single strain had inbreeding depression. At $F = 0.375$, the presumed family with more heterozygous population exhibited significant inbreeding depression. This translates to the conclusion that the more heterozygosity in the crossed strain population gave it one generation buffer compared with the other population from just one strain before the effect of inbreeding emerged (Dunham, 2011).

In tilapia, Ch'ang (1971b) compared progeny produced from one generation of full-sib (brother x sister) mating with inbreeding coefficient of 0.25 to crossbred controls in *O. mossambicus*. He observed lower survival and growth rate in the inbred population compared to the crossbred group during the first 2 months of life. Although he pointed out that the inbred population had been inbred for at least three previous generations which could be a reason for the high level of inbreeding observed in the population. Kocher (1997) reported several strains of farmed tilapia which have shown less than 10 percent heterozygosity to their wild counterparts.

Anderson & Hedgecock (2010) investigated inbreeding depression of crossbred and inbred larvae of the purple sea urchin, *Stronglyocentrotus purpuratus* (Stimpson) and discovered that there was a significant difference in the size of inbred larvae with greater coefficient of size variation than the crossbred larvae using the same culture system. This emphasises the

importance of outcrossing breeding stock at regular intervals in closed aquaculture systems to minimize the accumulation of inbreeding. Inbreeding experiment by [Bentsen & Olesen \(2002\)](#) using computer simulation to determine the effective number of breeders on the rate of inbreeding in fish populations concluded that to keep inbreeding rate to as low as about 1 percent per generation, a minimum of fifty pairs of breeders should be selected and not less than thirty - fifty progeny tested per pair. He further advised that reducing the number of broodstock pairs might increase the rate of inbreeding to as much as 6-8 percent per generation.

2.8 VULNERABILITY OF SMALL SCALE FARMERS TO INBREEDING

The lack of knowledge and access to genetically improved strains, poor management practices with non-random mating and the related exposure to inbreeding have been a major cause of low aquaculture productivity and production in Africa ([Dunham, 2011](#)). The developed countries (Northern Europe and United States) utilize highly productive genetically improved strains of farmed fish, whilst the developing countries (Asia, Africa) are still dependent on undomesticated wild genotypes of inferior genetic quality ([Eknath, 1991](#); [Bentsen & Olesen, 2002](#); [Brummet *et al.*, 2004](#); [McAndrew & Napier, 2011](#)).

Aquaculture in developed countries is mainly based on high value carnivorous species (e.g. salmonids, marine fish and crustaceans), filter feeders (oysters, mussels) and grazers (abalone) in highly intensive, technically advanced production systems. On the other hand, small and medium scale producers in the developing countries, including those in Africa, engage mainly in the production of low value herbivorous and highly fecund species such as carp, tilapia and catfish in mostly semi-intensive and extensive low-input production systems ([NACA/FAO, 2001](#)) with little or no knowledge of basic genetics and inbreeding. The low number of broodstock required (30 – 50 brood fish), along with unequal sex ratios commonly applied during mating (1 male : 4 females), followed by the high intensity of selection when selecting replacement animals from internal production systems, contributes significantly to

the susceptibility of small and medium scale farmers in Africa to the deleterious effects of inbreeding.

Similarly, even where genetically improved strains have been provided via development programs, a lack of knowledge of basic genetics and genetic management has led to an eventual loss of performance associated with inbreeding and the loss of genetic variation within the stock of this group of farmers.

In this regard, a sound knowledge of the causes and effects of inbreeding, with managerial practices to limit its occurrence remain a critical component to maintaining genetic integrity and performance of either wild, domesticated or genetically improved strains within the small and medium scale farming systems in developing countries including Africa. Nonetheless, along with maintaining a good pedigree record, keeping a large suitable population size of broodstock and ensuring random mating is key to ensuring genetic diversity and increasing productivity.

2.9 BASIC STRATEGIES TO CONTROL INBREEDING IN CLOSED AQUACULTURE SYSTEMS

As breeding of aquatic species becomes easier and more aquatic species become domesticated on the continent, genetically differentiated strains will undoubtedly increase and aquaculture development will be faced with the problem of how best to manage, promote and maintain the new genetic diversity.

In the natural environment, there are numerous empirical evidences which support that animals avoid close relatives as mating partners (Pusey & Wolf, 1996; Gerlach & Lysiak, 2006; Frommen & Bakker, 2006). However, in aquaculture where mating and reproduction is most often induced, there is always a fraction of inbreeding in every mating (Tave, 1999). Kincaid (1977) reported that inbreeding in trout did not become problematic until reaching an inbreeding level of 0.18 and proposed a rotational line crossing design to avoid mating full-sibs, but allow the crossing of first-cousins. Frankel & Soule (1981) suggested a more

conservative rate of inbreeding accumulation of 1 percent per generation, precluding the crossing of double first-cousins (resulting in offspring with $F = 0.125$) and first-cousins (resulting in progeny with $F = 0.0625$). The control of inbreeding and utilization of breeding strategies to avoid inbreeding depression are critical for the maintenance of genetic variance and diversity in aquaculture stocks, both in developed and developing countries, including Africa. This primary objective is a major task fish farm operative should ensure and incorporate into their management plan.

There are several management strategies to control inbreeding and inbreeding depression in aquatic populations, although the adoption of these strategies may depend on factors such as capital, available rearing space, labour, target species and perhaps the sensitivity of the species to levels of inbreeding. These strategies include:

Balancing number of sires and dams in breeding (Chevassus, 1989; Wang, 1997) - Standard management practices in small and medium scale aquaculture enterprises in Africa recommends equalizing family sizes, either one-on-one or maximum of one-on-two mating of males and females respectively to minimize inbreeding and random genetic drift (Doyle, 2003). Theoretically, this procedure can also reduce the intensity of natural selection for fitness as mutational load will accumulate over time. However, this equalization of parent size does not produce a high short-term threat to small domesticated populations for up to about twenty generations, and the more efficient preservation of genetic variability outweighs the disadvantages of this procedure.

Utilization of adequate numbers of broodstock, i.e. maintaining an adequate effective breeding population size, N_e to ensure that a large number of fish contribute progeny to the next broodstock generation (Kincaid, 1977; Tave, 1999; Bentsen & Olesen, 2002) – maintaining a larger breeding population size limits the chances of relatives being mated, and as such, the probability of inbreeding and resultant effect of inbreeding depression in the stock is contained. It is proposed by Tave (1993) that maintaining a total minimum of fifty

randomly mating breeding pairs per generation will retain inbreeding levels at acceptable levels for 25 to 50 generations in a particular population.

Keeping and maintaining updated pedigree information of stock – [Pante *et al.* \(2001b\)](#) concludes that maintaining full pedigree record of broodstock is necessary for accurate estimation of rate and level of inbreeding, and can significantly reduce inbreeding by ensuring that close relatives within the population do not mate. This procedure practically delays the first increment of inbreeding, but achieves very little with time depending on the population size. Pedigree records can be useful in making inbreeding levels more uniform among individuals within a generation ([Eknath, 1991](#)). This could be done by tagging breeding individuals and their progeny with tags and stamps, which makes this technique expensive with its effectiveness challenged by the problem of fish losing their tags to cannibalism within the pond.

Use of molecular or biochemical markers - Genetic markers are widely used in several applied breeding programs for parental assignment, pedigree, lineage analysis and control and monitoring levels of inbreeding in aquaculture stock ([Gjedrem, 2005](#)). This approach requires specialized facilities and equipment, more record keeping skills and involves significant operating costs that may not be accessible or affordable for small and medium scale farmers in Africa. It is therefore proposed that government fish seed producing centres, aquaculture extension officers and more sophisticated research facilities should assist with the monitoring of level of inbreeding in related stocks.

Rotational line-crossing system ([Kincaid, 1977](#)) – this is a simple, efficient, low cost method to control inbreeding in both small and large scale farming systems, and is particularly suitable for application in developing countries. It requires the systematic rotation of male and female broodstock fish into different cohorts or ponds after every spawning or mating season. The rotational breeding system prevents mating between close relative (e.g. father and daughter, mother and son, brothers and sisters) via physical separation from one

another. Although, this practice does not completely eliminate inbreeding from the stock, it is made less probable and the rate is thereby greatly reduced.

Periodic introduction of unrelated broodstock from external sources – this approach will eliminate existing levels of inbreeding with one generation of outcrossing, whilst further contributing to maintaining genetic variation and variability in the broodstock population as a whole (Kincaid, 1977). The harmful effect of inbreeding on the progeny is eliminated and inbreeding depression disappears within the next generation, as demonstrated in channel catfish by Bondari & Durham (1987). In combination with rotational line breeding, this approach offers the best approach in relation to small and medium scale farmers as it allows for the periodic supply of genetically improved material (brood or seed stock) from a central breeding program, whilst slowing down the rate of inbreeding in such populations.

Avoidance of full-sib mating – Mating strategies significantly have impacts on genetic variability. It was discussed by Dupont-Nivet & Vandeputte (2011) using stochastic simulations to compare genetic variability and inbreeding, if at all avoiding full sib mating is useful in a breeding program to minimize inbreeding depression. It was observed that within small populations with high selection intensity, avoiding full sib mating is a very useful way to manage genetic variance. However, it becomes less relevant as the population size and randomness of selection increases. Other experiments have reported likewise, emphasizing the exclusion of full-sib mating to maintain genetic diversity in aquaculture species (Waples & Do, 1994; Caballero *et al.*, 1996; Wang, 1997; Sekino *et al.*, 2004; Hansen & Jensen, 2005). For instance in tilapia, the number of effective breeders is often lower than expected (Fessehaye, 2006; Chatziplis *et al.*, 2007; Blonk *et al.*, 2009) therefore in this case, avoiding full-sib mating could help to obtain higher genetic response and reduce the occurrence of inbreeding. Although, this could be difficult for farmers in developing countries due to their economic and technical constrains.

2.10 CONSTRUCTIVE APPLICATION OF INBREEDING

Inbreeding is an important concept in animal husbandry that can add tremendous improvements to productivity but only few understand its application (Gjedrem, 2005). Generally, inbreeding is perceived as detrimental both to wild and aquaculture stocks, thus should be avoided and monitored in production environments. Equally, inbreeding can become a useful breeding tool if the number of individuals homozygous for some productive and desirable traits can be increased, since individual or mass selection is actually a mild inbreeding programme in itself.

Just like any other breeding technique, pure line breeding is a breeding strategy where fish that meet or exceed predetermined criteria for quantitative trait(s) or individuals that exhibit the desired qualitative trait(s) from the same population or family are mated to produce purebreds (Tave, 1999). Selective breeding, which is a pure breeding method, was adopted in the GIFT Project (Eknath *et al.*, 1998) based on their breeding values during selection. Inbreeding can be used to enhance stock genotypes by developing strains or lines which could produce super hybrids and demonstrate high heterosis by crossbreeding.

Line breeding has been extensively used in the plant and livestock industry e.g. on goats, sheep, cattle and pigs to increase the frequency of outstanding individual's gene in the population. Multiple methods exist for the development of inbred lines. However, the traditional way of producing inbred lines in animal is, of course, to mate relatives such as brother x sister or parent x offspring mating. With fish, there are other options to accomplish inbreeding because an individual can essentially be mated with itself through either gynogenesis or androgenesis. A meiotic gynogen has an essentially the same increase in homozygosity as with brother x sister mating. Furthermore, inbreeding could be efficiently useful in preparing aquaculture samples for progeny testing, where mostly homozygous samples are utilized.

2.11 LIMITATIONS OF INBREEDING EXPERIMENTS

It is important to mention that in inbreeding experiments, inbreeding depression may be underestimated or overestimated due to various factors. As a result, inbreeding and inbreeding depression values are not absolute values but estimates of the percentage of genes that are alike by descent in a population (Weaver & Hedrick, 1995; Falconer & Mackay, 1996). These limiting factors include:

The assumption that observed depression for a particular trait translates to resultant depression of several other associated traits, or if depression is not observed for one trait, it may likely not be observed for other associated traits as well.

Weaker individuals tend to die as a result of loss of fitness during the course of the experiment, and at the end when means are computed, some bias could exist because the worst performing individuals did not make it to the end of the experiment and also for many non-survival traits, such as growth rate and reproductive fitness, these mortalities are not included in the means, therefore, inbreeding depression would have been underestimated at the end.

In multiple generation experiments, weaker individuals are dying and are being eliminated in the course of the experiment over time which may not be considered in the multi-generational means. Subsequently in the experiment, low surviving families or groups are now at lower densities due to differential mortality rate, thus, their growth depression is further underestimated because they now have an environmental growth advantage over the high surviving higher density groups or families.

Literature suggests that inbreeding depression is ameliorated by captive conditions optimized for survival and growth, especially compared to harsh natural conditions. Inbreeding depression in artificial environments can be biased upwards by poor husbandry (Crnokrak & Roff, 1999). Hence, Individuals used in an inbreeding experiment are more often pampered and usually looked after better than in natural conditions which may

probably not reflect clearly their level of inbreeding depression as much as it would have in their natural environment where there is more survivor competitions, fluctuating environmental conditions, irregular nutrition and competition for mates. Therefore, Genotype X Environment interaction might probably play a huge role in underestimating inbreeding depression in controlled inbreeding experiments where competitions and natural challenges are close to zero. Inbreeding depression tends to be larger for traits closely related to fitness, such as survival and fecundity or fertility, and smaller for traits not too closely related to fitness, such as morphological traits (Falconer & MacKay, 1996; DeRose & Roff, 1999).

2.12 SUMMARY

Inbreeding has been extensively described to depress fitness in many aquatic species. The detrimental effect of inbreeding on production traits in aquaculture species is also widely reported in scientific literature, including for Tilapia (Ch'ang 1971a, b; Bentsen & Olesen, 2002; Fassehayé *et al.*, 2007). Literature further confirms the phenomenon that subsistence and small scale farming systems are particularly vulnerable and exposed to the effects of inbreeding (Brummett *et al.*, 2004; Brummett & Ponzoni, 2009). The main causes of inbreeding in these farming systems remain that of a small effective population size, the random nature of mating and internal replacement of broodstock. There is also a general lack of knowledge amongst such farmers and inadequate genetic management practices, such as controlled mating designs. Specific measures should therefore be put in place to reduce the impact of inbreeding on such farming systems.

In this present study, repeated full-sib mating between outcrossed Mozambique tilapia would be conducted to produce inbred progeny groups with different levels of inbreeding in order to investigate the implications of these levels of inbreeding on growth parameters, condition factor and yield of Mozambique tilapia. In addition, the occurrence of morphological deformities associated with increased inbreeding in Mozambique tilapia (*O. mossambicus*) will also be recorded and described.

3 RESEARCH DESIGN, MATERIALS AND METHODOLOGY

3.1 STUDY SITE

This research experiment and data collection was conducted in full at the Welgevallen experimental farm of Stellenbosch University, Stellenbosch, Western Cape. GPS coordinates: 33°56'33"S 18°51'56"E. No ethical clearance was required as all fish were reared in accordance with standard commercial operating procedures, including feeding, rearing conditions and husbandry methods.

3.2 STUDY POPULATION

The parental material of Mozambique tilapia, *O. mossambicus*, used in this study was obtained from two unrelated populations from two different experimental farms, namely the Elsenburg population maintained at the Elsenburg experimental farm of the Western Cape Department of Agriculture, and the Welgevallen population maintained at the Welgevallen experimental farm of the University of Stellenbosch. This outcrossing was done to ensure that genetically outbred parental pairs were used to produce the respective F₁ family groups. A total of twenty-five sexually mature male fish of average body weight of 40.00±5.00g were randomly selected from Welgevallen population together with a further twenty-five sexually gravid females from the Elsenburg population with average body weights of 55.00±5.00g.

3.3 CONDITIONING OF BROODSTOCK

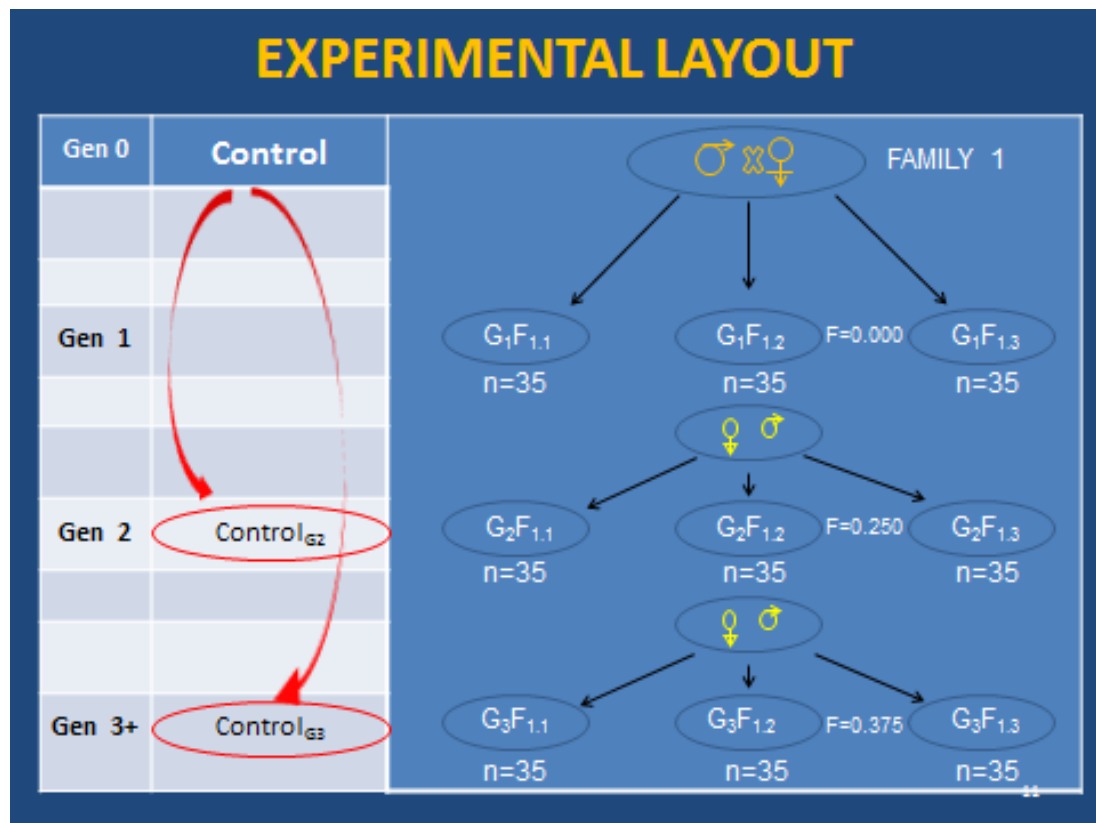
The Welgevallen and Elsenburg parental stocks were conditioned at Welgevallen experimental farm for a period of two weeks under standardized culture conditions, in two separate 1000 liter tanks with a shared water recirculation system, before the pairing off of the breeders.

3.4 MATING DESIGN

The base parent population (G₀) was formed consisting of 25 randomly paired single breeding pairs, similar to a procedure described by Charo-Karisa (2006). This mating design

allowed for seven full-sib family groups (G_1) to be collected for use as experimental material. The individual G_0 breeding pairs were kept in separate 60 liters glass aquaria, with standardized water recirculation via a shared 2500 liters bio filtration unit inside a glasshouse. The glass house together with the shared recirculation system ensured that a homogenous culture environment was maintained amongst the breeding pairs in relation to water temperature, pH, dissolved oxygen and other physico-chemical parameters including ammonia and total dissolved solids (TDS). The breeding pairs were closely monitored and fed *ad lib*, three times daily (i.e. morning, noon and evening) with a commercial tilapia formulated feed supplied by Aquafeeds (Pty) Ltd.

Figure 3.1: Overview of experimental design per family group over three generations.



3.5 CONTROL GROUPS

To estimate inbreeding depression amongst these seven families of *O. mossambicus*, an appropriate genetic control had to be established for each of the inbred families for each generation, other than a separate outbred family G_0 as an experimental control. This was

required to eliminate a possible source of experimental error as a separate randomly bred control family may not exactly match the beginning genotype of the other six inbred families (Vrijenhoek *et al.*, 1990).

The Control group for each subsequent generations in the experiment now consisted of a repeated mating of the original outbred parents used in G_0 . This produces genetic Controls at generations G_1 , G_2 and G_3 respectively which are however theoretically not inbred (i.e. $f \approx 0.000$) due to the non-relatedness of outbred parents. See Figure 3.1 and Glossary, pg xvii.

3.6 PROGENY GROUPS

The first seven family groups that were spawned within a fourteen days period that also provided adequate numbers of fry (≥ 75 per family) were selected as experimental material, i.e. the G_1 generation. A coefficient of inbreeding of $F \approx 0$ was assumed for the G_1 full-sib family groups on the basis as offspring from outbred G_0 parents. Each of the seven G_1 full-sib family groups were then randomly divided into three replicates containing thirty-five individuals each, to establish a total of twenty-one G_1 experimental groups including the genetic Control. See Table 3.1.

Generation two (G_2) offspring were produced from population G_1 through a random full-sib mating (i.e. a brother x a sister). After completion of the G_1 growth phase, a single random full-sib pairing was established from each of the six family groups excluding the genetic control, after combining all three replicates, to produce the inbred G_2 full-sib family groups. Each G_2 family group was further split into three replicates after spawning and had expected coefficient of inbreeding of $F = 0.250$, following Wright's (1922) approach of path counting to calculate the additive genetic relationship between parents.

The third (G_3) generation was produced in a similar manner from the G_2 family groups by repeated full-sib pairing of an inbred sire and a dam (inbred brother and sister) in all the six

families (excluding the Control), having an expected inbreeding level of $F = 0.375$ (Wright, 1922).

Table 3.1: Experimental Layout and Design

CONTROL			FAMILIES																	
P1	P2		P1	P2		P1	P2		P1	P2		P1	P2		P1	P2		P1	P2	
Control (G₀)			Fam 1 (G ₁)			Fam 2 (G ₁)			Fam 3 (G ₁)			Fam 4 (G ₁)			Fam 5 (G ₁)			Fam 6 (G ₁)		
R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =
35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35
Control (G₀)			Fam 1 (G ₂)			Fam 2 (G ₂)			Fam 3 (G ₂)			Fam 4 (G ₂)			Fam 5 (G ₂)			Fam 6 (G ₂)		
R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =
35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35
Control (G₀)			Fam 1 (G ₃)			Fam 2 (G ₃)			Fam 3 (G ₃)			Fam 4 (G ₃)			Fam 5 (G ₃)			Fam 6 (G ₃)		
R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =	n =
25	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35

3.7 SPAWNING OF BROODSTOCK

A total of seven breeding pairs were established by randomly selecting sexually mature male and female broodstock from the Welgevallen and Elsenburg populations of *O. mossambicus*. Each breeding pair was housed separately in a 60 liters glass aquarium, supplied by a water recirculation system via a central bio-filtration unit. The breeding pairs were fed to satiation three (3) times a day i.e. morning, mid-day and evening with a standard commercial tilapia feed supplied by Aquafeeds (Pty) Ltd. Water quality parameters were maintained within the prescribed optimal range for Tilapia through daily measurement of water temperature, dissolved oxygen and pH using a combined pH meter. Spawning behavior was observed on a daily basis to identify spawning females.

3.8 LARVAL REARING

Larval progeny groups were retained as full-sib families in the original tanks where they were spawned by simply removing both parents between seven to ten days after fertilization. The first seven full-sib family groups that spawned within a 14 day window period and provided

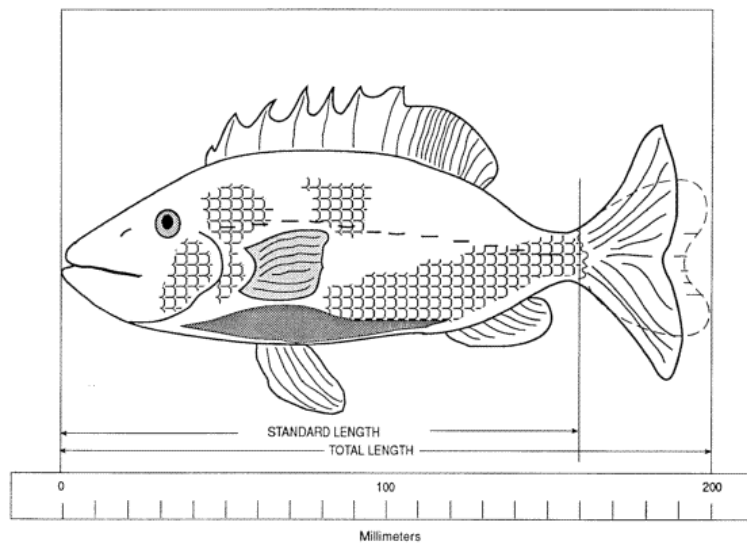
adequate numbers of fry ($n \geq 75$) were selected as the base parent population G_0 . At an average weight of $\geq 2g$, these progeny groups were randomly divided into three replicates of $n = 35$ fish each, to establish the full-sib population called generation one (G_1). The fish were fed to satiation three times daily with standard commercial tilapia feed supplied by Aquafeeds (Pty) Ltd, grinded to an appropriate crumble size for the fry to feed on.

In generations two (G_2) and three (G_3) respectively, the full-sib progeny groups obtained from each of the seven families were again randomly divided into three replicates of 35 fish per replicate when the families reached an average weight of $\geq 2g$. The number of 35 per replicate was decided on to allow for the expected growth during the 90 days growth period, at the same time avoiding overcrowding and cannibalism, yet providing adequate number of fish per replicate for reliable statistics at the end of the trial period.

3.9 SAMPLING

Sampling was carried out on a bi-weekly basis with a random selection of sixteen ($n = 16$) fish per replicate during the twelve (12) week growth period, at each generation. Sampled fish were anesthetized by submersion in Aqui-S™ at a dilution rate of 20mg/L of fresh water to reduce stress and injuries that could result from handling and sampling procedures. Measurements of body weight (BW) in grams, standard length (SL) and total length (TL) in centimeters (Figure 3.2) were recorded for each of the sixteen randomly sampled fish per replicate, at two weeks growth intervals for the period of 90 days at each generation.

Figure 3.2: Depiction of Standard length and Total length measurements in fish



Flesh yield in grams and number of deformities (D) were recorded at the end of the twelve weeks growth period for each generation. Flesh yield was determined through random sampling and slaughtering of twenty-five (25) fish per replicate at the completion of the final growth period, to determine the ratio of gutted weight to live weight at each generation.

3.9.1 GROWTH RATE – Specific Growth Rate (SGR) in gday^{-1}

Specific growth rate (SGR) in aquaculture refers to the estimate of growth of fish over a certain period of time. SGR measured in grams per day was calculated as:

$$\text{SGR} = \left[\frac{(\ln \text{BWF} - \ln \text{BWS})}{t} \right] \times 100 \text{ ----- (8) } \quad (\text{Elliott \& Hurley, 1995})$$

Where: BWS = body weight (g) at the start of the growth trial

BWF = body weight (g) at the end of the growth trial

t = the duration of the growth trial in days

3.9.2 CONDITION FACTOR (K)

A total of 48 individual fish, 16 per replicate were sampled at random in each family, at each growth interval, at each generation for the whole period of this study to make an aggregate sum of 336 fish at every generation. The total length (TL) of each fish was measured from the tip of the snout (mouth closed) to the end of the caudal fin using a measuring board.

Body weight (BW) was measured using a sensitive electronic digital scale. The condition factors of sampled fish were calculated by the formula:

$$\text{Condition Factor (K)} = \frac{W}{L^3} \times 100 \quad \text{----- (9) (Fulton, 1904)}$$

Where: W = Body weight (g)

L = Total Length (cm)

3.9.3 DEFORMITIES

Sampled fish were assessed in terms of morphological deformities including, shortened opercula covers, stunted body shape and fin abnormalities and numbers recorded.

3.10 DATA ANALYSIS

Test for normality of data was done for all performance traits using the Kolmogorov-Smirnov test and Shapiro-Wilk test for normality (SAS EG., 2014). Regression analysis was used to determine if the rate of phenotypic depression per unit increase in F was linear or quadratic and an analysis of variance, ANOVA, to establish the difference between the means of measured parameters (SAS EG., 2014).

Inbreeding depression was estimated for each family group, which are expected to respond in a variable manner as each family has its own genetic background and a different level of absolute starting heterozygosity in G_1 . The inbreeding depression for the experimental population was calculated as the mean of the inbreeding depression over all six individual families. However in a small inbred population such as in this case, each family had an internal experimental Control in as much as all G_1 offspring are considered not inbred as they are the first progeny from unrelated parents, G_0 . This approach theoretically makes the internal, outbred Control design the superior design in inbreeding experiments (Vrijenhoek *et al.*, 1990). The calculated condition factors (K) of individual fish sampled were recorded and the slope of K in all inbred families and the control was subjected to a one-way analysis of variance (ANOVA) using SAS EG. Software.

The possibility of tissue sampling of the Control and inbred offspring to confirm parental contribution and determine starting level and final homozygosity through molecular techniques was considered (McGoldrick & Hedgecock, 1997), though could not be afforded as part of this present investigation.

4 RESULTS

The main objective of this study is to quantify the effect of inbreeding and inbreeding depression on the growth performance and yield of Mozambique tilapia, *O. mossambicus*, over three generations of repeated full-sib mating. The resultant effects of acute inbreeding on growth performance of *O. mossambicus* are presented in [Tables 4.2, 4.3, 4.4 and 4.5](#) below. The data were statistically analysed using the statistical program, SAS Enterprise 5.1 (2014). ANOVA for Body weight (BW), Standard length (SL), Total length (TL), Specific Growth Rate (SGR) and Yield were computed to make evident comparisons within families and between generations.

4.1 TEST FOR NORMALITY

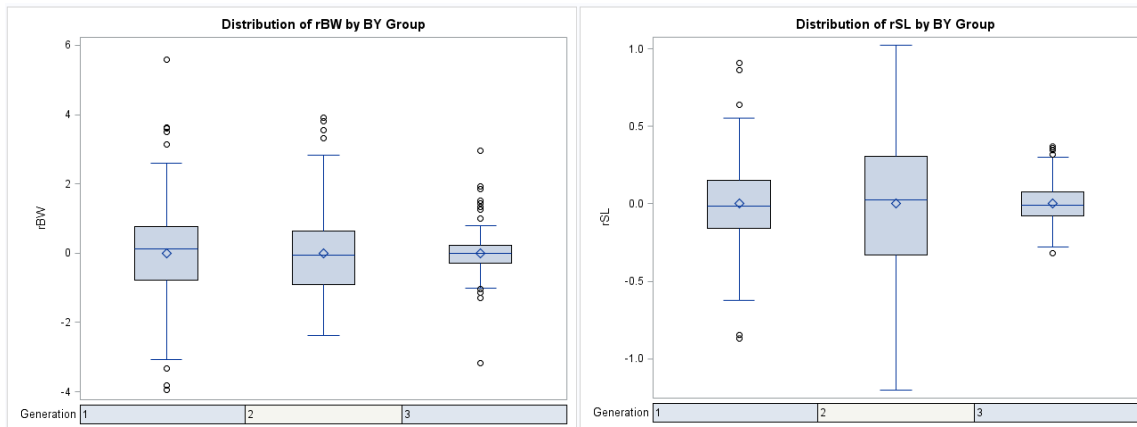
The tests for normality of data across the three generations and seven families measured, including the Control, for body weight (BW), standard length (SL) and total length (TL), justifies the underlying assumptions of normality with *P values* as listed in [Table 4.1](#);

Table 4.1: Test of normality of recorded data of growth traits: body weight (BW), standard length (SL) and total length (TL), of Tilapia (*O. mossambicus*) over three generations of full-sib mating.

Generation	Traits	Shapiro-Wilk (W)	p value	Kolmogorov-Smirnov (D)	p value
Gen 1	BW	0.9770	0.0304	0.102471	<0.0100
	SL	0.9808	0.0716	0.06442	>0.1500
	TL	0.9894	0.4505	0.063257	>0.1500
Gen 2	BW	0.9585	0.0007	0.066549	>0.1500
	SL	0.9914	0.6335	0.060782	>0.1500
	TL	0.9923	0.7279	0.036294	>0.1500
Gen 3	BW	0.8658	<0.0001	0.159366	<0.0100
	SL	0.9862	0.2341	0.054083	>0.1500
	TL	0.9696	0.0062	0.061229	>0.1500

Although, there was evidence of outlier measurements outside of the range of normally distributed values ([Figure 4.1](#)), such outliers were not removed from statistical analysis because they were often the more highly asymmetric individuals, as a purpose of the study was to determine if the frequency of such cases was related to inbreeding.

Figure 4.1: Distribution of body weight (BW) and standard length (SL) of Tilapia (*O. mossambicus*) over three generations of full-sib mating



4.2 OVERALL GROWTH PERFORMANCE

The results of the effect of inbreeding on growth performance of *O. mossambicus* are presented in Table 4.2 as a comparison between the non-inbred control and the average of six inbred families, which shows significant inbreeding depression ($P < 0.05$) at all inbreeding levels studied ($F = 0.250$ and $F = 0.375$).

Table 4.2: Effect of inbreeding on Body weight (BW), Standard length (SL), Specific growth rate (SGR), and Yield of inbred families of Mozambique tilapia, *O. mossambicus* against the Control over three generations of repeated full-sib mating.

TRAITS		Generation 1 ($F \approx 0.000$)		Generation 2 ($F = 0.250$)		Generation 3 ($F = 0.375$)	
		CONTROL	FAMILIES	CONTROL	FAMILIES	CONTROL	FAMILIES
BW (g)	LSM	19.330±1.416	17.715±1.831	23.390±0.805	12.503±1.384	17.560±1.137	9.382±0.102
	ID				46.544±5.916		46.574±0.580
	IDC				18.618±2.366		12.420±0.155
SL (cm)	LSM	7.710±0.188	7.143±0.349	8.080±0.478	6.613±0.236	7.380±0.137	6.055±0.033
	ID				18.152±2.917		17.954±0.443
	IDC				7.261±1.167		4.788±0.118
SGR (gday ⁻¹)	LSM	3.390±0.084	3.341±0.053	3.700±0.027	2.895±0.130	3.440±0.106	2.740±0.148
	ID				21.762±3.512		20.344±4.312
	IDC				8.705±1.405		5.425±1.150
Yield	LSM	0.785±0.004	0.818±0.026	0.830±0.001	0.782±0.024	0.796±0.020	0.72±0.005
	ID				5.752±2.894		8.940±0.670
	IDC				2.301±1.157		2.384±0.179

Where: **LSM** = Least Square Means; **ID** = Inbreeding Depression (%); **IDC** = Inbreeding Depression Coefficient per 10% increase in F (%)

The results in **Tables 4.3, 4.4 and 4.5** present the effect of inbreeding on growth performance of *O. mossambicus* as a comparison between the non-inbred Control and individual families, which confirms significant inbreeding depression ($P < 0.05$) at all inbreeding levels studied ($F = 0.250$ and $F = 0.375$) over all families. It should be noted that there were also significant differences amongst the family groups and the control in relation to Generation one ($F \approx 0.000$) as presented in **Table 4.3**. These differences could be ascribed to random genetic differences between the experimental family groups as well as standard experimental variance. From the tables below superscripts a, b, c, and d indicates significant differences with \pm SD (Standard deviation).

Table 4.3: Growth parameters and yield of Mozambique tilapia, *O. mossambicus* of the Control and six experimental families showing Body weight (BW), Standard length (SL), Specific growth rate (SGR), and Yield at F ≈ 0.000 inbreeding level.

TRAITS	C	FAM 1	FAM 2	FAM 3	FAM 4	FAM 5	FAM 6
BW (g)	19.33±1.42 ^{AB}	15.71±1.49 ^D	19.99±0.48 ^A	19.14±1.85 ^{AB}	17.87±2.26 ^C	18.17±0.55 ^{BC}	15.41±1.06 ^D
SL (cm)	7.71±0.19 ^A	6.77±0.16 ^D	7.75±0.05 ^A	7.08±0.41 ^{BC}	7.27±0.46 ^B	7.13±0.30 ^B	6.86±0.20 ^{CD}
SGR (gday⁻¹)	3.39±0.08 ^A	3.28±0.10 ^A	3.37±0.05 ^A	3.40±0.09 ^A	3.32±0.11 ^A	3.39±0.08 ^A	3.29±0.07 ^A
Yield	0.79±0.00 ^D	0.78±0.01 ^D	0.80±0.02 ^{CD}	0.81±0.02 ^C	0.82±0.02 ^{BC}	0.85±0.01 ^A	0.84±0.01 ^{AB}

Table 4.4: Effect of inbreeding on Body weight (BW), Standard length (SL), Specific growth rate (SGR) and Yield of six inbred families of Mozambique tilapia, *O. mossambicus*, at inbreeding level F = 0.250 against the Control (F ≈ 0.000).

TRAITS	C	FAM 1	ID	IDC	FAM 2	ID	IDC	FAM 3	ID	IDC	FAM 4	ID	IDC	FAM 5	ID	IDC	FAM 6	ID	IDC
BW (g)	23.39±0.81 ^A	11.92±1.59 ^{CD}	49.04	19.62	12.73±1.10 ^C	45.58	18.23	14.12±0.42 ^B	39.63	15.85	10.72±1.16 ^E	54.17	21.67	14.04±2.49 ^B	39.97	15.99	11.49±1.61 ^{DE}	50.88	20.35
SL (cm)	8.08±0.48 ^A	6.57±0.61 ^{CD}	18.69	7.48	6.55±0.54 ^{CD}	18.94	7.57	6.95±0.52 ^B	13.99	5.59	6.28±0.52 ^D	22.28	8.91	6.81±0.35 ^{BC}	15.72	6.29	6.52±0.04 ^{CD}	19.31	7.72
SGR (gday⁻¹)	3.70±0.03 ^A	2.85±0.11 ^{BC}	22.94	9.18	2.96±0.08 ^B	20.10	8.04	2.97±0.03 ^B	19.65	7.86	2.67±0.12 ^C	27.94	11.18	3.04±0.17 ^B	17.91	7.16	2.88±0.17 ^B	22.04	8.81
Yield	0.83±0.00 ^A	0.77±0.02 ^C	7.67	3.07	0.77±0.03 ^C	7.4	2.96	0.77±0.01 ^C	7.35	2.94	0.77±0.01 ^C	7.32	2.93	0.83±0.02 ^{AB}	0.41	0.16	0.79±0.03 ^{BC}	4.36	1.75

Table 4.5: Effect of inbreeding on Body weight (BW), Standard length (SL), Specific growth rate (SGR), and Yield of six inbred families of Mozambique tilapia, *O. mossambicus*, at inbreeding level F = 0.375 against the Control (F ≈ 0.000).

TRAITS	C	FAM 1	ID	IDC	FAM 2	ID	IDC	FAM 3	ID	IDC	FAM 4	ID	IDC	FAM 5	ID	IDC	FAM 6	ID	IDC
BW (g)	17.56 ±1.14 ^A	9.26±0.10 ^B	47.27	12.60	9.36±0.03 ^B	46.70	12.45	9.55±0.10 ^B	45.62	12.16	9.41±0.26 ^B	46.41	12.38	9.41±0.34 ^B	46.41	12.38	9.30±0.10 ^B	47.04	12.54
SL (cm)	7.38±0.14 ^A	6.03±0.07 ^B	18.29	4.88	6.04±0.02 ^B	18.16	4.84	6.11±0.03 ^B	17.21	4.59	6.07±0.02 ^B	17.75	4.73	6.06±0.11 ^B	17.89	4.77	6.02±0.02 ^B	18.43	4.91
SGR (gday⁻¹)	3.44±0.11 ^A	2.83±0.06 ^B	17.81	4.75	2.62±0.18 ^{BC}	23.93	6.38	2.87±0.07 ^B	16.72	4.46	2.50±0.11 ^C	27.23	7.26	2.77±0.26 ^B	19.56	5.22	2.86±0.08 ^B	16.81	4.48
Yield	0.80±0.02 ^A	0.72±0.02 ^B	9.17	2.44	0.72±0.01 ^B	10.20	2.72	0.73±0.03 ^B	8.45	2.25	0.73±0.02 ^B	8.59	2.29	0.73±0.02 ^B	8.48	2.26	0.73±0.03 ^B	8.75	2.33

4.2.1 GROWTH EXPRESSED AS BODY WEIGHT (BW)

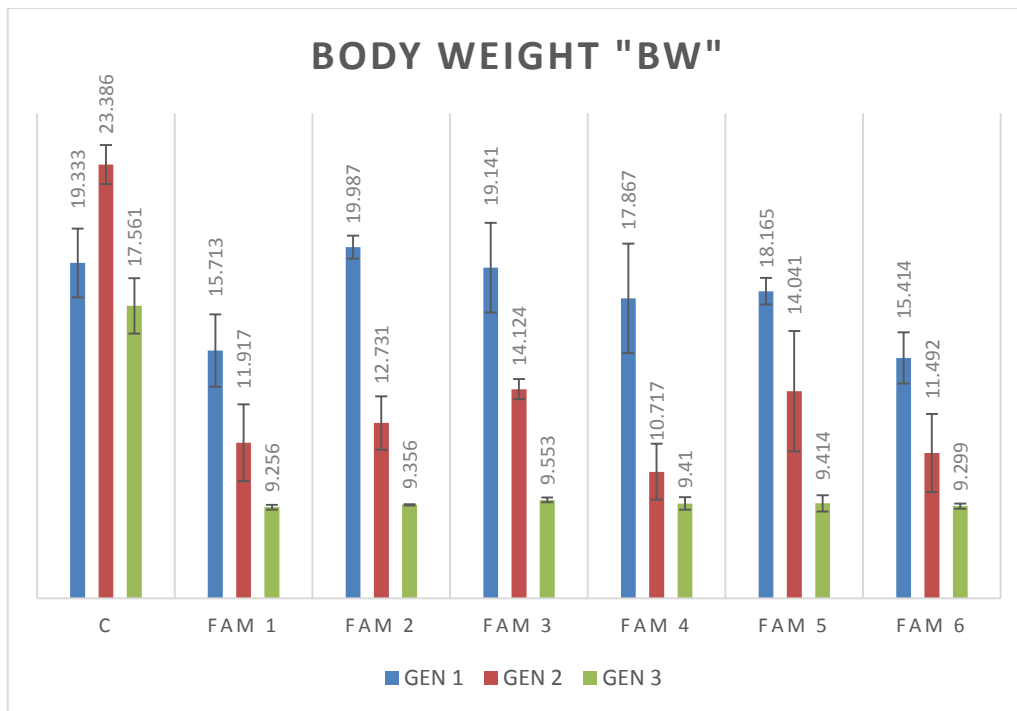
Table 4.6 shows the effect of inbreeding on body weight (BW) of individual inbred family groups of *O. mossambicus* against the non-inbred Control over three generations of repeated full-sib mating and graphically expressed in Figure 4.2 below. Average inbreeding depression for body weight ranged from 39.63 to 46.57 percent and the average inbreeding depression per 10% increase of inbreeding ranged from 12.42 to 18.62 percent. No significant differences in body weight were found between the control and other six family groups in G₁. However, families one and six were observed to have performed significantly lower than others with mean body weights of 15.71g and 15.41g respectively, considering the fact that they were from outcrossed parents and assumed not to be inbred with coefficient of inbreeding $F \approx 0.000$. This can be explained in relation to random genetic differences between the experimental families, including differences in individual starting heterozygosity for different families.

Table 4.6: Effect of inbreeding on body weight (BW) in gram of inbred families of *O. mossambicus* against the Control over three generations of repeated full-sib mating.

	C	FAM 1	FAM 2	FAM 3	FAM 4	FAM 5	FAM 6
Gen 1	19.333±1.416 ^a	15.713±1.492 ^a	19.987±0.478 ^a	19.141±1.848 ^a	17.867±2.257 ^a	18.165±0.547 ^a	15.414±1.059 ^a
Gen 2	23.386±0.805 ^a	11.917±1.589 ^b	12.731±1.105 ^b	14.124±0.419 ^b	10.717±1.157 ^b	14.041±2.487 ^b	11.492±1.614 ^b
Gen 3	17.561±1.137 ^a	9.256±0.096 ^b	9.356±0.026 ^b	9.553±0.099 ^b	9.410±0.257 ^b	9.414±0.336 ^b	9.299±0.101 ^b

The body weight of the Control group was significantly different ($P < 0.05$) from all inbred families in G₂ and G₃, at respective levels of inbreeding of $F = 0.250$ and $F = 0.375$. In generations two ($F = 0.250$) the mean body weight of the Control group was 23.39g compared to an average of 12.50g for the inbred families and 17.56g in G₃ ($F = 0.375$) compared to an average of 9.38g for the inbred families. The estimated inbreeding depression of body weight at $F = 0.250$ and 0.375 were 46.54 percent and 46.57 percent respectively. Inbreeding depression coefficients for body weight per 10% increase of inbreeding were -18.62 percent and -12.42 percent respectively in G₂ and G₃.

Figure 4.2: The effect of inbreeding on body weight (BW) in grams of *O. mossambicus* in terms of a non-inbred Control (C) and six inbred families, over three generations of repeated full-sib mating.



Of relevance to consider is that as inbreeding levels increase with resultant increase in inbreeding depression of trait(s), a level is reached where further inbreeding may not translate into higher depression of trait(s) in the inbred population. This point is referred to in literature as the inbreeding plateau (Dunham, 2011). This is however expected to set-in in generations beyond that of this present study.

4.2.2 GROWTH EXPRESSED AS STANDARD LENGTH (SL)

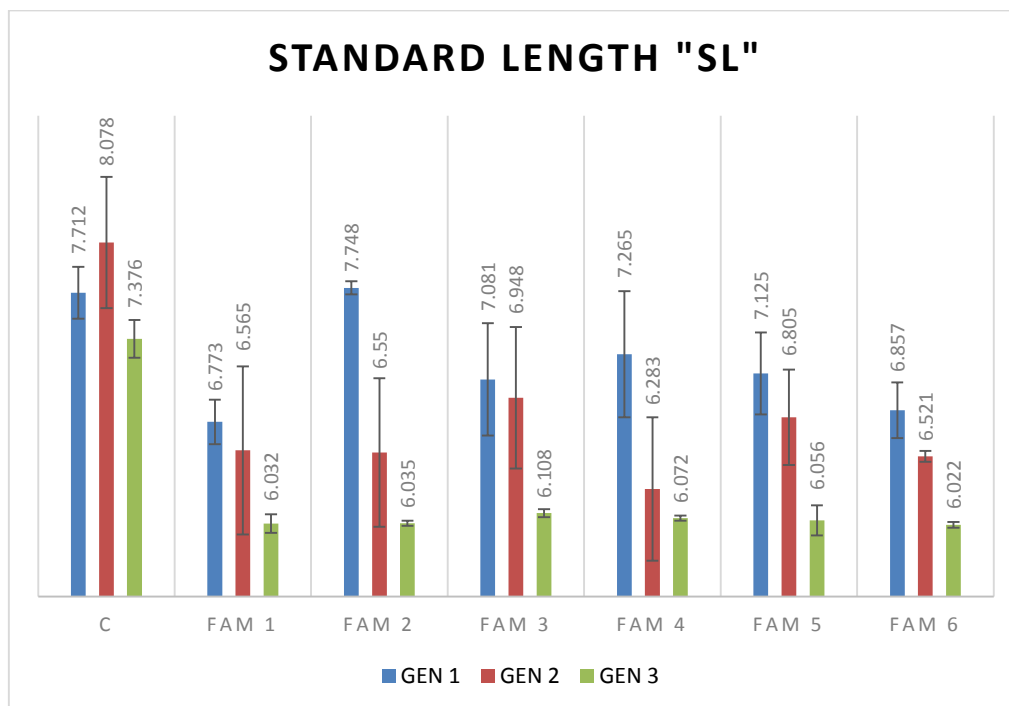
For standard length, the estimated mean inbreeding depression for inbred families was found to range between 13.99 percent in FAM 3 and 22.28 percent in FAM 4 at G_2 ; and a range between 17.21 percent in FAM 3 and 18.43 percent in FAM 6 at G_3 . Inbreeding depression coefficients were estimated to range from -5.59 percent to -8.91 percent at $F = 0.250$ in G_2 ; and from 4.59 percent to 4.91 percent at $F = 0.375$ in G_3 per 10% increase of inbreeding. Table 4.7 below shows the effect of inbreeding on standard length (SL) of individual inbred family groups of *O. mossambicus* against the non-inbred Control over three generations of repeated full-sib mating, graphically expressed in Figure 4.3 below.

Table 4.7: Effect of inbreeding on Standard length (SL) in cm of inbred families of *O. mossambicus* against the Control over three generations of repeated full-sib mating.

	C	FAM 1	FAM 2	FAM 3	FAM 4	FAM 5	FAM 6
Gen 1	7.712±0.188 ^a	6.773±0.162 ^a	7.748±0.048 ^a	7.081±0.409 ^a	7.265±0.459 ^a	7.125±0.299 ^a	6.857±0.202 ^a
Gen 2	8.078±0.478 ^a	6.565±0.611 ^b	6.550±0.541 ^b	6.948±0.515 ^{ab}	6.283±0.522 ^b	6.805±0.347 ^{ab}	6.521±0.040 ^b
Gen 3	7.376±0.137 ^a	6.032±0.067 ^b	6.035±0.019 ^b	6.108±0.029 ^b	6.072±0.019 ^b	6.056±0.109 ^b	6.022±0.021 ^b

Similar to observations in body weight, an expected non significance difference ($P>0.05$) was observed at G_1 for standard length of the control and all six individual family groups where $F \approx 0.000$. However, the mean standard length (SL) of the Control was the highest, measuring up to 8.08cm and 7.38cm at generations two (G_2) and three (G_3) respectively compared to an average of 6.61cm for the inbred families at G_2 where $F = 0.250$ and 6.06cm at G_3 where $F = 0.375$. However at G_2 , inbred families five and three were not significantly different from the Control, but different from all other inbred families at $F = 0.250$.

Figure 4.3: The effect of inbreeding on Standard length (SL) in cm of *O. mossambicus* in terms of a non-inbred Control (C) and six inbred families, over three generations of repeated full-sib mating.



Total length was measured during sampling and included in the provisional data analysis but was eventually discarded because of irregular physical damage of the caudal fins of fish caused by keeping the fish under high densities in a confined space for an extended period.

Damages to the tail fins seemed to be caused by the territorial behaviour and competition amongst tilapia fish as well as mechanical damage through excessive contact with the side panels of the holding aquaria. Hence, total length of fish was considered not to be an accurate indicator of growth under the specific conditions of this trial.

4.2.3 SPECIFIC GROWTH RATE (SGR)

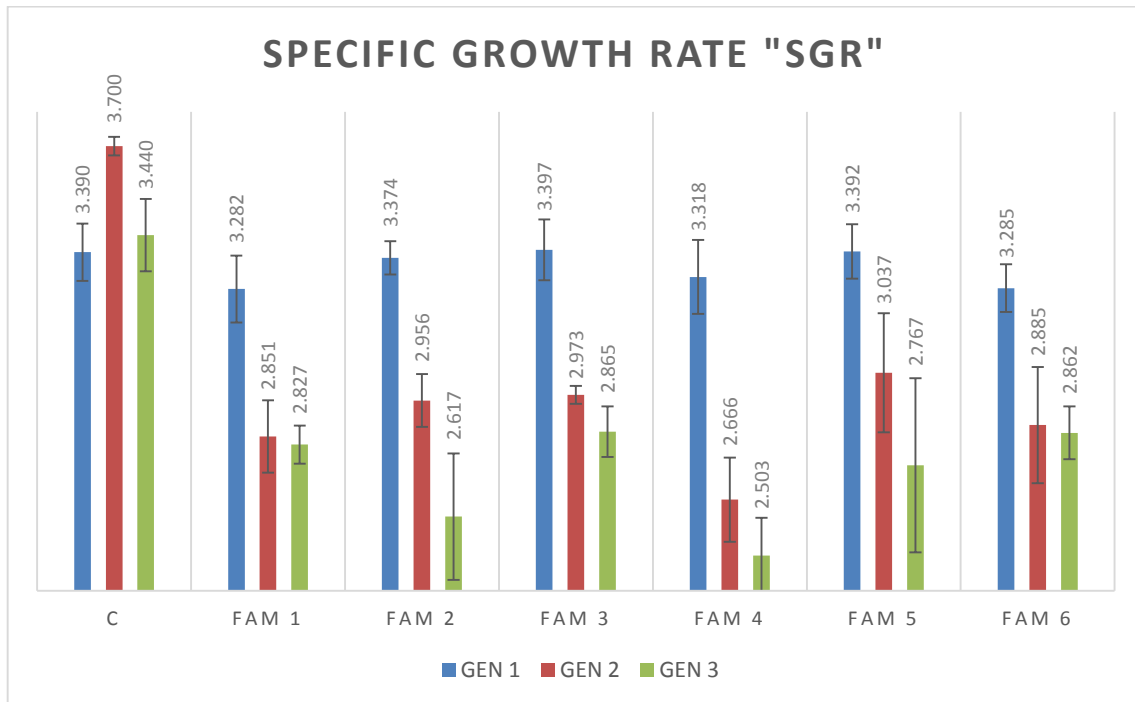
The results from [Table 4.8](#) indicates that mean specific growth rate (SGR) also decreases with increase in inbreeding level at each generation and is graphically expressed in [Figure 4.4](#) below. No significant difference was observed ($P > 0.05$) between the Control and all six individual families in the first generation where $F \approx 0.000$ in terms of specific growth rate. Although, family three (FAM 3) appears to have performed better than the Control and all other family groups with mean specific growth rate of 3.397gday^{-1} .

Table 4.8: Effect of inbreeding on the specific growth rate (SGR) in g/day of six inbred families of *O. mossambicus* at different inbreeding levels ($F = 0.250$ and 0.375) against the Control ($F \approx 0.000$) over three generations of repeated full-sib mating.

	C	FAM 1	FAM 2	FAM 3	FAM 4	FAM 5	FAM 6
Gen 1	3.390 ± 0.084^a	3.282 ± 0.098^a	3.374 ± 0.049^a	3.397 ± 0.089^a	3.318 ± 0.108^a	3.392 ± 0.080^a	3.285 ± 0.070^a
Gen 2	3.700 ± 0.027^a	2.851 ± 0.106^{bc}	2.956 ± 0.077^{bc}	2.973 ± 0.026^{bc}	2.666 ± 0.123^c	3.037 ± 0.174^b	2.885 ± 0.170^{bc}
Gen 3	3.440 ± 0.106^a	2.827 ± 0.056^b	2.617 ± 0.185^b	2.865 ± 0.074^b	2.503 ± 0.110^b	2.767 ± 0.255^b	2.862 ± 0.077^b

However, there was clear statistical difference ($P < 0.05$) between the specific growth rate of the Control and all six inbred families at generations two and three where $F = 0.250$ and 0.375 respectively. Mean specific growth rate of the Control was the highest at 3.695gday^{-1} in generation two and 3.442gday^{-1} in generation three. Family four (FAM 4) performed the least at both generations two and three for specific growth rate with mean SGR of 2.666gday^{-1} and 2.503gday^{-1} respectively. There were no statistical difference between the mean SGR of families one, two, three and six at generation two where inbreeding coefficient was estimated to be $F = 0.250$, however, at generation three, the Control significantly differs from all inbred families, but the inbred families does not differ from one another.

Figure 4.4: The effect of different levels of inbreeding ($F = 0.250$ and 0.375) on the specific growth rate (SGR) in g/day of six inbred families of *O. mossambicus* against the Control ($F \approx 0.000$) over three generations of repeated full-sib mating.



Inbreeding depression for specific growth rate was estimated to range from 17.91 percent to 27.94 percent at coefficient of inbreeding $F = 0.250$; and between 16.72 percent and 27.23 percent at $F = 0.375$. Per 10% increase in inbreeding, inbreeding depression coefficient was estimated to range from -7.16 to -11.18 percent at $F = 0.250$ and between -4.46 and -7.26 percent at $F = 0.375$ in all inbred families.

4.3 YIELD

Yield is broadly defined as the ratio of edible tissues available for consumption when fish are slaughtered to live weight of the fish. Yield in this study is expressed as the ratio of slaughtered weight, after the removal of the viscera and the gills, over live body weight (BW).

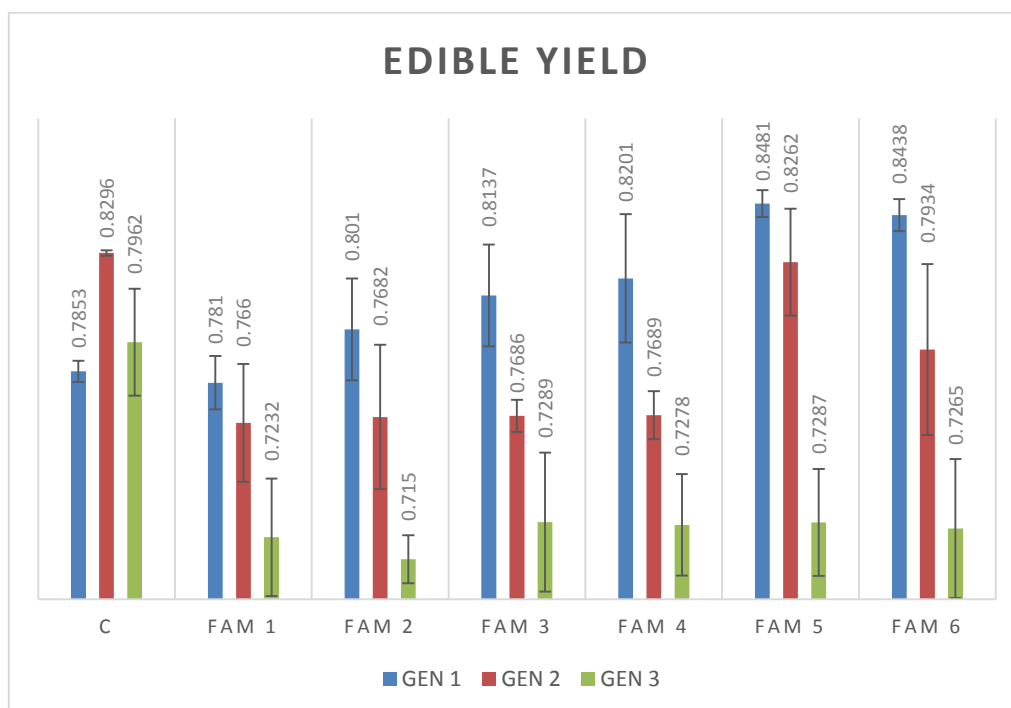
Table 4.9 below shows the differences in yield among seven families of Mozambique tilapia at different inbreeding levels ($F = 0.000, 0.250$ and 0.375) referred to by generations one, two and three respectively.

Table 4.9: Effect of inbreeding on the Yield of six inbred families of *O. mossambicus* at different inbreeding levels (F = 0.250 and 0.375) against the Control (F ≈ 0.000) over three generations of repeated full-sib mating.

	C	FAM 1	FAM 2	FAM 3	FAM 4	FAM 5	FAM 6
Gen 1	0.7853±0.004 ^c	0.781±0.010 ^c	0.801±0.019 ^{bc}	0.8137±0.019 ^{abc}	0.8201±0.024 ^{abc}	0.8481±0.005 ^a	0.8438±0.006 ^{ab}
Gen 2	0.8296±0.001 ^a	0.766±0.022 ^b	0.7682±0.027 ^b	0.7686±0.006 ^b	0.7689±0.009 ^b	0.8262±0.020 ^{ab}	0.7934±0.032 ^{ab}
Gen 3	0.7962±0.020 ^a	0.7232±0.022 ^b	0.715±0.009 ^b	0.7289±0.026 ^b	0.7278±0.019 ^b	0.7287±0.020 ^b	0.7265±0.026 ^b

Unlike in the specific growth rate, statistical differences were observed in the first generation among individual families for the yield trait. However, family group five (FAM 5) gave the highest mean yield value of 0.848 at G₁ where F is assumed to be 0.000 as a result of outcrossing of parents. The Control gave a mean yield of 0.785 at generation one which was not statistically different from the poorest performing family one (FAM 1) with a mean yield of 0.780 at generation one, where F ≈ 0.000.

Figure 4.5: The effect of different levels of inbreeding (F = 0.250 and 0.375) on the Yield of six inbred families of *O. mossambicus* against the Control (F ≈ 0.000) over three generations of repeated full-sib mating.



However, at generations two and three, the mean yield of the Control was highest with clear significant difference from all other six inbred families with mean yield values of 0.829 and 0.796 respectively. Inbreeding depression in yield of *O. mossambicus* in this study was found to range between 0.41 and 7.67 percent at generation two where F = 0.250 and between 8.45 and 10.20 percent at generation three where F = 0.375. Per 10% increase in

inbreeding, inbreeding depression coefficient was estimated to range from -0.16 to -3.07 percent at $F = 0.250$ while at $F = 0.375$, inbreeding depression coefficient was found to range between -2.33 and -2.72 percent per ten percent increase in inbreeding.

4.4 CONDITION FACTOR (K)

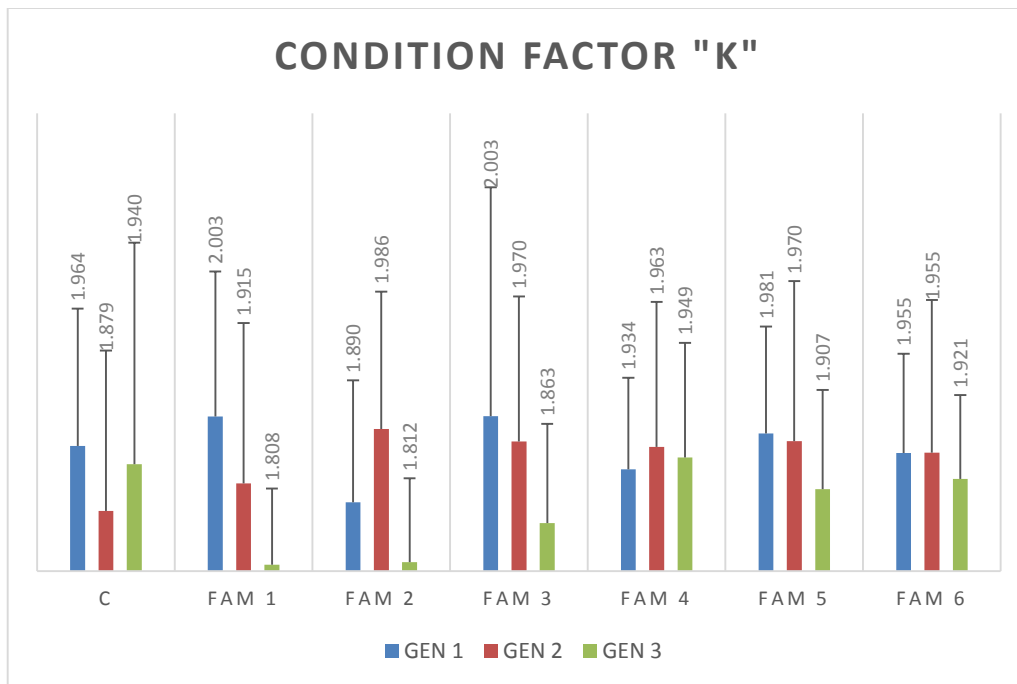
In this present study, a total of one thousand and eight ($n = 1\ 008$) fish were sampled from all seven families including the Control across three generations with three hundred and thirty eight (16 fish per repeat per family = 338) individual fish measured at each generation. The condition factor of individually sampled fish varies across families and generations (Table 4.10; Figure 4.6) ranging from the highest condition factor (K) of 2.85 recorded in family one (FAM 1) at generation one where $F \approx 0.000$, to the lowest condition factor of 1.42 recorded in the control group at generation three where $F = 0.375$.

Table 4.10: Effect of inbreeding on the Condition factor (K) of six inbred families of *O. mossambicus* at different inbreeding levels ($F = 0.250$ and 0.375) against the Control ($F \approx 0.000$) over three generations of repeated full-sib mating.

	C	FAM 1	FAM 2	FAM 3	FAM 4	FAM 5	FAM 6
Gen 1	1.964±0.18 ^a	2.003±0.19 ^a	1.890±0.16 ^a	2.003±0.30 ^a	1.934±0.12 ^a	1.981±0.14 ^a	1.955±0.13 ^a
Gen 2	1.879±0.21 ^a	1.915±0.21 ^a	1.986±0.18 ^a	1.970±0.19 ^a	1.963±0.19 ^a	1.970±0.21 ^a	1.955±0.20 ^a
Gen 3	1.940±0.29 ^a	1.808±0.10 ^a	1.812±0.11 ^a	1.863±0.13 ^a	1.949±0.15 ^a	1.907±0.13 ^a	1.921±0.11 ^a

It was observed that condition factor K was not necessarily the highest as expected at generation one where offspring are assumed not to be inbred, with inbreeding level $F \approx 0.000$. Thus, at generations one, two and three where inbreeding levels are estimated to be 0.000, 0.250 and 0.375 respectively, there was no significant difference ($P > 0.05$) between the Control and all inbred families (Table 4.10).

Figure 4.6: The effect of different levels of inbreeding ($F = 0.250$ and 0.375) on the Condition Factor of six inbred families of *O. mossambicus* against the Control ($F \approx 0.000$) over three generations of repeated full-sib mating.



As a result, it can be said that levels of inbreeding had no significant effect on the general condition of all inbred family groups, away from a normal physiological proportions.

4.5 MORPHOLOGICAL DEFORMITIES (D)

Further to the growth performance as presented there were some observations of morphological deformities occurring in different family groups at different generations and levels of inbreeding. The number of observed deformities presented in Table 4.11 provides no indication of significant differences both between the Control and inbred families or of an escalating trend of deformities over generations.

Table 4.11: Observed number of deformities in different family groups of the Control and inbred *O. mossambicus* over three generations of repeated full-sib mating.

DEFORMITY	C	FAM 1	FAM 2	FAM 3	FAM 4	FAM 5	FAM 6	TOTAL
Gen 1	1	-	-	-	2	1	-	4
Gen 2	-	1	-	-	1	-	1	3
Gen 3	-	-	2	-	-	1	1	4

Some examples of morphological deformities observed are presented in Figure 4.7 below;

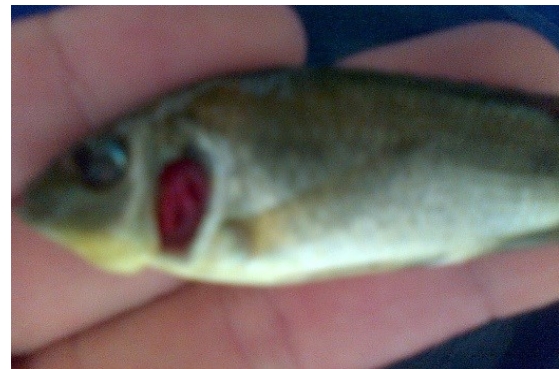
Figure 4.7: Examples of deformities observed in different inbred family groups and Control of *O. mossambicus* over three generations of repeated full-sib mating.



Fin Abnormality



Stunted Body Shape



Shortened Opercula Cover

5 DISCUSSION

5.1 INBREEDING DEPRESSION – GENERAL

Inbreeding usually leads to reduction in productive performance in naturally outbreeding species both in captivity and in the wild (Frankham *et al.*, 2001) and such decline in performance is referred to as inbreeding depression. The accumulation of inbreeding is a natural consequence in closed, but especially small populations undergoing, artificial selection (Pante *et al.*, 2001). Inbreeding is known to bring deleterious recessive alleles together in homozygous genotypes in an individual and exposing these recessive deleterious alleles to greater selective intensity that results in reduced productive fitness (Crnokrak & Roff, 1999). Traits closely associated with fitness such as reproduction and survival most typically exhibit significant depression in response to inbreeding (Falconer & Mackay, 1996; Lynch & Walsh, 1998).

Inbreeding and inbreeding depression have been of particular concern in most aquaculture species (Bucklin, 2002; Evans *et al.*, 2004; Keys *et al.*, 2004; Moss *et al.*, 2007) due to the high relative fecundity of species, manifesting in small effective population sizes associated with high levels of inbreeding. However, the magnitude of inbreeding depression may vary considerably depending on the species, the level of inbreeding and the trait under investigation (Gjerde *et al.*, 1983; Bondari & Dunham, 1987; Bentsen & Olesen, 2002). Published information on inbreeding and inbreeding depression in aquaculture species are mostly on salmonids (Wang *et al.*, 2002) and carp, with only a few on tilapia. Reports on traits related to the overall fitness in species such as Salmon (Gallardo *et al.*, 2004), Flat Oysters (Naciri-Graven *et al.*, 2000) and Bream (Li & Cai, 2003) have shown inbreeding depression in the range between 3-50 percent per 10% increase in inbreeding coefficient.

In this study, the significance of the effect of inbreeding on growth performance and flesh yield of Mozambique tilapia (*O. mossambicus*) was investigated over three generations of repeated full-sib mating. Results obtained confirmed the presence of significant inbreeding

depression observed at both levels of inbreeding studied (i.e. $F = 0.250$ and 0.375). These results correspond to those reported in terms of observed inbreeding depression in tilapia, salmonids and other aquaculture species. Although, no linear relationship between levels of inbreeding and inbreeding depression was observed in this study, similar to findings of Gjerde *et al.* (1983) and Pante *et al.* (2001).

5.2 EFFECTS OF INBREEDING ON BODY WEIGHT (BW)

The results of the effect of inbreeding on growth of *O. mossambicus* are presented in Table 4.3, 4.4 and 4.5 above. Growth rate as observed in terms of body weight (BW), standard length (SL) and specific growth rate (SGR), as expected, showed significant depression with increased levels of inbreeding. Body size, while considered a morphometric trait in endotherms, is more closely associated with fitness in ectotherms, including fin fishes, due to the strong relationship between body size and fecundity (Mousseau & Roff, 1987; Rawson & Hilbish, 1990). In this study values for inbreeding depression in relation to body weight in all six inbred families ranged between 39.63 percent in FAM 3 to 54.17 percent in FAM 4 at $F = 0.250$ (Gen 2) and 45.62 percent in FAM 3 to 47.27 percent in FAM 1 at $F = 0.375$ (Gen 3). These values appear to be higher in comparison to results obtained in Brook trout (*Salvelinus fontinalis*) after one generation of full-sib mating (at $F = 0.250$), with depression for body weight being 27.7 percent at age 7 months and 34.4 percent at 19 months (Cooper, 1961). Kincaid (1976b) also reported highly significant inbreeding depression values in Rainbow trout (*Oncorhynchus mykiss*) of 15.0 percent for body weight at $F = 0.250$ and 20.1 percent at $F = 0.375$. However, reports from this present study differs from report by Gjerde *et al.* (1983) who found no significant effect of inbreeding on body weight of rainbow trout after a 160-day growth period at inbreeding levels of $F = 0.250$ and $F = 0.375$. These results demonstrate to what extent inbreeding depression may differ for the same trait across species and even in relation to the same trait within a species as reported by Kincaid (1976b) and Gjerde *et al.* (1983) in Rainbow trout.

Inbreeding depression was found to be highly significant ($P < 0.001$) at each level of inbreeding studied, thus, per 10% of the inbreeding coefficient. The inbreeding depression coefficient for body weight was estimated to range between -15.85 percent in FAM 3 to -21.67 percent in FAM 4 at $F = 0.250$ and between -12.16 percent in FAM 3 to -12.60 percent in FAM 1 at $F = 0.375$. These results are higher than what was reported for body weight by Bridges (1973) who estimated a 5.12 percent inbreeding depression per 10% increase in inbreeding for rainbow trout; Gjerde *et al.* (1983) reported an estimate of 4.5 percent depression coefficient at $F = 0.250$ and 5.3 percent at $F = 0.375$ per 10% of the inbreeding in adult *Oncorhynchus mykiss*. Su *et al.* (1996) also found in rainbow trout a depression coefficient of between 2.26 and 5.77 percent per 10% increase in inbreeding and Evans *et al.* (2004) estimated an 8.8 percent inbreeding depression per 10% increase of inbreeding for Pacific oyster. In the Chinese shrimp, *Fenneropenaeus chinensis*, Luo *et al.* (2014) observed inbreeding depression coefficient estimate of 4.16 percent and 4.43 percent per 10% increase of inbreeding coefficient of F at 0.250 and 0.375 levels of inbreeding respectively.

5.3 EFFECTS OF INBREEDING ON STANDARD LENGTH (SL)

Growth rate as observed in terms of standard length also showed a significant inbreeding depression of between 13.99 percent in FAM 3 to 22.28 percent in FAM 4 at $F = 0.250$ and a range between 17.21 percent in FAM 3 and 18.43 percent in FAM 6 at $F = 0.375$ inbreeding levels. Per 10% increase in inbreeding, depression in standard length for all inbred families were estimated to range between 5.59 – 8.91 percent at $F = 0.250$ and 4.59 – 4.91 at $F = 0.375$ levels of inbreeding.

These results indicates that significant inbreeding depressions were observed in growth of *O. mossambicus*, measured in terms of both body weight and standard length at all inbreeding levels studied. The higher values of inbreeding depression in this experiment compared to those from literature possibly could be as a result of accumulated inbreeding in the base population used for this study. Such accumulated inbreeding in the base population

might also have reduced the starting heterozygosity in all seven families selected for use before the commencement of this experiment. Although, this effect was expected to have been negated by outcrossing of base parents at the beginning of the experiment. Nonetheless, the results agrees with different reports from literature which observed significant depression in growth measured in terms of body weight in various aquaculture species at all the levels of inbreeding studied (Kincaid 1976b; Gallardo *et al.*, 2004; Naciri-Graven *et al.*, 2000; Li & Cai, 2003).

5.4 EFFECTS OF INBREEDING ON SPECIFIC GROWTH RATE (SGR)

The relationship between specific growth rate and body weight in ectotherms is often described by a power function usually fitted by linear regression (Elliott & Hurley, 1995). In general, animal growth is affected by genetic, environmental and nutritional factors (Very & Sheridan, 2002). In this study, the genetic factor is of particular interest. Results presented in Table 4.2 demonstrated how inbreeding can depress productive traits such as growth in terms of body weight and length. The specific growth rate of each test family per day was calculated and analysed to estimate inbreeding depression at different levels of inbreeding and it was observed that the specific growth rate of Mozambique tilapia *O. mossambicus* was significantly affected at both levels of inbreeding studied in all inbred families. The inbreeding depression for SGR ranged between 17.91 percent in FAM 5 and 27.94 percent in FAM 4 at $F = 0.250$ and 16.72 percent in FAM 3 and 27.23 percent in FAM 4 at $F = 0.375$ inbreeding levels. Inbreeding depression coefficient per 10% increase in F was estimated to be between 7.16 – 11.18 percent at $F = 0.250$ and 4.46 – 7.26 percent at $F = 0.375$ levels of inbreeding. This high depression value observed in terms of SGR demonstrates the negative economic effect inbreeding may have on productivity and profitability of small and medium scale aquaculture in developing countries, where systems are particularly prone to inbreeding.

In this present study, it was interesting to observe that the inbreeding depression for growth traits in terms of body weight (BW), standard length (SL) and specific growth rate (SGR)

were lower in generation three with higher F value (i.e. $F = 0.375$) compared to generation two where $F = 0.250$. This was an unexpected observation as it was hypothesized that higher inbreeding levels should result into higher inbreeding depression. However, this can be explained by the high variation of these traits within the control over the three generations (Table 4.2), which could be as a result of varying environmental conditions over generations in terms of day length and water temperature. Also this could have been a result of purging which is usually a consequence of the dominance hypothesis of inbreeding depression.

Therefore it is recommended for future studies that, increasing the number of repeats for the control and/or improving on standardising environmental conditions over all inbreeding levels in terms of day length, water temperature, density, feeding etc. and if possible, same fish species of different inbreeding levels be grown together under same environmental condition and season could eliminate the effect of this variation.

5.5 EFFECTS OF INBREEDING ON YIELD

Harvest yield has been described to be a composite trait made up of two components – body weight and survival (Evans *et al.*, 2004). Therefore, the effect of inbreeding on harvest yield will be a product of the effects of inbreeding on body weight and on survival. In this study, inbreeding has been observed to depress individual body weight, and it should be assessed, if flesh yield of inbred families were likewise depressed at both levels of inbreeding under investigation.

At the end of each generation (12 weeks), the yield of all inbred families and control was calculated, and an analysis of variance conducted to find out if depression of yield was significant ($p < 0.05$) in each inbred family at inbreeding levels $F = 0.250$ and 0.375 . As expected, the effects of inbreeding depression on individual body weights and standard length of inbred families had a cumulative effect resulting also in depression of yield (Figure 4.5). Inbreeding level of $F = 0.250$ resulted in an inbreeding depression value of between 0.41 – 7.67 percent in yield of *O. mossambicus* amongst the inbred families.

Subsequently, in generation three where $F = 0.375$, inbreeding depression in yield was in the range of between 8.45 – 10.20 percent amongst the inbred families under investigation (Table 4.3, 4.4 and 4.5). Thus, 10% increase in F was estimated to result in an inbreeding depression coefficient ranging between 0.16 – 3.07 percent in generation two and between 2.33 – 2.72 percent in generation three for flesh yield of *O. mossambicus*. These levels are lower than those reported by Evans *et al.* (2004) who estimated a 12.2 percent depression in yield for adult Pacific oysters, *Crassostrea gigas* per 10% increase in F .

The genetic complexity of a composite traits such as yield had been recognized by Ceccarelli *et al.* (1991), Baker (1987) and Blum (1985), and any change in the sensitivity of individual body weight to inbreeding would directly affect a composite character like yield (Evans *et al.*, 2004). It was further pointed out that as the number of non-interacting causal components of a composite character increase, so does the likelihood of experiencing genotype x environment interactions (Baker, 1987). However, all inbred families including the Control in this study were reared in a homogenous environment with same water quality parameters, feed and feeding regimes; therefore we can conclude that the reduction in yield and observed inbreeding depression in all inbred families compared to the Control can be explained by the level of inbreeding in these test families.

5.6 EFFECTS OF INBREEDING ON CONDITION FACTOR (K)

The condition factor, K gives information on the physiological condition of fish. It is based on the hypothesis that heavier fish of a particular length are in a better physiological condition and it is regarded as a useful index for monitoring the effect of husbandry practices such as feeding, stocking density and health on the growth rate of fish (Abdullahi *et al.*, 2014). Maguire & Mace (1993) pointed out that an increase in K value is indicative of the accumulation of body fat hence nutritional status and sometimes also of gonadal development.

The K values of all the seven family groups in this study show no significant differences ($P > 0.05$) at all levels of inbreeding studied i.e. at $F = 0.000, 0.250$ and 0.375 (Table 4.10). It was observed in this study that all seven family groups as well as the Control recorded condition factors K ranging from 1.42 to 2.85 that is below the norm of 3.0 for Tilapia species. Perry *et al.* (1996) reported that, fishes with low condition index are presumed to have experienced adverse environmental or husbandry conditions. Angelescu *et al.* (1958) reported the highest K values are reached in a species when the fish are reproductively mature.

From all indications, a growth period of 90 days will be too short to conclude that the K values of inbred family groups in this study are of matured fish. Therefore, we cannot conclude that both inbreeding levels ($F = 0.250$ and 0.375) studied have no effect on the condition factor K of inbred *O. mossambicus* due to the fact that majority of the fish were still sexually immature at the end of the trial (90 days growth period).

5.7 INBREEDING LEVELS AND DEFORMITIES IN INBRED FAMILIES (D)

Morphological traits such as bilateral asymmetry which could be defined as the unbalanced numbers for meristic traits on the right and left halves of the body were observed to be an indirect indication of homozygosity of recessive genes in fish (Leary *et al.*, 1983, 1984, 1985a, b, c; Alibert *et al.*, 1994). Doyle (2003) reported that random asymmetry can be interpreted as failure of the genotype to adequately regulate the development of the phenotype in an individual. However on the contrary, Reale & Roff (2003) reported that inbreeding or heterozygosity does not affect developmental stability in fish. Fassehaye *et al.* (2007) suggested that the contribution of genetic factors to variation in fluctuating asymmetry is limited in the population of Nile tilapia studied in his experiment. Furthermore, there are increasing evidences that environmental stress may increase fluctuating asymmetry (Parsons, 1990, 1992 and Fassehaye *et al.*, 2007) but there are only few studies that clearly demonstrate the influence of genetic stress on developmental stability (Blanco *et al.*, 1990; Batterham *et al.*, 1996; Brakefield & Breuker, 1996). A meta-analysis by Vøllestad *et al.*

(1999) showed that heterozygosity explained only a very small amount (1%) of variation in fluctuating asymmetry, and most investigators now appear to agree that there is very little additive genetic variation for fluctuating asymmetry in most characters. My results support this conclusion, with a random, nonlinear occurrence of fluctuating asymmetry and deformities observed at different inbreeding levels in *O. mossambicus*. Four deformities were observed at random at the first generation in the offspring of families that were theoretically assumed not to be inbred with inbreeding coefficient $F = 0.000$; one in the Control group, two in FAM 4 and one in FAM 5 (see Table 4.11). Three fish were identified in generation two with inbreeding coefficient of $F = 0.250$ and another four fish from the third generation where inbreeding coefficient equals 0.375 (Table 4.11). With these random, nonlinear results, one might agree that, fluctuating asymmetry and deformities in *O. mossambicus* could be associated more with environmental stress than genetic variations in this study population.

6 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

Understanding the influence of inbreeding on aquaculture species is fundamental to the design of efficient long-term breeding strategies and proper genetic maintenance of improved fish strains within closed aquaculture systems. Small and medium scale farmers in developing countries, including Africa, are particularly exposed to inbreeding because of the high relative fecundity of aquaculture species and the small effective population size of broodstock required in a semi intensive to extensive farming system, limited by space and resources.

In developing countries, including Africa, a lack of knowledge about genetic management practices further increases the exposure of subsistence and small scale farming systems to inbreeding and the related effects. Tilapia species, which is a main contributor to aquaculture production in Africa through application in subsistence and small scale farming systems, have been demonstrated to be negatively affected by different levels of inbreeding in relation to body weight, survival, yield, reproduction and increases the occurrence of deformities in fish populations (Fassehaye *et al.*, 2007, 2009; Ch'ang, 1971b; Ponzoni *et al.*, 2010).

The amount of inbreeding that can be tolerated within a closed aquaculture system or breeding program depends on the species and the trait under consideration. Data presented in this study indicate significant inbreeding depression of productive traits in Mozambique tilapia, *O. mossambicus* and can occur when progenies share common ancestral genes which make them genetically alike by descent. The results from this study confirms the significance and detrimental effect of inbreeding on key production traits. Growth of Tilapia (*O. mossambicus*) expressed as body weight (BW), standard length (SL), specific growth rate (SGR) and flesh yield were all significantly affected by increasing levels of inbreeding, compared to a non-inbred Control group. However, the condition factor (K) and number of deformities seem not to be affected by different levels of inbreeding studied. There was no

statistical evidence to prove that the condition factor of all inbred families were different from the outbred Controls and also there was no relationship between levels of inbreeding and number of deformed individuals in this study. Hence, it is concluded that, deformities might be attributed more to physical environmental stress and nutrition than genetic stress in this population (Parsons, 1992; Vøllestad *et al.*, 1999; Fassehaye *et al.*, 2007).

The manifestation of high levels of inbreeding depression in a closed aquaculture system will ultimately result in genetic retrogression of stock, poor productivity and reduced profitability of aquaculture establishments. Various authors also emphasize that apart from reduced growth performance the negative impact of inbreeding on fitness, survival and reproduction also need to be taken into account (Bondari & Dunham, 1986; Falconer & Mackay, 1996; DeRose & Roff, 1999). Avoidance of mating close relatives through maintaining pedigree records or other management strategies is therefore essential to prevent the rapid accumulation of inbreeding in a population that would negatively affect the sustainability and profitability of tilapia and other aquaculture breeding programs and businesses.

However, the implementation of these procedures of monitoring and avoiding inbreeding in fish population would be a challenge due to the size of farm, level of education of farmers and commitment of government institutions and research establishments towards small and medium scale aquaculture farmers in most developing countries including Africa in terms of training and support.

6.2 RECOMMENDATIONS

The key recommendations stemming from this study are:

- Creation of awareness amongst subsistence and small scale fish farmers about the occurrence and detrimental effects of inbreeding on aquaculture production.
- Design and implementation of cost effective measures to limit and mitigate the effect of inbreeding on small scale farming systems, such as:
 - i. Maintenance of an adequate effective population size
 - ii. Implementation of a rotational mating system
 - iii. Regular outsourcing of broodstock
- Detailed assessments over time of the status and trends of inbreeding amongst small scale farming units in Africa, through the use of molecular markers.
- Detailed assessment of the maintenance of the genetic integrity of genetically improved material (selected lines) during dissemination amongst small scale farmers in Africa, over time.
- Training of small and medium scale farmers in Africa basic quantitative genetics and selection theories.

There are however a number of challenges to overcome in an effort to ensure effective uptake of the technology and knowledge systems amongst small scale farmers in Africa, these includes:

1. The large number species used in aquaculture.
2. Lack of relevant education and competence in basic quantitative genetics and selection theories among small and medium scale African fish farmers.
3. Large numbers of small and medium scale producers with low levels of integration into and connection within the industry.
4. Systematic genetic improvement and management is generally highly capital intensive and biologically vulnerable to conservation issues.

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