UNIVERSITY OF CAPE COAST

CROSSING, SEX REVERSAL AND REPRODUCTIVE CAPACITY OF

TWO POPULATIONS OF *SAROTHERODON MELANOTHERON*

(CICHLIDAE) FROM THE CENTRAL REGION OF GHANA

BY

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Aquatic Sciences, University of Cape Coast, in partial

fulfilment of the requirements for the award of Master

of Philosophy degree in Aquaculture

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this University or elsewhere.

Candidate's Signature:……………………   Date…………….

Name: Theophilus Apenuvor

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature …………………..   Date …………..

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ABSTRACT

The study investigated whether progeny of *Sarotherodon melanotheron* from two different populations would have higher reproductive capacity and also exhibit desirable aquaculture traits. Broodstock of *S. melanotheron* were collected from Kakum Estuary and Benya lagoon and bred to the second filial generation stage. Reproduction was higher in the parents from the Estuary than those from the lagoon. Water quality parameters were within the acceptable range for fish culture. Three different concentrations; 30, 60 and 120 mg/kg feed of 17 α-methyltestosterone and the control were administered for 30 days. Fry fed 120 mg MT/kg feed produced the highest proportion (92.70 %) of males which differ significantly (F = 266.22, $p < 0.05$) from 30 mg MT/kg feed and the control but was not significantly different ($p > 0.05$) from 60 mg MT/kg feed. A positive linear correlation was found between fecundity and total length, body weight, and ovary weight of *S. melanotheron* from Fosu lagoon with 'r' values as 0.58, 0.61 and 0.52 respectively. Relationship between brood size and fish length as well as body weight was also linear with 'r' as 0.85 and 0.86 respectively. The overall results indicated that none of the male hormone concentrations produced 100 % males and that the anabolic effect of the hormone was not significant in the growth of *S. melanotheron* fry in all the treatments.

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DEDICATION

In memory of my late daughter Princess Fafali Apenuvor who passed on when this work was in progress.

TABLE OF CONTENTS

Page

LISTOF TABLES

52

LIST OF FIGURES

LIST OF PLATES

CHAPTER ONE

INTRODUCTION

Background to the Study

Sarotherodon melanotheron (Rüppell, 1852) commonly called blackchin tilapia is one of the species of the subgenera *Sarotherodon* which belongs to the family, Cichlidae. The species of the subgenera are classified mainly according to differences in their mode of reproduction and to some extent, their feeding habits, morphology and biogeography (Lowe-McConnell, 1958; Trewavas, 1983). Cichlid fishes of the genus *Sarotherodon* contribute significantly to the fresh and brackish water fishery of West Africa (Lévêque, Pauly & Teugels, 1992).

They are generally found in estuaries, lagoons and occasionally in the mouth and the lower courses of the coastal basins of Senegal to the Congo (Trewavas, & Teugels, 1991). Teugels and Falk (2000) in their survey also observed that *S. melanotheron* is one of the major fish species that is frequently caught in West African coastal waters. In Ghana, it is the most abundant fish (65-98 %) in all the 60 lagoons and estuaries along the 550 km coastline (Abban, Asante & Falk, 2000 cited in Ofori – Danson & Kumi, 2006).

According to Ekau and Blay (2000) *S. melanotheron* is an important fish in the commercial and subsistence fisheries of many West African lagoons. Tilapia, as *S. melanotheron* are casually called, have certain favourable characteristics, like tolerant to adverse environmental conditions, survival at low dissolved oxygen level, relatively fast growth and efficient food conversion ability. All

these characteristics make tilapia one of the best choices for farmers (Yi, Lin & Diana, 1996; Penna - Mendoza, Gomez - Marquez, Salgado - Ugerte & Ramirez-Nogguera, 2005). Tilapias are among the most important warm water fishes used for aquaculture production and the culturing is practiced in over 80 countries in the tropical and subtropical regions of the world with a total production of 478,641 metric tonnes, with an average growth rate of about 12 % per annum since 1986 (FAO, 1997; Lovshin, 1997). They are described as plastic animals because their growth and maximum obtainable size can be seriously influenced by the physical and biological composition of their environment (Olurin & Aderibigbe, 2006).

Physico-chemical parameters

The success of a commercial aquaculture enterprise depends on providing the optimum environment for rapid growth at a minimum cost of resources and capital. Water quality affects the general condition of cultured organism as it determines the health and growth conditions of cultured organism. Quality of water is therefore an essential factor to be considered when planning for high aquaculture production. Although the environment of fish in aquaculture system is a complex one, consisting of several water quality variables, only a few of them play a decisive role. The critical parameters are temperature, concentrations of dissolved oxygen (DO), suspended solids and ammonia, nitrite, carbon dioxide and alkalinity.

Temperature tolerance

Temperature is one of the most important aquatic environmental variables. This is because all aquatic organisms are poikilothermic hence their body metabolic activity is closely related to water temperature (Brett, 1979). Growth in fishes is a complex process by which ingested energy is converted to biomass. The efficiency of this conversion is regulated by the growth potential of the organism and various abiotic factors such as food supply, temperature and adverse environmental factors brought about by the conditions in which the fish are cultured (Soderberg, 1997).

According to Woiwode and Adelman (1991) temperature is known to influence both ingestion and metabolism which, however, also affect growth rates. Temperature and other environmental factors dictate the progress of growth of the fish whether it will be at its optimum or not. When desirable levels of these factors are reached, growth will take place and if possible, optimum growth will be attained. Under conditions of unlimited food supply, an increase in temperature will lead to increase in food intake but at extremely high temperatures there would be an abrupt decline in rates of ingestion which would in turn affect growth (Woiwode & Adelman, 1991). The optimal temperature for growth of tilapia ranges from 29 to 31 ^{o}C and growth declines greatly with decreasing temperature and at 20 to 22 ^{o}C, growth is about 30 % of optimum (Teichert-Coddington, Popma & Lovshin, 1997). The lethal minimum temperature for most species of tilapia is 10 ^{o}C or 11°C while from 37 to 38 ^{o}C stress and diseases tend to attack most of them (Popma & Lovshin, 1995). Boyd (1998) reported that, warm water

species grow best at temperatures between 25 and 32 ºC. Tilapia does not thrive in low temperature but are very tolerant to high temperature with the optimum, usually at 28 to 32 ºC (FAO, 2005). Water temperatures are in this range all year-round at low altitude in the tropics, but they are too low in winter in temperate regions for rapid growth of warm water aquaculture species and their food organism (Boyd, 1998).

Wohlfarth and Hulata (1983) reported that the optimum temperature for the culture of *Oreochromis niloticus* is 27 to 30.5 °C. Bauer (1968) found 27 °C as a satisfactory temperature for *S. melanotheron melanotheron*. Tilapias do not grow well at temperature below 16 °C and cannot usually survive for more than a few days below 10 °C (Chervinski, 1982), but they are remarkably tolerant to high temperatures, up to 40 °C (Azaza, 2004). Banerjea (1967) stated that the significant effect of higher temperature is the increased rate of biochemical activity of the microbiota which would in turn release nutrients by decomposition of organic matter at the bottom with subsequent increase in the nutrient status of water.

Growth of fish is a complex process affected by many behavioural, physiological, nutritional, and environmental factors; however, temperature is recognized as one of the most important single abiotic factor affecting growth, food intake, and food conversion of fish (Martinez, Cristina & Ross, 1996). *O. niloticus* is known to tolerate high temperatures and therefore cannot tolerate for a long period, water temperature between 10 and 15 °C (Ballarin & Hatton, 1979) and does not survive below 10 °C (Chervinski & Lahav, 1976). The optimum

temperature for feeding, growth and reproduction is between 22 and 30 °C while good growth was recorded in the upper portion of this range (Hauser, 1977). Thus, at higher or lower temperatures, feeding and growth rates are reduced, and at 20 °C or less, feeding and growth cease (Caulton, 1982). Temperature is a critical factor which influences the solubility of oxygen in water.

According to Parker and Davies (1981) fish would grow best in the tropics at temperature between 25 – 32 °C. Chervinski (1982) observed that the tropical origin of *Tilapia* and *Sarotherodon* is reflected in their preferred temperature and that these genera do not tolerate temperatures below 12 °C but are remarkably tolerant to high temperatures up to 42 °C and additionally, the Nile tilapia (*Oreochromis niloticus*) in general do not grow at temperatures below 16 °C and exhibit poor survival if water temperatures fall below 10 °C for few days.

Studies on several fish species have revealed that in the temperature range tolerated by fish, growth rates increase with increasing temperature and show a parabolic pattern (Xiao-Jun & Ruyung, 1992; Watanabe, Mueller, Head & Ellis 1993; Larsson & Berglund, 2005). When experimental temperature reaches the upper extreme of the tolerance range, growth decreases. This depression of growth is due to the higher energy cost for maintenance, metabolism and seems to be related mainly to a loss of appetite. Watanabe, Takeuchi, Satoh and Kiron (1996) reported 25 °C to be the optimum temperature for nutrient digestibility in tilapia.

Dissolved oxygen tolerance

Dissolved oxygen is the most important and critical parameter, requiring continuous monitoring in aquaculture production systems. This is due to the fact that fish aerobic metabolism requires DO (Timmons, James, Fred, Sreven & Brian, 2001). Dissolved oxygen is critical for the survival of aquatic organisms for respiration (NERR, 1997). The amount of oxygen in water determines the species and abundance of organisms that can live in it. Mixing of surface waters by wind and waves increases the rate of absorption of atmospheric oxygen into water. The minimum DO requirements of tilapia species is 5 mg/l and if the concentration of DO decreases, respiration and feeding activities also decrease (Mallya, 2007). As a result, the growth rate is reduced and the possibility of disease outbreaks increases. Furthermore, fish are unable to assimilate the food consumed when DO is low (Tom, 1998). According to Phelps and Popma (2000), DO concentrations in aquatic environment should remain above 4 mg/1 for ideal fish culture.

Charkroff (1976) reported that oxygen level should be above 5 mg/l but not more than 15 mg/l to promote rapid growth of fish. lt was reported that at DO level below 2 mg/l, fish cease to feed, reduce locomotor activities and use the available oxygen for support system rather than growth (Parker & Davies, 1981), while fish mortality sets in at DO values less than 1mg/l (Ayinla, Oladoso, Ajiboye, & Ansa, 1994). The solubility of oxygen decreases with increasing salinity such that the solubility of oxygen in seawater is about 20 percent less than in freshwater of the same temperature (NERR, 1997). Boyd (2002) reported that,

DO concentration in intensive tilapia culture unit may be quite low at times. He noted that, tilapias are rather tolerant to low DO, and concentrations of 3 to 4 mg/l apparently are not extremely harmful to them even with long-term exposure.

The black-chinned tilapia tolerates salinity ranges of 0 – 45 ‰ and could live in an environment with DO level as low as 0.1mg/l (Pullin & Lowe – McConnell, 1982; Trewavas, 1983). Tilapias are, in general, highly tolerant of low DO concentration, even down to 0.1 mg/l (Magid & Babiker, 1975), but optimum growth is obtained at concentrations greater than 3 mg/l (Ross, 2000). However, when DO levels fall below the optimum, the fish are seen gulping air from the surface film of water in their holding facilities (Beamish, 1970; Magid & Babiker, 1975). According to Balarin and Hatton (1979) tilapia have a low oxygen demand and can survive at low levels and the lowest tolerance limits for *Oreochromis niloticus* ranges from 0.1 to 3 mg/l under different conditions.

Tolerance of pH

pH is a measure of whether water is acidic or basic (Boyd, 1998). The pH of water affects many water quality parameters and the rates of many biological and chemical processes. Thus, pH is considered an important parameter to be monitored and controlled in re-circulating aquaculture system (Losordo, Masser & Rakocy, 1998). Neess (1946) observed that fish under culture could tolerate pH of 12.0 for a few days. Typically, pH is highest at dusk and lowest at dawn because at night, respiration increases carbon dioxide concentrations that interact with water producing carbonic acid and lowering pH. This can limit the ability of fish blood to carry oxygen (Russell, 2011). On his part, Huet (1972)

recommended a pH of 7.8 as the best for fish culture. Additionally, Ross (2000) noted that tilapias can tolerate a pH range of 3.7 to 11, but best growth rates are achieved between pH of 7 to 9.

Popma and Masser (1999) reported that tilapia can survive at pH ranging from 5 to 10 but they do best at a pH range from 6 to 9. The recommended pH for maximum fish production is between 5 – 9 (Chakroff, 1976). Swingle (1961) stated that pH of 11.0 may be taken as the alkaline death point practically for all pond fishes. Because freshwater has low buffering effect, carbon dioxide can accumulate in the water, thus lowering the pH in ponds considerably and reducing the amount of un-ionized ammonia (Tucker, Lloyd, & Busch, 1984). The pH of pond water may increase above 9.0 during periods when photosynthesis is high (Tiechert- Coddington *et. al.,*1997).

Salinity tolerance

The average salinity of freshwater is less than 0.5 ‰ and that of seawater is 35 ‰. Salinity has been recognised as a key factor influencing the occurrence and composition of species in brackish water habitats in the tropics and subtropics (Little, Reay & Grove, 1988). Eyeson (1979) reported that hatching of eggs and survival of fry of *S. melanotheron* are very much favoured by brackish water conditions and concluded that maximum fish production by culture method can be obtained in a water system whose salinity is around 10 ‰. The fish produces viable fry in freshwater aquaria if the embryos are held in the mouth, but saline water is needed in artificial incubation; 40 % sea water giving

the best results (Trewavas, 1983). The fish occurs rarely in fresh water, and only when brackish water is nearby.

Pauly (1976) found *S. melanotheron*, the predominant fish in a closed lagoon where the salinity fluctuated from 0 to over 45 ‰. In the Niger Delta, the fish is most common in the middle salinity ranges (10 to 15‰) but is present in both higher and lower salinities (Marioghae, pers. comm.).

Reproductive biology of *S. melanotheron*

Reproduction is the process by which species are perpetuated. The success of any fish species is ultimately determined by the ability of its members to reproduce successfully in a fluctuating environment (Moyle & Czech, 2000). Therefore, the reproductive strategy as reflected in anatomical, behavioral, physiological, and energetic adaptations is an essential commitment to future generation. The reproductive behaviour in fishes is remarkably diversified. Most fishes lay a large number of eggs, fertilized in the aquatic environment. Studies of reproduction and growth of many species indicated that the reproductive cycle of fishes are closely tied to the environmental changes particularly temperature, day length and food supply. These environmental factors have the greatest influence upon the gonadal development initiation and fecundity of the species.

Reproductive parameters such as size at first maturity, spawning frequency, fecundity, sex ratio and recruitment are of great value in fishery prediction and formulation of management measures (Bal & Rao, 1984). The availability of quality seeds and the ability to control fish reproduction are limiting factors in the farming of any commercial species. This became an

9

important factor to fulfill the demand of continuity in supply of table fish and fish seeds throughout the year.

Eyeson (1979) observed that in the natural lagoon population, the opercular breeding colour develops in fish of about 4.5 cm standard length (SL) and established that in a confined environment, S. *melanotheron* can be sexually active at 4 to 6 months old at a size as small as 4 to 5cm (SL). Eyeson (1983) reported that since some spermatozoa had developed in the testes of certain individuals within four months and maturing oocytes were also present in the female, it can be predicted that breeding could occur within six months. Similarly, Semakula and Makoro (1967) have reported that under pond conditions, *Tilapia nilotica*, *Tilapia zillii,* and *Tilapia leucosticta* mature in about six months.The operculum colour of the male is golden yellow whilst that of the female is purplish.

Populations in Ghana belong to the subspecies *Sarotherodon melanotheron melanotheron.* Eyeson (1992) found two strains of *S. melanotheron* in Cape Coast coastal lagoons. According to Aronson (1949) these species of tilapia are principally paternal oral brooders but there are reported cases of maternal brooders. Specker, Eales, Tagawa and Tyler (2000) reported that in captivity, the blackchin tilapia exhibits maternal as well as paternal mouth brooding behaviour. Although in equatorial waters, tilapias tend to spawn throughout the year, in the tropics, there are seasons when increased breeding activity has been observed and are usually correlated with the two rainy seasons in equatorial East Africa (Lowe

– McConnell, 1955). According to Aronson (1951) spawning by tilapia depends on changes in light intensity and temperature.

Condition factor of fish

Condition factor (K) is used in fisheries science in order to compare the "condition", "fatness" or wellbeing of fish. It is based on the hypothesis that heavier fish of a particular length are in a better physiological condition (Bagenal, 1978). Condition factor is also a useful index for the monitoring of feeding intensity in fish (Oni, Olayemi & Adegboye, 1983). The condition factor in fish serves as an indicator of physiological state of the fish in relation to its welfare (Le Cren, 1951). Condition factor provides information when comparing two populations living in certain feeding area, climate and other conditions (Weatherly and Gills, 1987).

The value of K is influenced by age of fish, sex, season, stage of maturation, fullness of gut, type of food consumed, amount of fat reserve and degree of muscular development. With females, the K value will decrease rapidly when eggs are shed. The K value can also be used in determining the stocking rate of fish in particular water. If the K value reaches an unacceptably low level in water which is totally or partly dependent on stocking, the stocking rate can be reduced accordingly until the K value improves and reaches an acceptable level. Thus, condition factor is important in understanding the life cycle of fish species and it contributes to adequate management of these fish species, hence, maintaining the equilibrium in the ecosystem. Condition factor is strongly influenced by both biotic and abiotic environmental factors and can be used as an

index to assess the status of the aquatic ecosystem in which fish live (Abowei, 2010).

Oni *et al*. (1983) noted that condition factor is not constant for a species or population over time and might be influenced by both biotic and abiotic factors such as feeding regime and state of gonadal development. In the West-Africa sub-region, the mean condition factor values calculated for *S. melanotheron* were between 4.1 and 5.7 in Lagos lagoon (Fagade, 1979), 3.85 in a man-made Lake Ayamé (Koné & Teugels, 1999). Legendre and Hem (1989) however recorded an average condition factor for *S. melanotheron* under culture to be 2.09 and for the same species from the wild as 2.34.

Anene (2005) recorded relatively lower condition factors for relatively large sizes of *Chromidotilapia guntheri, Tilapia cabrae and Tilapia mariae* while relatively higher condition factors were recorded for rather smaller fish. Condition factor is calculated by using Fulton's equation, $K= 100\ W/\ L^3$ (Bagenal & Brawn, 1978) where, W is the whole body weight and L, the standard length of fish.

Fecundity of fish

Fecundity is defined as the number of ripe ova in the female prior to spawning (Bagenal & Braum, 1978). Similarly, Allee, Emerson, Park and Schmidt 1949, as cited in Welcomme, 1967, defines fecundity as the number of eggs (or sperm) produced and fertility as the number of eggs that develop into living young. Fecundity is one of the best criteria for determining the reproductive potential of fish species, and therefore an important parameter which enables

estimation of total population and forecasting of fish productivity. The assessment of fecundity (egg production) and fertility (young produced) of fishes is of special importance in biological studies, particularly in so far as the management of heavily fished commercial species is concerned (Welcomme, 1967). It has been recorded for several species of tilapia that the numbers of fry brooded are less than the egg production of fish of the same size. Eyeson (1979) agrees with this view as he found out that as the fry grow, the mouth of the brooding parent cannot accommodate all of them and it is possible that some die as a result of overcrowding, some are swallowed by the brooder or some escape into surrounding waters.

Fecundity increases with age and size of the fish (Bone *et al.,* 1995). But according to Bagenal and Braum (1978) fecundity in fish species characteristically varied among individuals of the same size and age. As female fish grow, the number of eggs produced increases. On average, larger female will produce more eggs than smaller female. Therefore, a larger female has an expectation of a greater number of eggs than a smaller female (Galvani & Coleman, 1998). The best growth performances have in fact been obtained with *S. melanotheron* males when reared in a monosex culture. It is a biological principle that fecundity is inversely proportional to the degree of parental care in species (Atz, 1958; Largler, Bardach, & Miller, 1962).

It has been shown that there is a direct relationship between potential fry production and body length; the square of the standard length approximates the number of eggs that a female of a particular size can incubate (Welcomme, 1967).

In *S. melanotheron*, the growth rates reverse when one goes from mixed to monosex culture, this shows clearly that the lower growth of males under mixed culture is a consequence of mouth-brooding. It is indeed, known that under mixed culture *S. melanotheron* reproduces actively, and that the males do not feed during brooding (Legendre & Ecoutin, 1989).

Legendre and Ecoutin (1989) further reported that the number of eggs or fry brooded is positively related to the male body weight in *S. melanotheron*. Ecological studies conducted on population of *S. melanotheron* by Kone and Teugels (1999) shows that they had a lower fecundity, larger oocyte diameter and a good condition factor, indicating a good adaptation to pure freshwater conditions. Fryer and IIes (1972) established that in tilapia as in other fish, egg production tends to be lower in species showing parental care and that mouth brooders lay fewer eggs than the guarder species.

Trewavas (1983) established that matured female tilapia produce anywhere from 200 to 900 eggs. In similar vein, Eyeson (1979) found that in *Tilapia melanotheron* fewer eggs are spawned than may be found in the ripe ovaries and observations conducted on laboratory samples indicate that no spawning occurs in the absence of male partner. Marshall (1979) found no relationship between the number of eggs in the mouth and the egg stage at harvest of *Oreochromis machrochir*.

Again, Eyeson (1979) reported that under laboratory conditions, *Tilapia melanotheron* spawned up to 6 times in six months at an average of one in 22 days. On his part, Specker *et al*. (2000) observed that in the black-chin tilapia, the

male picks up the eggs in his mouth within minutes after he fertilizes them and churns them there for about 2 weeks (14 to 18 days). Eyeson (1979) noted that in *S. melanotheron* the eggs survived better in salty water and that salinity of 10 ‰ was the most favourable medium for hatching, in terms of survival and rate of embryonic development.

Fryer (1961) established that the size at which the fish matures determines its initial egg production and brooding efficiency. Welcomme (1967) however noted that *Tilapia leucosticta* can breed at least twice in succession during one season, but the frequency may be more than this. Cridland (1961) recorded *Tilapia esculenta* as spawning seven times in 24 months with individual broods as close together as 39 days and Aronson (1951) observed between one and fourteen spawnings per annum in *Tilapia macrocephala*. *Tilapia mossambicus* was recorded by Reidel (1965) as having four to five spawnings per season, separated by some 30 days. Fryer (1961) produces evidence for three and possibly five broods over eight months in *Tilapia variabilis*. In a similar vein, crosses involving female *Tilapia aurea* and male *Tilapia nilotica* in the Philippine yields a higher percentage male in progeny than the reciprocal cross (Prunet & Bornancin, 1989).

Sex ratio

Sex ratio provides information on the proportion of male and female fish in a population and indicates the dominating sex of fish in a population which constitutes basic information in assessing reproductive potentials and estimating stock size in fish population (Vicentini & Araujo, 2003). In order to determine

female spawning biomass, estimates of reproductive potential can be added to sex ratio information to give a better understanding and assessment of stock status relative to a biological indicator, which has been observed for some fish stocks (Morgan & Pavely, 1996).

The near equal production of males and females in most organisms has fascinated biologists since the days of Darwin (Khallaf, Galal & Authman, 2003). Sex ratio, gonadosomatic index, stages of gonadal development and fecundity are some important aspects of fish reproductive biology, which give information necessary for successful fisheries management and recruitment in natural water bodies and aquaculture of fish species. According to Romer and Beisenherz (1995) a correlation between sex ratio of offspring of 33 different species of *Apistogramma* and temperature at certain pH could be demonstrated with high significance. At low temperature (23 ºC) the sex ratio of offspring of 32 *Apistogramma* species was skewed towards females, whereas at high temperature (29 ºC) it was directed towards males and at 26 ºC, the number of males and females was approximately equal in proportion.

In many fish and amphibians, sex determination is genetic but reversible by environmental factors during a sensitive period that is typically very early in their life history. Environmental sex reversal can be induced by various factors, including temperature changes or exposure to hormone active substances (Wallace, Badawy, & Wallace, 1999; Devlin & Nagahama, 2002; Baroiller, D'Cotta & Saillant, 2009). It is nowadays even used in fish farming to produce more profitable one-sex cultures (Pandian & Sheela, 1995; Piferrer, 2001; Cnaani

& Levavi-Sivan, 2009). Distorted sex ratios in the wild could potentially be caused by environmental sex reversal (Olsen, Miller, Harper, Nagler & Wenburg, 2006; Brykov, Kukhlevsky, Shevlyakov, Kinas & Zavarina, 2008; Alho, Matsuba & Merila, 2010). Sex hormones, hormone-active substances, and endocrine disrupting chemicals are frequently released into natural water courses, for example, in effluents from domestic and industrial sources (Larsson, Hallman & Forlin, 2000; Parks, Lambright, Orlando, Guillette, Ankley & Gray, 2001; Jobling & Tyler, 2003).

Fish exposed to endocrine disrupting chemicals often display reduced reproductive performance (Vos *et al*., 2000), and exposure to such chemicals could well be responsible for gonadal malformations if, for example, sex reversal was incomplete leading to individuals that display gonadal characteristics of both sexes. A sudden increase in the prevalence of intersex or of other gonadal malformations is indeed frequently observed in natural populations (Harries *et al*., 1997; Bernet, Wahli, Kung & Segner, 2004; Penáz, Svbodova, Barus Prokes & Drastichova, 2005; Jobling *et al*., 2006; Bernet *et al*., 2008; Bittner *et al*., 2009). Other possible consequences of exposure to hormones or hormone-active substances may include reductions in gonadal growth, a delayed onset of sexual maturity, inhibition of spermatogenesis, lower egg production, or reduced egg quality (Sumpter & Jobling, 1995; Vos *et al*., 2000).

However, sex ratios in the wild can be skewed for many reasons (Palmer, 2000) and environmentally induced sex reversal is often difficult to prove (Nagler *et al*., 2001; Chowen & Nagler, 2004, 2005; Williamson, Phillips & May, 2008).

The prevalence and significance of environmental sex reversal in the wild is therefore still unclear (Wedekind, 2010). So far, the consequences of environmentally induced sex reversal have only been analyzed in theoretical studies (Kanaiwa & Harada, 2002; Hurley, Matthiessen & Pickering, 2004; Cotton & Wedekind, 2009). These studies suggest that environmentally induced sex reversal can change population growth and population sex ratios in ways that may sometimes be counter-intuitive.

A moderate rate of feminization, that is of an environmentally - induced development of the female phenotype despite male sex chromosomes, could sometimes be beneficial for population growth, especially in the absence of strong viability effects of the sex reversal. However, most possible outcomes of environmental sex reversal are negative with regards to population growth or the persistence of sex chromosomes.

Source of samples

Kakum estuary

The Kakum estuary in Ghana (approximately 5° 6' N, 1° 18' W) is located along the Cape Coast – Takoradi trunk road near Iture, a village in the Cape Coast metropolis, Central Region. The estuary is about 2 km west of the University of Cape Coast and about 3 km east of Elmina. It is formed by two rivers, namely, the Kakum and the Sweet (Sorowie) rivers. Average annual rainfall in the area is about 1,000 mm and the vegetation type is coastal savannah with grassland and few trees (Government of Ghana Official Portal – Central Region, n. d.). The average annual temperature, salinity, DO and pH were 27.9

^{o}C, 0.7 ‰, 3.5 mg/l and 6.9 respectively (Okyere, Blay & Aggrey- Fynn, 2011). The inhabitants depend on the estuary as their major source of fisheries and water for domestic activities. The dominant cichlid species in the estuary are *Hemichromis fasciatus, Sarotherodon melanotheron* and *Tilapia zillii.*

Benya lagoon

Benya lagoon (approximately 5° 7' W, 1° 17' N) is an open lagoon located in the Central region of Ghana. It has a surface area of about 192 ha and is fringed by red and white mangroves. As an open lagoon in contact with the sea throughout the year, it is mostly under tidal influence. Annual temperature of the lagoon ranged from 27 to 31.5 ^{o}C with an average of 29.8 ^{o}C. The DO content of the lagoon for the year ranged between 1.29 and 5.47 mg/l with an average value of 3.23 mg/l whilst pH ranged from 7.17 to 8.05 and average of 7.45. The annual salinity was between 30 to 40 ‰ but values greater than 35 ‰ were recorded from February to April, August and December (Obodai, Yankson & Blay, 1994). According to Biney (1982) physico – chemical parameters of the lagoon in the rainy season were 7.28 g/l, 7.54 and 26.8 ‰ for DO, pH and salinity respectively, whilst DO, pH and salinity for the dry season were 6.30 g/l, 7.33 and 27.7 ‰ respectively. The main cichlid species in the lagoon is the *Sarotherodon melanotheron.*

Fosu lagoon

The Fosu lagoon (5° 7' N, 1° 06' W) described as a closed lagoon, is located at Bakano in the Cape Coast municipality. It is a shallow brackish body of water separated from the Gulf of Guinea by a sand bar, which is usually removed

by heavy rainfall or manually as part of annual rituals. Annual temperature ranged between and 27 and 34 °C and salinity ranged from 27 to 70 psu but hyperhaline condition results from evaporation during the dry season (Yankson, 1982). Biney (1982) measured some physico – chemical parameters of the lagoon in the rainy season and recorded values, 7.38 g/l, 8.15 and 9.3 ‰ for DO, pH and salinity respectively and for the dry season, 7.50 g/l, 7.12 and 0.5‰ for DO, pH and salinity respectively.

The main economic activity that goes on in the lagoon is fishing and the commonest and most abundant fish in the lagoon is the black chin tilapia (Blay & Asabere-Ameyaw, 1993). Biney (1982) reported that Fosu lagoon was classified as moderately polluted by the end of the 1980s. Over the years, the lagoon has faced severe environmental crisis brought on by pollution and this deteriorating condition has led to a decline in the productivity of fish and other resources within the lagoon (Ghana Environmental Protection Agency, EPA, 2004).

Importance of fish

Fish is known to be a cheap source of nutritious food (Onumah & Aquah, 2010) and it is consumed by the majority of people in Ghana, from the rural poor to the urban rich. Fish and fishery products constitute an extremely important source of protein and nutritional security for many people all over the world (FAO, 2012). Fish are low in collagen content, have a nutrient profile superior to all other sources of animal protein and its digestibility is high - as almost every nutrient is absorbed (WHO/FAO, 1999). Consumption preference for fish in Ghana is 82 % compared to livestock and poultry products (Nketsia – Tabiri,

1993). It is estimated that 60 % of the total animal protein requirement in the Ghanaian diet comes from fish (Sarpong, Quaatey & Harvey, 2005).

The average per capita fish consumption in Ghana is 23.7 kg (FAO, 2012) and 25 kg (Awity, 2005) per annum; higher than the world's average of 18.6 kg. It has been established that fish and fishery products are now the country's most important non – traditional exports, accounting for over 50 % of earnings from non – traditional exports (Bennett, 2002; Sarpong et al., 2005).

Wim, Issabelle, Stefan and John (2007) reported that the global consumption of fish and fish products has generally increased during recent decades. However, there has been a decline in capture fisheries (Braimah, 2001; Anon, 2003 in FAO, 2004). Fish culture has been identified worldwide as an important option for increasing fish production (Bardach, Ryther & Mclarney, 1972). However, the impact of fish culture on the total fish output of Ghana is negligible considering the annual fish demand of 968,000 metric tonnes, as against the total national fish production from both capture fisheries and aquaculture as 482,000 metric tonnes. (MoFAD, 2012).

In an effort to increase fish production to meet national demand, the government of Ghana has been encouraging potential investors to go into commercial aquaculture in view of the favourable culturing climate that exist for the industry in Ghana. Tilapia farming is expanding world-wide in both developed and developing countries because tilapia can be cultured under very basic conditions and so it is ideal for rural subsistence farming (Varadaraj & Pandian, 1987). In reaction, many individuals, groups and corporate bodies are currently

investing in tilapia culture in the country but the species being largely cultured is *Oreochromis niloticus* which is known to perform better in freshwater. Legendre and Ecoutin (1989) reported that *O. niloticus* is not suited for intensive aquaculture in Ivory Coast brackish waters. Doudet (1988) affirmed this by stating that the culture of *O. niloticus* is restricted to totally desalinated lagoon area in Ivory Coast.

However, the aquaculture potential of *S. melanotheron* had been studied in Ebrie lagoon, Ivory Coast (Gilles, Amon - Kothias & Agnese, 1998; Legendre & Ecoutin, 1989) but the results obtained were not impressive as growth was between 0.32 and 0.50g per day and therefore do not favour commercial culture. In addition to that, reports by several rearing trials have shown that the species may not be suitable for intensive pond culture but does well in acadjas (Welcomme, 1972; Legendre, 1992 cited in Ekau & Blay, 2000).

Ghana is reported to have along its 550 km coastline over 100 lagoons and estuaries (Yankson & Obodai, 1999) most of which have the potential for brackish water fish culture (Pillay, 1962; Pauly, 1976). A recent survey on the proportion of *S. melanotheron* in Ghana's subsistence fishing showed that 60 - 80 % of all fish caught in the lagoons were tilapias and among the tilapias, *S. melanotheron* constituted between 85 and 98 % of catch in various lagoons (Abban *et al*., 2000). Although the commercial culture of this fish in Ghana is not popular, recent trials on the fish by the Water Research Institute of the Council for Scientific and industrial Research (CSIR), Ghana, have yielded positive results as to its potential as a cultured food fish (Entsua-Mensah & Dankwa, 1997).

Pillay (1965) reported that experiment to assess the aquaculture potential of *Tilapia melanotheron* was abandoned for lack of market for this relatively small fish but contrary to this assertion, there exist a huge market for this fish species in Ghana (Awumi, pers. Comm.) hence the need to study its culture potential with regards to breeding and subsequent sex reversal practices as is done in the case of *O. niloticus* which is widely cultured in Ghana.

In a bid to culture tilapia, one major drawback is their tendency to overpopulate ponds. Tilapias are known to be highly fecund and able to reproduce at a relatively small size (Lowe - McConnell, 1955). One way to control population is to use the male hormone, 17 α-methyltestosterone (MT) which, when administered to the fry at the right time before sex differentiation leads to the production of all- male population. According to Macintosh (2000) artificial sex reversal is the process by which the physical sex direction (male or female) can be manipulated through the feeding of synthetic sex hormones (e.g., methyltestosterone) prior to and during the "sexless stage" of the fry and added that, sex reversal of tilapia is now applied worldwide. Hickling (1960) and Swingle (1961) observed that the excessive reproduction of tilapia minimizes the yield of harvestable sized fish.

According to Phelps, Salazar, Abe & Argue (1995) numerous methods are used to distort the sex ratios and increase the percentage of males in a population and the most common method of generating mostly male populations is through the feeding of steroid hormones to sexually undifferentiated fry. Anabolic steroids are potentially useful compounds in aquaculture due to their ability to increase

weight gains and muscle deposition of treated fish (Khalil *et al*., 2011). Ostrowski and Garling (1988) reported that steroid treatment may produce fish of more robust size containing more muscle per unit length than untreated fish.

Hickling (1968) reported that tilapia is known to attain sexual maturity at an early age and breed repeatedly at short intervals which results in stunted growth since energy is expended for reproduction rather than for growth. Within a limited environment, uncontrolled multiplication of the fish not only reduces the faunal diversity of the system but also produces dwarf fish population of poor market value (Hepher & Pruginin, 1982; Coleman, 2001). The small individuals that are produced in a mixed population are unsuitable for marketing and interest in trying to prevent stunting has concentrated on the search for ways to prevent reproduction (Meschkat, 1968).

In tilapia, sex steroid hormones play a promising role in directing the gonadal differentiation process (Piferrer, 2001). This hormone promotes both muscle growth and development of male sexual characters. However, it is important to identify the optimal level of MT as well as duration and timing of treatment for consistent, successful sex reversal (Dunhan, 1990). Tilapia is known to have sexual growth dimorphism in which males grow faster and have more standard size than females (Mair & Little, 1991).

High rate of masculinization in tilapia can be influenced by some important factors like hormone concentration, treatment duration, age and size of fry, availability of natural feed, stocking density and feeding frequency (Mair & Little, 1991). Hanson, Evmanm, Shelton and Dunham (1984) reported that 10 –

60 mg MT treatment showed the best growth than control. On the other hand, Dan and Little (2000) who compared the culture performance of different species of strains of *O. niloticus* found that, MT treatment resulted in final size of fish 10.7 % larger than the mixed sex fish. Jae -Yoon *et al.* (1988) obtained 97 % *O. niloticus* males at dose rate of 10 mg MT/ kg of diet. Vera–Cruz and Mair (1994) in their study obtained 95 to 98 % males with 40 mg MT/kg of diet and 99 % with 60 mg MT/kg of diet fed at 20 % body weight for 25 days. Abucay and Mair (2000) produced 100 % male sex population of Nile tilapia at 40 mg MT/kg of feed for each 15, 20 and 25 days of treatment. Romerio, Frencrich - Verani, De - compmus and Pasilva (2000) obtained 98 % male population at dose rate of 60 mg MT/kg of feed.

Smith and Phelps (2001), reported 99 -100 % male proportion of Nile tilapia when given MT at 60 mg/kg of feed. Bhandari, Nakamura, Kobayashi and Nagahama (2006) achieved 100 % masculinization of *O. niloticus* at the dose rate of 50 mg MT/kg of feed. Okoko (1996) obtained 99.3 % males at 30 mg/kg MT feed, while 97 and 71.9 % males at the dose rates of 60 and 120 mg/kg MT feed respectively. Marjani, Jamili, Mostafavi, Ramin and Mashinchian (2009) found that the dose rate of 75 mg MT/kg feed gave the maximum gain in body weight, which was 1.2 times greater than the control. Also, Rizkalla, Haleem, Abdel – Halim and Youssef (2004) found that whole body samples of normal fish and those treated for 28 days with 17 α - methyltestosterone contained detectable amounts of testosterone only in the first five months after the termination of feeding. Additionally, Rizkalla *et al*. (2004) found that, muscle samples taken

from the monosex fish at marketable size, did not differ from the untreated controls and testosterone concentrations were below the detectable level. On their part, Curtis, Diren, Hurley and Tubb (1991) and Ahmad *et al*. (2002) found that plasma testosterone concentration is rapidly metabolized and excreted.

Male tilapias naturally grow faster than the females (Hanson *et al*., 1983; Toguyeni *et al*., 1997) making them the better choice for commercial tilapia farming. Similarly, Bardach *et al*. (1972) contended that male tilapias grow two or three times faster than females. Hickling (1960) indicated that the growth rate of female tilapias is greatly reduced upon attaining sexual maturity. It therefore reasons that the culture of all - male tilapias will eliminate reproduction so that more tissues are deposited to increase yield. In addition, Soderberg (1997) stated that fish typically have sexual differences in growth, like the male tilapias grow to larger sizes than the females. In addition, methyltestosterone has been reported to enhance growth of various fish species such as the Nile tilapia, *Oreochromis niloticus* (Tayamen & Shelton, 1978). Oral administration of the synthetic androgen, 17 α-Methyltestosterone at 60 mg/kg feed fed to newly hatched tilapia fry 9-11mm total length for a period of 28 days resulted in populations comprising 97 to 100 % phenotype males (Popma & Green, 1990).

Okoko (1996) obtained 71.9 % males at the dose rate of 120 mg kg^{-1} MT of feed. According to Mair and Little (1991) and Macintosh and Little (1995) sexually active tilapia channels more energy into reproduction rather than somatic growth and also added that faster growth of monosex tilapia has been related to

the lack of energy expenditure in egg production and mouth brooding by females and lower energy expenditure on courtship by male.

Among the benefits derived from the use of male hormone, 17α-methyltestosterone to obtain monosex males include high stocking density which increases yield if 100 % sex reversal is achieved (Guerrero, 1975). All - male populations have also greater growth potential as well as attaining uniform sizes (Kirk, 1972). At the time of hatching, tilapia fry are sexually undeveloped, therefore, during the early period of gonadal differentiation, changes in sex hormone level can affect the final sex independently of the genetic sex (Andersen, Holbech, Gessbo, Norrgen & Peterson, 2003).

Yashouv and Eckstein (1965) indicated that tilapia fry treated with male hormone during the first month of life had lower mortality rates than fish treated with female hormone and added that maintaining tilapia fry in water with male hormone increases growth. Guerrero (1975) observed that the mean weights of fish treated with 17 α- methyltestosterone, at harvest, were generally higher than the untreated ones. Fagerlund and McBride (1973) reported a significant increase in weight and length of juvenile coho salmon fed 17 α- methyltestosterone.

Yamamoto (1958) on the other hand, concluded that 15 µg/g diet of methyltestosterone was adequate for inducing 50 % sex reversal of genetic females and that 25 µg/g diet was adequate for almost 100 % sex reversal in the *Oryzias latipes* (medaka). Similarly, Yamamoto and Kajishima (1968) found 25 µg/g diet of methyltestosterone effective for inducing sex reversal in goldfish. Whereas ethynyltestosterone became more effective with increasing

concentration, methyltestosterone was less effective at 60 than at 30 µg/g diet. Clemens and Inslee (1968) found 40 µg/g and 50 µg/g diet of methyltestosterone less effective than 30 µg/g diet for inducing sex reversal in *T. mossambicus.* Tayamen and Shelton (1978) found that diethylstilbestrol (25 mg/kg) caused an increased growth rate in *O. niloticus*. In contrast, Majumdar and McAndrews (1984) reported a decline in growth of 28 to 42 % in *O. mossambicus* and *O. aureus* after treatment with estradiol and increase of over 20 % in *O. niloticus.*

Ghana is known to have a coastline favourably dotted with numerous lagoons and estuaries which can provide the ideal culture environment for *S. melanotheron* and also for the fact that works reported on this species so far centered mostly on its biology and considering the huge market for this fish, it is imperative to study how amenable the species will be in terms of its breeding and subsequent culture on commercial scale in Ghana.

The present study is therefore aimed at breeding *S. melanotheron* species and subsequently adopting all the necessary nursery practices including sex reversal of the fry to obtain fingerlings that may possibly manifest superior qualities for commercial culture.

Problem statement

Fish is an important source of protein (Aggrey-Fynn, 2001) contributing almost 82 % of total animal protein supply (FAO, 2001) but there has been a decline in capture-fisheries (Braimah, 2001; Anon, 2002 in FAO, 2004) in Ghana in particular and the world as a whole due to rapid growth in human population

that has put a huge pressure on fisheries resources in the country. It is however predicted that aquaculture has the potential to offset the imbalance (FAO, 2000).

The species of tilapia being cultured commercially in Ghana is the *Oreochromis niloticus* which is known to thrive better in freshwater. It is therefore obvious that only the fingerlings of this species are being produced in hatcheries for commercial aquaculture in the country. Considering the popularity of S. *melanotheron* especially along the coastal towns and villages of the country, it is very important to start commercial production of the fingerlings of *S. melanotheron* in order to promote brackish water aquaculture with which the species is associated. This will not only reduce the price of fingerlings for culture but also make S. *melanotheron* seeds readily available for brackish water aquaculture in the country.

Justification for the study

Efforts at increasing fish production in the country through fish culture have so far concentrated on freshwater environments even though the coastline of Ghana has numerous lagoons and estuaries most of which have the potential for brackish water fish culture (Pillay, 1962; Pauly, 1976). It has been established by many researchers including Philippart and Ruwet (1982) that *S. melanotheron* is a brackish water tilapiine species found in the estuaries and lagoons of West Africa. The species has been known to exhibit desirable aquaculture traits (Legendre & Ecoutin, 1989). However, siltation, pollution, fishing pressure and habitat destruction is threatening the survival and genetic quality of the species. This calls for selective breeding of the fish to improve its genetic material, enhance the

performance of the fish under culture condition and also to provide scientific information on the culture characteristic of the species.

Objective

The overall objective of this study was to obtain progeny of *S. melanotheron* that will have higher reproductive capacity and also exhibit desirable aquaculture traits in terms of better growth performances.

The specific objectives were:

i. To assess the fertility and the sex ratio of the progeny of the two brackish water population of *S. melanotheron*

ii. To assess the response of the species to different concentrations of the male hormone, 17 α-methyltestosterone (MT)

iii. To evaluate the growth rates of the fish under different concentrations of the hormone

iv. To assess the fertility and fecundity of the wild population of *S. melanotheron*

CHAPTER TWO

MATERIALS AND METHODS

Preparation of experimental ponds

Experiments on crosses and sex reversal were set up in two separate concrete ponds each measuring $3.7 \times 3.7 \times 0.77$ m and filled with tap water to a depth of 0.58 m. The ponds for the crossing of the black-chinned tilapia populations were allowed to stand for two weeks to allow for breakdown of chlorine used to treat the water and also to ensure the growth of plankton. The pond used for sex reversal trials was filled with water a day before the trial began to ensure absence of plankton in the pond before stocking. Twelve cages, each of dimensions $0.5 \times 0.5 \times 0.6$ m were suspended in the ponds such that the cages floated 0.1 m above the water surface.

Occasionally, the outlet pipes of the ponds were opened to allow some of the pond water to drain out while the inlets were opened for inflow of fresh water to replenish the drained water. Whenever excreta from the fish were spotted on the surface of the water, they were removed with a scoop net, and excess algae which formed algal mats at the bottom of the pond were removed occasionally to maintain good pond water quality.

Measurement of physico - chemical parameters in breeding pond

Temperature, dissolved oxygen and pH in the breeding pond were measured three times daily at 08:00 GMT, 12:00 GMT and 16:00 GMT from October, 2012 to September, 2013 with a water quality checker (WQC Model

22A) and the weekly and monthly means of these physico-chemical parameters were calculated.

Measurement of physico - chemical parameters in sex reversal pond

From July, 2013 to October, 2013, temperature, dissolved oxygen and pH in the pond for the sex reversal trials were measured thrice daily at about 08:00 GMT, 12:00 GMT and 16:00 GMT and the measurements repeated three times in a week. The weekly and monthly means of the physico-chemical parameters were computed.

Breeding of Kakum river estuary and Benya lagoon fish

Brood fish were netted with cast nets by fishermen from Kakum River Estuary and Benya lagoon in September, 2012. Samples from the two populations were sent to the experimental site in plastic buckets and released into two separate cages which served as conditioning facility and the fish were acclimatized for 7 days. The breeding experiment began in October, 2012 after the total length (TL) and body weight (BW) of the fish had been taken. A thirty – cm ruler, graduated 0.1cm was used to measure the (TL) to the nearest 0.1 cm and an electronic balance [Scout Pro (SPU) 402] was used to measure the BW of fish to the nearest 0.01g.

Before the measurements, the brood fish were immersed in Tricaine Methanesulfonate (MS 222) solution to anaesthetize them to reduce the stress associated with handling of fish (Harms & Bakal, 1995; Ross 2001; Stetter, 2001). The TL and BW of the females from Kakum river estuary were 10.0 ± 0.03 cm and 18.17 ± 0.84 g respectively while the TL of males from the same

population was 11.37 ± 0.20 cm and the BW was 17.79 ± 0.29 g. The TL of males from the lagoon was 11.59 ± 0.47 cm and their BW was 18.82 ± 0.37 g. These measurements were taken to determine if the female *S. melanotheron* of approximately the same size have the same absolute fecundity. Pairing of a male and a female brood fish from Kakum and Benya lagoon was done and each pairing was triplicated.

The stocked cages were examined after ten days for any breeding activity, and whenever fry were spotted, the parents were removed to avoid predation. Observation on breeding activity of the estuary stock ended in December, 2012 whilst that of the lagoon continued up to February, 2013. Fry produced were counted and feed administered to the fry thrice a day at 08:00 GMT, 12:00 GMT and 16:00 GMT with prepared feed at 20 % body weight for two weeks, and thereafter fry were fed 10 % body weight till the end of the breeding period.

Crossing of Kakum River Estuary and Benya lagoon offspring

Sexually mature F_1 female offspring measuring 7.00 ± 0.01 cm TL and weighing 10.27 ± 0.02 g BW from the parent stocks of Kakum estuary and Benya lagoon, and sexually mature males of TL 9.53 ± 0.15 cm and mean BW of 15.62 ± 0.52 g were crossed on 15th June, 2013 when the offspring from the lagoon were five months old and those from estuary were six months old. Three replicate pairings of Estuary male and Estuary female and lagoon male and lagoon female were done. Reciprocal crosses, each of which was in triplicate were done for

Estuary male and lagoon female and lagoon female and Estuary male. Twelve pairings were done in all.

The fish were fed twice daily with a local feed at 10 % body weight at 09:00 GMT and 16:00 GMT. Feeding rings made from Styrofoam material were used to confine the feed in the cages. Unfed feed particles in the cages were removed with a dip net to avoid deterioration of the water. The experiment lasted 145 days and was meant to determine the reproductive capacity of the two populations in captivity.

Determination of reproductive capacity of *Sarotherodon melanotheron* from Fosu lagoon

Mature female *S. melanotheron* from Fosu lagoon (Cape Coast) were obtained from fishermen in August and September, 2013 and transported in plastic buckets to the laboratory. Females with ripe ovaries were sorted after an incision was made on the abdomen of each. The total length (TL) and standard length (SL) of the ripe fish were measured using a 30 cm ruler, graduated in 0.1cm and body weight (BW) of the fish determined with an electronic balance. Ripe ovaries were then preserved in 10 % formalin for five days in order to harden the ova so that they could easily be separated for counting (Bagenal & Braum,1978; Hunter 1983; Cailliet, Love & Ebeling, 1986). Prior to counting the eggs, the formalin was decanted and the ovary rinsed in water and weighed. The clumps of eggs were gently teased with a dissecting needle and all eggs counted (Bagenal & Braum,1978) to determine the fecundity of the fish. The whole count method was used due to the relatively small number of eggs in the ovary of the

samples. The relationship between fecundity and total length, fecundity and body weight and fecundity and ovary weight were determined using a regression analyses.

Plate 1: Matured ova of *Sarotherodon melanotheron* from Fosu lagoon

Plate 2: Measuring body length of *S. melanotheron*

Brood size of Fosu lagoon *Sarotherodon melanotheron*

Brooding males from the Fosu lagoon were sampled based on the extent of bulging of the buccal pouch from the catches of fishermen and the brood

35

(fertilized eggs or fry) in the mouth of each fish was gently squeezed out of the mouth of the parent into a container for detailed examination in the laboratory. The TL, SL and BW of brooding fish were measured. To estimate the fertility of the brooders, the broods (eggs or fry) were spread in a petri dish and counted. Regression analyses were conducted to establish the relationship between brood size and total length of the fish as well as the relationship between brood size and body weight of the brooding fish.

Sex reversal of *Sarotherodon melanotheron* from Fosu lagoon

Preparation of hormonal feed

The various concentrations of the hormonal feed for the study were prepared as described by Killian and Kohler (1991). Thirty milligrams, 60 mg and 120 mg of the hormone, 17 α – methyltestosterone (MT) were weighed with an electronic balance and 250 ml of 95 % ethyl alcohol was used to dissolve the hormone. Each of the hormone solutions was mixed with 1 kg of feed until a homogenous sample was obtained. The feed used as the control (0 mg MT/kg feed) was mixed with only alcohol without the hormone. The treated feeds were spread on trays to dry at room temperature for 4 hours. The dried hormonal feeds, designated 0 mg MT /kg feed (control), 30 mg MT /kg feed, 60 mg MT /kg feed and 120 mg MT /kg feed were bagged and stored in a refrigerator.

Collection of fry for sex reversal trials

Swim up fry of *S. melanotheron* for the study were collected by the researcher from Fosu lagoon (Cape Coast) with a scoop net and transported in plastic buckets to the experimental site where the fry were released into a cage for

conditioning. The fry were acclimated for five days after which they were sorted to eliminate weak fish and individuals longer than 11mm. A total of fifty fry each measuring between 9 and 11 mm were counted and stocked in each of the 12 cages floated in the pond.

Fry were fed at 20 % BW in four portions and administered at 08:00 GMT, 12:00 GMT, 15:00 GMT and 17:00 GMT for the first fourteen days. The feeding rate was reduced to 10 % body weight from day 15 to day 30. Feeding of juveniles was continued with locally prepared feed until day 86 when the trial ended. Sampling of the fish was done fortnightly to measure growth of 20 fish from each cage or 60 fish from every treatment and at each sampling, the fish were anaesthetized using Methanesulphonate (MS 222) to reduce stress.

Plate 3: Cages floated by tying on bamboo poles in sex reversal pond

Plate 4: Fry used for stocking in sex reversal trial

Evaluation of growth of sex – reversed fry

The parameters chosen for the evaluation of growth of the sex – reversed fish were according to De Silva and Anderson (1995).

Absolute growth (AG) was estimated by the relation $AG = W_1 – W_0$, where $W_1 =$ final wet weight and $W_0 =$ initial wet weight.

Specific growth rate [(SGR) % /day] was calculated using the formula, $SGR = (\ln W_1 - \ln W_0) \times 100/ t$, where, $W_1 =$ final wet weight, $W_0 =$ initial wet weight, t = time interval in days.

Condition factor which refers to the well-being of fish was calculated by using the relation $K = 100 \times BW / SL^3$, (Tesch, 1971; Weatherley, 1972), where $K =$ condition factor, BW = body weight of fish (g), SL = standard length of fish (cm).

Plate 5: Anaesthetized *S. melanotheron* being recovered in fresh water

CHAPTER THREE

RESULTS

Physico - chemical factors in the pond used for crosses

Temperature

Mean monthly temperature measured in the pond for the twelve – month study period ranged between 27.33 ± 0.40 ° C and 31.13 ± 0.65 ° C (Figure 1). The minimum temperature was recorded in August, 2013 whilst the maximum was recorded in April, 2013. Mean temperature recorded in October, 2012 was 28.20 ± 0.50 ° C and temperature of the pond water was almost constant until April, 2013 when it rose sharply to a peak of 31.13 ± 0.65 ° C and decreased thereafter to 27.33 ± 0.40 ° C in August, 2013.

pH

The hydrogen ion concentration of a substance (pH) ranged between 5.52 ± 0.19 and 7.79 ± 0.05 during the study period (Figure 1). The lowest pH value which was slightly acidic and the highest pH value which was almost neutral (7.0 on the pH scale) were recorded in January, 2012 and September, 2013 respectively. The pH value which was 7.64 ± 0.18 in October, 2012 declined to the minimum value of 5.52 ± 0.19 in January, 2013 after which it rose to 6.83 ± 0.58 in April, 2013. A steady increase was observed from May, 2013 until the end of the study in September, 2013 when a value of 7.79 ± 0.05 was recorded.

Dissolved Oxygen

The mean dissolved oxygen values recorded during the study period ranged from 1.57 ± 0.57 mg/l to 3.70 ± 0.26 mg/l (Figure 1). The minimum DO

value was recorded in May, 2013 and the maximum value recorded in September, 2013. The DO value registered in October, 2012 was 2.60 ± 0.30 mg/l and was almost constant until May, 2013 when DO values decreased sharply to 1.63 ± 0.14 mg/l in June, 2013 before rising to 3.70 ± 0.26 mg/l in September, 2013.
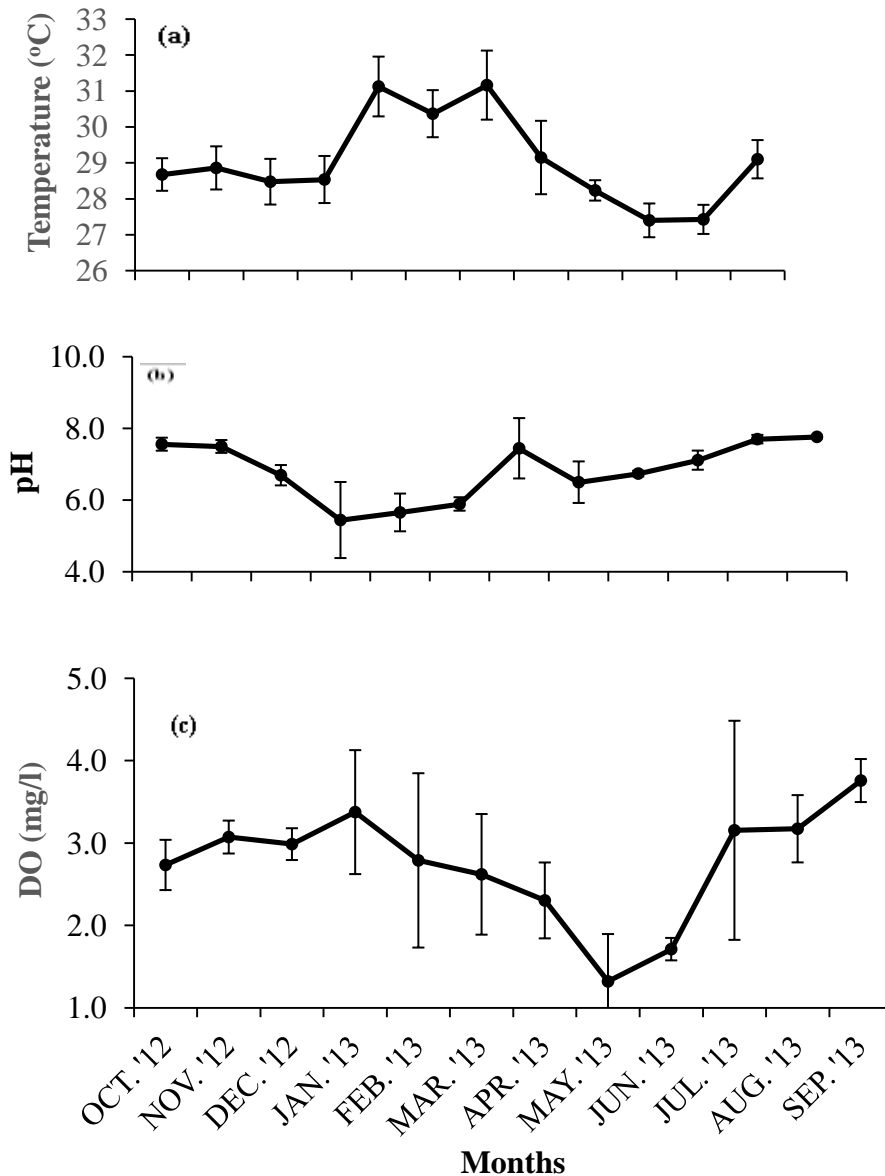
Figure 1: Monthly variations in physico-chemical conditions of pond used for breeding *Sarotherodon melanotheron* (Vertical bars represent standard deviation)

Physico - chemical factors in the pond used for sex reversal trial

Temperature

The minimum temperature value of 27.32 ± 0.12 ° C was measured in August, 2013 while the maximum of 28.97 ± 0.20 ° C was recorded in October, 2013 (Table 1). Generally, temperature values recorded in the pond were similar for the four – month trial period.

pH

The maximum and the minimum pH readings were 7.16 ± 0.08 and 7.76 ± 0.02 respectively and these were recorded in July, 2013 and October, 2013 (Table 1). The general trend was that pH values were almost neutral throughout the trial.

Dissolved Oxygen

Dissolved oxygen values varied from 3.12 ± 0.17 mg/l in September, 2013 to 4.12 ± 0.08 mg/l in October, 2013 (Table 1). The general pattern was that DO values were similar from July, 2013 to September, 2013 but increased slightly in October, 2013

Table 1: Monthly mean pond physico - chemical conditions of sex reversal pond

Month, 2013	Mean ± Standard Error		
	Temperature (^{o}C)	pH	DO (mg/l)
July	27.55 ± 0.10	7.16 ± 0.08	3.70 ± 0.29
August	27.32 ± 0.12	7.64 ± 0.03	3.16 ± 0.16
September	27.50 ± 0.11	7.61 ± 0.04	3.12 ± 0.17
October	28.79 ± 0.20	7.76 ± 0.02	4.12 ± 0.08

Fingerlings from Kakum river estuary and Benya lagoon brood fish

A total of 352 fry were produced from the three pairings for the brood stocks from Kakum estuary with the mean number of offspring as 117 ± 30.00 (Table 2). Total number of females produced was greater than that of males giving a sex ratio of 1: 1.3 (males: females) which did not differ significantly ($\chi^2 = 2.75$, p > 0.05) from the expected 1: 1. Only one replicate from Benya produced 34 fish comprising 15 males and 19 females in the ratio of 1: 1.3 (M: F) out of the three pairings done for the lagoon parents. Similarly, the ratio of males was not significantly different ($\chi^2 = 0.12$, p > 0.05) from that of females and did not depart from the hypothetical ratio of 1: 1.

Table 2: Number and sex ratio of fingerlings produced by Kakum river estuary and Benya lagoon brood fish in ponds

| | | Number | | Sex Ratio | |
| | Brood | Male | Female | (M : F) | χ^2 |
Broodstock	number ($\pm$ se)				
Kakum estuary	117 ± 30.4	154	198	1 : 1.3	2.75^{NS}
Benya lagoon	34*	15	19	1 : 1.3	0.12^{NS}

*indicates value from one replicate only. NS = not significant at 5 % significant level

Proportion of males of *Sarotherodon melanotheron* produced by different concentrations of 17 α- methyltestosterone after 30 – day treatment period

None of the hormone concentrations yielded 100 % male population of S. *melanotheron* (Figure 2). The maximum male population of 92.70 % was obtained in fish fed 120 mg MT/kg feed while the minimum male proportion of 39.3 % was recorded for fish fed 0 mg MT/kg feed (control). The hormone

concentrations of 30 mg MT/kg feed and 60 mg MT/kg feed resulted in 84 % and 89 % male respectively. Statistical analysis showed a significant difference (F = 266.22, $p < 0.05$) between the MT treated groups and the control. Proportion of male in the group of fish fed 30 mg MT/kg feed (84 %) was significantly lower ($p < 0.05$) than percentage males produced in the group of fish fed 120 mg MT/kg feed. There was however, no significant difference ($p > 0.05$) between proportion of male produced in fish fed 60 mg MT/kg feed and those fed 120 mg MT/kg feed.
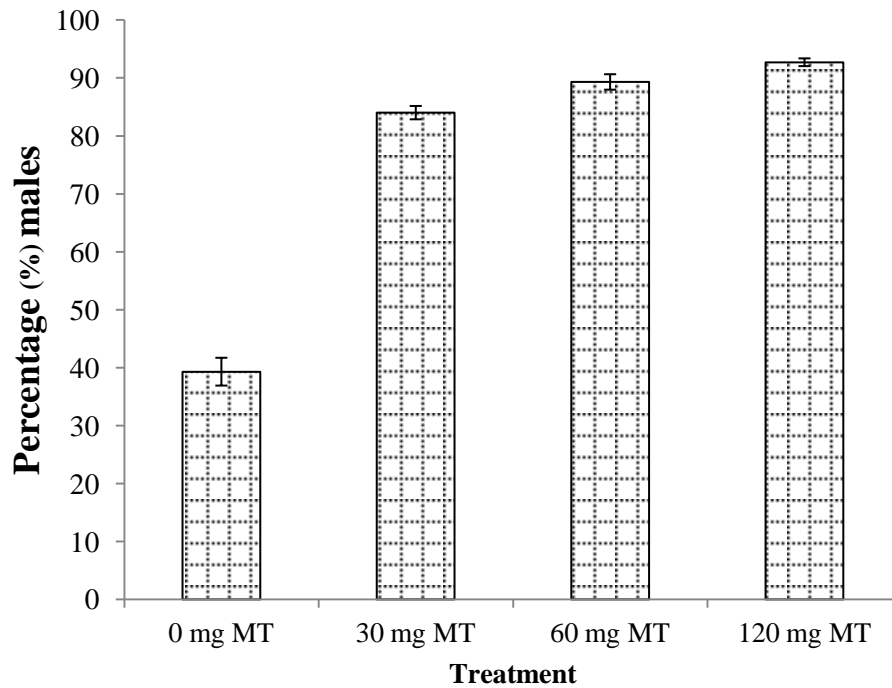
Figure 2: Percentage males of *Sarotherodon melanotheron* produced by different concentrations of 17 α- methyltestosterone (MT) (Vertical bars represent males standard error)

Growth of sex reversed fry

Changes in the size of hormone – treated fry

Fish fed 120 mg MT/kg feed grew from 0.02 g to 0.57 ± 0.38 g while fry fed 30 mg MT/kg feed grew from 0.02 g to 0.41 ± 0.17 g by the end of the 30-day hormone treatment period (Figure 3). Growth from 0.02 g to 0.51 ± 0.26 g was observed by fish fed 60 mg MT/kg feed while the fry fed 0 mg MT/kg feed grew from the initial 0.02 g to 0.43 ± 0.49 g at the end of the treatment period. By the end of the twelve - week trial period, the heaviest weight of 3.14 ± 2.05 g was attained by the fish fed 120 mg MT/kg feed whilst the least body weight of 2.82 ± 1.78 g was attained by fish fed 0 mg MT/kg feed. Weights of 3.07 ± 2.11 g and 3.01 ± 1.43 g were attained by fish fed 30 mg MT/kg feed and 60 mg MT/kg feed respectively. The ANOVA test indicated no significant difference (F = 0.904, p > 0.05) among the various treatments.
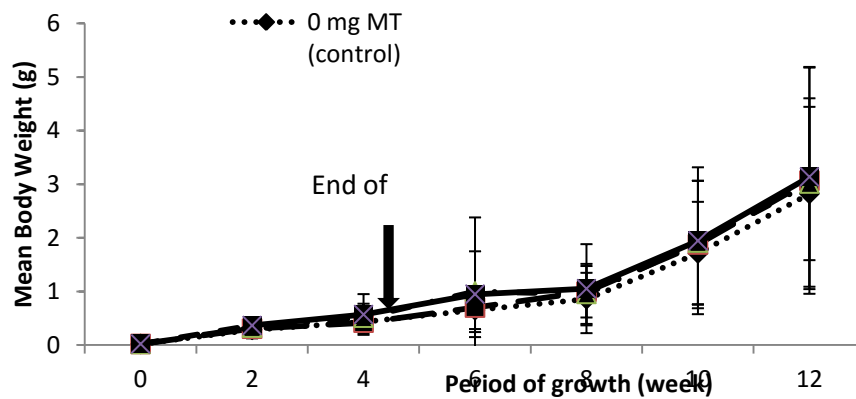
Figure 3: Changes in body weight of *Sarotherodon melanotheron* fry fed different concentrations of 17 α- methyltestosterone during sex reversal treatment (Vertical bars represent mean body weight standard error)

In terms of length, fry fed 120 mg MT/kg feed increased from 1.1 cm to 3.04 ± 0.53 cm while the fish fed 0 mg MT/kg feed grew from 1.1 cm to 2.65 ± 0.39 cm by the end of the hormone treatment period (Figure 4). The fish group that were fed 30 mg MT/kg feed and 60 mg MT/kg feed grew from 1.1 cm to 2.73 ± 0.41cm and 2.94 ± 0.44 cm respectively. At the end of the trial, fish fed 120 mg MT grew to 5.40 ± 1.27 cm while fish group fed 0 mg MT/kg feed attained the length of 5.03 ± 1.15 cm. Equal lengths of 5.41 ± 1.27cm and 5.41 ± 0.99 cm were attained by fish fed 30 mg MT/kg feed and 60 mg MT/kg feed respectively. ANOVA test indicated no significant difference (F = 0.02, p > 0.05) in length of fish among the treatments at the end of the trial.
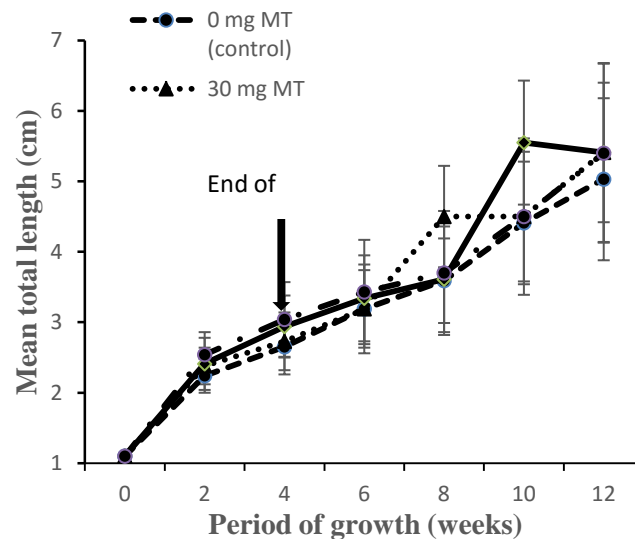

Figure 4: Changes in body length of *Sarotherodon melanotheron* fry fed different concentrations of 17 α- methyltestosterone (Vertical bars represent mean length standard error)

Absolute and Specific Growth Rates of hormone- treated fry

Absolute Growth Rate

Absolute growth rate of hormone - treated fry ranged from 0.34 ± 0.10 g /day to 0.41 ± 0.11g /day (Table 3). The highest absolute growth of 0.41 ± 0.11 g/day was recorded in the fish fed 120 mg MT/kg feed while the fry fed 0 mg MT/kg feed were observed to have the least absolute growth of 0.34 ± 0.10 g /day. Absolute growth values of 0.35 ± 0.09 g/day and 0.37 ± 0.14 g/day were obtained from fish fed 30 mg MT/kg feed and 60 mg MT/kg feed respectively. ANOVA test indicated no significant difference (F = 0.063, p > 0.05) in absolute growth of fry fed different concentrations of 17 α- methyltestosterone.

Specific Growth Rate (SGR)

Fish fed 0 mg MT/kg feed showed the highest specific growth rate (9.70 ± 4.31 % BW per day) while the lowest specific growth (9.01 ± 4.16 % BW per day) was recorded in fish fed 60 mg MT/kg feed (Table 3). Specific growth rates of 9.45 ± 3.81 % BW per day and 9.29 ± 4.13 % BW per day were recorded in 30 mg MT/kg feed and 120 mg MT/kg feed respectively. ANOVA test indicated no significant difference (F = 0.005, p > 0.05) in the specific growth rate of fry under the different concentrations of 17 α- methyltestosterone.

Table 3: Absolute growth rates [(AGR (g day^{-1})] and specific growth rate [(SGR) % day^{-1}] of fry of *Sarotherodon melanotheron* treated with 17α - methyltestosterone for 30 days

Treatment	AGR (g day^{-1} ± SD)	SGR (% day^{-1} ± SD)
0 mg MT/kg	0.34 ± 0.10	9.70 ± 4.31
30 mg MT/kg	0.35 ± 0.09	9.45 ± 3.81
60 mg MT/kg	0.37 ± 0.14	9.01 ± 4.16
120 mg MT/kg	0.41 ± 0.11	9.29 ± 4.13

Fingerlings fed 120 mg MT/kg feed grew better and had the highest absolute growth of 1.57 ± 0.04 g/d while the fish fed 0 mg MT/kg feed (control) were observed to have the lowest absolute growth of 1.43 ± 0.10 g/d at the end of the twelfth week (Table 4). Absolute growth values of 1.54 ± 0.09 g/d and 1.51 ± 0.25 g/d were obtained for fish fed 30 mg MT/kg feed and 60 mg MT/kg feed respectively. Differences in absolute growth of fish under the various treatments was not significant (F = 0.206, p > 0.05).

Specific growth rate of hormone – treated fingerlings ranged between 7.95 ± 2.59 % per day and 10.79 ± 6.92 % per day after 84 days (Table 4). The fish fed 60 mg MT/kg feed had a specific growth rate of 10.79 ± 6.92 % per day whilst 7.95 ± 2.59 % per day was recorded in fish fed 30 mg MT/kg feed. Specific growth rate of 8.71 ± 3.94 % per day and 8.36 ± 3.46 % per day were recorded in fingerlings fed 120 mg MT/kg feed and 0 mg MT/kg feed respectively. The ANOVA test conducted indicated that specific growth rate of fingerlings raised under different treatments was not significantly different (F = 0.078, p > 0.05).

Table 4: Absolute growth rate (g day^{-1}) and specific growth rate (% day^{-1}) of hormone - treated fingerlings of *Sarotherodon melanotheron* from day 31 to day 84

Treatment	AGR (g day^{-1} ± SD)	SGR (% day^{-1} ± SD)
0 mg MT/kg	1.43 ± 0.10	8.36 ± 3.46
30 mg MT/kg	1.54 ± 0.09	7.95 ± 2.59
60 mg MT/kg	1.51 ± 0.25	10.79 ± 6.92
120 mg MT/kg	1.57 ± 0.04	8.71 ± 3.94

Condition factor of hormone - treated fish

Fish were fed different concentrations of hormone – treated feeds for 30 days after which the condition of the fish was assessed (Table 5). The fish fed control feed attained the highest condition factor (2.10 ± 0.34) at the end of the hormonal feed administration while fish fed 120 mg MT/kg feed had the lowest condition factor (2.04 ± 0.44). Condition factor values of 2.07 ± 0.58 and 2.09 ± 0.35 were recorded for fish fed 60 mg MT/kg feed and 30 mg MT/kg feed respectively.

The results show that condition factor of the fish during the treatment period was slightly higher for fish fed 30 mg MT/kg feed, 120 mg MT/kg feed and 0 mg MT/kg feed than the period after the treatment (Table 5). Condition factor for fish fed 60 mg MT/kg feed was however higher at post treatment period than the treatment period. Using paired- sample student *t*- test, the difference between the condition factor values for treatment and post treatment periods for fish fed 0 mg

MT/kg feed was significant ($t = 6.362$, $p < 0.05$). Significant difference ($t = 3.593$, $p < 0.05$) was observed in the condition factors between treatment and post treatment for fish fed 30 mg MT/kg feed.

Table 5: Comparison of mean condition factor of *Sarotherodon melanotheron* after 30-day hormone treatment and post treatment period

Treatment	Condition	Factor ± (S. D)		
	Treatment	**Post treatment**	**t- statistic**	**p- value**
0 mg MT/kg	2.10 ± 0.34	1.86 ± 0.13	6.362	0.000*
30 mg MT/kg	2.09 ± 0.35	1. 90± 0.53	3.593	0.000*
60 mg MT/kg	2.07 ± 0.58	2.15 ± 2.29	- 0.378	0.706
120 mg MT/kg	2.04 ± 0.44	2.00 ± 1.70	0.229	0.819

*Significant level at $p < 0.05$

Fecundity of Fosu lagoon *Sarotherodon melanotheron*

Relationship between fecundity and body length of S. melanotheron

The relationship between fecundity and total length of *S. melanotheron* was linear and was described by the equation F = 28.58L – 72.97 with coefficient of correlation value 'r' as 0.58 (Figure 5a). Absolute fecundity was determined for 204 *S. melanotheron* specimens and fecundity ranged from 55 ova for fish measuring 5.10 cm TL to 351 ova for fish measuring 10.9 cm TL with the mean fecundity as 128.78 ± 2.98 ova.

Relationship between fecundity and body weight of S. melanotheron

A positive linear relationship was observed between fecundity of *S. melanotheron* and body weight which was expressed by the relation F = 5.80 BW + 57.39 (Figure 5b). The coefficient of correlation value 'r' was 0.61. Fecundity of fish ranged from 55 ova to 351 ova for fish weighing 5.29 to 42.32 g respectively.

Relationship between fecundity and ovary weight of S. melanotheron

A linear relationship was observed between fecundity and ovary weight of the fish and was described by the equation, F = 52.62W + 76.74 (Figure 5c). Coefficient of correlation is positive with the value of 'r' as 0.52. Fecundity ranged from 55 ova for fish of gonad weight 0.43 g to 351 ova for fish of gonad weight 1.14 g.
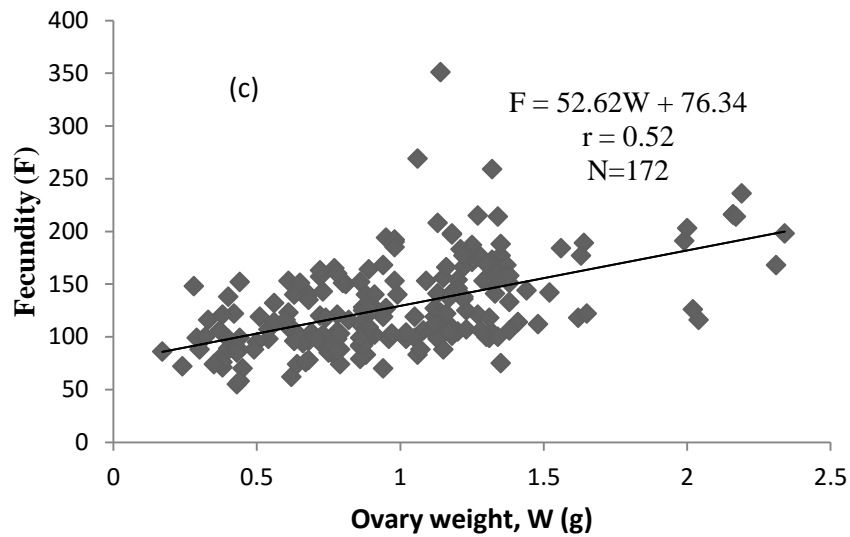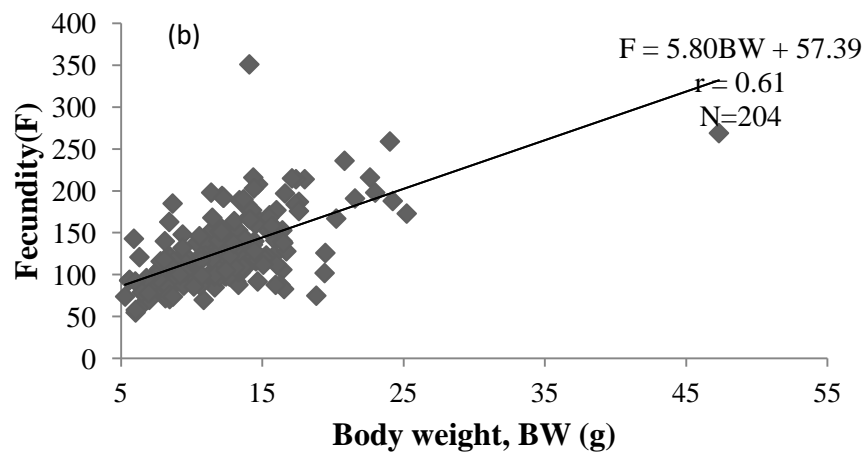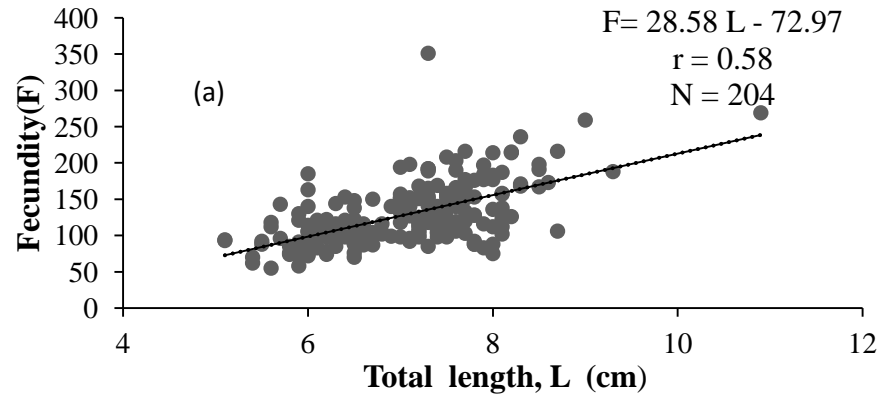
Figure 5: Relationship between fecundity and (a) total length, (b) body weight and (c) ovary weight of *Sarotherodon melanotheron* from Fosu lagoon

Brood size of *Sarotherodon melanotheron* from Fosu lagoon

Relationship between brood size and fish length

A linear relationship expressed by the equation, B = 20.50L – 70.48 was observed between fish length and the number of fry or egg brooded by the male *S. melanotheron.* (Figure 6). A strong positive correlation with 'r' value of 0.85 for 172 specimens was observed. Fish in the range 3 – 3.9 cm TL had 18 fry in the mouth whilst those in the range 8 – 8.9 cm TL had a total of 103 fry in the mouth.

Relationship between brood size and body weight

The relationship between brood size and body weight of *S. melanotheron* was determined for 172 individuals and the result established a strong positive linear relationship expressed by the equation, B = 6.00BW + 6.26 with coefficient of correlation, 'r' of 0.86 (Figure 6). Brooding males that weighed between 2 – 3.9 g BW were observed to have an average of 24 fry in the buccal cavity whilst those that weighed between 14 – 15.9 g BW had an average of 94 fry in the mouth.
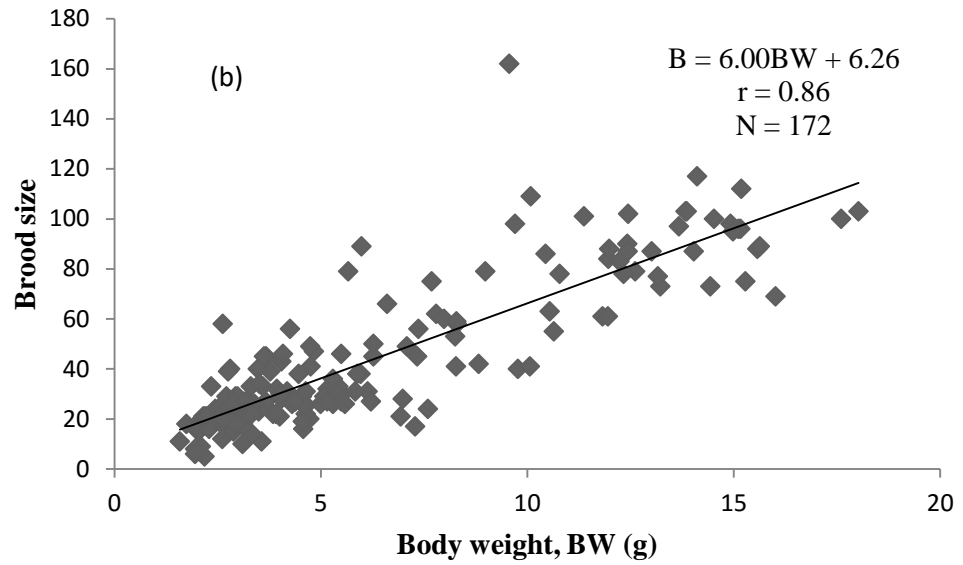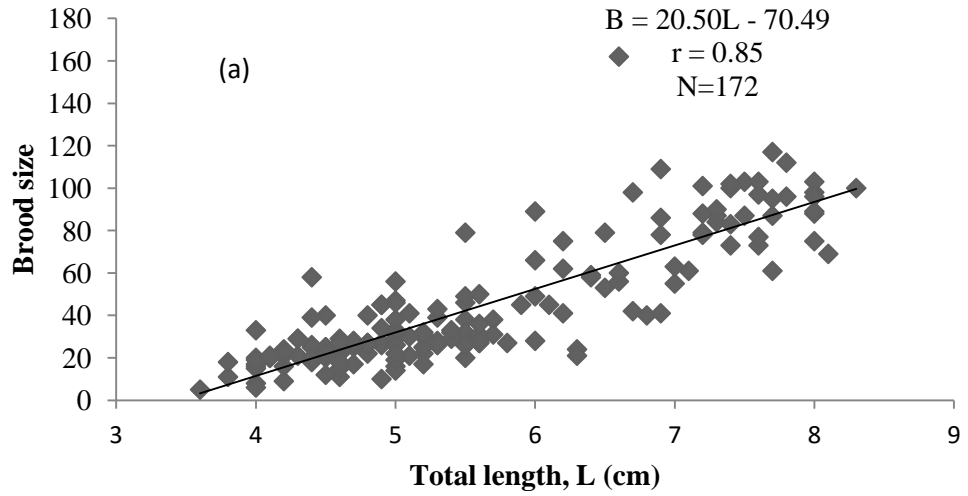
Figure 6: Relationship between brood size and (a) total length and (b) body weight of *S. melanotheron* from Fosu lagoon

CHAPTER FOUR

DISCUSSION

Physico- chemical conditions in ponds

One of the crucial physico-chemical parameters in aquatic environment is temperature since it determines the health and growth conditions of cultured organism. The mean monthly temperature variations in the pond used for crosses ranged from a minimum of 27.33 ± 0.40 ºC measured in August, 2013 to a peak of 31.13 ± 0.65 ºC measured in April, 2013. The temperature range reported in the breeding pond in the present study falls within the range of 25 to 32 ºC reported by Boyd (1998) and Parker and Davies (1981) as the range within which warm water species grow best. The minimum temperature recorded in the breeding pond is consistent with the temperature of 27 °C reported by Bauer (1968) as satisfactory for *S. melanotheron melanotheron*. In his work, Hauser (1977) reported a temperature range between 22 and 30 ºC as the optimum temperature for feeding, growth and reproduction in *Oreochromis niloticus* and added that good growth was recorded in the upper portion of this range. This finding is seen to be in agreement with the temperature range established in the present study. On their parts, Popma and Lovshin (1995) noted that tilapia prefer waters with temperature 29 - 31 ºC. Although the minimum temperature in the present study was lower than what was recommended by Popma and Lovshin (1995) no adverse finding was detected in the culture system in the present study. It is interesting however, to note that majority of these workers worked with the Nile tilapia and the fact that the species of interest in the present study survived and grew normally under similar temperature, is an indication that *Sarotherodon*

melanotheron could be cultured under lower temperatures than what may be optimum for the Nile tilapia.

FAO (2000) gave the optimum temperature range for tilapia culture as 28 to 32 ºC and the temperature range for the four - month sex reversal trial which was between 27.32 ± 0.12 ºC and 28.97 ± 0.20 ºC was comparable to the range considered to be ideal for fish culture. Wohlfarth and Hulata (1983) reported that the optimum temperature for culture of *Oreochromis niloticus* is 27 ºC to 30.50 ºC and the finding of the present study although on *S. melanotheron* is consistent with the temperature range reported by Wohlfarth and Hulata (1983). The mean temperature range registered in the present work also fits into the findings of Chervinski (1982) who reported that the tropical fish *Tilapia* and *Sarotherodon* do not tolerate temperatures below 12 ºC but are remarkably tolerant to high temperatures up to 42 ºC. The peak mean temperature of 31.13 ± 0.65 ºC was registered in the pond used for breeding in April, 2013 which is within the hottest period in the southern part of Ghana. The lowest mean temperature of 27.33 ± 0.40 ºC was measured in August, which fell in the rainy season in southern Ghana hence the lower temperature. Considering the temperature ranges proposed by different workers for tilapia culture, it could be concluded that artificial culture of *S. melanotheron* could be done throughout the entire year since temperatures in the tropics do not vary significantly throughout the year due to high solar insolation throughout the year.

The trend from the present study was that pH values declined between January, 2013 and March, 2013 whilst temperature at the same period was on the

rise in the breeding pond. This was in agreement with the observation made by Russell (2011) that as temperature increases, respiration by living organisms increases thereby raising carbon dioxide concentrations that interact with water producing carbonic acid and lowering pH. The trend could also be linked to the rate of carbon dioxide consumption which depends on phytoplankton density. For the fact that carbon dioxide is acidic, its accumulation in the pond could result in reduced pH values from the neutral region to acidic zone as observed in the present study.

The hydrogen ion concentration measured in the pond used for crosses of *S. melanotheron* ranged between 5.52 ± 0.19 and 7.79 ± 0.05 and the pH range in the pond used for sex reversal was from 7.16 ± 0.08 to 7.76 ± 0.02. The ranges for the two ponds were within the pH range of $5 - 9$ recommended by Chakroff (1976) as the range for maximum fish production. It is worth-mentioning that the neutral values recorded as the minimum and maximum pH in the sex reversal pond, may be attributed to the relatively clearer water devoid of algae that was maintained in the pond resulting in lower carbon dioxide accumulation which in turn, elevated the pH values. Huet (1972) also recommended a pH of 7.8 as the best for fish culture. The pH of 5.61 ± 1.06 to 5.52 ± 0.19 resulting in slightly acidic condition between January, 2013 and March, 2013 might be due to absence of fresh water inflow into the pond as a result of the long period of drought that led to closure of taps in and around Cape Coast township. This situation encouraged heavy phytoplankton growth in the pond and its concomitant

increased carbon dioxide levels which subsequently increased the acidic content of the pond water.

The decrease in pH values from January, 2013 – March, 2013 could also be attributed to the aerobic decomposition and reducing substances in the mud at the bottom of the pond as observed by Wetzel (1983). As the flow of the water into the pond resumed in April, 2013, the pH appreciated to 6.84 ± 0.84 in April, 2013 suggesting that as good water is maintained in fish ponds, neutral pH is provided for optimum fish culture. On his part, Swingle (1961) observed that pH of 11.0 may be taken as the alkaline death point practically for all pond fishes but the maximum pH of the current finding was nowhere near what Swingle (1961) proposed.

Among the abiotic factors in fish ponds, the most important and critical one requiring continuous monitoring in aquaculture production systems is dissolved oxygen (DO). The dissolved oxygen range in the pond used for crosses was from 1.57 ± 0.57 to 3.70 ± 0.26 mg/l. On the other hand, the range registered in the pond used for sex reversal trials was 3.12 ± 0.17 mg/l to 4.12 ± 0.08 mg/l. According to Balarin and Hatton (1979) tilapia have a low oxygen demand and can survive at low levels and the lowest tolerance limits for *Oreochromis niloticus* ranges from 0.1 to 3 mg/l under different conditions. The DO range obtained in the present study was higher than what was proposed by Balarin and Hatton (1979) for *Oreochromis niloticus* but was seen to favour the growth of *S. melanotheron* which is the subject for the present study.

The minimum and maximum DO values measured in the breeding pond in the present study were lower than values measured in the sex reversal pond. This could be attributed to the limited quantity of phytoplankton in the sex reversal pond which might have resulted in low dissolved oxygen consumption by the algae hence relatively higher DO values in the pond. In their study, Phelps and Popma (2000) found out that dissolved oxygen concentrations should remain above 4 mg/1 for ideal fish culture whilst Ayinla *et al*. (1994) reported that fish mortality sets in at dissolved oxygen levels less than 1mg/l. In the case of the present study, the DO values were above 1mg/l and therefore no adverse findings were observed in the culture system.

The present result also deviates from the findings of Charkroff (1976) who reported that oxygen level in a fish pond should be above 5 mg/l but not more than 15 mg/l. On the other hand, the assertion by Parker and Davies (1981) that at dissolved oxygen level below 2 mg/l, fish cease to feed, reduce locomotor activities and use the available oxygen for support system rather than growth was in disagreement with the minimum DO values of 1.57 ± 0.57 mg/l which was measured in May, 2013 in the present study but fish did not display the signs observed by Parker and Davies (1981) when DO values were below 2 mg/l. The difference in behavior of the fish might be attributable to species differences. Although fish was occasionally seen in the breeding pond gasping for air in early hours of the day, the minimum DO recorded was 1.57 ± 0.57 mg/l but there was normal growth and development of the fish species.

The low level of dissolved oxygen in the crossing pond between May and June, 2013 may be attributed to the shading effect by phytoplankton which limits the penetration of sunlight thereby reducing photosynthetic oxygen production in the bottom of the water column. This finding however contradicts the result of Boyd (1998) who reported that diffusion of atmospheric oxygen into water bodies reduces with higher temperatures. The months of May and June, 2013 did not witness any intense sunlight and as such, temperatures were on the decline and therefore the lower DO values could not have been assigned to higher temperatures in the pond. The generally low DO levels in the ponds may be attributed to the small surface area of the holding facility which hindered diffusion of atmospheric oxygen into pond.

Fingerlings from Kakum river estuary and Benya lagoon brood fish

The low number of fry observed confirms the findings of Kone and Teugels (1999, 2003) who in their study observed that *Sarotherodon melanotheron* had a lower fecundity and this might be attributed to the mouth breeding habits of the species and the limited space available for incubation of eggs and rearing of alevins in the buccal cavity. Panfili *et al*. (2004b) also observed that the fecundity of black-chinned tilapia seemed to be more affected by low salinity whereby in less saline environment, the relative fecundity was lower with larger oocytes. Kone and Teugels (1999) made the same observations in a freshwater lake in the Ivory Coast.

The low fecundity observed in the present study also corroborates Fryer and IIes (1972) assertion that in tilapia and other fish species, mouth brooders lay

fewer eggs than the guarder species. Sex ratio is an important aspect of fish reproductive biology, which gives information necessary for successful fisheries management and recruitment in natural water bodies and in aquaculture. The observed sex ratio of 1:1.3 in favour of females for both offspring by Kakum estuary and Benya lagoon parents was not significantly different from the expected 1: 1 (see Table 2). The preponderance of female offspring over males in the present study is similar to the observation made by Getahun *et al.* (2014) who worked on *Clarias gariepinus* and attributed the skewed ratio in favour of females to sexual segregation during spawning. Barioller *et al.* (1995) however attributed the occurrence of unbalanced sex ratios to environmental influences such as temperature and that, the observed sex ratio in the present study could be as a result of environmental factors. Additionally, variation in sex ratio in the present study may probably be due to the paternal brooding characteristics of the estuarine dwelling species. The skewed sex ratio in favour of females might also be due to genetic factors of the species.

According to Trewavas (1983) members of *Sarotherodon melanotheron* practice mouth brooding and the limited number of brood observed in the present study agrees with this observation. Biogeographically, *S. melanotheron* are typical estuarine fish, and are therefore naturally suited to the lagoon environment (Daget & Iltis, 1965; Payne & Collinson, 1983; Albaret, 1987). Although the *S. melanotheron* can easily adapt and survive in fresh water, breeding them in shallower cages in fresh water might possibly be the cause of the

reduced fry number. This goes to support Gerking (1980) who suggested that abrupt changes in water levels may reduce fecundity in fish.

According to Siddiqui, Al - Harbi, and Al – Hafedh (1997) variation in fecundity may be attributed to differential abundance of food within members of a population. Despite the fact that equal amount of feed was fed to the fish based on their body weights, the quantity fed by individuals might be different. The disparity in the brood size of the parents of approximately the same size from the two populations conforms to the finding of Bagenal and Braum (1978) who documented that fecundity in fish species characteristically varied among individuals of the same size and age. Witthames *et al.* (1995) reported that fecundity may vary within species as a result of different adaptations to environmental habitats.

Eyeson (1983) indicated that fecundity of *Sarotherodon melanotheron* under pond condition was generally lower as compared with the natural. Indeed, in his study, a lower fecundity of 14 eggs for each centimetre increase in length of the mature fish was registered under pond conditions whilst the increase was about 35 eggs per centimeter for the natural population, this is reflected in the low fecundity recorded in the present finding. The unusually low brood size noticed in Benya lagoon crosses and the failure of two out of the three replicates to breed could be as a result of the transfer of brood stock from hypersaline condition with which Benya lagoon is associated especially in the dry season to fresh water. It is based on differences in the performance of *Sarotherodon melanotheron* in different habitats with varying salinity levels that

Omoyi and Agbon (2008) recommended that for breeding purposes, brood stock should be collected from fresh water as against brackish water because of the higher body and caudal peduncle depth recorded for the fresh water fish. The relatively higher fry produced by the Kakum parents is consistent with Omoyi and Agbon (2008). Obodai *et al.* (1994) recorded sanility of more than 39 ‰ for Benya lagoon between February and April whilst Okyere (2010) registered salinity of 6 ‰ for Kakum Estuary wetland in the dry season.

It has been established by Faruk, Mausumi, Anka and Hassan (2012) that egg production decreases with increases in temperature and that maximum number of eggs were produced at temperature of 25 ℃ and the minimum number of eggs at 33 ℃ and considering the mean temperature of 28.68 ± 0.60 registered from November to December, 2012 within which the crosses were done, it could be inferred that the temperature probably was too high for increased egg production. This might have accounted for the low fecundity and the subsequent low fry production. The differences in the breeding figures from the brood stock from the two populations have a strong reason to suggest that differences in salinity might have influence on fertility in *S. melanotheron*.

The annual reproductive cycle in tilapia is known to be dependent on temperature and photoperiodicity of the seasons and the peak breeding period associated with the rainy season. The role of rainfall in fish spawning is well documented (Fryer and Iles, 1972; Balarin & Hatton, 1979; Lowe-McConnell, 1982). Blay (1974) recorded a progressive increase from 10 % in December to 28.8 % in May of brooding male of *Tilapia melanotheron* in Fosu lagoon. The

difficulty of breeding in the lagoon brood stock from November, 2012 to January, 2013 as observed in the present finding might be as a result of the fact that it was off-season for tilapia breeding.

Moreover, Jennings and Williams (1992) observed that *Sarotherodon melanotheron* can tolerate salinity of 100 ‰ and reproduce in salinity up to 35 ‰. This therefore suggests that broodstock of *Sarotherodon melanotheron* collected from brackish or saline water for breeding purposes could perform better in brackish water. Poor reproduction observed in the breeding of brood stocks from Benya in the present work could be as a result of collecting from high saline natural environment into fresh water. This assertion agrees with Eyeson (1979) who reported that eggs of *Sarotherodon melanotheron* survived better in salty water and that salinity of 10 ‰ was the most favourable medium for hatching, in terms of survival and rate of embryonic development.

Sex ratio should be a consideration when choosing species of freshwater fishes for culture and reproduction (Mekkawy & Hassan, 2011; El-Kasheif, Authman & Ibrahim, 2012). In fishes, the sex ratio varies from one species to another (Khallaf & Authman, 2003). Environmental factors can trigger or determine the process of gonad development in some fishes and can lead to skewed sex ratios in wild or farmed fish (Siegfriend, 2010). The sex ratio of (M : F) of all the offspring of the Kakum and Benya lagoon parents were skewed towards females but the ratio did not depart from the expected 1 : 1. Statistically, the present result is in line with Okorie (1973) who documented a sex ratio of 1 : 0.8 (male to female) for *O. niloticus* from Opa reservoir in Nigeria which shows

no significant difference ($p > 0.05$) from the hypothetical distribution of 1:1. The preponderance of females in the present study contrasts the result of Fryer and Iles (1972) who pointed out that in African lakes it is common that in the populations of cichlids, that the males dominate because they generally present more growth than the females. Due to environmental differences between natural aquatic environment and artificial pond system, caution would have to be exercised in relating the breeding activities in the two environments.

Yankson (1996) also reported that the preponderance of females in a population prior to onset of favourable conditions is a strategy to improve breeding success as few males could contribute to fertilization of numerous eggs. Therefore the dominance of female *S. melanotheron* in the crosses in the present study could be a strategic approach by the fish to enhance breeding success having being in confined environment. Fawole and Arawomo (2000) also indicated that for every female, there is a male specimen in *Sarotherodon galilaeus*. Babiker and Ibrahim (1979) reported for *Tilapia nilotica* that the two sexes are present in an almost 1:1 ratio in populations of middle-age fish. Nikolsky (1963) reported that the sex ratio varies considerably from species to species, but in the majority of cases it is close to 1:1. However, studies conducted on *Oreochromis niloticus* in some water bodies in Ethiopia and elsewhere in the world (Babiker & Ibrahim, 1979; Demeke, 1994; Gómez-Márquez *et al.,* 2003) revealed that females are more numerous than males. The present finding concurred with these observations.

It has been established that temperature can induce sex ratios of some fish species including *Oreochromis niloticus*. Baroiller *et al*. (1999) discovered that in most thermo sensitive fish species, increases in environmental temperature results in sex ratio skew from male to female while ovarian differentiation is favoured by low temperatures. Azaza, Dhraief & Kraiem (2008) performed an experiment to assess the effect of ambient temperature during the period of sex differentiation on sex ratio. The result showed that high temperature of about 36.90 ℃ yielded significant higher proportion of males (64 - 80%) but with lower survival rates (60 – 81%). On the other hand, the progenies reared at temperature below 36 ℃ never deviated significantly from the balanced sex ratio. But caution will have to be exercised in discussing the present finding in line with environmental influence on the sex ratio. This is because inasmuch as temperatures are always higher in the tropics and subtropical regions, the value of 36.90 ℃ as used by Azaza (2004) had not been recorded in the present study to warrant skewed sex ratio in favour of females.

The sex ratio of 1: 1 (Male: Female) in the breeding of *S. melanotheron* could be considered advantageous to tilapia breeders since the species is largely a male mouth brooder and that equal pairing of male and female could be done to avoid wasting some of the favoured sex since it was reported by (Eyeson, 1979) that no spawning was observed in *S. melanotheron* in the absence of a male fish.

Hormone administration

In the present finding, the highest proportion of *S. melanotheron* males produced after post yolk-sac fry measuring 11mm and weighing 0.02 g were fed

with 120 mg MT/kg feed for 30 days was 92.70 % and this was the highest among the other hormone treated groups and the control. This did not differ significantly ($p > 0.05$) from fish fed 60 mg MT/kg feed but differ significantly ($p < 0.05$) from the control group. There appear to be some evidence to agree that the administration of hormone had some influence on the skewed sex ratio in favour of males and judging from the sex ratio of the control group which was 1:1.5 and skewed towards females. The present finding deviated from the findings of Popma and Green (1990) and Romerio *et al.* (2000) who obtained 97 - 100 % phenotype males and 98 % male population respectively after feeding fry of the same length as in the present study with the synthetic androgen, 17 α-methyltestosterone at 60 mg/kg feed for 25 days. It may be argued that Popma and Green (1990) and Romerio *et al.* (2000) worked on *Oreochromis niloticus* and the species of interest in the present work is *S. melanotheron.* It therefore suggests that perhaps, higher concentrations of the hormone, 17 α-methyltestosterone may be required in masculinizing the salt–tolerant species, *S. melanotheron* under similar environmental conditions (see Figure 2). The present finding further deviates from the result obtained by Jae -Yoon *et al.* (1988) who recorded 97 % male population after feeding 10 mg MT/kg of diet to *Oreochromis niloticus* fry for 28 days.

It has been reported by Barry *et al.* (2007) and Green and Teichert-Coddington (2000), which over 95 % of the population was masculinized in 21–28 days when 30-60 mg 17α-MT/kg feed was applied orally to the tilapia larvae (7–12 days of age, 9–11 mm total length and 10–15 mg of total weight). It was

obvious that the concentration of the hormone used to achieve the masculinization rate of 92.70 % in the present work was higher but with reduced masculinization rate as compared with Barry *et al*. (2007) and Green and Teichert-Coddington (2000). An interesting observation in their finding was that although the length of their sample was similar to what was used in the present work, the weight of their sample was between 0.01 and 0.015 g far lower than the 0.02 g in the present work. The deviation may be ascribed to the difference in the species of tilapia used by these workers.

The disparity between the results of earlier workers and the present study may be due to differences in environmental conditions or species differences. Although natural production was discouraged in the sex reversal pond by regular replenishment of pond water, damselflies and dragonflies of the order Odonata were common in the water and the nymphs of these insects may have been fed by the fry of *S. melanotheron* which is known to be carnivorous at the juvenile stage. This observation agrees with Luna (2012) who reported that *Oreochromis mossambicus* which bears likeness with the species under study is an omnivore and consumes among other things, insects and that juveniles tend to be carnivorous. Vera–Cruz and Mair (1994) obtained 95 to 98 % males with 40 mg MT/kg of diet and 99 % with 60 mg MT/kg of diet fed at 20 % body weight for 25 days. Okoko (1996), obtained 71.9 % males at the dose rate of 120 mg MT/ kg of diet and further reported that higher dose rates of MT/kg of feed resulted in no increase of male percentage.

It was reported that methyltestosterone suppresses the oogenesis and this inhibitory effect on the development of oocytes is dependent on the dose of methyltestosterone. It is therefore important to consider the dose of the hormone to avoid the problems related to overdoses. Goudie *et al*. (1983) documented that excessive doses of hormone lead to sterility or paradoxical feminization following aromatization of androgens to estrogens. It is therefore imperative for the optimum dose of the hormone to be administered to avoid overdose or under dose since according to Popma and Green (1990) sub-optimal treatments resulted in intersexes.

Evaluation of growth indices indicated that the highest mean specific growth rate (SGR) of 9.70 ± 4.31 was recorded in 0 mg MT/kg feed (control) whilst the highest mean absolute growth value of 0.41 ± 0.11g was recorded in fry fed 120 mg MT/kg feed. The difference was statistically not significant ($p > 0.05$) between the control and the hormone treated group for both parameters (Table 4). The result that the control group having higher specific growth rate could possibly be ascribed to species difference since most earlier workers used *Oreochromis niloticus* while the present work was on *Sarotherodon melanotheron*. The present finding agrees with the findings of Vera Cruz and Mair (1994) who reported that androgen had no significant effect on growth and survival of fry of *Oreochromis niloticus* during the treatment period and attributed the growth of fry and monosex tilapia to the effect of temperature. Oluwatoyin and Akapo (2012) on the other hand, reversed the sex of *Sarotherodon melanotheron* and reported better mean weight gain of 10.86 ± 0.18 g and specific growth rate of 17.58 ± 1.55 % per day

in the treatment tanks under a temperature of 27.80 ± 0.70 ºC and dissolved oxygen of 8.46 ± 0.05mg/l as opposed to the temperature range between 27.32 ± 0.12 and 28.97 ± 0.20 ºC and dissolved oxygen values of 3.12 ± 0.17 - 4.12 ± 0.08 mg/l in the present study.

A careful consideration of differences in the environmental conditions of the present study and Oluwatoyin and Akapo (2012) cannot therefore be taken as the factor for the disparity in the growth performance as suggested by Vera Cruz and Mair (1994) and judging from the similarity in the temperature values in the Oluwatoyin and Akapo (2012) and the present study. This present findings however, contrast the observations of several authors (Guerrero, 1975; Katz *et al*. 1976; Owusu Frimpong and Nijjhar, 1981) who found that there was improved size of MT treated male fish as compared to untreated males. According to Jo *et al*. (1995) the highest weight gain was obtained in the fry treated with 60 mg of 17α - MT/kg of feed compared to that of the control, 40, and 80 mg of 17α - MT/kg of diet and added that the treatment can be attributed to the anabolic effect of 17α- methyltestosterone. The anabolic effect of the 17α-MT could possibly be the cause of higher weight gain in the treated group. Mair *et al*. (1995), Dan and Little, (2000) and Little and Edwards (2003) all corroborated the anabolic effect of 17α-MT and ascribed it to the higher weight gain of *Oreochromis niloticus.* Chakraborty and Banerjee (2010) also attributed the higher mean weights to posssible improvement of food conversion efficiency of sex-reversed fry of *Oreochromis niloticus*.

The highest and the best mean specific growth rate of fingerlings measured at the end of the trial was obtained in 60 mg MT/kg of feed whilst the highest mean absolute growth was recorded in 120 mg MT/kg feed but the differences were not statistically significant ($p > 0.05$) from other groups (Table 5). Fingerlings in the hormone- treated group expectedly recorded relatively higher values in body weight and total length. This could be attributed to the anabolic effect of the17α- methyltestosterone which might have positively influenced the growth of the fish after the hormonal feed administration. The finding is in agreement with Khalil *et al*. (2011) who also reported that administration of 17 α - methyltestosterone induced significant increase in fish growth of treated Nile tilapia. Hanson *et al*. (1984) reported that 10 - 60 ‰ methyltestosterone treatment showed the best growth than control, these are also consistent with Dan and Little (2000) who compared the culture performance of different strains of *Oreochromis niloticus* and found that considering all strains, MT treatment resulted in a final size of fish 10.7 % larger than mixed sex fish.

The Condition Factor (K) of a fish is a measure of the state of the general well being of that fish. The condition factor is used to assess the 'fatness' of a fish. This is based on the hypothesis that a heavier fish of a particular length is in a better physiological condition than a lighter fish of the same length (Bagenal, 1978). Changes in condition factor of fishes could be used to interpret various biological features such as fatness, food availability, reproductive activities and environmental health (Le Cren, 1951; Dadzie *et al*., 2000).

The condition factor (K) was calculated a day after hormonal feed administration ended (treatment) and after the end of the trials (post- treatment) and the results revealed that condition of the fish fed 30 mg MT/kg and 120 mg MT/kg feed were 2.09 ± 0.35 and 2.04 ± 0.44 respectively and were better at treatment period than post- treatment period. The post treatment condition factors were 1.09 ± 0.53 and 2.00 ± 1.70 respectively. This suggests that the hormone might have caused the improvement in the condition of these groups of fish at treatment period. The result corroborated the finding of Tayamen and Shelton (1978) who reported that methyltestosterone enhanced the growth of various fish species including *Oreochromis niloticus.*

Bagenal and Tesch (1978) on their part described condition factor values ranging between 2.9 - 4.8 as the ideal K value for the normal growth and utilization of nutrient by a normal freshwater fish. Growth promoting potential of 17α- methyltestosterone was also reported by previous authors (Woo, Chung & Ng, 1993; Satpathy, Mukhopadhyay, & Ray, 1995; Sambhu & Jayaprakas, 1997; Ahmad, Shalaby, Khattab & Abdel- Tawwab, 2002). The increase in the condition factor of the untreated fish at the end of treatment period may be due to natural increase in the growth parameters of this group of fish.

Condition factor for fish fed 60 mg MT/kg feed was on the other hand, better at post treatment period than treatment period (see Table 5). This may probably be attributed to a case where the anabolic effect of dietary steroids become more visible later in the growth of the fish as suggested by (Abdelghany,1995).

Crab *et al*. (2009) suggested that K values of less than 1.8 is an indication of poor condition and K values greater than 2 as fish in good condition, the present K values may be rated good condition and may be attributable to better feed nutrient utilization by the control group. Ayode (2011) however described the mean condition coefficient (K) of 1.64, 1.86, 1.74, 1.72 and 1.79 for *Oreochromis niloticus* as above average and indicated that the fish were in good health and added that these condition factor values are desirable for fish in fish farms. It may be concluded that the fish in the present study were in better condition in the pond, re-emphasizing Fagade (1979) assertion that condition factor greater than 1, implies that fish is in good condition and healthy.

Reproductive potential or fecundity is defined as the number of ripe ova in the female prior to spawning (Bagenal & Braum, 1978). Fecundity is one of the best criteria for determining the reproductive potential of fish species, and therefore an important parameter which enables estimation of total population and forecasting of fish productivity in a particular water body.

Assessing the fecundity of *Sarotherodon melanotheron* species which is the dominant fish species in the Fosu lagoon, it was found that fecundity increased linearly with total length, body weight, and ovary weight of the fish species with correlation coefficient (r) values of 0.58, 0.61 and 0.52 respectively. Among the (r) values, body weight seems to be better correlated with fecundity than other parameters, suggesting that body weight was a better predictor of fecundity in this study. Similar finding was reported by Ikomi and Odum (1998) in *Chrysichthys auratus.* The 'r' values in the present work implies that the

number of eggs produced by the fish is dependent on body size suggesting that the larger the fish, the higher the number of eggs it can produce and this may be due to more available visceral volume for holding the eggs. These findings are supported by the findings of previous researchers, Jhingran (1968), Raina (1977), Pathani (1981), Sunder (1984), Islam and Hossain (1990), Hussain *et al*. (2003), Mohan (2005), Offem, Ayotunde and Ikpi (2008), Bahuguna and Khatri (2009). Other workers who reported significant relationships between fecundity and these variables were Bone, Marshall and Blaxter (1995), Galvani and Coleman (1998) and Olurin and Aderibigbe (2006).

The relationship between brood size and total length as well as brood size and body weight were also established for *Sarotherodon melanotheron* in Fosu lagoon. A strong positive linear correlation was found between brood size and total length with correlation coefficient (r) values as 0.85 and 0.86 respectively (Figure 6). The finding implies that the number of eggs brooded by the fish is dependent on the length and weight of the brooding parent. This suggests that the larger the fish the greater the number of eggs or fry it can brood. This is in line with the observation by Eyeson (1979) who reported that the number of eggs brooded in *Tilapia melanotheron* tends to increase with the size of the fish.

Sarotherodon melanotheron as a paternal mouth brooder, broods the eggs in the mouth, it is therefore obvious that the size of the mouth will determine the number of eggs it can accommodate. Evidence abounds from the present study that the number of fry spawned by fish of a particular size are fewer than the number of ripe eggs found in the ovary of the same fish. This suggests that as

74

development proceeds in the buccal cavity, the number of fry decreases. This confirms the observations of Aronson (1949) and Welcomme (1967).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

Conclusion

In the face of Ghana's dwindling fisheries resources due to factors such as over - exploitation and irresponsible fishing practices, conflict over fisheries resources, weak fisheries management measures, weak monitoring, control and surveillance, overcapitalization of the sector and population pressure due to migration to fishing areas in search for jobs, it is obvious that aquaculture needs to be stepped up to bridge the gap between fish demand and supply. Current statistic from the Ministry of Fisheries and Aquaculture Development (MoFAD) shows that the national fish production was 482,000 metric tonnes, while total fish requirement was 968,000 metric tonnes, showing a deficit of about 50.2 % (MoFAD, 2012).

Currently in Ghana, aquaculture is being practiced but largely on the Volta Lake on small to medium scale, using *Oreochromis niloticus* which is known to be less- tolerant to high salinity which is the feature of most water bodies in the southern part of the country, by virtue of their proximity to the sea. It is therefore imperative on the stakeholders including the academia to investigate and come out with a suitable breed of the Cichlidae family which will be able to perform well in brackish to saline water environments and also to assess its cultural potential in these areas. It is against this backdrop, that this investigation was carried out to elucidate if *S. melanotheron* can produce seeds with improved aquaculture traits that can be harnessed just as its counterpart, *Oreochromis*

niloticus for culture on commercial scale. This was done and the outcomes discussed below.

All the water quality parameters measured in the ponds used for the breeding and sex reversal were within the range that could be used for warm water fish. Temperature range in the pond used for crosses was between 27.33 ± 0.40 ℃ and 31.13 ± 0.65 ℃ measured from October, 2012 to September, 2013 whilst the temperature range in the pond used for sex reversal was 27.32 ± 0.12 ℃ to 28.97 ± 0.20 ℃ recorded between July, 2013 and October, 2013. The pH, as measured in the pond used for the crosses ranged from 5.52 ± 0.19 to 7.79 ± 0.05 and the range of 7.16 ± 0.08 to 7.76 ± 0.02 measured in the pond used for sex reversal trials. Dissolved oxygen ranged from 1.57 ± 0.57 to 3.70 ± 0.26 mg/l in the pond used for breeding while DO values ranged from 3.12 ± 0.02 to 4.12 ± 0.78 mg/l in the pond used for sex reversal. There was a high level of survival of the fish cultured under these environmental conditions and comparing these values with earlier works, the conclusion was that the physico - chemical conditions in the ponds are optimum for *S. melanotheron* culture in Ghana.

Fish of the same size do not have the same fecundity or produce the same number of broods. This has been observed in the offspring produced by the brood stocks collected from Kakum River Estuary and Benya lagoon.

S. melanotheron from the wild bred under captivity; however, the progeny did not breed. This was evident as all the crosses from Kakum River Estuary bred with a total fry population of 352 while only one replicate out of the three from Benya lagoon bred with difficulty, producing only 34 fry. The development of

reproductive material (gonads) under captivity is sensitive to phyisco-chemical parameters as well as nutrition of the fry.

The difficulty of breeding of the brood stock from the Benya from November to December/ January, which is known to be the harmattan season with its associated lower ambient temperature has brought to the fore the conclusion that breeding of *S. melanotheron* should be done in the wet season which is known to be the peak of the breeding season for the tilapiine fishes.

It was realized from the sex ratio of the fry produced from the crosses that, the females outnumbered the males but the result from chi-square test on the sex ratios of the offspring revealed that the differences were not significant (see Table 2) suggesting that the sex ratios conform to the expected 1: 1.

With regards to sex reversal, the fry fed 120 mg MT/kg feed gave the highest proportion (92.70 %) of male but the percentage conversion was not large enough to encourage monosex culture of *S. melanotheron* with this concentration. This is because the 7.30 % being female will be capable enough to breed among the population thereby defeating the purpose of monosex culture.

Evaluation of growth parameters of the sex reversed fish showed that the hormone, 17 α- methyltestosterone did not have positive effect on growth of the species. This is because the difference in growth indices as calculated for the treated fry were not significant ($p > 0.05$) from the control. This however suggests that the anabolic effect of the hormone did not impact on the growth of the fish.

A regression analysis of fecundity and total length and body weight of *S. melanotheron* showed that fecundity was positively correlated with the total

length and body weight suggesting that the larger or heavier the fish, the higher the number of eggs it can produce.

A regression analysis of brood size and total length and body weight of the species also indicated that fecundity was positively correlated with the total length and body weight implying that the larger or heavier the fish, the higher the number of eggs or fry it can brood. It was also observed that the number of eggs brooded is lower than the number of fry or larvae produced.

Recommendations

➢ The breeding aspect of the study should be replicated in the wet season which is documented to be the peak reproductive season for tilapia.

➢ The study should be replicated with broodstock of *S. melanotheron* collected from lakes and reservoirs.

➢ Since the progeny did not breed, further work should be done on the effects of DO and feed quality on gonadal development of *S. melanotheron*.

➢ A study should be done on characterization of the species to ascertain the genetic differences if any, in the populations.

➢ For seed production, larger sizes of both sexes of the fish must be preferred to small ones.

➢ Since none of the hormone concentrations gave a 100 % sex - reversal success, further researches are required in this regard to

establish the optimum hormone concentration that will give a 100

% sex - reversal success in *S. melanotheron*.

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APPENDICES

Appendix 1: Sizes of broodstocks of *S. Melanotheron* from the two populations for crosses

Population	Sex	Total length (TL) cm ± SD	Body weight (BW) g ± SD
Benya lagoon	Female	10.85 ± 0.41	17.79 ± 0.29
Benya lagoon	Male	11.59 ± 0.47	18.82 ± 0.37
Kakum Estuary	Female	10.0 ± 0.03	18.17 ± 0.84
Kakum Estuary	Male	11.37 ± 0.20	23.78 ± 1.90

Appendix 2 : Crossing of broodstock from the two populations

CROSSING OF BROODSTOCK

Kakum   Benya

M_K X F_K M_B X F_B

(3 Replicates)   (3 Replicates)

CROSSING OF PROGENY

M_K X F_K (3 Replicates) M_B X F_B (3 Replicates)

M_K X F_B (3 Replicates) M_B X F_K (3 Replicates)

Appendix 3: Average size of fry at fortnights sampling in sex reversal trials

Week	Parameter	0 mg MT/kg			30 mg MT/kg			60 mg MT/kg			120 mg MT/kg		
		Replicates											
		I	II	III	I	II	III	I	II	III	I	II	III
2	TL	2.25	2.46	2.36	2.32	2.27	2.54	2.30	2.44	2.49	2.61	2.59	2.42
	BW	0.38	0.34	0.31	0.23	0.26	0.38	0.32	0.33	0.32	0.39	0.37	0.33
4	TL	2.77	2.86	2.95	2.56	2.82	2.79	2.87	2.88	3.08	3.12	2.86	3.13
	BW	0.41	0.46	0.54	0.34	0.44	0.45	0.47	0.50	0.55	0.64	0.44	0.61
6	TL	3.10	3.37	3.37	3.13	3.39	3.05	3.53	3.13	3.35	3.55	3.55	3.17
	BW	0.63	0.73	0.85	0.66	0.78	0.63	0.84	1.03	1.12	1.18	0.98	0.67
8	TL	3.61	3.71	3.66	3.59	5.97	3.94	3.98	3.46	3.40	4.0	3.43	3.71
	BW	0.89	0.88	1.02	0.90	0.99	1.08	1.15	0.87	0.82	1.24	0.91	0.99
10	TL	4.0	4.51	4.71	4.88	4.41	4.28	4.71	4.48	4.44	4.54	4.33	4.62
	BW	1.43	1.89	2.00	2.11	1.81	1.69	2.01	1.89	1.82	2.01	1.86	1.95
12	TL	2.25	5.08	5.41	5.62	5.37	5.23	5.63	5.09	5.49	5.63	5.09	5.49
	BW	4.79	2.67	3.66	3.33	2.99	2.86	3.09	2.79	3.14	3.09	2.79	3.14

Appendix 4: ANOVA for proportion of males of *S. melanotheron* produced in sex reversal trial.

One-way ANOVA: 0 mgMT, 30mgMT, 60mgMT, 120mgMT

```
Source  DF    SS     MS     F     P
Factor   3  5590.67  1863.56  266.22  0.000
Error    8   56.00   7.00
Total   11  5646.67
```

$S = 2.646$ $R\text{-}Sq = 99.01\%$ $R\text{-}Sq(adj) = 98.64\%$


```
                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Level    N   Mean   StDev  --------+---------+---------+---------+-
0 mgMT   3  39.333  4.163  (--*-)
30mgMT   3  84.000  2.000                        (--*-)
60mgMT   3  89.333  2.309                           (-*-)
120mgMT  3  92.667  1.155                              (-*-)
                           --------+---------+---------+---------+-
                               48      64      80      96
```

Appendix 5: ANOVA table for absolute and specific growth rate of fry under sex reversal trial

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Absolute	Between Groups	.010	3	.003	.063	.978
	Within Groups	.606	12	.050		
	Total	.615	15			
Specific	Between Groups	1.003	3	.334	.005	.999
	Within Groups	808.582	12	67.382		
	Total	809.585	15			

Appendix 6: ANOVA table for Specific and absolute growth rates of fingerlings under sex reversal trial

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Absolute	Between Groups	.021	3	.007	.206	.887
	Within Groups	.138	4	.034		
	Total	.159	7			
Specific growth rate	Between Groups	9.604	3	3.201	.078	.969
	Within Groups	164.184	4	41.046		
	Total	173.789	7			

Paired Samples Test-30 mg MT/kg feed

Condition factor	Paired Differences				t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			

Appendix 7: T- statistics tables for different hormones concentrations

Paired Samples Correlations—0 mg MT/kg feed (control)

Condition factor		N	Correlation	Sig.
Pair 1	Treatment – Post treatment	120	.186	.042

Paired Samples Test-0 mg MT/kg feed (control)

Condition factor		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Treatment – Post treatment	.24100	.41494	.03788	.16600	.31600	6.362	119	.000

127

					Lower	Upper			
Pair 1	Treatment – Post Treatment	.19346	.58989	.05385	.08683	.30009	3.593	119	.000

Paired Samples Statistics-30 mg MT/kg feed

Condition factor	Mean	N	Std. Deviation	Std. Error Mean
Pair	2.0888	120	.35471	.03238
1T	1.8953	120	.53244	.04861

Paired Samples Statistics- 60mgMT/kg feed

Condition factor		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Treatment – Post Treament	2.0701	120	.58230	.05316
		2.1512	120	2.29045	.20909

Paired Samples Test- 60 mg MT/kg feed

Condition factor		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Treatment – Post Treatment	-.08108	2.34761	.21431	-.50543	.34327	-.378	119	.706

Paired Samples Test for 120 mg MT/kg feed

Condition factor		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Treatment – Post treatment	.03601	1.72026	.15704	-.27494	.34696	.229	119	.819

Paired Samples Statistics for 120 mg MT/kg feed

Condition factor		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Treatment-Post Treatment	2.0369	120	.44099	.04026
		2.0009	120	1.70256	.15542