PRODUCTION OF MONOSEX TILAPIA AND ITS' GROWTH PERFORMANCES IN FARMERS' FIELD

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in
AQUACULTURE

By

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Dedicated
to
my beloved parents and almighty

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CERTIFICATE

This is to certify that the work recorded in this thesis entitled "Production of Monosex Tilapia and its' Growth Performances in Farmers' Field" submitted by **Mr. Sourav Dhabal**, in partial fulfillment of the requirements for the Degree of **Master of Fishery Sciences (Aquaculture)** in the Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Chakgaria, Kolkata, West Bengal, India, is a faithful and bonafide research work carried out by him under my supervision and guidance. The results of the investigation reported in this thesis have not so far been submitted for any other degree or diploma. The assistance and help received during the course of investigation have been duly acknowledged.

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APPROVAL SHEET

Approval of Examiners for the Award of the Degree of Master of Fishery Science (Aquaculture)

We, the undersigned have been satisfied with the performance of **Sourav Dhabal** in the viva – voce examination, conducted today, the 6th October, 2015 recommend that the thesis be accepted for the award of the Degree of **Master of Fishery Science** in Aquaculture.

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List of Abbreviations

17αMT	17α-methyltestosterone	MT	Methyltestosterone
ANOVA	One way analysis of variance	mm	milimeters
APHA	American public health association	MS	Microsoft
cm	Centimeter	ml	mili litre
D.O	Dissolved oxygen	N	Normal
DWG	Daily weight gain	No	Number
EDTA	Ethylene Di-amine Tetra acetic aci	ng	Nano gram
FCR	Feed conversion ratio	pH	Potential of Hydrogen ion concentration
Fig	Figure	ppm	Parts per milion
GH	Growth hormone	PER	Protien efficiancy ratio
g	gram	UV	Ultra violet
ha	hectares	SGR	Specific growth weight
IGFs	Insulin-like growth factors	SPSS	Statistical Package for Social Science
kg	Kilo gram	SRT	Sex reversal trough
kms	Kilometer	µm	Micro meter

ABSTRACT

The Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) popularly known as aquatic chicken is a widely cultured species because it grows and reproduces in a wide range of environmental conditions and tolerates stress induced by handling. The major drawback of pond culture of Tilapia is uncontrolled breeding, which led to excessive recruitment, stunted growth and a low percentage of marketable-sized fish. Monosex culture of male tilapia is postulated to solve this problem and several potent methods are there for production of all-male tilapia population. Little is known about the growth performance of sex-reversed, all male *Oreochromis niloticus* under different traditional culture methods practiced in India. 17α methyltestosterone treated monosex tilapia was produced in hatchery. To avoid heterogeneous growth, eggs were collected from females' mouth and incubated in hatchery. Three days old hatchlings (avg wt. 0.02 g, avg. length 0.97cm) were stocked in hatching trough and treated with high protein commercial feed incorporated with 17α methyle testosterone hormone @ 60mg/kg of feed with Ethanol (as hormone carrier solvents). The administration of hormone continued for 30 days with 100% water exchange system. Monosex seed were distributed among nine fish farmers for culture in earthen tanks following same type of culture procedure except stocking density. Three different stocking density viz. 20,000/ha (T_1), 30,000/ha (T_2) and 40,000/ha (T_3) was selected having three replicates. Commercial grade fish feed @ 10% for first 60 days and 5% for next 30 days was applied twice a day. Water and soil samples were collected fortnightly along with growth parameters of tilapia. The result was analysed statistically and maximum production was achieved in T_1 ponds. The final mean length and weight in T_1, T_2 and T_3 was 19.3 cm, 18.7 cm, 17.9 cm and 153.67g, 139.34g, 128.56g respectively. The major growth parameters including final weight gain (g), average daily weight gain (g/day) and specific growth rate (SGR %) of tilapia were analysed and T_1 exhibited significantly highest value than other ponds.

Chapter 1

Introduction

Tilapia is native to Africa and Middle East, and has emerged from mere obscurity to one of the most productive and internationally traded food fish in the world. The farming of tilapias, especially of Nile tilapia (*Oreochromis niloticus*) in its crudest form is believed to have originated more than 4000 years ago from Egypt. The first recorded scientifically oriented culture of tilapia was conducted in Kenya in 1924 and soon spread to many parts of the world.

The last three decades have seen significant developments in farming of tilapias worldwide. They are being farmed in about 85 countries worldwide (FAO, 2008) and about 98% of tilapia produced in these countries are grown outside their original habitats (Shelton, 2002).

Tilapia production may top 4.5 million tons by 2014. Worldwide tilapia production exceeded more than 4.2 million metric tons in 2012, a 6 % increase from 2011, but experts say by 2014 it will exceed 4.5 million as demand continues to grow (IntraFish, 2013).

It is growing at 9% annually and is projected to contribute 41% (53.6 million tonnes) of the world's fish production by 2020 (Krishen *et al.*, 2009).

According to FAO (2007), Farmed tilapia production throughout the world increased dramatically in recent years, increasing from 383,654 mt in 1990 to 2,326,413 mt in 2006 (FAO, 2007).

The reason behind the increasing trends of tilapia culture is not only for its extraordinary growth performances but also its resistivity towards diseases and capability to survive in relatively bad environmental conditions such as high stocking density, lower water quality, organically pollutant water, and low dissolved oxygen level of the water (< 0.5 mg l^{-1}). They have tolerance to salinity in wide range and are suitable for maintaining and feeding conditions in culture (Cruz and Ridha, 1994).

Tilapia has been widely introduced in the shallow and seasonal ponds of eastern region of India. The fish can form a readily available source of animal protein in the diets of rural and urban dwellers belonging to the lower socio-economic strata.

Among the wide variety of cultured tilapias, the most widely farmed and popular species is the Nile tilapia (*O. niloticus*) for their better growth performance acquired the second rank only to carps in the basis of global production (Ridha, 2006).

The Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) is a widely cultured species because it grows and reproduces in a wide range of environmental conditions and tolerates stress induced by handling (Tsadik and Bart 2007).

Scientists began their research by focusing on Nile Tilapia because of its ability to breed and produce new generations rapidly, its tolerance for shallow and turbid waters, its high level of disease resistance and its flexibility for culture under many different farming systems (Yosef, 2009; Soto- Zarazúa *et al.*, 2010).

The Nile tilapia was introduced to India during late 1970s. In 2005, River Yamuna harboured only negligible quantity of Nile tilapia, but in two years' time, its proportion has increased to about 3.5% of total fish species in the river. Presently in the Ganges River system, proportion of tilapia is about 7% of the total fish species. However, tilapia holds vast promise to become an important species for aquaculture in India, considering the demand for more fish.

The major drawback of pond culture of Tilapia is uncontrolled breeding, which led to excessive recruitment, stunted growth and a low percentage of marketable-sized fish, dampened the initial enthusiasm for tilapia as a food fish.

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 and Pruginin 1981; Coleman 2001; Lèveque, 2002).

However, the development of hormonal sex-reversal techniques in the 1970s, followed with research on nutrition and culture systems, along with market development and processing advances, led to rapid expansion of the industry since the mid-1980s.

Monosex culture of male tilapia is postulated to solve this problem and several potent methods are there for production of all-male tilapia population (Guerrero 1982; Macintosh *et al* 1985; Gale *et al* 1999; Beardmore *et al* 2001; Smith and Phelps 2001).

Males are preferred because they grow almost twice as fast as females, which may be caused by a sex-specific physiological growth capacity, female mouth-brooding or the

more aggressive feeding behaviour of males and expected survival for all-male culture is 90% or greater.

All-male populations have greater growth potential because no energy is shunted toward reproduction and no competition with younger fish occurs (Green *et al.*, 1997).

The density for male monosex culture varies from 10,000 to 50,000/ha or more, at proper feeding rates. Densities of around 10,000/ha allow the fish to grow rapidly without the need for supplemental aeration. About six months are required to produce 500 g fish from 50 g fingerlings, with a growth rate of 2.5 g/day (Fortes, 2005).

Male Tilapia production has an economic importance to its producers and sellers. The increase in employment in the sector outpacing world population growth and employment in traditional agriculture is a crucial source of income and livelihood for hundreds of millions of people around the world (Soto-Zarazúa *et al.*, 2011).

It could play an important role to provide food security for the general population as an excellent source of high-quality protein (FAO, 2010; Soto-Zarazúa *et al.*, 2010).

Moreover, sex-specific differences in growth were significant in *O. niloticus* where males grow significantly faster, larger and more uniform in size than females (Bwanika *et al.*, 2007).

Monosex seed production technique includes manual separation of sexes, environmental manipulation, hybridization, hormone augmentation (sex reversal) and genetic manipulation methods such as androgenesis, gynogenesis, polyploidy and transgenesis.

Although monosex male population can be obtained by direct or indirect methods, oral administration of *Oreochromis niloticus* has been reported to be the most preferred method in commercial uses (Green and Teichert-coddington, 2000; Wahby and Shalaby, 2010; Celik *et al.*, 2011).

Oral administration of exogenous male sex steroid hormones before the differentiation of primal gonadal cells can cause reversal of phenotypic sex (Smith and Phelps, 2001; Bhandari *et al.*, 2006). In an earlier study, almost 100% all-male monosex tilapia population was produced by treating 3 days old fry with a synthetic male hormone 17α-methyltestosterone (17αMT) at a treatment regime of 10 mg kg^{-1} food for 30 days (Chakraborty *et al.*, 2007).

Besides Ahmad *et al.*, (2002) reported that 17α-MT (at the dose of 5 mg/kg of feed) promote total weight gain and SGR of Nile tilapia. So it is clear that the 17 αMT has definite role in the enhancement of the somatic growth in a unit time.

So, there will be a synergistic effect for the farmers to culture all male population originated from the 17αMT treatment.

After evaluating all the information incorporated here, it was obvious to produce all male tilapia seed and its growth performance in field condition. For this reason the present research work was selected with the following objectives:

i) Production of monosex tilapia seeds using androgenic hormones and
ii) It's growth performances in farmers' field

Chapter 2

Review of Literatures

The Nile tilapia, *Oreochromis niloticus* (Linnaeus) is one of the most important species of fish in tropical and sub-tropical aquaculture (FAO, 2007). It provides one of the major sources of animal protein and income throughout the world (Sosa *et al.*, 2005). It is currently ranked second only to carps in global production and is likely to be the most important cultured fish in the 21st century (Ridha, 2006).

2.1. Production of tilapia: India and Global:

Farmed tilapia production throughout the world increased dramatically in recent years, increasing from 3, 83,654 mt in 1990 to 23, 26,413 mt in 2006 (FAO, 2007). Tilapia has been widely introduced in the shallow and seasonal ponds of eastern region of India. But, its performance in open water ponds of the country has been discouraging over the years (Jhingran, 1991). Increase in yields can result from the development and adoption of new technologies and improved farming operations (Coelli, 1995).

2.2. Culture practices:

The success of the culture methods applied for tilapia farming depends on various factors and determination of the optimal method under a certain condition can be quite complex (Graaf *et al.*, 2005). Various traditional and non-traditional tilapia farming methods are adapted in different countries in accordance with the socioeconomic and ecological condition of that place (Lèveque, 2002).

It is often cultured in earthen ponds without supplemental feeding (Liti *et al.*, 2005). Pond culture provides an opportunity to balance the use of supplementary feeding in correlation with the natural food availability. Intensive culture of tilapia in tanks has been globally expanding (El-Sayed, 2006).

2.3. Constrains of mixed sex tilapia:

Tilapia grows and reproduces in a wide range of environmental conditions and tolerates stress induced by handling (Tsadik and Bart, 2007). However, this efficiency of reproduction in tilapia has undesirable consequences. Problems common for many tilapia culture systems are the reduction of growth rates at the onset of sexual maturity and

precocious and excessive reproduction, leading to various sizes of small fish production (Lèveque, 2002). There are a number of ways to control reproduction in mixed-sex population. One of these is the culture of all-male tilapia (Phelps and Popma, 2000).

2.4. Why mono sex:

Moreover, sex-specific differences in growth were significant in *O. niloticus* where males grow significantly faster, larger and more uniform in size than females (Bwanika *et al.*, 2007).

Male monosex tilapia cultures are preferred to females because of the differential growth in favour of males. In males, the metabolic energy is channelled towards growth. They benefit from anabolism enhancing androgens (Tran-Duy *et al.*, 2008; Angienda *et al.*, 2010).

In females, there is a greater reallocation of metabolic energy towards reproduction. Methyltestosterone suppresses the oogenesis. This inhibitory effect on the development of oocytes is dependent on the dose of methyltestosterone. The ovary is almost occupied by somatic elements (Wolf *et al.*, 2004).

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 males (Dan and Little, 2000; Tran-Duy *et al.*, 2008). The males exhibited a growth rate 2.5 times more than female tilapia (Thomas, 2003).

2.4.1. More production:

According to Hanson *et al.* (1984) 10-60 mg methyltestosterone /kg feed treatment showed the best growth than control.With the purpose of achieving more productivity in growing tilapia, *Oreochromis niloticus* , at the unit time, it is important to produce monosex culture that constitutes totally of males (Mair and Little, 1991). Dan and Little (2000) observed that on average, monosex tilapia grew more than 10% faster than mixed-sex fish in cages.

2.4.2. More growth rate:

Several studies are in agreement that testosterone produces muscle hypertrophy by increasing muscle protein synthesis (Bhasin *et al.* 2001). A few studies have

demonstrated the enhanced yield of monosex male Nile tilapia populations under experimental conditions (Mair *et al.* 1995). In *Oreochromis mossambicus* (Peters, 1852) also, 17α-methyltestosterone (17α-MT) treated fish is reported to show higher growth compared to the untreated fish reared under similar conditions (Macintosh *et al.* 1985).

O. niloticus grows more than 600 g within 6 months and it attains sexual maturity on reaching 10-17 cm length in 4 months (Thomas, 2003). Mono-sex exhibited very fast growth rate i.e. 500-600 g within 5 months and up to 1200g in 18th month (Thomas, 2003).

2.4.3. Low FCR:

Chakraborty *et al.* (2011) reported that there was a general decrease in FCR and increase in PER for monosex fish compared to the mixed-sex fish. Such observation may be related to the fact that FCR decreases while PER increases with increased feeding rate (Pechsiri and Yakupitiyage, 2005).

2.4.4. Less management is required:

The desirability of monosex male populations of tilapia is well established for increased production potential and low management requirements (Pillay, 1993; Beardmore *et al.*, 2001; El-Sayed, 2006).

2.5. Developed process for mono sex tilapia:

Several techniques have been adopted for production of monosex (all-male) tilapia (Phelps and Popma, 2000), and hormonal sex reversal of tilapia has been an active area of research for the past three decades (Pandian and Varadaraj, 1988; Gale *et al.*, 1999; Carrasco *et al.*, 1999; Afonso *et al.*, 2001). Within the contemporary atmosphere of increasing governmental regulation on the use of chemicals on food fish, continued dependency on steroid-induced monosexing places the culture of tilapias in a precarious position. The recently developed protocol to develop YY-male tilapia relies on estrogen treatment, and even though fish to be cultured are one generation removed from the treatment, the protocol still depends on progeny testing to identify target brood stock (Scott *et al.*, 1989; Mair *et al.*, 1997).

Although monosex male population can be obtained by direct or indirect methods, oral administration of feed incorporated with androgen provided to

Oreochromis niloticus has been reported to be the most preferred method in commercial uses (Green and Teichert-coddington, 2000; Wahby and Shalaby, 2010; Celik *et al.*, 2011).

2.5.1. Environmental manipulation:

Tilapia is a thermo-sensitive species and it's male to female ratio increases with temperature. Temperature treatments must be applied at a critical sensitive period, relatively similar to the hormone sensitive period. Excess high and low temperature also reduces the success rate of sex reversal (Baroiller and D Cotta, 2001). Temperature of the culture environment has been shown to affect sex ratios of some fish, tilapia inclusive. The change in sex of tilapia from female to male increase with high temperature while ovarian differentiation is induced by low temperatures (Baroiller and D Cotta, 2001).

Gonad differentiation of progenies of some fish species can be directed toward testicular differentiation by high temperature treatment applied at 10 days post fertilization (Tessema *et al.*, 2006). Increased water temperature during sex differentiation favours testicular development hence a greater male population with the aid of hormonal activity (Rahma, 2015).

2.5.2. Manual segregation:

The sexing of small Tilapias, although feasible, is tedious and not entirely reliable (Hickling, 1963). While it is an easy technique, it is extremely laborious and human accuracy varies from 80 to 90%, which leads to the presence of females in the pond. Therefore, this method is rarely used (Penman and McAndrew, 2000).

2.5.3. Genetical manipulation:

Research in the 1960s revealed that certain interspecific crosses in tilapia produced all or nearly all male progeny, leading to a number of follow-up studies and the eventual commercial production of some hybrids. *(Wohlfarth and Hulata, 1991;Trombka and Avtalion, 1993)*

Research studies in tilapia have demonstrated that sex determination is predominantly monofactorial, similar to that in humans. In the two important cultured species *O. niloticus* and *O. mossambicus* the female has the homogametic genotype XX,

and the male is heterogametic XY. It has been demonstrated that a breeding programme combining hormonal feminization and progeny testing, can result in the production of novel YY male genotypes, which sire all- or nearly all-male progeny (Varadaraj and Pandian, 1989; Mair *et al.*,1995).

2.5.4. Hybridization:

Research in the 1960s revealed that certain interspecific crosses in tilapia produced all or nearly all male progeny, leading to a number of follow-up studies and the eventual commercial production of some hybrids. (Wohlfarth and Hulata, 1991;Trombka and Avtalion, 1993)

Crosses (female x male)	**Result**
O. niloticus × *O. variabilis* *O. nigra* × *O. urolepis hornorum* *O. vulcani* × *O. urolepis hornorum* *O. vulcani* ×*O. aureus*	98 to 100% (Fishelson, 1962; Pruginin, 1967; Pruginin *et al.*, 1975; Hsiao, 1980; Hulata *et al.*, 1983, 1993)
O. niloticus × *O. urolepis hornorum*	All-male progeny (Wohlfarth *et al.*, 1990)
O. niloticus × *O. aureus*	All-male progeny (Wohlfarth, 1994)

2.5.5. Hormonal sex reversal:

Oral administration of exogenous male sex steroid hormones before the differentiation of primal gonadal cells can cause reversal of phenotypic sex (Smith and Phelps, 2001; Bhandari *et al.*, 2006). Different steroids have been used over the years to induce sex reversal even if 17α-methyltestosterone is the most common (Pandian and Varadaraj, 1990) for *Oreochromis mossambicus*; 17α-ethynyltestosterone (Shelton *et al.*, 1981) with *O. aureus*; 17α-methylan-drostendiol (Varadaraj and Pandian , 1987) with *O. mossambicus*; mibolerone (Torrans *et al.*, 1988) with *O. aureus*; norethisterone acetate (Pandian and Varadaraj, 1990) with *O. mossambicus*; fluoxymesterone with *O. niloticus* (Phelps *et al.*, 1992); trenbolone acetate with *O.aureus* (Galvez *et al.*, 1996).

2.6. Effects of 17α-methyltestosterone on sex reversal of tilapia:

In an earlier study, almost 100% all-male monosex tilapia population was produced by treating 3 days old fry with a synthetic male hormone 17α-methyltestosterone (17α MT) at a treatment regime of 10 mg/ kg food for 30 days (Chakraborty *et al.*, 2007).

17α-methyltestosterone (17α MT) is a synthetic male hormone which closely mimics the naturally-produced hormone testosterone. The most common sex-reversal treatment involves giving a powdered fish feed to the first-feeding (and still sexually undifferentiated) tilapia fry. This diet contains 30–60 mg 17α MT/kg of feed until the 25–60th days post hatching (Macintosh and Little, 1995).

Macintosh (2008) reported that the legal status of methyltestosterone (MT) hormone use in aquaculture may vary from country to country, the main conclusions reached from the available scientific evidence is that MT treatment of tilapia carries no human health risks, provided it is applied only during the early fry stage, at the recommended dosage.

Homklin *et al.*,(2012) isolated, identified and characterized MT degrading bacteria in the sediment and water from a masculinizing pond of Nile tilapia fry and they reported that androgenic activity of MT degraded (using betagalactosidase assay) by all strains to the products with no androgenic potency.

Based on the phylogeny, physiological properties and cell morphology, the three isolated MT degrading bacteria were related closely to Rhodococcus equi, Nocardioides aromaticivorans, and Nocardioides nitrophenolicus (Homklin *et al.*,2012). Vera-cruz and Mair (1994) evaluated the effect of dietary administration of the androgen MT on the growth and survival of *O. niloticus* fry. They found that the androgen had no significant effect on growth and survival of fry during the treatment period and produced a mean sex ratio of 98.4% male in ponds and 95.4% in hapas.

2.7. Dosage & treatment:

Nile tilapia and the other *Oreochromis* species that dominate commercial tilapia farming are mouth brooders. After their eggs are released and fertilized, the female brood fish carry the eggs orally until they develop into fry (Macintosh and Little, 1995).

Treatment with 17α MT should begin from the second or third day after the fry are released from maternal care. However, other studies recommended the treatment to start from the seventh day post hatching until 30th day (Nakamura and Iwahashi, 1982).

The higher values of weight of the fish 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 after 15, 35 and 75 days of the treatment can be attributed to the anabolic effect of 17α MT (Jo *et al.*, 1995).

According to Greisy and Gamal (2012), the best dosage was 60 mg/ kg of 17α MT among the different dosage of 40, 60 and 80 mg of 17α MT/kg of feed on sex reversal of *Oreochromis niloticus* fry. It revealed that the higher values of mean length, weight and survival rates were recorded in fish treated with 60 mg of 17α MT/kg of feed.

Greisy and Gamal (2012) sprayed the hormone dissolving in 50 ml of 95% ethyl alcohol and mixed well in fine granules of feed. Glycerine was added at 0.5% / kg by volume to render the harmful effect of the alcohol. The mixture of feed has been completely dried at room temperature and then sealed in air tight black container and stored in refrigerators until use to retard bacterial or fungal contamination (Celik*et al.*, 2011).

Romerio *et al.*, (2000) used different dose rates of 17α-methyltestosterone in two diets having 40% crude protein for a total of 9600 tilapia fries. Seven days post hatching fry received the MT orally mixed in diets for 45 days. The result of chi-square test of the frequency data of males and females after the treatment and of analysis of histological and macroscopic characteristics showed that the numbers of males obtained by all the MT treatments was higher than the control groups and the dose of 60 mg MT/kg of diet were more efficient, resulting in 98% of males.

Mainardes-Pinto *et al.*, (2000) were compared the efficiency of 2 diets: 1 (NUTRAVIT) and 2 (IP), both with 40% of crude Protein, containing the synthetic androgen hormone MT and were analysed the most effective dose of this hormone on the sex reversal of Nile tilapia (O. niloticus). They showed that the number of males obtained by A (30 mg MT/kg diet 1), B (60 mg MT/kg diet 1), and C (30 mg MT/kg diet 1) and D (60 mg MT/kg diet 2) treatments were higher than the controls groups and the dose of 60 mg MT/kg of diet as for the diets 1 and 2 was more efficient resulting in 98% of males.

Smith and Phelps (2001) studied the sex reversal and growth of tilapia as influenced by seven feed storage regimes, using 17α-methyltestosterone for 28 days. There was no significant difference among the stored MT treated diets and ability to sex-reverses the fry. They got 99-100% male populations under all the treatments. They also reported a non-significant difference in fry growth survival or feed conversion ratio of different treatments.

Ferdous (2011) was designed with various doses of hormone to find out the most effective one. The treatments were designated as 10 mg MT/kg, 40 mg MT/kg, 50 mg MT/kg, 60 mg MT/kg and 70 mg MT/kg using nursery feed and ethanol for 28 days in hapa. She carried out MT receiving treatments showed a significantly higher male proportion (94.84%) at dose of 60 mg MT/kg of feed resulted in maximum production.

Greisy and Gamal (2012) characterized the diets containing 17α MT as follows:

Diet (1): Control (untreated).

Diet (2): control diet + 40 mg of 17α MT/kg of diet.

Diet (3): control diet + 60 mg of 17α MT/kg of diet.

Diet (4): control diet + 80 mg of 17α MT/kg of diet.

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

Sex reversal of newly hatched tilapia generally is accomplished via oral administration of 17-methyltestosterone (MT), which has been incorporated into a starter fish feed at 60 mg MT/kg feed (Popma and Green, 1990). Although the use of the 60 mg MT/kg feed dose consistently yields populations comprised of less than 5% females (i.e., > 95% males), this has not been shown to be the optimal dose.

Different doses of 17 α– methyltestosterone hormone (MT) used as a growth promoter was administrated to Nile tilapia; *Oreochromis niloticus* (L.) in fishmeal based pelleted diet for 90 days (Ahmad *et al.*, 2002).

Rizkalla *et al.*, (2004) found that whole body samples of normal fish and those treated for 28 days with 17 α-methyltestosterone (17 α-MT) contained detectable amounts of testosterone only in the first five months after the termination of feeding.

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 (3ng/g).

2.8. Stocking:

Different authors had found limited effect of stocking density on fish survival and demonstrated that cannibalism could be a main cause of tilapia fry mortality at high stocking densities (El-Sayed 2002).

Chakraborty and Banerjee (2012) stated that a) The hormone treated males show higher growth rates than their control counterparts, b) Additional advantages of larger fish and higher yields are gained through culture of such sex reversed fish, c) Tilapia shows poor growth potential at very high and low stocking densities, d) Culture of tilapia at density of 20 fish/m^3 shows the highest growth among all density classes. Thus, this study enables us to postulate an optimum stocking density level of tilapia for maximum utilization of food and space with minimum stress and energy expenditure resulting in higher growth potential of the fish.

It was found that the highest weight, length, daily weight gain, growth rate and protein content were observed for the 20000 fish/ha density class. Thus, culture of monosex tilapia at a density of 20000 fish/ha can be considered ideal for augmented production of the fish under Indian context.

2.9. Feeding:

Mono sex tilapia were fed twice daily with an artificial diet (Uno Feeds, India) containing 32% crude protein at a rate of 10% body weight day-1 for the first two months and 5% body weight day^{-1} for the rest three months.(Chakraborty *et al.*, 2011).

The better growth of fish in pond culture system compared to the other three culture methods can be facilitated by the additional availability of relatively energy-rich natural food materials that may confer an energetic advantage for increased growth (El-Sayed, 2002, Bwanika *et al.*, 2007).

2.10. Culture management:

The better growth of fish in pond culture system compared to the other three culture methods can be facilitated by the additional availability of relatively energy-rich natural food materials that may confer an energetic advantage for increased growth (El-Sayed, 2002, Bwanika *et al.*, 2007).

2.11. 17 α– Methyltestosterone in Hormone-Treated Feed Used as Growth promoter:

Ahmad *et al.*, (2002) reported that 17α MT (at the dose of 5 mg kg^{-1}) promotes total weight gain and SGR of Nile tilapia. So it is clear that the 17α MT has definite role in the enhancement of the somatic growth in a unit time.

Shepherd et al. (1997) suggested that the growth-promoting actions of 17α MT in tilapia were linked to elevations in growth hormone (GH) metabolism and consequently to insulin-like growth factors (IGFs).

Several studies are in agreement that testosterone produces muscle hypertrophy by increasing muscle protein synthesis (Bhasin *et al.*, 2001).

2.12. Water quality parameters:

Bahnasawy (2009) recorded the water quality parameters for mono-sex Nile tilapia (*Oreochromis niloticus*) reared in fertilized tank. He found temperature ranging from 18 to 32 °C dissolved oxygen 5.4 to 7.8 mg l^{-1} and pH 6.8 to 7.9.

Swann (2009) described the suitable ranges of water quality parameters for aquaculture. Water temperature suitable for warm water species would be 24 to 32°C, dissolved oxygen content of water would be between 5 mg l^{-1} and saturation level, pH would be 6.5 to 9.0, alkalinity would be at least 20 mg l^{-1} for recirculation system, nitrite-nitrogen would be 0.03 to 0.06 mg l^{-1} and nitrate-nitrogen would be 0.0 to 3.0 mg l^{-1}.

Different physicochemical parameters of water like temperature, DO, free CO_2, transparency and pH are generally considered to have primary importance in fish culture. The optimum temperature for tilapia culture is reported to be 20- 30°C or above (Islam *et al.*, 2006).

The ideal DO level for tilapia culture is 4-5 mg l^{-1} and the present study showed higher DO values for all the density class ponds attributing good environment for tilapia culture. Free CO_2 is another factor that negatively affects feed intake and therefore fish growth. But, Nile tilapia can tolerate CO_2 concentration above 20 mg l^{-1} and is unlikely to have an adverse effect on fish in intensive culture systems unless free CO_2 concentration reaches 100 mg l^{-1} (Tran-Duy *et al.*, 2008).

Chapter 3

Materials and Methods

3. Seed production of mono sex tilapia:

3.1 Selection of sites for experiment:

It is very important to select appropriate site for the experiment because the site should be properly be fitted with the desired objective of the experiment. As the experiment was on the production of all male seeds from a tilapia seed production unit (Hatchery) and observation on the growth performances in the farmer's field, the location of the hatchery and the monosex culture ponds were selected in a suitable area.

Shubhas Gram (S-24 Parganas, West Bengal) was selected as experimental site, 7-8 kms away from fishery faculty campus. The area has vast potentiality in aquaculture with numerous seasonal and perennial ponds and hatcheries.

3.2. Selection of hatchery and potential farmers:

A well reputed hatchery at Subhas Gram was selected for conducting the experiment. The hatchery facilitated with all scientific facilities *viz.* water and soil analysis laboratory, breeding tanks, nursery tanks, effluent treatment tanks, quarantine tanks, aeration unit, oxygen packaging unit, water treatment plant, water supply facility, generator, skilled manpower and good drainage system. Nine progressive fish farmers were selected (Table-1) for field trial of monosex tilapia in ponds. Monosex seeds were generated and distributed from the hatchery for evaluation of growth performance in different ponds.

3.3. Selection of brood raising ponds:

The brood raising pond (0.09 hectares) is situated in the hatchery premises with a well-constructed dikes and inlet-outlet system. Facility like deep tube well for watering the pond was installed.

3.4. Preparation of brood raising ponds:

As the brood rearing pond was a perennial in nature, common pond preparation practices was followed viz. eradication of unwanted fishes by applying Mohua oil cake @

250kg/ha and aquatic weeds by Taficide-80 @ of 5-8kg/ha with 0.1% detergent. The pond was then left for two weeks to remove the toxicity of mohua oil cake.

Agricultural lime was applied @ 250kg/ha to adjust the pond pH near about neutral (7.0). Raw cow dung was also applied @ 5000kg/ha to enhance the productivity of the pond. The brood pond became ready for stocking of brood fish after 7 days of fertilization.

3.5. Collection of fishes to be raised as brood stocks:

A number of healthy Nile tilapia species of both sexes were collected from a pond of a reputed tilapia farmer. The fishes were introduced in the brood pond after quarantine. The brood fish consist of 50 male fish with 100 female fish in the ratio of 1:2. The weights of the brood fishes were about 250 g.

3.6. Brood fish management:

Water quality of brood raising pond was analysed fortnightly and brood fish were reared for a period of 30 days. The water quality was maintained hygienically to avoid disease. Formulated feed enriched with protein and vitamin E enhances the gonad maturation in tilapias. Feed was applied @ 4 % of the biomass of tilapia. Total 150 brood fish (50 male and 100 female) having average body weight of 250 g i.e. 37.5kg fish were stocked in the brood fish pond. Daily ration requirement was 1.5kg which was applied twice in a day at 9:00 am and 04:00 pm respectively.

3.7. Selection of the breeding period:

As the main objective of the experiment was to produce mono sex seeds of tilapia, so it was important to determine the ideal time of the year for breeding. Spawning frequency of tilapia is 10-12 batch/year (Philippart and Ruwet, 1982), though temperature plays a vital role in production of all male population. Tilapia is a thermo-sensitive species and it's male to female ratio increases with temperature. The early days of April were selected as seed production period because water temperature near 32°C induces better production of all male population. Excess high and low temperature also reduces the success rate of sex reversal (Baroiller and D'Cotta, 2001).

3.8. Preparation of feeds for fries:

The formulated feed (FCR) was collected from the market and grinded finely in a grinder. The hormone 17aMT@ 60mg per kg feed was dissolved in 50 ml of 95% ethyl alcohol and mixed in the feed by spraying. The Glycerine was added @ 0.5%/kg of feed by volume to render the harmful effect of the alcohol. The mixture of feed had been completely dried at room temperature and then sealed in air tight black container and stored in refrigerators until use to retard bacterial or fungal contamination (Celik et al., 2011). The feed contains 35% of protein, 10% of lipid, 3% crude fibre, 10% moisture, carbohydrates, vitamins and minerals.

3.9. Breeding/ induced breeding:

3.9.1 Natural breeding:

The selected brood fishes were transferred to the hapa. One week before breeding, five hapas were arranged in brood pond and each contained 10 male and 20 female brooders (1:2). Since tilapia is mouth brooder, natural breeding was confirmed by randomly opening some mouth of female brooder.

3.9.2 Induced breeding:

Induced breeding was also done when reddish colour doesn't appear around the genital aperture of the females. Induced breeding was done by synthetic inducing hormone WOVA-FH at rate of 0.1ml/ 250 g of fish. The dosage of both male and female was same.

3.9.3. Egg collection:

One week before the breeding, hapas were arranged in the brood raising pond in such a way so that bottom of the hapa touches the pond bottom. Some earthen pots (4-5/hapa) were placed in tilted position as hide out of the mouth brooding females. All the flower pots were tied with thin nylon rope so that they could be pulled out easily from water to examine the readiness of the brood fishes. The prepared female cleans the inner portion the flower pots, a unique nature observed in the experiment.

When breeding activity advances female takes their fertilized eggs in their mouth and broods for hatching. To avoid heterogeneous growth of larvae all mouth brooded

females were collected from the hapa and eggs were collected carefully and incubated in glass jar hatchery otherwise the larvae in the hapa may be of different age group and may start taking natural food from fish pond.

It is very cumbersome job to collect eggs from the mouth of the females as they quickly engulf their eggs if any disturbance occurs in the system or if they get any type of stress.

In this experiment, it was observed that the collection of egg was most appropriate during night time. The whole hapa, containing the mouth brooded female were lifted out of the water quickly and the eggs were collected.

It was also seen that they could not able to engulf the fertilized eggs when lifted quickly out of water. The fertilized eggs were then transferred to well sanitize glass jar hatchery. Out of 50 female tilapias, 40 spawned and 35000 eggs (approx.) were collected.

3.9.4 Egg incubation:

The fertilized eggs in the glass jar hatchery started hatching after 3 days of transfer. Water flow along with aeration through air pipe was facilitated in the glass jars from the bottom which led to continuous movement of the eggs inside the jar. The glass jar hatchery was placed in a trough for collection of hatchlings.

The hatchlings after absorption of their yolk-sac left the glass jars with the water flow and were collected in the trough. The hatchlings were reared in the trough for a month in flow through system.

3.9.5 Administration of hormones:

3.5ft x 1.5ft x 1.5ft troughs were employed for rearing the hatchlings. About 10,000 hatchlings were placed in each sex reversal trough (SRT). Hatchlings were fed with 17 alpha-methyl testosterone @ 60 mg / kg mixed with powdered feed immediately after absorption of yolk sac. Desirable concentration of dissolved oxygen (6-8 mg l^{-1}) and gentle water flow was maintained throughout the experimental period (30 days). Cleanness and hygienic condition in troughs was strictly maintained to avoid infection. Hatchlings were fed hormone fortified feed @ 30 %, 20 %, 18 % and 12 % of their body mass for first, second, third and fourth week respectively.

3.9.6 Distribution of seeds:

After one month rearing in SRTs, the monosex fingerlings (0.20 g, 0.97cm) were distributed to fish farmers of which nine of them were selected.

3.2. Observation of growth performances:

3.2.1 Pre stocking management of the culture ponds (Table 2):

3.2.1.1 Eradication and control of aquatic weeds and algae:

The aquatic weeds are removed manually but in 3 cases weeds were controlled by chemical treatment. Taficide-80 @ of 5-8kg/ha with 0.1% detergent was applied.

3.2.1.2 Eradication of unwanted fish:

All unwanted fishes in the pond were removed by application of Mohua oil cake (*Basia latifolia*) @ 250 mg l^{-1}.

3.2.1.3 Liming:

Lime was applied @ 250kg/ ha in culture ponds.

3.2.1.4 Manuring:

Organic manure like cow dung was applied @ 5000kg/ha in all culture ponds. A standard fertilizer dose of 60 kg/ha of superphosphate (11kg/ha of P_2O_5) and 60kg/ha of ammonium sulphate (13kg/ha of N) was periodically applied to give maximum concentration of nitrogen and phosphorus.

3.2.1.5 Stocking of seeds:

After 7 days of manuring, all-male seeds are stocked in different stocking density. Three different stocking density *viz.* @ 20,000, 30,000 and 40,000 individuals/ha were selected. Each stocking density was supported by triplicate. So, total nine ponds of different socking densities were taken under observation.

3.2.2 Post stocking management of the culture ponds (Table 3):

3.2.2.1 Feeding:

In farmer's ponds fish were fed twice daily (morning and afternoon) with an artificial diet (Grobest Feeds, India) having 32% crude protein @ 10% body weight /day for the first two months and @ 5% body weight/ day for the rest one month.

3.2.2.2 Netting:

Netting was done twice a month, to remove obnoxious gases, better growth performance and for sampling.

3.2.2.3 Health management:

Periodical health management like liming in pond, application of $KMnO_4$ etc. was applied as preventive measure against diseases.

3.2.2.4 Checking of sex:

After two months of culture fishes were sexed by gonad squashing and aceto-carmine staining method (Guerrero and Shelton, 1974). The fish was killed and then viscera was removed to reveal the two thread like gonads lying along the surface of the body cavity on either side of the kidney. The gonads were removed and placed on a clean glass slide. A few drops of aceto-carmine stain were added and the gonad squashed with a cover slip. The male sex of the fish was identified by examining the slide under a microscope. No female fish was found during examination.

3.2.2.5 Sampling of the mono sex tilapia for growth performances:

Experimental ponds were regularly netted, either by drag net or caste net and 10 samples were collected randomly from each pond for experimental purposes. Length (in centimetre) and weight (in g) of the fishes were taken by using measuring scale and digital balance.

3.2.2.6 Sampling of the physical and chemical parameters of water and soil:

Water and soil samples were collected from each experimental pond in 15 days interval. Water and soil quality parameters like Temperature, pH, D.O., free CO_2 total alkalinity,

hardness, nitrate nitrogen, orthophosphate, soil available phosphorus and soil organic carbon were tested in laboratory of the hatchery and faculty.

3.3. Water and Soil collection and analysis:

3.3.1 Collection of water samples:

Water samples were collected at 15 days interval from each of the tanks at a fixed hour of the day (9:00 a.m.). During collection of water samples, cautions were taken so as to prevent air bubbling, which might influence water parameters such as dissolved oxygen.

3.3.2 Collection of soil samples:

Soil samples from were collected from three different places of the culture ponds and the sample then mixed together, air dried, pulverized with pestle and mortar and sieved through 150 μm mesh sieve and proceed for analysis.

3.3.3 Water quality analysis:

3.3.3.1Temperature:

The water temperature was measured using a centigrade thermometer on spot and expressed as ^{o}C.

3.3.3.2 pH:

pH of water samples was estimated by a digital pH meter at the site.

3.3.3.3 Dissolved oxygen:

For estimation of dissolved oxygen content of water, the samples were collected with all necessary precautions. Winkler's method was followed for the same (APHA, 1995).

3.3.3.4 Total Alkalinity:

Carbonate alkalinity of water samples were analysed by titrating the samples against N/50 H_2SO_4 using phenolphthalein as indicator. Bicarbonate alkalinity was determined against N/50 H_2SO_4 using methyl orange indicator (APHA, 1995).

3.3.3.5 Total hardness:

Total hardness of water samples was measured on the sampling day by titrating the samples against EDTA (Ethylene Di-amine Tetra Acetic acid) after adding ammonia buffer and Eriochrome Black T (APHA, 1995) as indicator.

3.3.3.6 Orthophosphate:

The orthophosphate level of water was determined colorimetrically through a double beam UV-vis-Spectrophotometer (CECIL CE-4002) at 690 nm wavelengths following the stannous chloride method (APHA, 1995).

3.3.4 Soil quality analysis:

3.3.4.1 pH

The pH was determined with a digital pH meter (Systronics-VI) using 1:2 suspensions of soil and water (APHA, 1995).

3.3.4.2 Organic carbon:

For estimation of organic carbon, air-dried powdered sediment sample (1 g) was digested with 1 N $K_2Cr_2O_7$ (10 ml) and concentrated H_2SO_4 (20 ml) and kept for 30 minutes at dark. The digested sample was then diluted with 200 ml distilled water and 10 ml ortho-phosphoric acid and 1 ml diphenyl amine indicator was added. It was the titrated against 1 N ferrous ammonium sulphate (Mohr's salt) until brilliant green colour appeared (Walkey and Black, 1934).

3.3.4.3 Soil available P:

Soil available P was determined using 1:20 soil to Olsen's extractant (0.5 $NaHCO_3$ adjusted to pH 8.5) (Olsen *et al.*, 1954) followed by Dickman and Bray's (1940) Chlorostannous reduced molybdophosphoric blue colour method in hypochloric acid system as described by Jackson (1967).

3.3.5. Fish growth:

Fish growth was recorded at 15 days intervals from each pond. Length and weights of the fishes were recorded for estimation of Average weight gain, Daily weight gain (DWG) and Specific growth rate (SGR).

The following estimates were done as:

Average length gain = (Final length - Initial length)

Average weight gain = (Final weight - Initial weight)

Average Daily Weight Gain = (Mean Final Wt. – Mean Initial Wt.)/T_2-T_1

Body weight gain (%) = Final wt. – Initial wt. / Initial wt. x 100

Specific growth rate (SGR) = 100 x [ln. (W_t)– ln. (W_O)/ T_2-T_1]

3.4. Statistical analysis:

All the results were subjected to statistical analysis. One way analysis of variance (ANOVA) was performed to test the significance of variation among the treatment. Statistical tests were performed by computer based statistical software SPSS (Statistical Package for Social Science) programme and MS Excel Programme.

Plate I. Arrangement of hapa in brood-stock pond

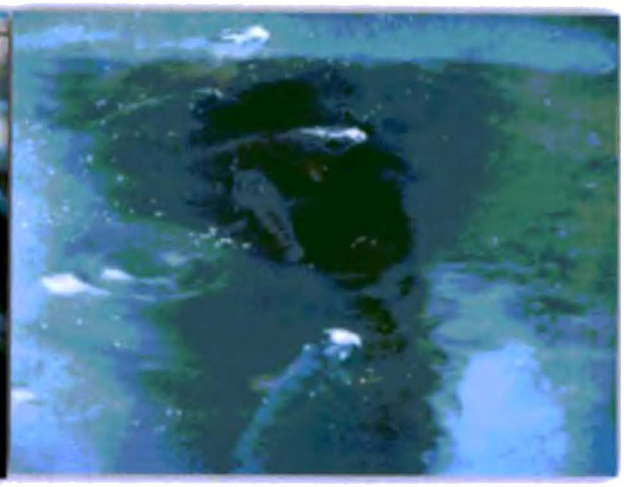


Plate II. Brood fishes in the hapa kept for breeding.

Plate III. Selected brood fishes for breeding.

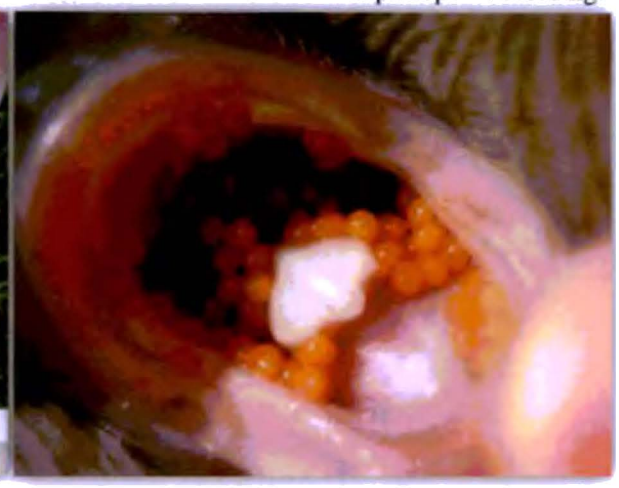


Plate IV. Egg mass in mouth of a mouth brooding female.

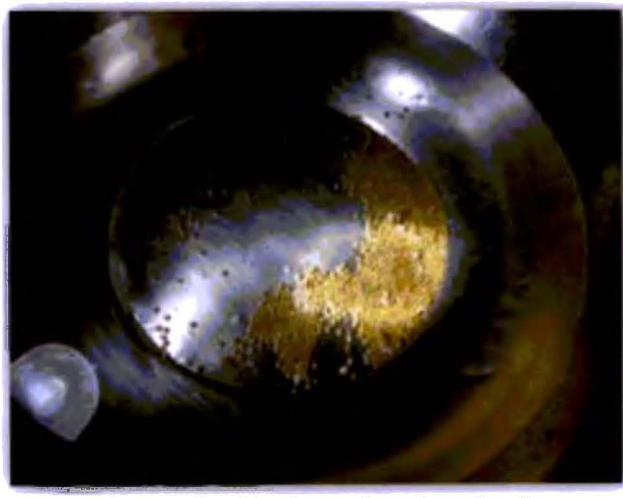


Plate V. Fertilized eggs in a clean bowl.

Plate VI. Incubation in glass pot hatchery with aeration.

Plate VII. Hatchlings placed in sex reversal troughs.

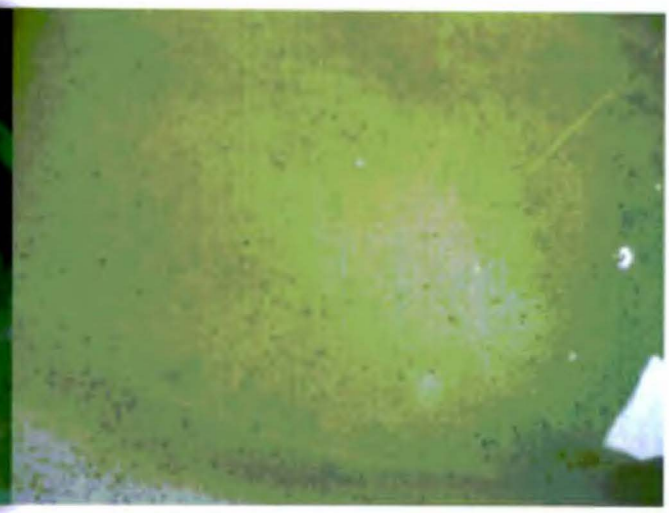


Plate VIII. Hatchlings of tilapia before yolk absorption.

Plate IX. Formulated feeds fortified with 17α MT.

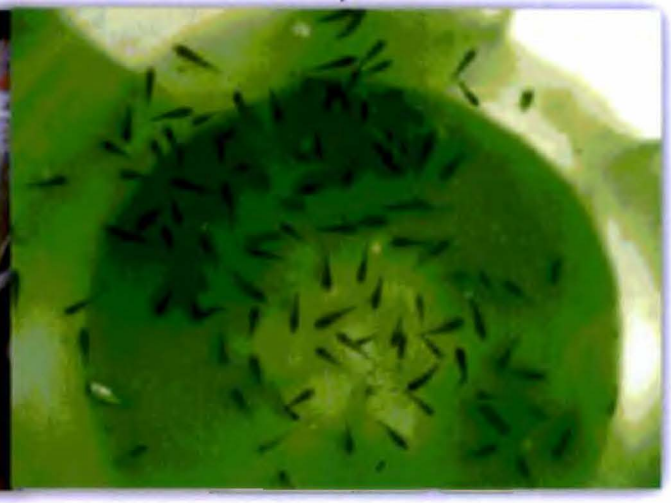


Plate X. 17α MT treated tilapia fries.

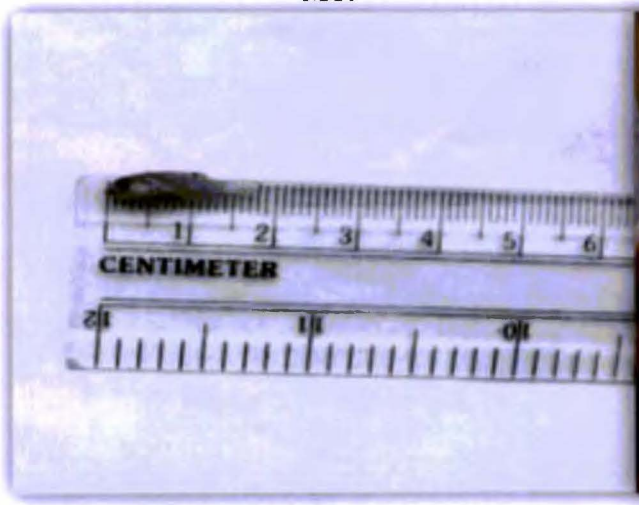


Plate XI. Initial length measurement of 17α MT treated tilapia fries.

Plate XII. Weight measurement of 17α MT treated tilapia seed by electronic balance.

Plate XIII. Length measurement of 17α MT treated tilapia seed by measuring scale.

Plate XIV. Eradication of aquatic weeds in culture ponds.

Plate XV. Prepared culture pond before stocking

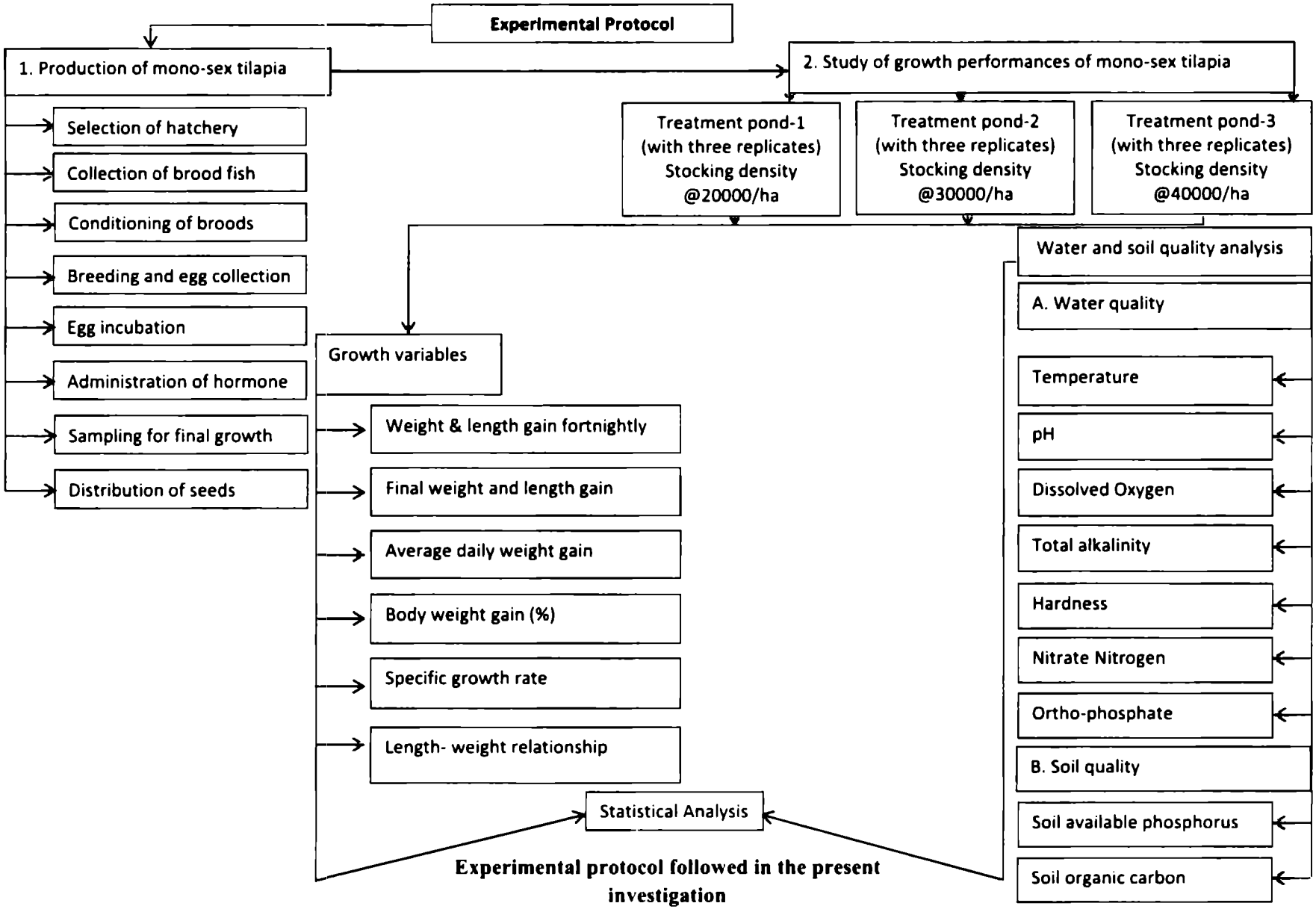
Plate XVI. Application of feeds in culture ponds.

Plate XVII. Collection of fish by cast net for periodic sampling

Plate XVIII. Length measurement of 17α MT treated mature tilapia.

Plate XIX. Fish samples in 90th day of sampling.

XX. one year old monosex tilapia



Experimental protocol followed in the present investigation

Chapter 4

Results and Discussion

4.1. Result:

Brood fish of tilapia (both sexes) were raised in the hatching complex of the selected hatchery. Special care was taken for proper nourishment of the brood fish. No mortality was observed during the culture period. Fish were healthy, brightly coloured and active in behaviour. Genital papilla of the female was examined in the month of April and reddening of genital papilla indicated readiness of the female. Water temperature at the period was 32°C which was ideal for breeding. Water quality parameters of brood fish pond was analysed fortnightly for a period of 30 days.

The mean values (range) of water quality parameters of brood raising pond.

Temperature	pH	D.O.	Free CO_2	Alkalinity	Hardness	Ortho-Phosphate	NO_3-N
30-32°C	6.5-7.5	6-8 ppm	0.05- 1.5 ppm	50-60 ppm	250-280 ppm	2-3 ppm	0.4-0.5 ppm

Water and soil samples were collected fortnightly from nine experimental ponds for a period of 90 days culture. Growth parameters like weight and length of cultured fish and growth variables viz. body weight gain percentage, average daily weight gain, specific growth rate, length weight relationship were examined fortnightly.

4.1.1. Water quality:

All the nine experimental ponds were sampled for water and soil analysis fortnightly. The result obtained throughout the experimental period are mentioned bellow:

4.1.1.1. Temperature:

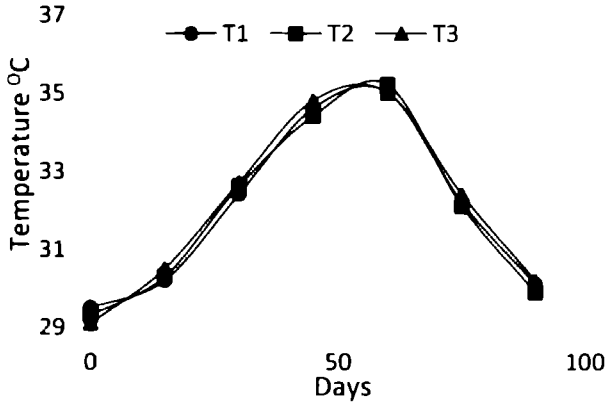


Fig-4.1.1: Temporal variation of temperature (^{0}C) in all ponds throughout the experiment.

Maximum mean temperature (35.2°C) was recorded in T_2 ponds on 60th day and minimum mean temperature (29.1°C) was recorded in T_3 on 1st day of sampling.

Temporal variation of water temperature in all ponds was analysed and it was observed that temperature increased gradually up to 60th day of sampling and then the value decreased due to rain fall. At the end of the experiment, the temperature in all ponds ranged between 29.9-30.2°C.

4.1.1.2.pH:

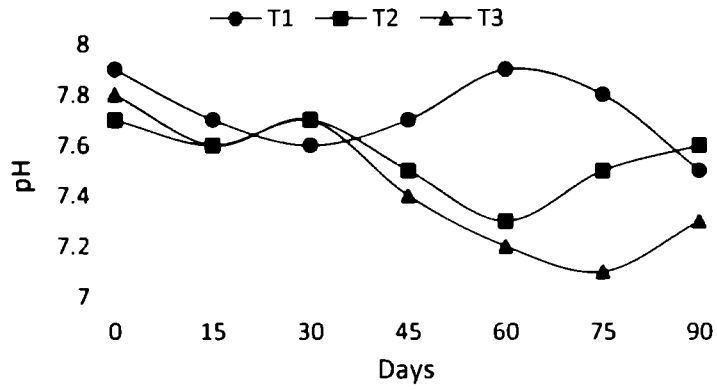


Fig-4.1.2: Temporal variation of water pH in all ponds throughout the Sampling period

The pH value in T_1 ponds decreased gradually up to 30th day of sampling and it exhibited increasing trend and reached to the value of 7.8 on the 60th day. Afterward the value decreased and exhibited minimum value on the 90th day of sampling.

In T_2 the highest value was 7.7 recorded on 30th day and then it decreased to its lowest value (7.3) on the 60th day. The value again increased and attained the value of 7.6 on 90th day of sampling.

In T_3 ponds the pH value decreased to 7.6 on 15th day from its initial value 7.8 on day 01. Afterwards the value increased on the 30th day and then decreased drastically till 75th day of sampling (7.1). The value again increased and touched (7.3) on 90th day of sampling.

One Way Analysis of Variance (ANOVA) revealed that the difference among the ponds was highly significant ($F_{2, 12} = 4.68$; $P < 0.05$).

4.1.1.3. D. O.

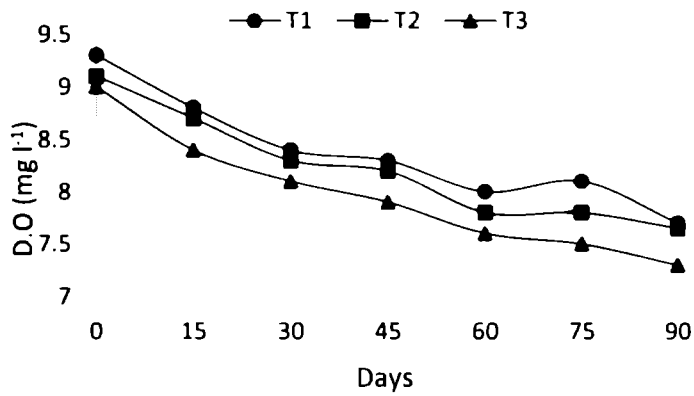


Fig-4.1.3: Temporal variation of dissolved oxygen (mg l^{-1}) in all ponds throughout the experiment.

In case of T_1 ponds the dissolved oxygen (D.O) of water showed a declined trend from the 1st day to 60th day (9.3 to 8.0 mg l^{-1}) of sampling except the 45th day and gradually it increased on 75 day and exhibited decreased trend on 90th day of sampling.

A declining trend of D.O was observed in T_2 pond from day 01 to 60th day (9.1 to 7.8 mg l^{-1}) of sampling except 45th day (8.2 mg l^{-1}). Later the value slightly increased on 75th day (8.2 mg l^{-1}) and again declined (7.65 mg l^{-1}) on 90th day of sampling.

The T_3 exhibited a smooth declined trend of D.O level throughout the entire culture period. The maximum and minimum values of D.O recorded in T_3 ponds were 9.0 mg l^{-1} and 7.3 mg l^{-1} on 1st and 90th day of sampling respectively.

The values were estimated through One Way Analysis of Variance (ANOVA) and observed that difference among the D.O. concentrations in all ponds were significant ($F_{2, 12} = 68.6$; $P < 0.001$).

4.1.1. 4. Free CO_2:

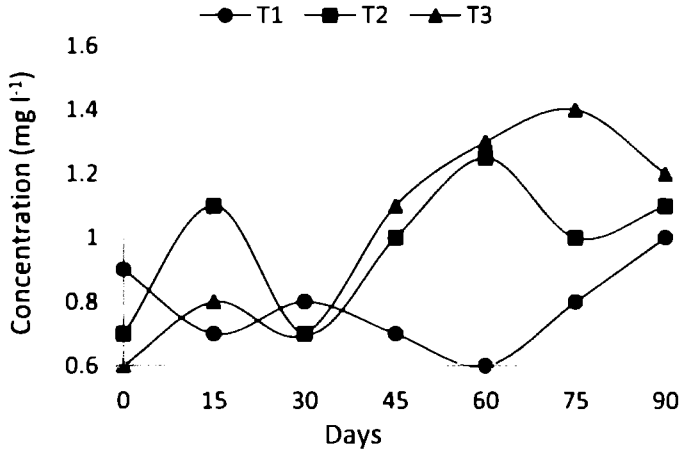


Fig-4.1.4: Temporal variation of free CO_2 of water (mg l^{-1})in all ponds throughout the experiment.

In T_1 ponds the value of free CO_2 declined on 15th day and increased on 30th day of sampling and again exhibited declined trend till 60th day of sampling which was recorded as minimum (0.6 mgl^{-1}) value. After 60th day, the value of free CO_2 again increased till 90th day of sampling.

Free CO_2 of water in T_2 ponds increased on 15th day of sampling and minimum value was recorded on 30th day of sampling. After 30th day, the free CO_2 value increased till 90th day of sampling except the 75th day.

Like T_2 ponds, the value of free CO_2 in T_3 increased on 15th day and then decreased on 30th day. Later the value showed an increasing trend up to the 75th day of sampling and then it decreased again on the 90th day of sampling. The values were estimated through One Way Analysis of Variance (ANOVA) and observed that difference among the free CO_2 concentrations in all ponds were insignificant ($F_{2, 12} = 2.41$).

4.1.1. 5. Total Alkalinity:

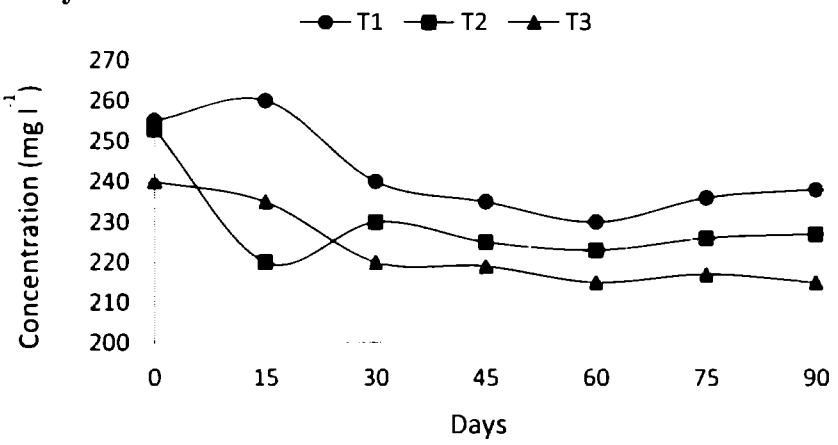


Fig-4.1.5: Temporal variation of total alkalinity (mg l^{-1}) in all ponds throughout the experiment

In T_1, minimum and maximum values of alkalinity were 260mg $l^{-1}$and 230mg l^{-1} throughout the experiment. Maximum value in T_1 was observed on 15th day after word the value decreased gradually till 60th day of sampling and again it exhibited higher value till the end of experiment. Mean minimum alkalinity in T_2 was 220 mg l^{-1} and maximum value was 253 mg l^{-1}. Alkalinity in T_2 exhibited lowest value (220 mg l^{-1}) on 15th day of sampling and later it increased slightly till end of experiment (227mg l^{-1}) except 60th day of sampling (223mg l^{-1}).

In T_3 minimum value was 215mg l^{-1} on 60th day of sampling and maximum value (240mg l^{-1}) was recorded on 1st day of sampling. After 30th day of culture the alkalinity value fluctuated every alternate day of sampling though the magnitude of fluctuation was small.

The values were estimated through One Way Analysis of Variance (ANOVA) and it was observed that total alkalinity in all ponds varied significantly ($F_{2, 12} = 15.13$; $P < 0.001$).

4.1.1. 6. Total Hardness:

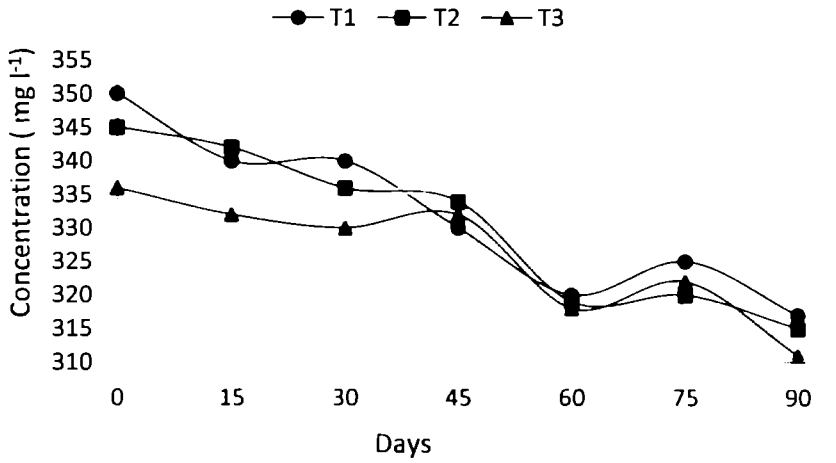


Fig-4.1.6: Temporal variation of hardness (mg l^{-1}) in all ponds throughout the experiment.

The total hardness value in T_1 ponds exhibited highest value (350 mg l^{-1}) on the 1st day of sampling and afterwards the value gradually declined till 60th day except the 30th day of sampling. Later the value increased up to 75th day and again it declined.

In T_2 ponds the value declined gradually from its highest value (345 mg l^{-1}) and reached its lowest (315 mg l^{-1}) value on the 90th day of experiment.

In T_3 highest value (336 mg l^{-1}) and 2nd highest value (332 mg l^{-1}) was recorded on 1st and 45th day of sampling and lowest value (311 mg l^{-1}) was observed on 90th day of sampling.

The values were estimated through One Way Analysis of Variance (ANOVA) and it was observed that difference among the total hardness in all ponds were significant($F_{2, 12} = 6.64$; $P < 0.05$).

4.1.1.7. NO_3-N:

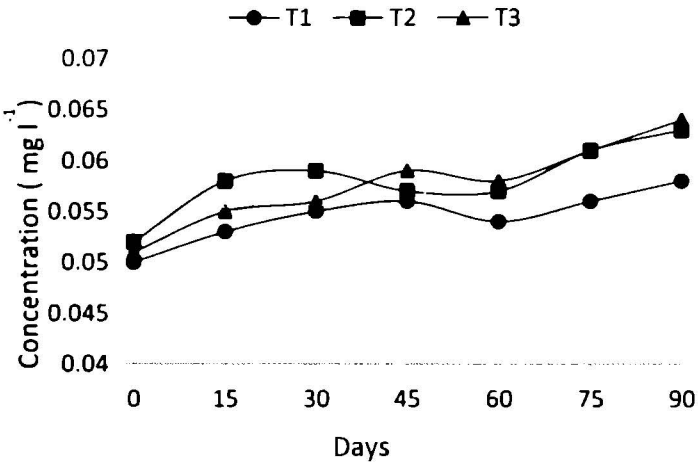


Fig-4.1.7: Temporal variation of nitrate-nitrogen (mg l^{-1}) in all ponds throughout the experiment

In T_1 ponds, the initial concentration of Nitrate nitrogen (NO_3-N) was 0.05 mg l^{-1} on the 1st day of sampling and gradually it increased up to 45th day (0.057 mg l^{-1}) and after that it was declined on 60th day (0.054 mg l^{-1}) but reached the highest value on 90th day (0.058mg l^{-1}) of sampling.

The T_2 ponds showed the initial concentration of 0.052 mg l^{-1} and slightly increased up to 30th day of sampling (0.059 mg l^{-1}) and reached the highest value on 90th days of sampling (0.064 mg l^{-1}).

In case of T_3 ponds, the initial concentration were found as 0.051mg l^{-1} (1st day) and after that the value increased (0.059 mg l^{-1}) up to 45th days of sampling except the 30th day (0.056 mg l^{-1}). The value of NO_3-N concentration decreased on 60th day (0.058 mg l^{-1}) but later the trend was higher (0.064 mg l^{-1}) till 90th day of sampling.

It was estimated through One Way Analysis of Variance (ANOVA) and it was observed that difference among the NO_3-N concentration in all ponds were significant($F_{2, 12} = 13.68$; $P < 0.001$).

4.1.1.8. Ortho-P:

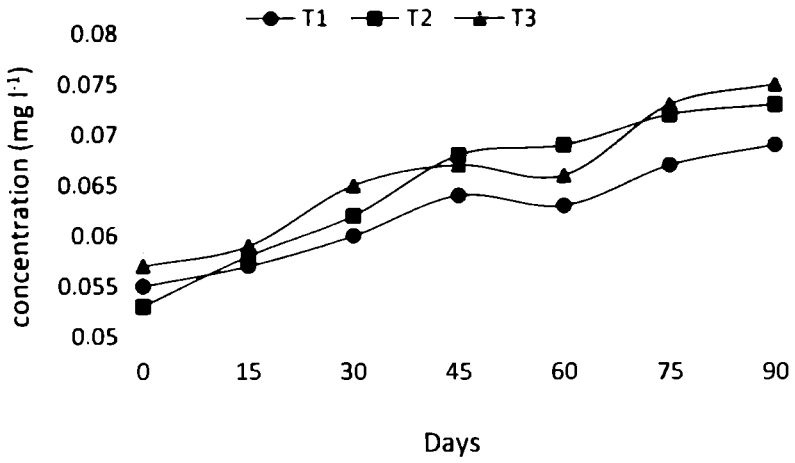


Fig-4.1.8: Temporal variation of Ortho-Phosphate (mg l^{-1}) in all ponds throughout the experiment.

The ortho phosphate value of water in T_1 ponds increased gradually througthout the entire culture period except the 60th day of sampling and in case of T_2 ponds the trend was upward till the end of experiment. In T_3 ponds the increasing trend continued till 90th day of sampling except 60th day.

It was estimated through One Way Analysis of Variance (ANOVA) and it was observed that difference among the Ortho-Phosphate level in all pond ponds were significant ($F_{2, 12} = 10.33$; $P < 0.01$).

4.1.1.9 Available soil P:

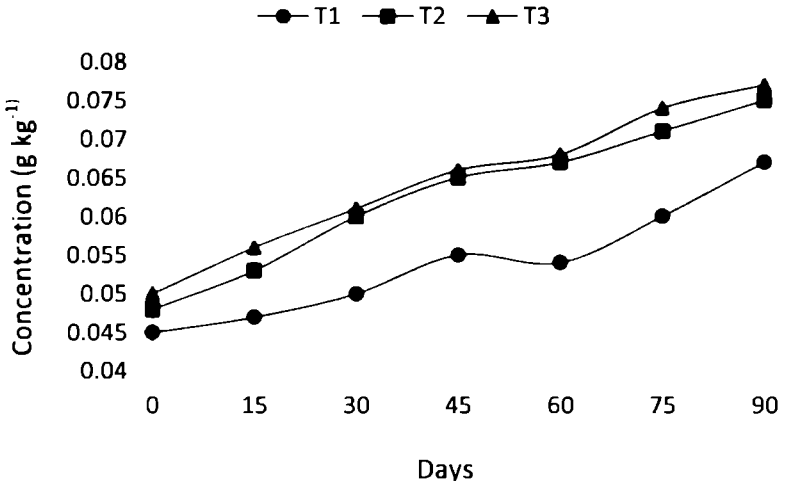


Fig-4.1.9: Temporal variation of available soil phosphorus (g kg^{-1}) in all ponds throughout the experiment.

Likewise orthophosphate, an increasing trend was also found in available soil phosphorous in all ponds throughout the experimental period. The average mean value of available phosphorus was highest in T_3 (0.077 g kg^{-1}) on 90th day and lowest in T_1 (0.045 g kg^{-1}) on day 01 sampling. In case of T_1 and T_3 ponds, the increasing trend of available soil phosphorus concentration continued till the end of experiment (90th day) except 60th day of sampling.

It was estimated through One Way Analysis of Variance (ANOVA) and it was observed that difference among the available soil phosphorus in all ponds were significant($F_{2, 12}$ = 61.75; $P < 0.001$).

4.1.1.10. Organic-carbon:

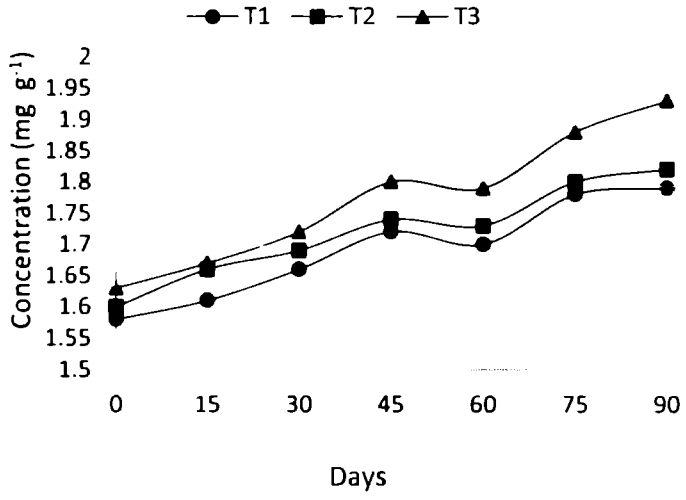


Fig-4.1.10: Temporal variation of soil organic carbon (mg g^{-1}) in all ponds throughout the experiment.

The concentration of organic carbon in T_1 and T_2 ponds exhibited an increasing trend, except on 60th day where the value decreased and later it again increased till 90th day of sampling. In T_3 ponds, the value of organic carbon exhibited increasing trend throughout the experiment except day 60 where it showed less value.

It was estimated through One Way Analysis of Variance (ANOVA) and it was observed that difference among the organic carbon level in all ponds were significant ($F_{2, 12}$ = 33.31; $P < 0.001$).

4.1.2.1.Mean final body weight:

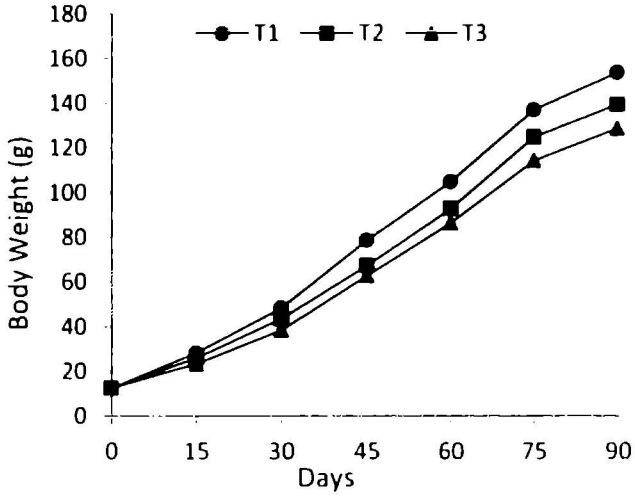


Fig-4.1.11: Mean body weight (g) of mono-sex tilapia in different sampling days

In T_1 ponds (stocking density @20,000/ha), fishes exhibited highest mean body weight (153.67 g) on 90th day of sampling. Likewise in T_2 ponds (stocking density @30,000/ha) fishes exhibited second highest mean body weight (139.34 g) on 90th day and in T_3 ponds (40,000/ha) fishes exhibited third highest mean body weight (128.56 g.) on same sampling day.

The T_1 ponds exhibited 10.28% and 19.53% more mean weight compared to T_2 and T_3 respectively on 90th day of sampling. The T_2 pond showed 9.32 % less mean weight than T_1 and 8.30 % more than T_3 ponds on same sampling day. The T_3 pond exhibited 16.33% and 7.66% less mean weight than T_1 and T_2 ponds respectively on 90th day of sampling.

The values were estimated through One Way Analysis of Variance (ANOVA) and it was observed that difference among the mean weight in all ponds were significant ($F_{2, 12} = 15.15$; $P < 0.001$).

4.1.2. 2.Mean weight gain:

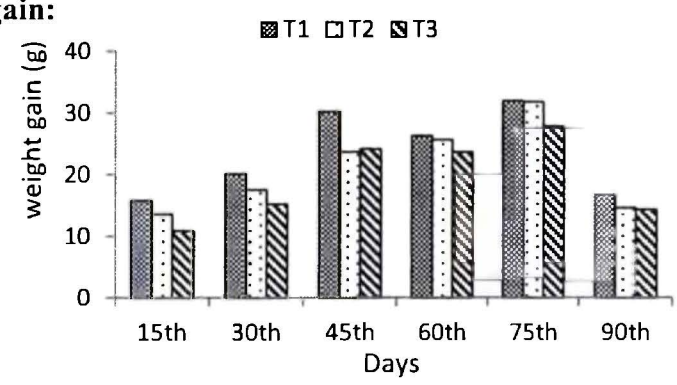


Fig-4.1.12: Mean weight gain (g) of mono-sex tilapia in different sampling days

In T_1 ponds (stocking @20,000/ha) the maximum weight gain (31.96 g) was observed on 75th day of sampling and minimum weight gain (15.78 g) on 15th day of sampling. In T_2 ponds (stocking @30,000/ha), it was observed that maximum (31.77 g) and minimum

(13.57 g) weight gain was observed on 75th day and 15th day of sampling. Likewise in T_3 ponds (stocking @40,000/ha), maximum (27.8 g) and minimum (10.89 g) weight gain was also showed on 75th and 15th day of sampling.

The values were estimated through One Way Analysis of Variance (ANOVA) and it was observed that difference among the mean weight gain in all ponds were significant ($F_{2, 12} = 16.49$; $P < 0.001$).

4.1.2.3. Average Daily Weight Gain:

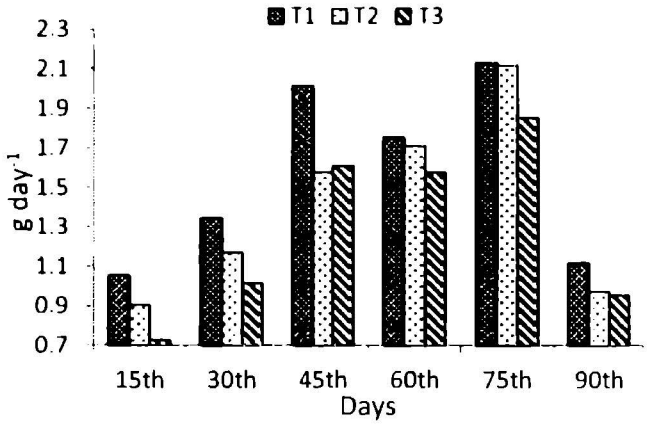


Fig-4.1.13: Average daily weight gain (g day^{-1}) of mono-sex tilapia in different sampling days

In case of T_1 ponds (stocking density @ 20000/ha) the average highest daily weight gain (2.13g/day) was observed on 75th day of sampling. And the average lowest daily weight gain (1.05 g/day) was observed on 15th day of sampling.

Likewise in T_2 ponds (stocking density @ 30000/ha) the average highest daily weight gain (2.1g/day) was observed on the 75th day of sampling and lowest average daily weight gain (0.90g/day) was recorded on 15th day of sampling.

Similarly in T_3 ponds (stocking density @ 40000/ha) the average highest daily weight gain (1.85g/day) was during 75th day of sampling. And the average lowest daily weight gain was 0.72 g/day on the 15th day of sampling.

It was estimated through One Way Analysis of Variance (ANOVA) and was observed that difference among the mean daily weight gain in all ponds were significant ($F_{2, 12} = 16.50$; $P < 0.001$).

4.1.2.4.Body weight gain (%):

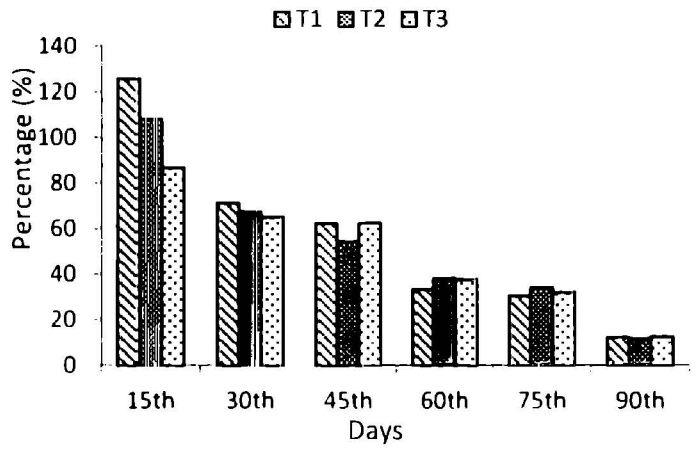


Fig-4.1.14: Body weight gain percentage (%) of mono-sex tilapia in different sampling days

In T_1 ponds (stocking@20000/ha), fishes exhibited the mean highest growth rate on 15th day of sampling (125.63%) and average lowest growth rate (12.23%) was observed on 90th day of sampling.

The mean highest growth rate in T_2 ponds (stocking @30000/ha) was 108.04% observed on 15th day of sampling and average lowest growth rate (11.69%) was observed on 90th day of sampling. And in T_3 ponds (stocking @40000/ha) fishes exhibited the mean highest growth rate 15th day of sampling (86.70%) and average lowest growth rate (12.52%) was observed on 90th day of sampling.

It was observed that, there was no significant (P>0.05) difference in the average growth rate of the monosex tilapia among all ponds.

4.1.2.5. Specific growth rate:

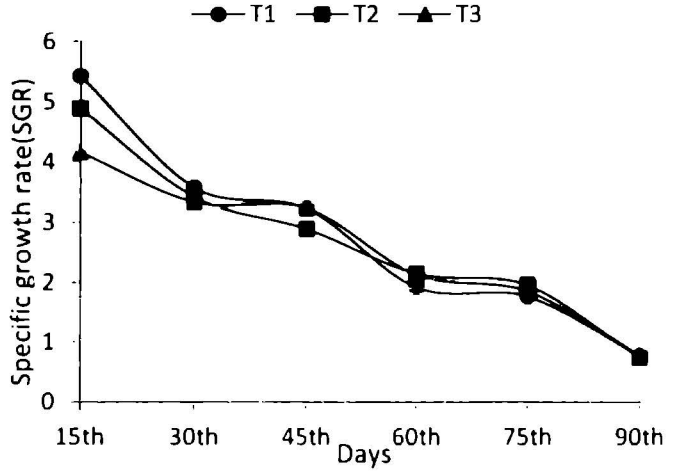


Fig-4.1.15: Specific growth rate (%) of mono-sex tilapia in different sampling days

In T_1 pond (stocking @20000/ha) fishes exhibited the mean highest specific growth rate on 15th day of sampling (5.42%) and average lowest growth rate (0.76%) was observed on 90th day of sampling.

The mean highest specific growth rate in T_2 pond (stocking @30000/ha) was 4.88 % observed on 15th day of sampling and average lowest growth rate (0.73%) was observed 90th day of sampling.

And in T_3 pond (stocking @40000/ha) fishes exhibited the mean highest growth rate on 15th day of sampling (4.16%) and average lowest growth rate (0.78%) was observed on 90th day of sampling.

It was estimated through One Way Analysis of Variance (ANOVA) and observed that, there were no significant ($P>0.05$) difference in the specific growth rate of in all ponds.

4.1.2.6. Mean total length (TL) gain:

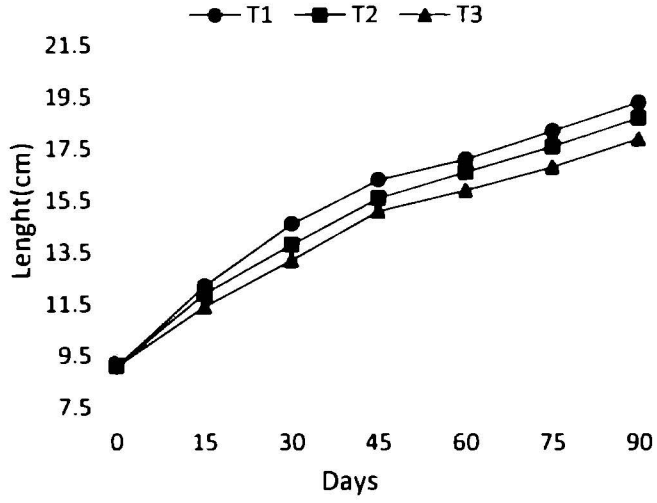


Fig-4.1.16: Mean total length gain (cm) of mono-sex tilapia in different sampling days

In T_1 ponds (20,000/ha) fishes exhibited highest mean total length (19.3 cm) on the 90th day of sampling. In T_2 ponds (30,000/ha) fishes exhibited highest mean total length (on the 90th day of sampling.) on the 90th day of sampling. And in T_3 ponds (40,000/ha) fishes exhibited highest mean total length (17.9cm.) on the 90th day of sampling.

It was observed that the total length in T_1 ponds was 3.20 % and 7.82 % higher than the mean total length in T_2 and T_3 ponds (on 90th day of sampling) respectively.

The mean total length in T_2 ponds was 3.10 % lower than T_1 ponds but 4.46% higher than T_3 ponds (on 90th day of sampling).

And the T_3 pond exhibited 7.25 % and 4.27 % less mean total length compared to the mean total length of T_1 and T_2 ponds on 90th day of sampling.

It was estimated through One Way Analysis of Variance (ANOVA) and observed that difference among the mean length gain in all ponds were significant ($F_{2, 12}$ =28.49; $P < 0.001$).

4.1.2.7. Length weight relationship:

The relationship between total length (L) and total weight (W) for nearly all species of fish is expressed by the equation:

$W = a L^b$

Values of W usually been calculated from the logarithmic (base 10) equivalent: $\log W = \log a + b \log L$

A graph of log W against log L forms a straight line with a slope of b and a Y-axis (log W) intercept of log a. Invariably, b is close to 3.0 for all species.

In the experiment, length weight relationship of mono-sex Nile tilapia in all the ponds (T_1, T_2 and T_3) during 90 days culture period was done to evaluate the relationship (fig-3.17, 3.18 and 3.19).

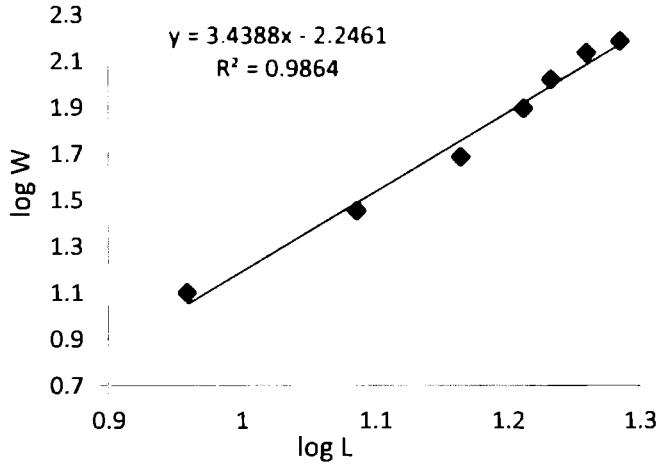


Fig-4.1.17: Length-weight relationship of mono-sex tilapia (T_1 ponds)

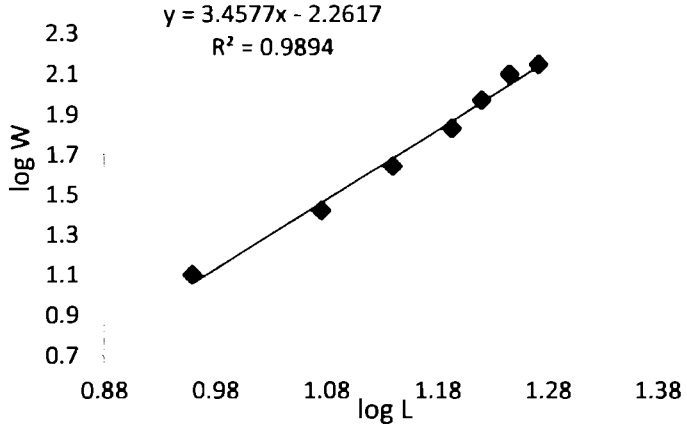


Fig-4.1.18: Length-weight relationship of mono-sex tilapia (T_2 ponds)

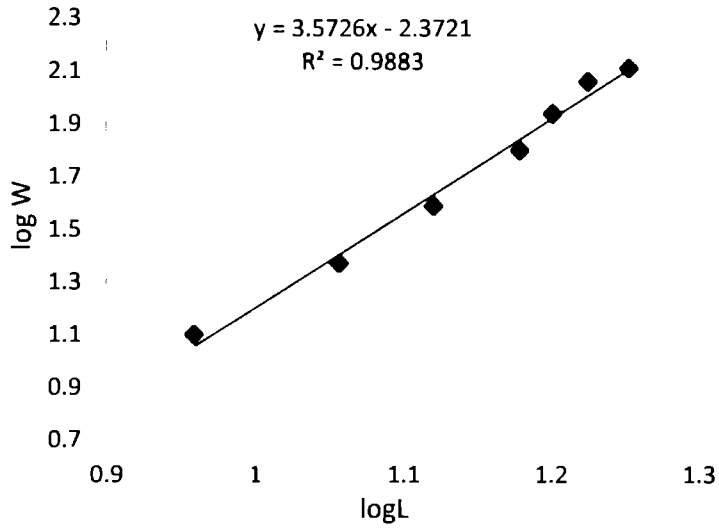


Fig-4.1.19: Length-weight relationship of mono-sex tilapia (T_3 ponds)

Length-weight relationship of mono-sex Nile tilapia at the 90th day of sampling in all ponds exhibited linear relationship and such relationship was explained by 98% validity. The value of R^2 in T_1, T_2 and T_3 ponds was 0.9864, 0.9894 and 0.9883 respectively.

4.2 Discussion:

The reason behind the increasing trends of tilapia culture is not only for its extraordinary growth performances but also its resistivity towards diseases and capability to survive in relatively bad environmental conditions (Cruz and Ridha, 1994).

Tilapia is a prolific uncontrolled breeder and breeds 10-12 times in a year (Philippart and Ruwet, 1982), which led to excessive recruitment, stunted growth and a low percentage of marketable-sized fish. 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 and Pruginin 1981; Coleman 2001; Lèveque, 2002).

Monosex culture of male tilapia is postulated to solve this problem and several potent methods are there for production of all-male tilapia population (Guerrero 1982; Macintosh et al 1985; Gale et al 1999; Beardmore et al 2001; Smith and Phelps 2001).

Although monosex male population can be obtained by direct or indirect methods, oral administration of Oreochromis niloticus has been reported to be the most preferred method in commercial uses (Green and Teichert-coddington, 2000; Wahby and Shalaby, 2010; Celik et al., 2011). Different steroids have been used over the years to induce sex reversal even if 17α-methyltestosterone is the most common (Pandian and Varadaraj, 1990) for Oreochromis mossambicus.

4.2.1. Section I (all male seed production):

Brood fish of tilapia (both sexes) were raised in the hatchery complex of the selected hatchery. Special care was taken for proper nourishment of the brood fish. Ready to spawn female tilapia was identified by examining genital papilla. Reddening of genital papilla indicated readiness of the female. Aggressiveness and territorial behaviour (nesting) indicated maturation of the male (PenaMendoza et al., 2005).

Tilapia is a thermo-sensitive species and it's male to female ratio increases with temperature (Baroiller and D Cotta, 2001). The early days of April were selected as seed production period because water temperature near 32°C induces better production of all male population (Baroiller and D'Cotta, 2001). Temperature of the culture environment has been shown to affect sex ratios of tilapia. The change in sex of tilapia from female to

male increase with high temperature while ovarian differentiation is induced by low temperatures (Baroiller and D Cotta, 2001). Increased water temperature during sex differentiation favours testicular development hence a greater male population with the aid of hormonal activity (Rahma et al., 2015). Excess high and low temperature also reduces the success rate of sex reversal (Baroiller and D Cotta, 2001).

Nile tilapia establishes social hierarchies in which the dominant males have priority for both food and mating. Once the social hierarchy is established within a group, the dominant males enjoy the benefits of both increased access to food and an increased number of mates. Circular nests are built predominantly by males through mouth digging to become future spawning sites. These nests often become sites of intense courtship rituals and parental care (Castro et al., 2009). Observing the breeding behaviour of Nile tilapia, earthen flower pots were provided in the hapa as there was no provision of nest building through mouth digging. Earthen flowerpots acted as nest and inner side of the pots were cleaned for breeding activity. Nile tilapia is a maternal mouth breeders thus females only provide parental care. When breeding activity advances female take their fertilized eggs in their mouth and for hatching. They take shelter in the flower pots and remain confined for 3-4 days in the shelter.

Mouth brooding females can be identified either by observing cleanliness of the earthen pots or by non-accepting feeding behaviour. They have the tendency to swallow entire eggs kept in mouth when frightened in water. Care was taken and the whole hapa was lifted suddenly out of water in night time to avoid such incidence.

Treatment with 17α MT was started from the second or third day after the fry are released from maternal care. However, other studies recommended the treatment to start from the seventh day post-hatching until 30th day (Nakamura and Iwahashi, 1982).

Oral administration of feed incorporated with 17α MT @ 60mg /kg of feed. The principle behind this method lies on the fact that at the stage when the Tilapia larvae are said to be sexually undifferentiated (right after hatching up to about 2 weeks or up to the swim-up stage), the extent of the androgen (male hormone) and the estrogen (female hormone) present in a fish is equal. Thus, augmenting one of the hormones that is originally present in the fish will direct the fish to either male or female depending upon the hormone introduced. As the Tilapia larvae were fed with feeds that were incorporated with male hormone (17α-methyltestosterone), the fish developed into phenotypic male

physically and functioned as male but possessed the female genotype (XX) (Silva et al., 2013). This is commonly referred to as "sex reversal". However, the technique has some limitations such as the uniform age of fish that should be used at the first feeding stage to ensure high reversal rate and less control of reversal efficiency especially when done in the natural environment where natural feed is present. This technique has achieved successful results up to 100% and feed with the male hormone is commercially available or can be prepared.

The limitations regarding uniform age of fish was solved by collecting eggs from the mouth of the females and incubated in glass pot hatchery to avoid heterogeneous growth of larvae.

4.2.2 Section B (growth performance of all male tilapia):

Three stocking density @ 20000, 30000 and 40000 per hectare was tested in farmers field for evaluation of growth performance of all male tilapia. From the experiment it was observed that stocking density @ 20,000 per hectare produced higher yield than 30,000 and 40,000 per hectare stocking.

According to Chakraborty and Banerjee (2012), hormone treated males show higher growth rates than their control counterparts. Additional advantages of larger fish and higher yields are gained through culture of such sex reversed fish. Tilapia shows poor growth potential at very high and low stocking densities. Culture of tilapia at density of 20 fish/m^3 shows the highest growth among all density classes. Thus, this study enables us to postulate an optimum stocking density level of tilapia for maximum utilization of food and space with minimum stress and energy expenditure resulting in higher growth potential of the fish.

It was observed that the highest weight, length, daily weight gain, growth rate and protein content were observed for the 20,000 fish/ha density class. Thus, culture of monosex tilapia at a density of 20,000 fish/ha can be considered ideal for augmented production of the fish under Indian context. Different authors had found limited effect of stocking density on fish survival and demonstrated that cannibalism could be a main cause of tilapia fry mortality at high stocking densities (El-Sayed 2002).

The maintenance of good water quality is essential for optimum growth and survival of tilapia. The levels of physical and chemical parameters control the quality of pond

waters. The level of metabolites in pond water can have an adverse effect on the growth. Good water quality is characterized by adequate oxygen and limited level of metabolites. Water quality not only affects the growth and survival rate of culture organism, but also affects the accuracy of the experiment result (Chim et al., 2008). Excess feed, faecal matter and metabolites will exert tremendous influence on the water quality of the fish ponds. Hence critical water quality parameters are to be monitored carefully as adverse conditions may be disastrous effect on the growing fish (Ramanathan, et al., 2005).

Water temperature is probably the most important environment variables in tilapia culture, because it directly affects metabolism, oxygen consumption, growth, moulting and survival.

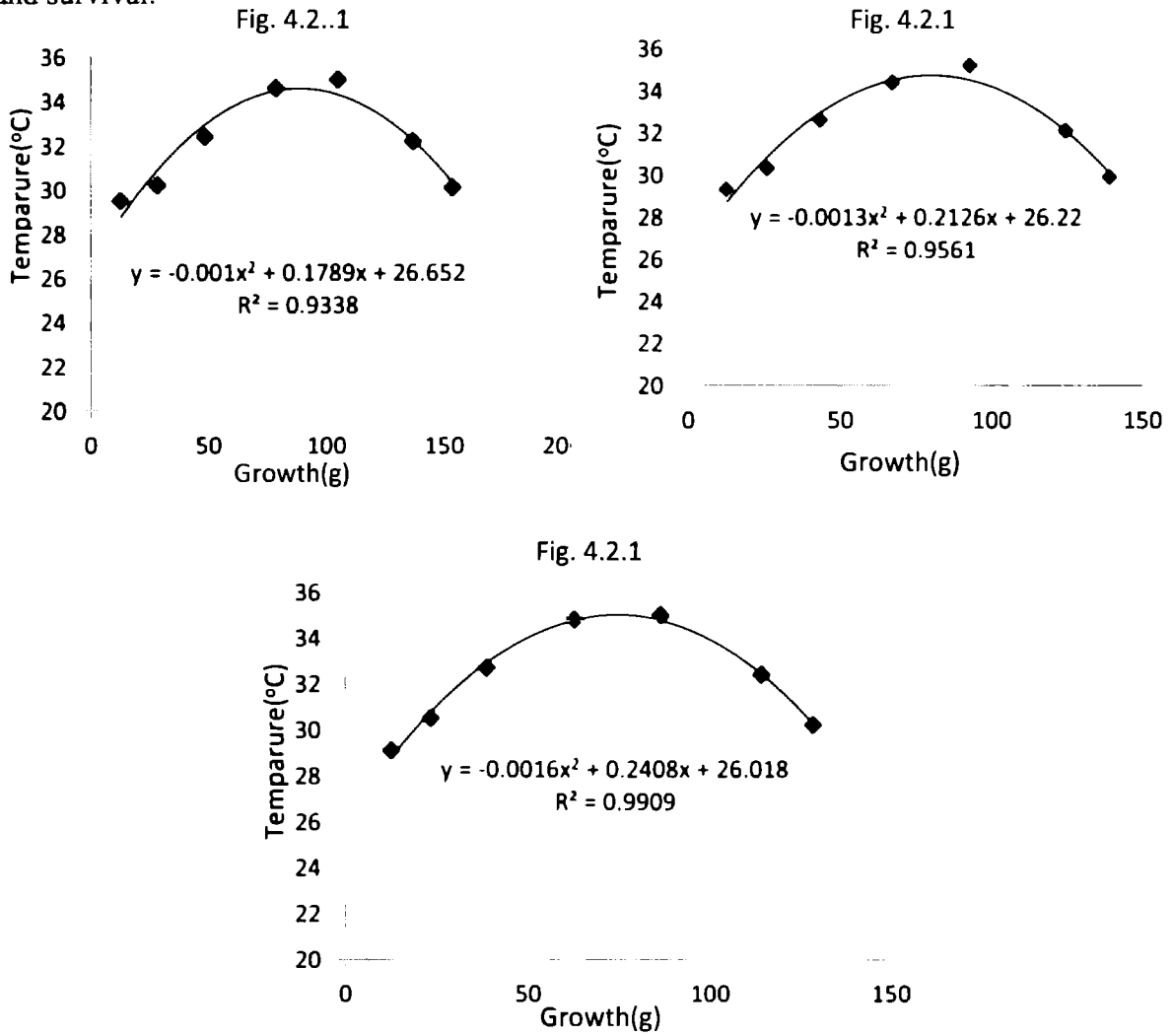


Fig.4.2.1: Relationship between temperature and growth rate in (a) 20,000, (b) 30,000 and c) 40,000 per ha stocking density

The polynomial relationship between growth rate and water temperature [Fig- 4.2.1 a, b, and c] in different stocking densities was established. Values of R^2 in a) 0.9338, b)

0.9561 and c) 0.9909 respectively were highly significant. The overall relationship between growth rate and water temperature established polynomial relationship with 93% validity (4.2.1a), 95% (4.2.1b) and 99% (4.2.1c).

pH is one of the vital environmental characteristics, which decides the survival and growth; it also affects the metabolism and other physiological process of tilapia. The pH of water affects many water quality parameters and the rates of many biological and chemical processes. Thus, pH is considered important parameters to be monitored and controlled aquaculture system (Losordo et al., 1998). In the present study, the range of pH was 7.1-7.9. Pompa 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.

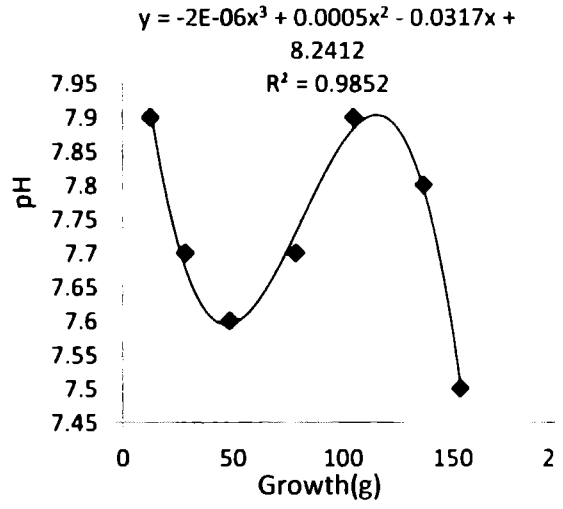


Fig. 4.2.2a

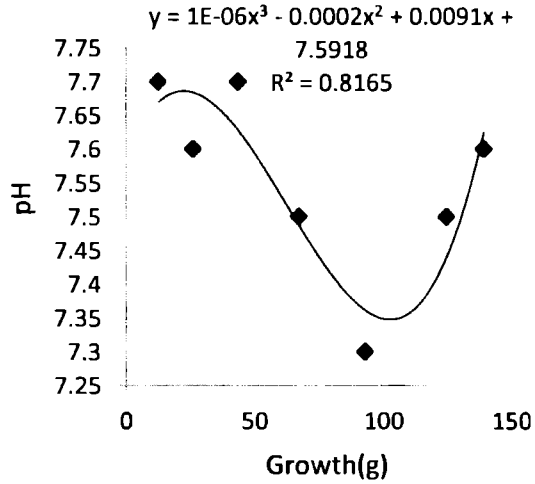


Fig. 4.2.2b

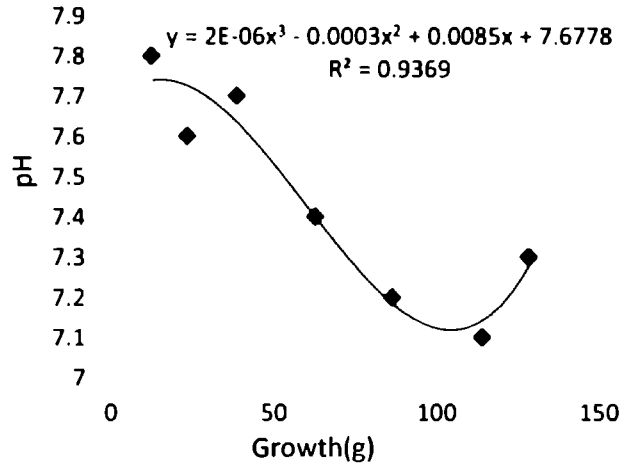


Fig. 4.2.2c

Fig.4.2.2. Relationship between pH and growth rate in (a) 20000, (b) 30000 and c) 40000 per ha stocking density

The polynomial relationship between growth rate and water pH [Fig- 4.2.2 a, b, and c] in different stocking densities was established. Values of R^2 in a) 0.9852, b) 0.8165and c) 0.9369 respectively were highly significant in 3rd degree except the figure b.

The overall relationship between growth rate and water pH established polynomial relationship with 98% validity (4.2.2a), 81% (4.2.2b) and 93% (4.2.2c) . Such strong correlation between growth rate and water pH was also reported by Bahnasawy (2009) and Swann (2009).

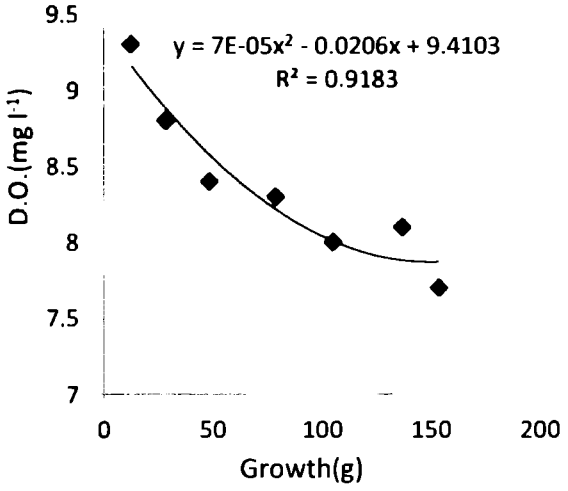


Fig. 4.2.3a

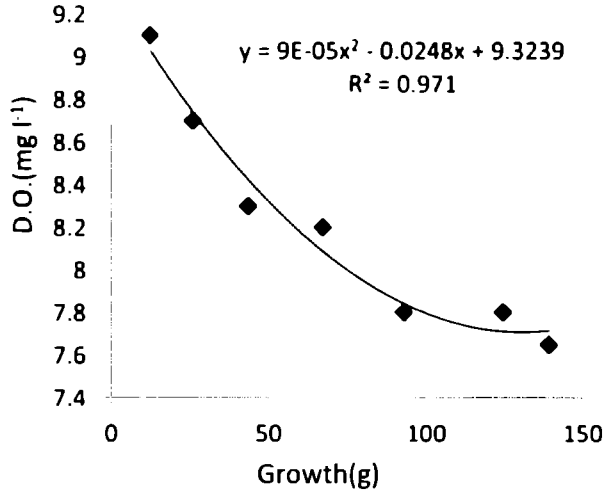


Fig. 4.2.3b

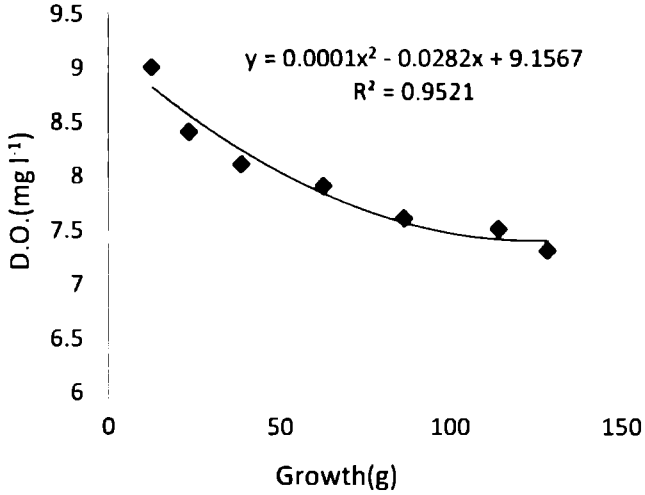


Fig. 4.2.3c

Fig.4.2.3. Relationship between D.O and growth rate in (a) 20000, (b) 30000 and c) 40000 per ha stocking density

A higher degree of polynomial relationship between two variables *viz.* DO and growth rate in tilapia (Fig- 4.2.3a, Fig.4.2.3b and Fig4.2.3c) was established. And the vales of R^2 were 0.9183 in 4.2.3a, 0.971 in 4.2.3b and 0.9521 in 4.2.3c respectively. The overall relationship between growth rate and water pH established polynomial relationship with 91% validity (4.2.3a), 97% (4.2.3b) and 95% (4.2.3c). Such strong correlation between growth rate and water pH was also reported by Bahnasawy (2009) and Swann (2009).

Successful fish production depends on good oxygen management. Oxygen is essential to the survival (respiration) of fish, to sustain healthy fish and bacteria which decompose the waste produced by the fish, and to meet the biological oxygen demand (BOD) within culture system. Dissolved oxygen levels can affect fish respiration, as well as ammonia and nitrite toxicity. When the oxygen level is maintained near saturation or even at slightly super saturation at all times it will increase growth rates, reduce the food conversion ratio and increase overall fish production (Mallya, 2007).

Oxygen is important in respiration and metabolism processes in any animal. In fish, the metabolic rate is highly affected by the concentration of oxygen in the rearing environment. As the dissolved oxygen concentration decreases, respiration and feeding activities also decrease. As a result, the growth rate is reduced and the possibility of a disease attack is increased. However, fish is not able to assimilate the food consumed when DO is low (Tom 1998). Overall health and physiological conditions are best if the dissolved oxygen is kept closer to saturation. When the levels are lower than those mentioned above, the growth of the fish can be highly affected by an increase in stress, tissue hypoxia, and a decrease in swimming activities and reduction in immunity to diseases. However, there is a need to maintain the level of dissolved oxygen at the saturation level which will not affect its physiological or metabolic activities, so as to have high production in any culture system (Wedemeyer 1996). More than that, one has to keep in mind that the oxygen level requirement depends on the species, but also on fish size and activity of the fish. In case of tilapia it was established that the fastest rate of growth was at high D.O. and the slowest growth in the low DO (Mallya, 2007

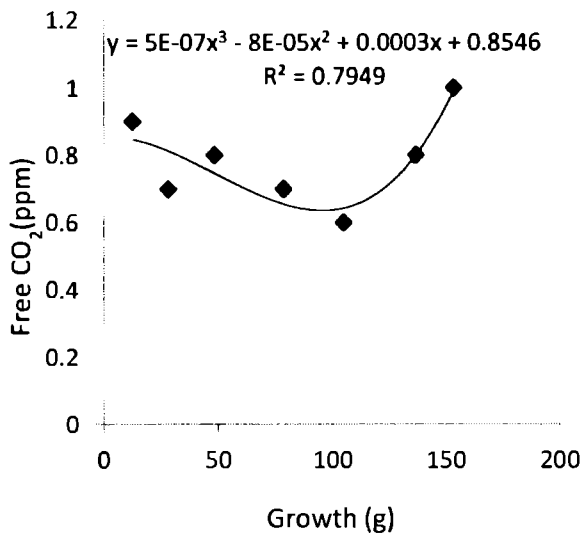


Fig. 4.2.4a

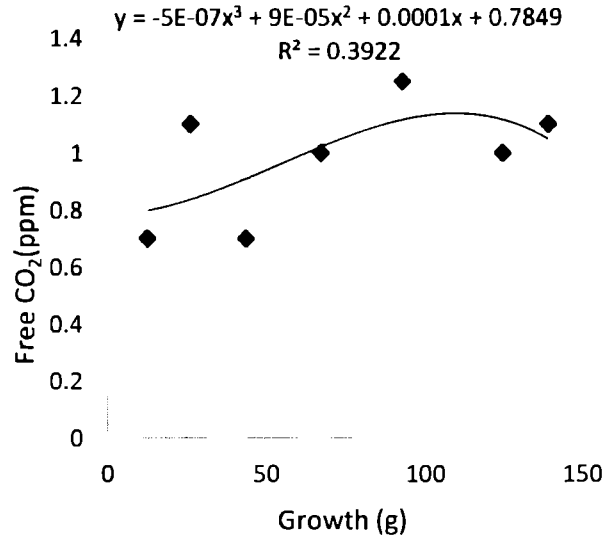


Fig. 4.2.4b

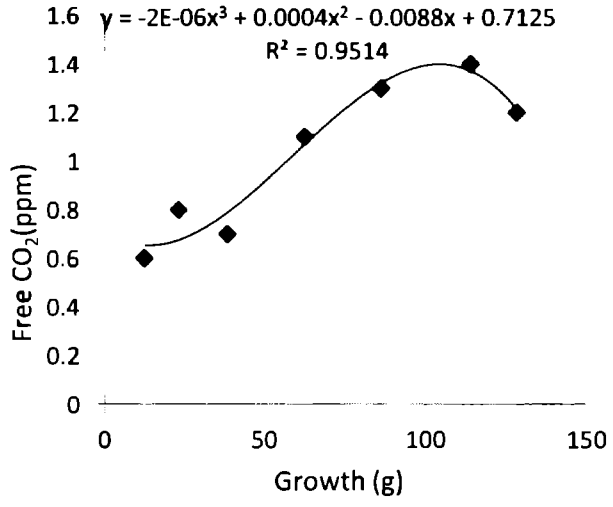


Fig. 4.2.4c

Fig.4.2.4. Relationship between free CO_2 and growth rate in (a) 20000, (b) 30000 and c) 40000 per ha stocking density

In heavily stocked fish ponds, carbon dioxide (CO_2) concentrations can become high as a result of respiration. The free CO_2 released during respiration reacts with water, producing carbonic acid (H_2CO_3), and pH is lowered ($H_2O + CO_2 = H_2CO_3 = H^+ + HCO_3$). Carbon dioxide rarely causes direct toxicity to fish. However, high concentrations lower pond pH and limit the capacity of fish blood to carry oxygen by lowering blood pH at the gills (Tucker, 1984). The polynomial relationship between growth rate and free CO_2 [Fig- 4.2.4 a, b, and c] in different stocking densities was established. Values of R^2 in a) 0.7949, b) 0.3922 and c) 0.9514 respectively

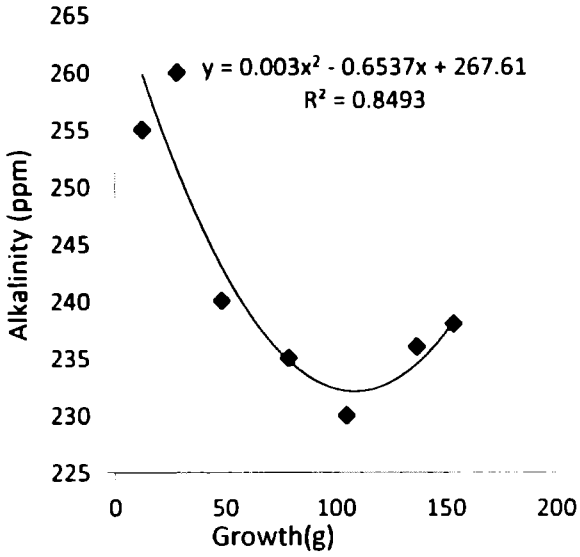


Fig.4.2.5a

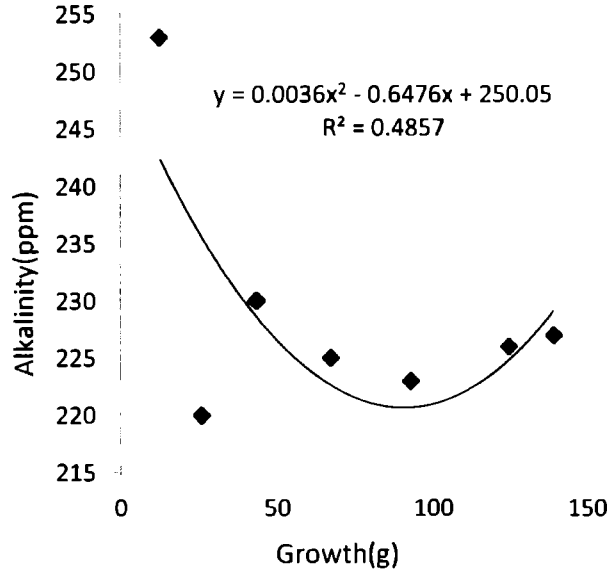


Fig.4.2.5b

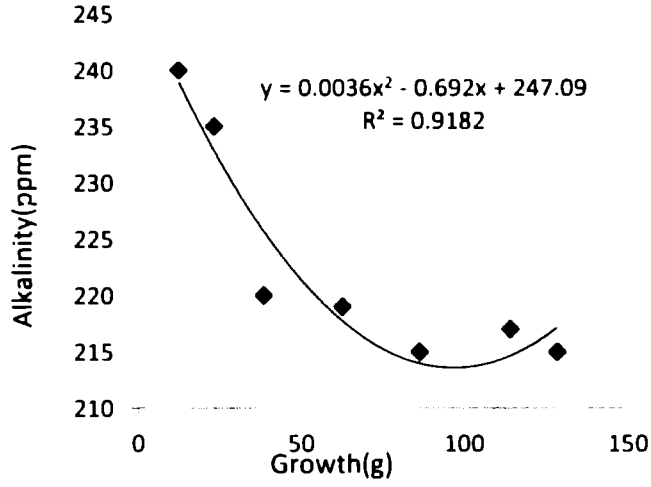


Fig. 4.2.5c

Fig.4.2.5. Relationship between total alkalinity and growth rate in (a) 20000, (b) 30000 and c) 40000 per ha stocking density

The total alkalinity concentration should be no lower than 20 mg/L CaCO3 in production ponds. Pond pH can swing widely during the day, measuring from 6 to 10, when alkalinity concentrations are below this level. Large daily changes in pH can cause stress, poor growth and even death of the farmed animals. Most aquatic organisms can live in a broad range of alkalinity concentrations (Wurts, 1992). The polynomial relationship between growth rate and total alkalinity [Fig- 4.2.5 a, b, and c] in different stocking densities was established. Values of R^2 were a) 0.8493, b) 0.4857 and c) 0.9182 respectively.

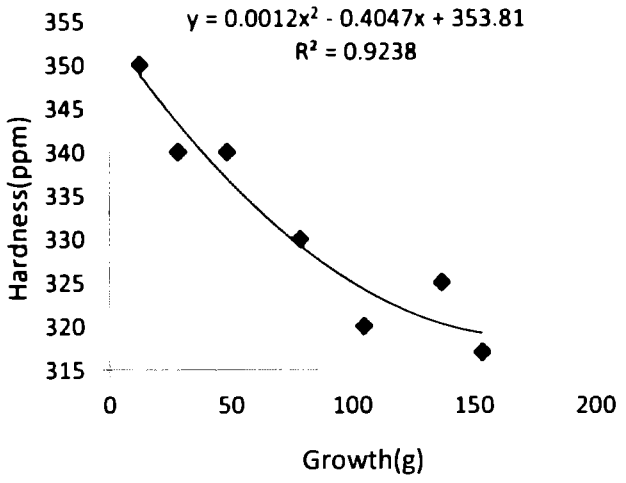


Fig. 4.2.6a

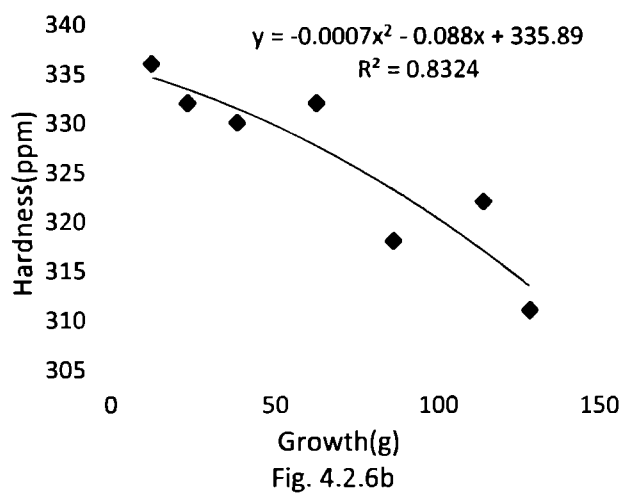


Fig. 4.2.6b

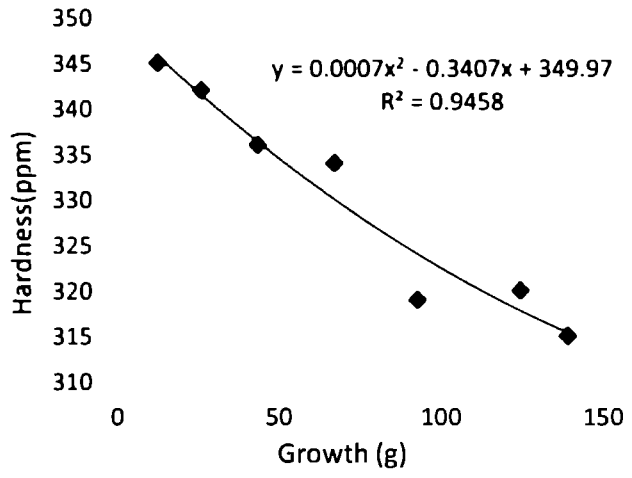


Fig. 4.2.6c

Fig.4.2.6. Relationship between Hardness and growth rate in (a) 20000, (b) 30000 and c) 40000 per ha stocking density

Numerous inorganic (mineral) substances are dissolved in water. Among these, the metals calcium and magnesium, along with their counter ion carbonate (CO_3^{-2}) comprise the basis for the measurement of 'hardness'. Optimum hardness for aquaculture is in the range of 40 to 400 ppm of hardness. Hard waters have the capability of buffering the effects of heavy metals such as copper or zinc which are in general toxic to fish. The hardness is a vital factor in maintaining good pond equilibrium. Calcium and magnesium are essential in the biological processes of aquatic animals. Environmental calcium is crucial for osmoregulation, which is, maintaining precise levels of internal salts for normal heart, muscle and nerve function (Wurts, 1992).

The polynomial relationship between growth rate and hardness [Fig- 4.2.6 a, b, and c] in different stocking densities was established. Values of R^2 were a) 0.9238, b) 0.8324 and c) 0.9458 respectively.

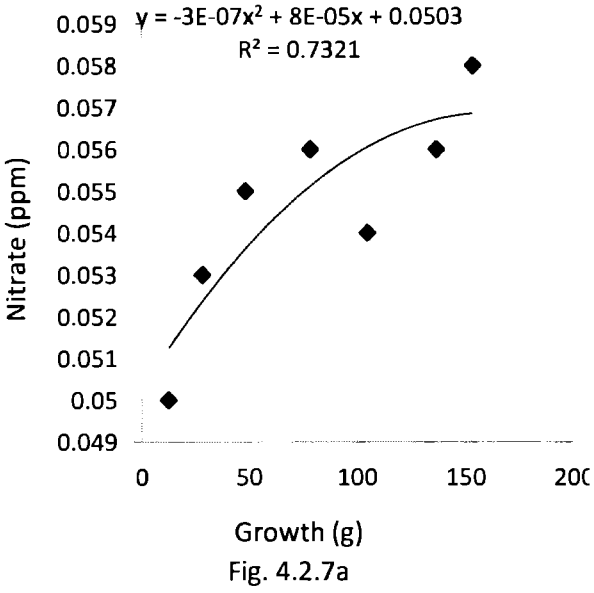


Fig. 4.2.7a

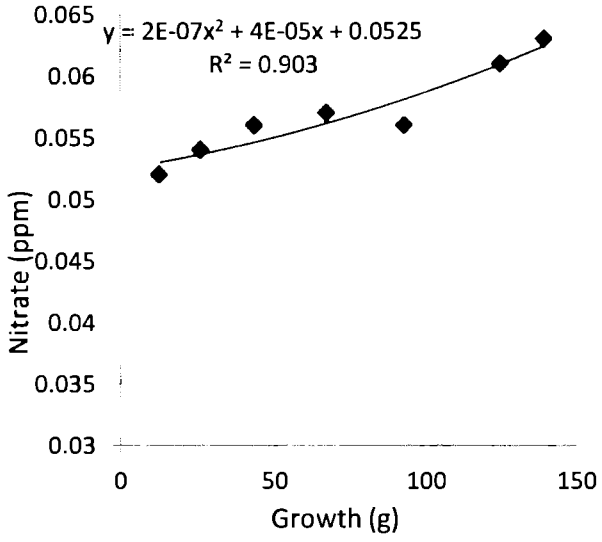


Fig. 4.2.7b

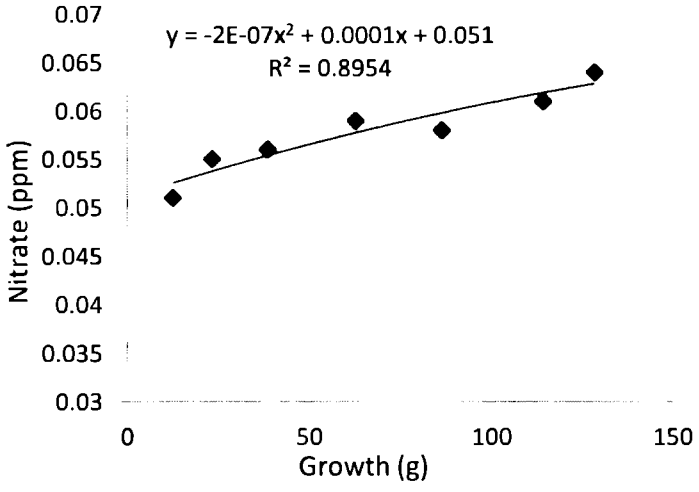


Fig.4.2.7c

Fig.4.2.7 Relationship between nitrate nitrogen and growth rate in (a) 20000, (b) 30000 and c) 40000 per ha stocking density

Nitrate is formed through nitrification process, i.e. oxidation of NO_2 into NO_3 by the action of aerobic bacteria. Nitrate not taken up directly by aquatic plants is denitrified in anaerobic sediments and microzones. In tropical systems, denitrification will be most intense in the following areas: (a) where detritus accumulates; (b) in water bodies subject to enhanced nutrient loading from pollution; (c) in water bodies with long residence

times; and (d) in wetland ecosystems subject to periodic drying, where oxygen inputs during drying periods stimulate coupled mineralization-nitrification-denitrification within organically rich sediments (Furnas, 1992). Generally, it is stable over a wide range of environmental conditions and is highly soluble in water. Compared with other inorganic nitrogen compounds, it is also the least toxic. However, high levels can affect osmoregulation, oxygen transport, eutrophication and algal bloom (Lawson, 1995).

The polynomial relationship between growth rate and NO_3-N [Fig- 4.7 a, b, and c] in different stocking densities was established. Values of R^2 were a) 0.7321, b) 0.903 and c) 0.8954 respectively.

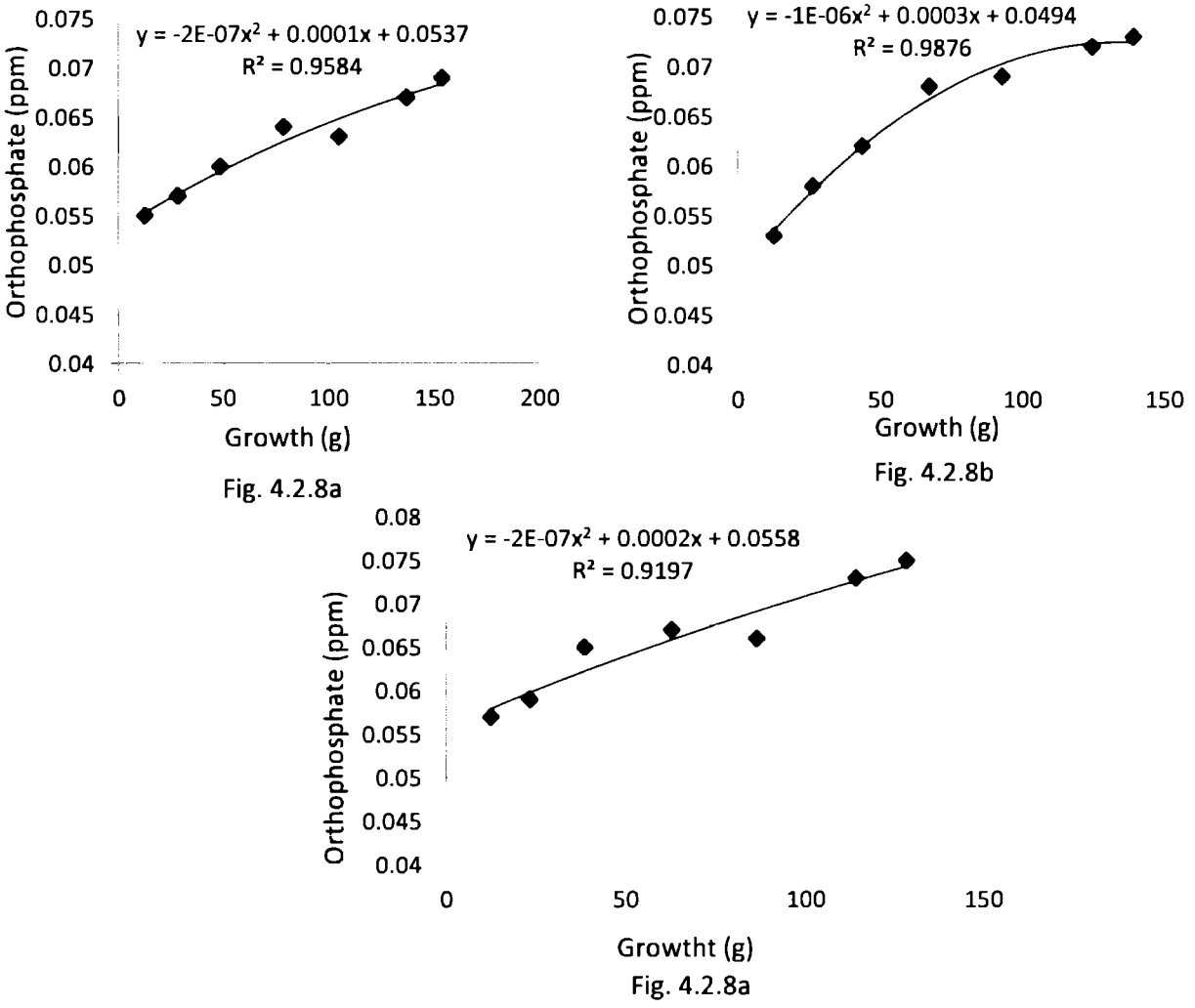


Fig.4.2.8. Relationship between ortho-phosphate and growth rate in (a) 20000, (b) 30000 and c) 40000 per ha stocking density

The polynomial relationship between growth rate and orthophosphate [Fig- 4.2.8 a, b, and c] in different stocking densities was established. Values of R^2 in a) 0.9584, b) 0.9876 and c) 0.9197 respectively were highly significant.

The overall relationship between growth rate orthophosphate established polynomial relationship with 95% validity (4.2.8a), 98% (4.2.8b) and 91% (4.2.8c). In all experimental tanks increased trend of orthophosphate concentration and growth rate of tilapia was highly significant.

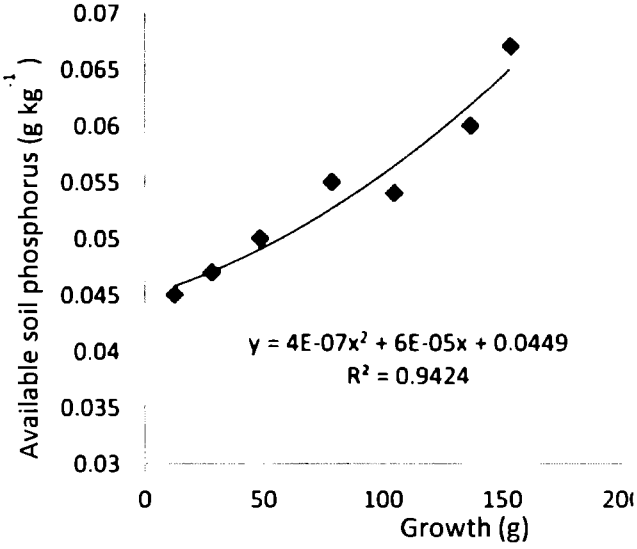


Fig. 4.2.9a

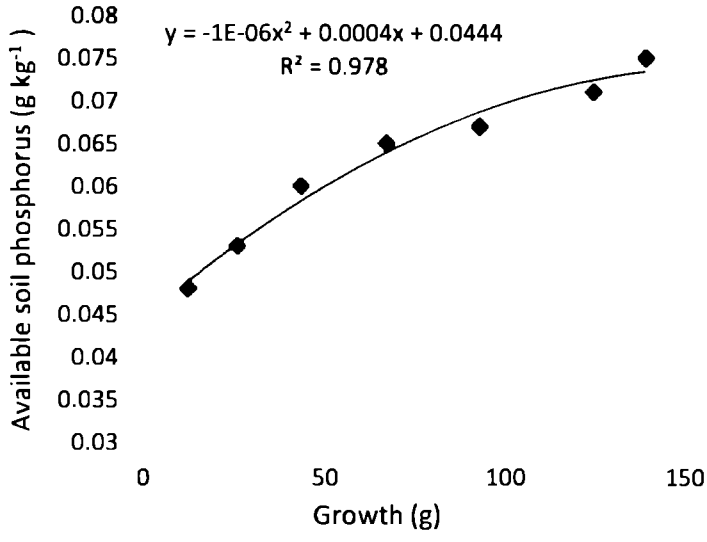


Fig. 4.2.9b

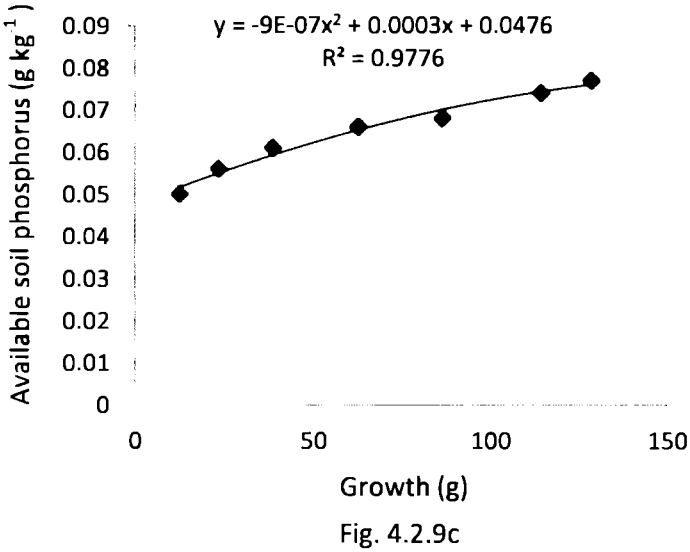


Fig. 4.2.9c

Fig.4.2.9. Relationship between available soil phosphorus and growth rate in (a) 20000, (b) 30000 and c) 40000 per ha stocking density

The polynomial relationship between growth rate and available soil phosphorus [Fig-4.2.9 a, b, and c] in different stocking densities was established. Values of R^2 in a) 0.9424, b) 0.978 and c) 0.9776 respectively were highly significant.

The overall relationship between growth rate and available soil phosphorus established polynomial relationship with 94% validity (4.2.9a), 97% (4.2.9b) and 97% (4.2.9c). In all experimental tanks the relationship of available soil phosphorus and growth rate of tilapia was similar to orthophosphate concentration where the value of available soil phosphorus influenced the growth of tilapia.

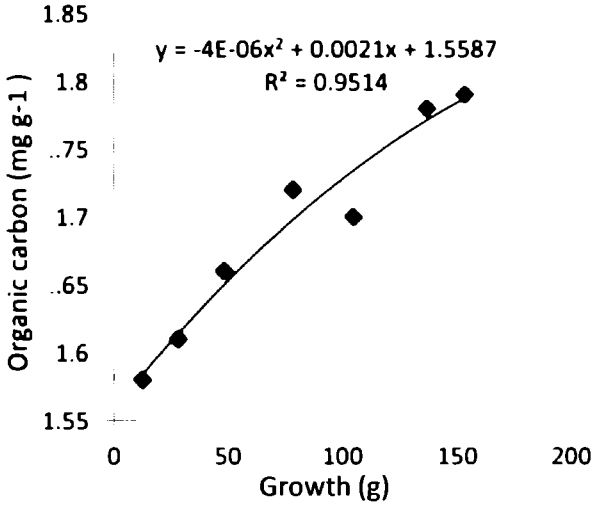


Fig. 4.2.10a

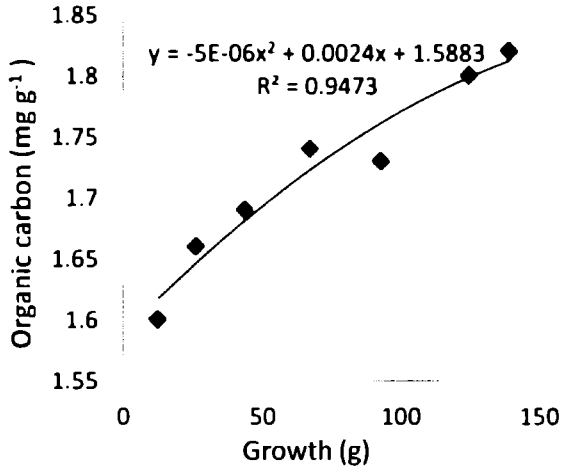


Fig. 4.2.10b

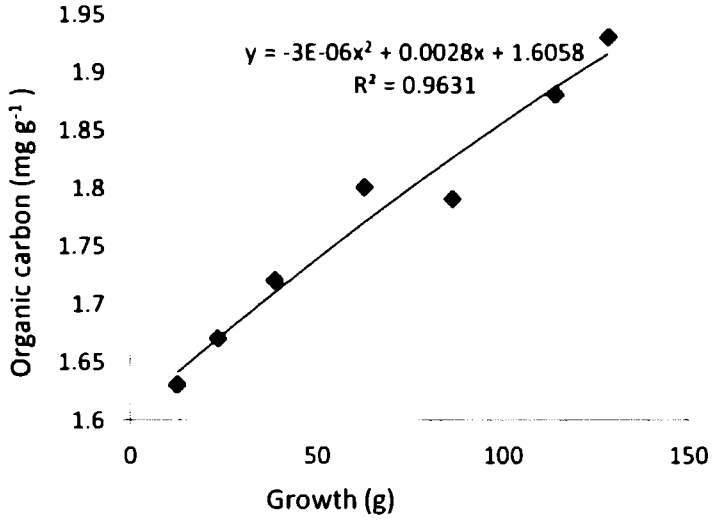


Fig. 4.2.10c

Fig.4.2.10. Relationship between organic carbon and growth rate in (a) 20000, (b) 30000 and c) 40000 per ha stocking density

Banerjee (1967) reported that range of organic carbon for pond productivity for aquaculture is 0.5-1 % (average fish production), 1.5-2.5% (optimum fish production) and >2.5% (declining fish production).

The polynomial relationship between growth rate and organic carbon [Fig- 4.2.10a, b, and c] in different stocking densities was established. Values of R^2 in a) 0.9514, b) 0.9473 and c) 0.9631 respectively were highly significant.

The overall relationship between growth rate and soil organic carbon established polynomial relationship with 95% validity (4.2.10a), 94% (4.2.10b) and 96% (4.2.10c). Such strong correlation between growth rate and soil organic was also reported by Banerjee (1967). In all experimental tanks soil organic load increased along with the duration of culture and thus the relationship was highly significant.

In the present experiment it was observed that all the experimental tanks were prepared as per standard recommendation of organic manure, inorganic fertilizer, feeding rate and water quality management but only stocking density differed. The highest yield was obtained in T1 tanks where monosex tilapia was stocked @ 20,000/ ha. It was also observed that the highest weight, length, daily weight gain, growth rate and protein content were observed for the 20,000 fish/ha density class. Thus, culture of monosex tilapia at a density of 20,000 fish/ha can be considered ideal for augmented production of the fish under Indian context.

Length-weight relationships give information on the condition and growth patterns of fish (Bagenal, T.B. and F.W. Tesch, 1978). Fishes are said to exhibit isometric growth when length increases in equal proportions with body weight for constant specific gravity. The exact relationship between length and weight differs among species of fish according to their inherited body shape, and within a species according to the condition (robustness) of individual fish. Length-weight relationship of tilapia on 90^{th} day was measured and it was observed that in all experimental ponds the correlation was highly significant and value of R^2 were 0.9864, 0.9894 and 0.9883.

Linear regression of length-weight relationship is an indication of growth of fishes. In this experiment the length-weight relationship exhibited highly significant value in all the culture ponds with overall 98% validity.

Chapter 5

Summary and Conclusion

5.1. Summary:

The whole work embodied in the thesis "Monosex tilapia production and its growth performances in farmers' field" has been documented in seven chapters *viz.* i) Introduction, ii) Review of Literatures, iii) Materials and Methods, iv) Result and Discussion, v) Summary and Conclusion, vi) Future Scope of Research and vii) Bibliography. The major finding of this work are presented here in:

Among the wide variety of cultured tilapias, the most widely farmed and popular species is the Nile tilapia (*O. niloticus*) for their better growth performance and acquired the second rank only to carps on the basis of global production.

The major drawback of pond culture of Tilapia is uncontrolled breeding, which led to excessive recruitment, stunted growth and a low percentage of marketable-sized fish, dampened the initial enthusiasm for tilapia as a food fish.

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.

Monosex culture of male tilapia is postulated to solve this problem and several potent methods are there for production of all-male tilapia population. Males are preferred because they grow almost twice as fast as females, which may be caused by a sex-specific physiological growth capacity, female mouth-brooding or the more aggressive feeding behaviour of males and expected survival for all-male culture is 90% or greater.

As little is known about the growth performance of sex-reversed, all male *Oreochromis niloticus* under different traditional culture methods practiced in India, the present study focused on the growth performances of 17α methyltestosterone treated monosex tilapia in farmers' field.

A hatchery in Subhas gram (South 24 Parganas) was selected for conducting the experiment as it was facilitated with all scientific facilities *viz.* water and soil analysis laboratory, breeding tanks, nursery tanks, effluent treatment tanks, quarantine tanks, aeration unit, oxygen packaging unit, water treatment plant, water supply facility,

generator, skilled manpower and good drainage system. Nine progressive fish farmers were selected for field trial of monosex tilapia in ponds.

A brood raising pond (0.09 hectares) was selected in the hatchery premises and common pond preparation practices was followed viz. eradication of unwanted fishes and aquatic weeds, liming, manuring, etc. Healthy brood (average 250 g) of both sexes were stocked (male : female :: 50:100) in the brood pond after quarantine. The water quality was maintained hygienically to avoid disease. Formulated feed enriched with protein and vitamin E was applied @ 4 % of the biomass for enhancing the gonad maturation in tilapias.

The ideal time of the year for breeding was selected on April because this poses the ideal temperature for sex reversal because tilapia is a thermo-sensitive species and its' male to female ratio increases with temperature.

Tilapia is mouth brooder, natural breeding was confirmed by randomly opening some mouth of female brooder and induced breeding (by WOVA-FH at rate of 0.1ml/ 250 gram of fish) was done when reddish colour doesn't appear around the genital aperture of the females.

Observing the breeding behaviour of Nile tilapia, earthen flower pots were provided in the hapa as there was no provision of nest building through mouth digging. Earthen flowerpots acted as nest and inner side of the pots were cleaned for breeding activity. Nile tilapia is a maternal mouth breeder thus females only provide parental care. When breeding activity advances, females take their fertilized eggs in their mouth for hatching. They take shelter in the flower pots and remain confined there for 3-4 days.

Mouth brooding females can be identified either by observing cleanliness of the earthen pots or by non-accepting feeding behaviour. They have the tendency to swallow entire egg mass kept in mouth when frightened in water. Care was taken and the whole hapa was lifted suddenly out of water in night time to avoid such incidence.

The fertilized eggs were collected from the mouth of the females and transferred to a glass jar hatchery and hatching was started after 3 days of transfer. The hatchlings were then reared in the trough for a month in flow through system with a formulated feed (35% of protein, 10% of lipid, 3% crude fibre, 10% moisture, carbohydrates, vitamins

and minerals) incorporated with 17α methyltestosterone hormone @ 60mg/kg of feed with Ethanol (as hormone carrier solvents). Hatchlings were fed hormone fortified feed @ 30 %, 20 %, 18 % and 12 % of their body mass for first, second, third and fourth week respectively. After one month rearing in troughs, the monosex tilapia seeds (12.56 g, 9.1 cm) were distributed to nine selected farmers. Pre-stocking management like eradication and control of aquatic weeds and algae, eradication of unwanted fish, liming, manuring (cow dung along with super phosphate and ammonium sulphate), etc. were done following same protocols. Three different stocking density viz. 20,000/ha (T1), 30,000/ha (T2) and 40,000/ha (T3) was selected and each had three replicates. Commercial grade fish feed (32% crude protein) @ 10% for first 60 days and 5% for next 30 days was applied twice a day. Water and soil samples were collected fortnightly along with growth parameters of tilapia.

Water and soil quality parameters (temperature, pH, D.O., free CO_2 ,total alkalinity, hardness, nitrate nitrogen, orthophosphate, soil available phosphorus and soil organic carbon) and growth parameters (Length and weight of the fishes) were tested and analysed by using standard methods and all the results were subjected to statistical analysis using one way analysis of variance (ANOVA).

All male seed of homogenous size and age was developed using 17α methyltestosterone hormone in hatchery. Seeds were reared in hatchery for a period of 30 days and were distributed among 9 fish farmers.

Maximum growth rate was achieved in T_1 ponds where 20000/ha stocking density was maintained. Minimum yield was obtained in ponds having 40000 fish /ha.

5.2. Conclusion:

Genuine monosex seed of tilapia may not be available in market. So there is a possibility of cheating by dishonest traders which causes serious problem in tilapia farming. Farmers may lose interest of monosex tilapia farming. Production of quality monosex seeds is an important input for this farming which was achieved in this experiment. Monosex seed was produced in hatchery. Heterogeneous growth and age of tilapia poses problem in hormone manipulation. To avoid such problem egg mass was collected from mouth of females and incubated in glass pot hatchery. Stocking density is considered as most critical factor in fish farming. Higher stocking density leads to competition, higher accumulation of metabolites in culture system and causes stress which ultimately reflects upon the poor growth and outbreak of diseases. Stocking density @20,000/ha proved most suitable as it exhibited highest growth rate, daily weight gain and specific growth rate.

Chapter 6

Future Scope of Research

The dissertation work on "Production of monosex tilapia and its performance in famers' field" incorporated in this thesis has been able to meet the objectives of the study. The findings of this study will help the farmers to propagate mono sex tilapia seed production and farming in the areas suitable for this culture.

Tilapia is popularly known as 'aquatic chicken' because it provides one of the major sources of animal protein and income throughout the world (Sosa *et al.*, 2005). Tilapia is currently ranked second only to carps in global production and is likely to be the most important cultured fish in the 21st century (Ridha, 2006).

As the market price of carp is increasing day by day, people largely rely on tilapia. Monosex tilapia grows faster and fetches high market demand. It can be cultured in various aquatic systems. Therefore production of monosex tilapia seed and its farming deserves huge potentiality. **Seed production by hybridization is an effective alternate method and research on this aspect can expand the horizon of tilapia farming.**

Chapter 7

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Chapter 8

Appendix

Table 1. Measurement of culture ponds with different stocking density:

Pond	Replicates	Area(ha)	Depth(m)	Stocking density
T_1	T_1R_1	0.086	1.50	20,000/ha
	T_1R_2	0.113	1.53	
	T_1R_3	0.070	1.35	
T_2	T_2R_1	0.146	1.39	30,000/ha
	T_2R_2	0.072	1.43	
	T_2R_3	0.061	1.47	
T_3	T_3R_2	0.114	1.60	40,000/ha
	T_3R_2	0.077	1.56	
	T_3R_3	0.104	1.67	

Table.2. Pond preparation procedure for all culture ponds:

Day 1	Day 2	Day 7	Day 14	Day 21
Eradication of aquatic weeds	Application of MOC	Liming	Application of cow dung	Stocking
Taficide-80 @ of 5-8kg/ha	250 kg/ ha	250 kg/ ha	5000kg/ ha	

Table 3. Post stocking management for all culture ponds:

Feeding rate(% of body weight)			Feeding time per day	Netting per month	Health check-up per month	Physiological and chemical parameter analysis/ month
1st month	2nd month	3rd month				
10%	10%	5%	twice	twice	twice	twice

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EDUCATIONAL BACKGROUND

Serial No.	Education / Degree	Subjects	Board / University	Percentage of Marks / 10 point grade	Class
1.	Madhyamik Pariksha (2007)	Bengali, English, Mathematics, Ph. Science, Lf. Science, History, Geography.	W.B.B.S.E	87. 5	First
2.	Higher Secondary (2009)	Bengali, English, Physics, Chemistry, Biology, Mathematics & Env. Science	W.B.C.H.S.E	78.60	First
3.	B.F.Sc (Bachelor of Fishery Sciences) (2013)	Fishery Sciences	West Bengal University of Animal & Fishery Sciences	80.10	First
4.	M.F.Sc (Master of Fishery Sciences) (2015)	Aquaculture	W.B.U.A.F.S	Appearing	

PROFESSIONAL QUALIFICATIONS:

1	One week training at **Kakdwip Reaserch Centre on Brackish Water Aquaculture** (CIBA, ICAR) in 2012.
2	**Training at Fish Technological Station** (FTS, Junput) under State Govt. in 2012
3	**Training at Freshwater Fisheries Research Training Centre** (FFRTC, Kulia, Kalyani) under State Govt. in 2012.
4	**Block Survey on Status & Types of Fisheries** in Nandakumar block under the

	Fishery Extension Officer and Block Development Officer, Nandakumar block in 2012.
5	Training **"Fisheries Work Experience Program"** at North 24 Perganas Krishi Vigyan Kendra (K.V.K), Ashokenagar, held during 17th to 23th August, 2012.
6	**"On Farm Training of Various aspects of prawn breeding and hatchery management"** on 29.09.12 at Experimental Prawn Hatchery, Dept. of Fisheries, Digha, Purba Medinipur.
7	Experimental learning on **"Hands on Training"** programme held in Dept. of Fish Processing Technology F.F.Sc., W.B.U.A.F.S. Kolkata in 2013.
8	**"On Farm Training on "Integrated Duck-cum Fish Farming"** from 08.12.12 to 11.12.12 under the DST Project, Dept. of Fishery Extension, F.F.Sc., W.B.U.A.F.S. Kolkata.
9	Training on different Central Institute like CIFE, Saltlake; CIFA, Rahara; CIFRI, Barrackpore and BENFISH, Kolkata in 2012.
10	Basic knowledge in **Computer operations & programming. (2004 - 2007)**
11	Training on **"Sustainable Brackish Water Aquaculture Practices"** from 21-25 July 2015 at CIBA Kakdwip Reasearch Centre.

PERSONAL DETAILS

Date of birth	15.01.1992
Age	23+
Birth place	Bankura
Gender	Male
Marital Status	Unmarried
Blood group	B^+
Category	General
Religion	Hinduism
Nationality	Indian
Correspondence Address	Faculty of Fishery sciences, 5,budherhatroad, Chakgaria,panchasayar,kol-94
Permanent Address	Namo Anchuri, Bankura,722102
Hobbies / interests	Reading Books

Languages known:

Languages	Read	Speak	Write
Bengali	Yes	Yes	Yes
English	Yes	Yes	Yes
Hindi	No	Yes	No

I do hereby declare that the above mentioned particulars are true to the best of my knowledge and belief.

Place: Kolkata
Date: 06.10.15

Signature