Aspects of Reproductive Biology, Growth Performance and Survival of the African Catfish, *Clarias gariepinus* (Burchell, 1822) in Captivity for Enhancing Aquaculture

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This is to certify that the thesis entitled, "Study on the Reproductive and Growth Performance of the African Catfish, *Clarias gariepinus* (Burchell, 1822) in captivity; for enhancing aquaculture", prepared and submitted by Alayu Yalew in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biology (Fisheries and Aquatic Sciences) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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DECLARATION

I hereby declare that this work is the product of my own research efforts undertaken under the supervision of Prof. Abebe Getahun and has not been presented elsewhere for the award of a degree or certificate. All sources have been duly distinguished and appropriately acknowledged.

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Certification

This is to certify that the research for this thesis and subsequent preparation of this thesis by Alayu Yalew Teferra (GSR/0821/06)) were carried out under our supervision.

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Co-advisor

Acknowledgement

In the name of the Father, Son, and Holy spirit, Glory be to God in the highest for His mercies, wisdom and grace that keep me safe and awaits me to this time.

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Dedication

I dedicate this work to my daughters, Betemariam and Tsedey Alayu and my son Kirubel Alayu.

Abstract

Being a potential species for the development of Ethiopian aquaculture, some aspects of the reproductive biology, larval rearing and growth performance of the African catfish, C. gariepinus had never been addressed. Hence this research aims to compare the performance of different size classes on their reproductive biology different z the artificial propagation, larval rearing and table size production of the African catfish, C. gariepinus in captivity. Different sizes of broodstock groups were injected with pituitary extract and compared for fecundity, number of fertilized eggs and the number of survived early larvae produced. The larvae were provided with zooplanktons multiplied in ponds at different feeding intervals to determine the best feeding frequency. The African catfish were cultured in extensive, semi intensive and intensive systems at different stocking densities and the best performing stocking rate was determined for each system. The income return of the different practices was compared and evaluated t identify the best practice for Ethiopia. The result indicated that fecundity of 500-600g weighed broodstocks was 51,800 eggs/kg bodyweight but only 47,000 eggs/kg was spawned from 1000-1200 g weighed broodstocks. The percentage survival of early larvae from small sized broodstocks and larger ones was 81% and 76%, respectively. The larvae fed on local zooplankton performed successfully and zooplankton multiplied in ponds replaced imported Artemia nauplii effectively. At feeding frequency of four times a day (every three hrs), larvae showed an average daily weight gain of 18.9 mg/day which was 19% more compared to those fed fewer times and less frequently. The percentage survival was 95% for the group fed four times a day and 75%, 93% and 87% for larvae fed twice, three times and five times a day, respectively. The most preferred stocking density in terms of weight gain and survival was 1fish/m^2 for extensive, 5 fishes/m^2 for semi-intensive and 90

fishes/m^3 for intensive system. And the final harvest or yield was better in ponds or tanks at higher stocking density; 2fish/m^2 for extensive, 10 fishes/m^2 for semi-intensive and 100 fishes/m^3. The average yield was 0.66kg/m^2 for extensive pond culture, 4.77kg/m^2 asemi-intensive pond culture and 53.4 kg/m^3 in intensive culture during 8 months period. Hence, for a better productivity, fish should be stocked at a rate of 2 fishes/m^2 in extensive and 10 fishes/m^2 in semi-intensive pond culture and 100 fishes/m^3 in intensive tank culture. Comparison of the different culture practices for economic benefit (considering fish sale), indicated that semi intensive culture system benefitted more with a profit index value of 13 compared to intensive culture with 9.28. In conclusion, African catfish female parent with 500g and more live weight can be used as a broodstock in a hatchery rearing. Feeding live zooplankton cultured in plankton ponds could be the most consistent technique for Ethiopia since importation of Artemia cyst is not sustainable. For the sake of alternative fisheries and to run profitable business, catfish farming in a semi intensive pond using feed formulated from local ingredients would be a solution.

Key words*: Artificial spawning, Copepods, Diversity, Fertilization, Incubation, Latency period, Livefood, Milt, Multiplication pond, Parent stock, Pituitary extract, Profit index, Stripping.*

Table of contents

List of Figures

List of Tables

Abbreviations/Acronyms

ADP	=	Acetone Dried Pituitary
ADPE	=	Acetone Dried Pituitary Extract
ANOVA	=	Analysis of variance
ARARI	=	Amhara Region Agricultural Research Institute
ORARI	=	Oromia Region Agricultural Research Institute
asl	=	above sea level
BFALRC	=	Bahir Dar Fisheries and other Aquatic Life Research Center
BW	=	Body weight
cm	=	centi meter
DO	=	Dissolved Oxygen
EC	=	Electrical Conductivity
FALRC	=	Fisheries and Aquatic Life Research Center
FDRE	=	Federal Democratic Republic of Ethiopia
FPME	=	Fish Production and Marketing Enterprise
FR	=	Fertilization Rate
g	=	gram

GTP	=	Growth and Transformation Program
ha	=	hectare
HR	=	Hatching rate
IBM	=	International Business Machine
Kg	=	Kilogram
L	=	Liter
m	=	meter
mg	=	milligram
mm	=	millimeter
MoA	=	Ministry of Agriculture
NI	=	Net Income
PI	=	Profit Index
PIF	=	Fish Performance Index
PS	=	Percentage Survival
RAS	=	Recirculation Aquaculture System
SD	=	Standard Deviation
SDGs	=	Sustainable Development Goals

SE	=	Standard Error
SPSS	=	Statistical Package for Social Sciences
SSA	=	Sub Saharan Africa
TC	=	Total Cost
TDS	=	Total Dissolved Solids
TL	=	Total Length
TR	= Total Return	
TW	=	Total Weight
USA	=	United States of America
USD	=	United States Dollar

Chapter 1. Introduction

1.1 Aquaculture Research and development in Ethiopia

Ethiopian economy is mainly based on livestock - crop mixed farming system using both rain fed and irrigation agriculture, though not supported by modern technologies. Fish farming have been considered as an integral part of livestock production and still officially under the ministry of livestock at the national level. Fish farming started in 1950s using small backyard fish ponds (≤ 100 m^2) stocked mostly with fingerlings of tilapia species.

The modern aquaculture production was introduced to the country through establishing a facility at Sebeta by the financial and technical assistance of the Japan International Cooperation Agency - JICA (Rothuis, *et al*., 2012). The center had been breeding tilapia and common carp for stocking ponds and enhancing several lakes and reservoirs. Sebeta as a national coordinator, the Bahir Dar Fishery and other Aquatic Life Research center under Amhara Region Agricultural Research Institute (ARARI) and Ziway Fisheries Resource Research Center under Oromia Region Agricultural Research Institute (ORARI) were established since 2000. These research centers have been conducting research on Tilapia culture, fish breeding, fish feed formulation, hatchery rearing and other issues of aquaculture. Atleast recently, researchers considered African catfish as a potential species for aquaculture and conduct experiments on artificial propagation (Alemayehu Wube, 2015; Belay Abdisa and Alayu Yalew, 2015) and feeding (Tarekegn Arage, 2015).

Aquaculture has been proposed as a good supplement and alternative means to increase fish production in Ethiopia. The country's Fisheries Development and

Utilization Proclamation (No. 315/2003) also set articles on aquaculture development. Ministry of Agriculture took an action and develop the National Aquaculture Development Strategy of Ethiopia (NADSE) document in 2009 through the financial support of Food and Agricultural Office Sub Regional office for Fisheries (FAO-SFE). This strategic document (NADSE) comes with short (5 years) and medium (10 years) action plan which was supposed to be active from 2010 to 2020.

The output from fish farming, however is still at a lower quantity and had never been a good experience for Ethiopia. The overall effect of underdevelopment was typically because of poor extension service provided to the people towards aquaculture. Scarcity of appropriate inputs (mainly quality fish feed and seed), lack of skill and knowledge on fish farming is also other key problems.

However, things could never continue as they had been before. Atleast the demand for more food, interests to improve the lifestyles and livelihoods, and an increasing number of jobless citizens is becoming apparent. These all are opportunities requiring a coordinated effort to improve the aquaculture situation in Ethiopia. The government is looking for different opportunities and means, designed different strategies and starting to implement them in coherence. The initiation of different investments, attention given for the forgotten and marginalized sectors among which the fisheries and aquaculture sector is one, proclamation of the different development policies and programs, establishing and (re) structuring institutions, insisting and motivating the investors to establish industries and companies and so on. One among the sectors which gets attention back since 2016 is the Livestock and Fisheries sector in which aquaculture seems to get a better attention.

1.2 . Potentials on Aquaculture Development in Ethiopia

Ethiopia has a very high potential for developing fish farming in terms of natural resources (land and water), suitable climatic condition (with diverse agro-ecological zones) and huge human resource. With regard to land, for instance, a GIS analysis based on water availability, temperature, topography, soil texture, land use and cover, and economic factors, studies (Fig 1.1) indicated that 15,158 km^2 of land is identified as highly suitable for earthen pond Tilapia fish farming and 871,731 km^2 is moderately suitable (Eshete Dejen and Zemenu Mintesinot, 2012). This can work also to the other commercial species mainly for African catfish, *Clarias gariepinus* farming.

The current waterbodies have an estimated surface area of 7,740 km^2 that include major lakes and reservoirs, small water bodies of 275 km^2 and rivers stretch of 8,065 km (FAO, 2011c; Gashaw Tesfaye and Wolff, 2015). When the great renaissance dam with reservoir area of 1680 km^2 (Chen and Swain, 2014) and other reservoirs under construction, nearly 400 km^2 (MoWRD, 2012), become active the volume of reservoirs is expected to increase to more than 2,000 km^2.

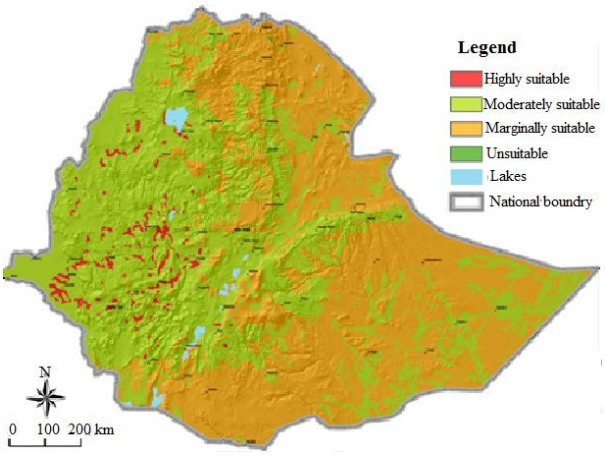

Figure 1. 1. Aquaculture suitability map using earthen ponds

(Source: Eshete Dejen and Zemenu Mintesinot, 2012)

The current annual per capita fish consumption is expected to grow to 1kg and Ethiopia needs 125,586 tons of fish for the population in 2025 (FAO, 2018). Other than population, the comparatively low price of fish or the increasing prices of its substitutes; improvements on fish eating habit, a rise in income; improvement in fish product quality and enhancement and expansion in fish distribution or supply networks expected to trigger future fish demand (FAO, 2011b). The traditional beef eating habit of Ethiopians is shifting in favor of fish, irrespective of species preference, in areas and communities where there is appropriate supply of fish.

Aquaculture is increasingly recognized as an alternative means of achieving food security and poverty reduction in the rural and considered as an integral part of rural and agricultural development policies and strategies of Ethiopia. Aquaculture policy and strategy of the country and the legal framework on fishery development are also very supportive and looking for problem solving research outputs. The Growth and Transformation Plan (GTP II) encourages the private sector to invest in aquaculture and consider it as untapped agribusiness open for domestic and foreign investors.

Like for other priority areas, investors in aquaculture subsector are also eligible for comprehensive incentive packages in accordance with the Investment Proclamation No. 769/2012 as amended in 2014, Investment Incentives and Areas Reserved for Domestic investors Regulation No. 270/2012 as amended in 2014 and other relevant legislation frameworks. The major benefits include, Customs duty payment exemption on capital goods and construction materials, and on spare parts whose value is not greater than 15% of the imported capital goods' total value.

An inclusive countrywide study initiated and funded by the government of the Netherlands identified key business opportunities for the rapid development of

aquaculture in Ethiopia through the private sector. According to the findings of this study, the lagging trend of the fish supply from capture fisheries and the growing demand in Ethiopia offers opportunities for aquaculture businesses to play a role in improving food security (Rothuis *et al*., 2012). There is a huge area of land suitable mainly for earthen ponds fish farming (Eshete Dejen and Zemenu Mintesinot, 2012). A study suggested that the involvement of the private sector is the best option if aquaculture is going to develop so fast in Ethiopia.

Otherwise, aquaculture in Ethiopia remains more potential than actual practice although investment startups are apparent. This opens wider room for commercial investment in a range of possible enterprises. Based on these motives, Dutch company establishes commercial fish farm ("Africa Sustainable Aquaculture -ASA") in West - Northern Ethiopia using cages at Koga Reservoir. Others are also establishing fish farm and related businesses in different parts of the country, but emphasis is give only for tilapia farming. This is mainly due to lack of sufficient scientific knowledge on the culture of other potential aquaculture species.

1.3 Research questions, rationales and significance

As *C. gariepinus* is the fastest growing freshwater aquaculture species, farming both at subsistence and commercial level can play a role in securing food and improve the livelihood of the people. But its production in a farm have never been tested due to a number of challenges; mainly lack of scientific knowledge to manage catfish in captivity, insufficiency in recruiting appropriate broodstocks which can produce viable eggs, scarcity of practical skill on live food production and larval feeding to rear quality fingerling; shortage of information on catfish farming in different culture systems using locally available feed sources; and techniques to manage the optimum

condition for this fish (Adewumi and Olaleye, 2011). Hence, to address all the above issues, there were questions to be answered. The major ones among others are; whether it is possible to stimulate the small sized mature female broodstock and produce viable larvae using locally available inputs and facilities? Would it be possible to use local feed resources to culture *C. gariepinus*? Is that possible to manage and grow *C. gariepinus* in different production systems? which production system produces better yield and generate more income? How it is possible to grow *C. gariepinus* in impoundments so that these water bodies can supplement fisheries around the Blue Nile basin? which production system produces best performing *C. gariepinus* and generate more income?

1.4 Ethical considerations

Animal welfare principles applied to other farm animals have not yet deeply been adapted to fish, and there exists a lack of scientific information on fish wellbeing (Chandroo *et al*., 2004; Braithwaite and Boulcott, 2008). However, consumers and consequently fish farmers are increasingly aware of the health and cheerfulness of reared animals (Braithwaite and Huntingford, 2004). Fish welfare is influenced by a range of biotic and abiotic (environmental) factors. The other factor like different physiological parameters, food quality and distribution, and anthropogenic influences such as sorting and handling affects the fish welfare (Ashley, 2007; Barber, 2007; van de Nieuwgiessen *et al*., 2008; van de Nieuwgiessen *et al*., 2009).

The physiological conditions mainly stress hormones and metabolites together with the external body conditions are often considered for welfare assessments. Once an organism is exposed to a stressing agent and there exist changes on seasonal, feeding condition and photoperiod then hypothalamus produces cortisol (stress hormone) and

the animal goes out of homeostasis (Barton and Iwama, 1991; Bonga, 1997; Barton, 2002; Flik *et al*., 2006; Schram *et al.*, 2010; Pankhurst, 2011). If stress exceeds the homeostatic state, it most likely negatively affects the wellbeing and welfare of fish (Conte, 2004).

In this study, external injuries of body, fins and barbells of *C. gariepinus* from the recirculation aquaculture system (RAS) were assessed since they appear often on scaleless fish at higher stocking density. Hence, regular observations of the external body was taken as the most likely useful indication for the stress and welfare of *C. gariepinus* as it has been commended by other studies (Huntingford *et al*., 2006; van de Nieuwgiessen *et al*, 2008). In addition, the movement of experimental fishes was inspected frequently mainly during morning, afternoon and while feeding.

1.5 Null Hypothesis

- African Catfish (*C. gariepinus*) female broodstock size has no direct effect on the spawning, fertilization and hatching of eggs as well as survival of early larvae of *C. gariepinus.*
- Feeding frequency of locally available zooplanktons cannot affect the growth performance of early larval stages of *C. gariepinus* in a hatchery.
- There is no significant interactive effect between stocking density and fish growth performance in grow out production of *C. gariepinus*.
- There is no significant difference in income return and growth rates of *C. gariepinus* among the different culture systems.

1.6 Scope and limitation of the study

As the price of *Artemia nauplii* is costly and unable to get easily, zooplankton culture was recommended as an alternative means. But, the culture of zooplankton species in the hatchery could not be managed with the existing capacity and facility in a research center. Hence, zooplankton were multiplied on fertilized outdoor concrete ponds and fed to *C. gariepinus* larvae. Semi-intensive culture was conducted using the usual small farmers' backyard ponds of ≤ 100m^2 which represent the current fish farming practice existing in many places of Ethiopia. The study of intensive *C. gariepinus* fish production was conducted using small culture tanks of 750L total volume due to lack of a recirculation system with bigger culture tanks. For extensive farming system experiment, impounded waters in high flooding areas were selected considering the future plan to stock these water bodies for alternative fisheries.

1.7 Objectives of the study

The general objective is to investigate some reproductive features, growth and survival of *C. gariepinus* in captivity.

Specific objectives

- To study the effect of *C. gariepinus* broodstock size on spawning, fertilization and hatching of eggs and larvae survival.
- To multiply zooplanktons in a pond and determine the appropriate feeding frequency of zooplanktons for *C. gariepinus* early larvae.
- To study effect of stocking density on the growth of *C. gariepinus* in semi intensive and extensive pond culture as well as intensive tank culture.
- To compare the different culture systems for income return and yield.

Chapter 2. Literature Review

2.1. History and importance of aquaculture

Fish farming can significantly contribute vital role in the supply of food for the growing population in the world (White *et. al*., 2004) and the potential to become a sustainable means to mitigate the declining fish production in capture fisheries. The status of fisheries are in serious crisis and in a state of dramatic decline in the world (Pauly *et. al*., 2002), aquaculture offers an alternative solution to meet the ever growing demand for food and nutrition (Meyers and Worm, 2003). In terms of global production volume, that of farmed fish and aquatic plants combined exceeds that of capture fisheries in 2013 (FAO, 2016). The growth in fish production is due to increased developments of aquaculture practices, and the need for aquaculture arose from a decrease in fish supply from capture fisheries as a result of overfishing, habitat and spawning ground destruction and pollutions (Emmanuel *et al*., 2014).

The contributions of fish farming is making valuable economic improvements both at local, national and regional level through the supply of valuable products and services for both local and export markets. Small scale subsistence fish farming contributes directly to poverty reduction and achievement of food security (FAO, 2014). Aquaculture can generate employment opportunities in the art of doing fish farming, processing, value addition and marketing.

Compared with agriculture and other food production sectors, fish farming is the fastest growing food production sector and achieved an annual increase of 10% since 1984 and the annual growth rate of fish is more than meat production (Foresight, 2011). It increases economic growth, tax revenues generation and foreign-exchange

earnings (Cai *et al*., 2009). The farm gate value of food fish production from aquaculture is estimated at US$119.4 billion for 2010 (FAO, 2014). Aquaculture also contributes to the Sustainable Development Goals (SDG) by providing protein and increasing the availability of food.

2.2. Global production and contribution of aquaculture

According to the global aquaculture production statistics dataset released in 2017, a total of 201 existing countries and territories culture 591 aquatic species and species groups ever farmed in different water sources (FAO, 2017). Aquaculture production in the world has been steadily increasing over time.

Global food fish (both finfish and shellfish) production through aquaculture has increased by almost 12 times within 20 years, during 1982 to 2012 and the global food fish production rose to 76.6 million tons in 2015 (FAO, 2017) on which, finfish production contributed 67.8% of this total aquaculture output. Interms of value, the 2015 produced food fish has a farm gate value of 157.9 billion USD. The world average supply of food fish for human consumption increased from 10.14 kg in 2014 to 10.42 kg in 2015 (FAO, 2017).

Aquaculture practice and production still concentrated on few countries of the world, and most of them are in Asia. But the percentage growth varies between continents. African aquaculture grown at a rate of 10.4% followed by Asia (6%) during 2001-2015 (FAO, 2016). The global finfish aquaculture production raised to 51 million tons (Table 2.1). The sequential list of 5 top countries in aquaculture production in 2016 were China, India, Indonesia Vietnam and Bangladesh which accounted 82% of the total global production (FAO, 2018).

Table 2. 1. World fin fish production by continent from inland aquaculture (million tons live weight)

Continent	**Production year**							
	2004	***2006***	***2008***	***2010***	***2012***	***2013***	***2015***	***2016***
Africa	0.546	0.739	0.928	1.274	1.468	1.594	1.75	1.954
N & S America	0.733	0.752	0.826	0.977	0.960	0.986	1.01	1.110
Asia	22.792	26.045	30.187	34.065	39.065	41.645	41.850	47.765
Europe	0.468	0.443	0.479	0.467	0.461	0.456	0.475	0.502
Oceania	0.002	0.002	0.002	0.004	0.004	0.004	0.005	0.005
Global	24.541	27.981	32.422	36.787	41.958	44.685	45.197	51.376

Source: FAO, 2016; FAO, 2017; FAO, 2018.

By production volume, aquatic animals culture has been dominated by finfish farming, which represents the major aquaculture product in many countries. The composition of major groups of species of farmed aquatic animals vary greatly across the world. Farmed fish and shellfish are mostly grown in traditional small-scale systems that benefit local communities and the impact on the environment is minimum. The net contribution of these traditional aquaculture systems can be great as they offer many benefits, including food security in developing countries.

World annual per capita food fish consumption increased from an average of 9.9 kg (live weight equivalent) in the 1960s to 18.4 kg in 2009, and still increased further in 2010 to 18.6 kg (Foresight, 2011). However, the global population is increasing and, in order to maintain at least 18.6kg per-capita consumption of aquatic foods, the world will require an additional 23 million tons by 2020 (FAO, 2016) and the global aquaculture production is projected to rise to 94 million tons in 2030 (World Bank, 2014).

2.3. Aquaculture in Africa

The Sub-Saharan Africa (SSA) began aquaculture in the 1950s with the main objectives of food security, income and creation of jobs for the rural poor (Hecht, 2006). Environmentally, the continent is friendly for tilapia, African catfish and carp farming (Ridler and Hishamunda, 2001). The African aquaculture has been affected by a number of external problems that have prevented proper management and development despite the investment (Emmanuel *et al*., 2014). However, the presence of extensive flood plains provided environment for growth and reproduction of indigenous species during the rainy season and concentrating them in depressions or marshes during the dry season.

Many projects have been considered in West African countries like Gambia, Ghana, Gabon, Senegal and Guinea Bissau to integrate fish/shrimps culture with rice farming (Trottier, 1987). West African Aquaculture limited company started the culture of shrimp (*Peneaus monodon)* in the coastal regions in 1982 and established well in Gambia in 2000 ((Jallow, 2009). Women's groups practiced integrated rice and fish farming in Senegal and traditional fish culture using wild fingerlings in Gabon to stock ponds and cages (Trottier, 1987).

Ghana adopted a national policy to develop fish ponds for fish farming within all irrigation schemes (White *et al*., 2004; FAO, 2009) and covered a total area of 242.7ha (Amisah and Quagrainie, 2007). Most SSA aquaculture operations, particularly inland aquaculture, are still subsistence and small scale market driven ones (Satia, 2017). The major species grown were Nile tilapia (*Oreochromis niloticus),* African catfish *(C. gariepinus) and Heterotis niloticus* (Mboge, 2010).

Egypt became highest producer of aquaculture and the production increased from 919,585 tons in 2010 to 1.3 million tons (FAO, 2018).

The reported food fish consumption in 2009 was only 9.1 kg per capita per year in Africa (FAO, 2012). Anyways, fish consumption in developed countries was projected to increase from 28.1 million tons in 1997 to 29.2 million tons, at a rate of 4%, in 2020. Currently, there are many initiatives for aquaculture development in several countries of the continent like Nigeria, Egypt, Ethiopia, Kenya, Lesotho, Rwanda, Tanzania and Uganda.

2.4. Contribution of catfishes in African aquaculture development

The Clarrids constitute an excellent food fish of high commercial value. A total of 15 catfish species belonging to 7 families are exploited for aquaculture production (Adah *et al*, 2014). The fishers elsewhere in the world, including Ethiopia, target this species during the main rainy season while the runoff water flooded as the fish migrates to the field for spawning. Catfishes are good sources of high value and quality animal protein with appreciable quantity of essential amino acids and micro nutrients (Legendre *et al*., 1992).

The African catfish, *C. gariepinus*, are fast growers in captivity even at a higher stocking rates, lays up to 40 thousand eggs in one spawning season, disease resistant, tolerant to a range of extreme environmental conditions (De graaf, 1994). This species has good meat quality and smoking characteristics and possible to produce in 2-3 cycles in a year (Fagbuaro, 2010).

The overall aquaculture production of African catfish during 2015, officially reported by FAO, was 246,476 tonnes and Nigeria was top producer of *C. gariepinus* in Africa

as well as worldwide (FAO, 2017). Recently, catfish farming is leading the growth of commercial aquaculture production in Nigeria and playing a very great contribution for the development of aquaculture. A kilo of fresh catfish in Nigeria was sold for about 3.80 USD and 1.50 USD above the price of tilapia and chicken, respectively (Cai *et al*., 2009). The attractive market value of catfish encouraged the development of catfish farming industry to an extent of a commercial enterprise scale and advancing so rapidly and establishing an overriding aquaculture business (Hishamunda and Ridler, 2004). With the increasingly popular roadside shops and restaurants, the development of commercial catfish farming in Nigeria is leading to flourishing catfish specialized restaurant industry (FAO, 2011a).

2.5. Catfishes and their biological features

Catfish is a diverse group of ray-finned fishes representing more than 3000 species, 478 genera and 36 families (Ferarris and Pinna, 1999). The Clariid freshwater fishes belong to the order Siluriformes (ray-finned fishes) with a wide geographical distribution in Africa. It consists of 13 genera and 34 species in Ethiopia and two of these species, *Amphilius lampei* and *Chiloglanis modjensis,* are endemic (Fishbase, 2014).

The fish is very strong and adapts very diverse environments even with poor water quality and lower oxygen level (Hecht *et al.,* 1996) due to its ability to breath atmospheric air outside water as it possesses a pseudo lung. The African catfish, *C. gariepinus,* has appreciably high growth rate attaining marketable size of 1 kg within 5–6 months under intensive management conditions, highly adaptable and resistant to handling and stress. This fish can effectively be propagated artificially by induced

spawning techniques to supply fingerlings in mass and tips a very high commercial value and return for an enterprise (Olaleye, 2005).

Characteristically, the African catfish, *C. gariepinus*, is insistent predatory omnivore that hunts at night using non-visual primary sense organs especially the senses of touch through the barbells and tactile organs found on the mouth and skin (Bruton, 1996). The yields of catfish culture ponds could be as much as 2.5 times higher than those of tilapia in one harvesting cycle (Abdelhamid *et al*, 2010). Among the freshwater fishes, *C. gariepinus* is the species with the widest latitudinal range in the world (about 70 degrees latitude) (de Moor and Bruton, 1988; Na-Nakorn and Brummett, 2009).

2.5.1 Reproduction in African Catfish

Naturally, *C. gariepinus,* shows a seasonal gonadal maturation which is usually associated with the onset of the rainy season (de Graaf and Janssen, 1996). In nature, catfish does not become sexually mature until it attains an age of one year, depending on the availability of feed in nature (de Graaf *et al*., 1995). Mostly they reach sexual maturity after 2-3 years of age (Bruton, 1978) and between 7-10 months of age in captivity, where males mature earlier than females (Legendre *et al*., 1996). The annual changes in water temperature and photoperiodicity influences the maturation process of *C. gariepinus* and the final triggering agent for spawning is a rise in water level due to runoff water (de Graaf *et al*., 1995) as the hormone responsible for spawning changes and its level elevates during this season (Goos and Richter, 1996).

African catfish makes large migrations in rivers before spawning, although large migration for spawning (potamodresis) is not mandatory (Merron, 1993). Normally,

this species makes a lateral migration towards the inundated plains to breed and the juveniles remain in the flooded areas (Witte and van Densen, 1995). There is no parental care for ensuring the survival of the progenies of *C. gariepinus* but selection of suitable site secluding them and huge number of eggs spawned (fecundity) compensates the non parental care of eggs and larvae (Hogendoorn, 1977). The development of larvae is rapid and enable to swim within 48–72 hours after fertilization and the larval period lasts for 7–10 days. Juveniles return to the lake or river when they attain a length of 15mm to 25mm (Witte and van Densen, 1995).

In captivity, broodstock transferred from nature to the hatchery maintains their annual reproductive cycle for about 1 year, after which spawning can be induced artificially using variety of hormonal treatments to the superior parents (Hogendoorm and Vismans, 1980; Adeyemo *et al*., 2007). Male brood fish must be slaughtered for testes and the semen collected from the lacerated testes (Steyn and van Vuren, 1987).

2.5.2 Food and feeding habits in catfishes

Catfish are extremely social and tend to live and hunt in firmly organized groups(Hecht and Uys, 1997). The species can eat various kinds of food, true euryphagus, and regarded as an opportunistic, omnivorous predator (Elias Dadebo, 2000). It has the ability to utilize and/or switch efficiently between alternative food sources such as plants and detritus when prey animals become rare and scarce (Potts *et al*., 2008). Food type and availability and feeding method can mitigate agonistic behavior and cannibalism in larvae and early juveniles (Almazan *et al.*, 2004; Carter and Davies, 2004).

Catfish in culture ponds have been observed to seize sinking pellets before reaching the substratum. Once they grabbed and consumed the pellets, then feed all at the substratum and finally surface to feed on the floating fines using the gillrakers as a mechanism to filter out smaller ones (Hecht *et al*., 1988). Predation is most efficient on moderately slow moving organisms at the substratum, but fast prey such as other fishes can also be caught independently or by using group hunting tactics (Merron, 1993). The proportion of natural food for African catfish is dependent on the load and accessibility of various food items within systems.

In Africa, the soaring cost of formulated fish feeds is foremost constraint to the expansion and development of the aquaculture sector (Hecht, 2007), and this has provoked to look for suitable alternative feed ingredients. The main obstacle facing fish culturists is the need to seek a balance between rapid fish growth and optimizing the utilization of supplied feed (Gokcek *et al*., 2008). There is also a need to establish the consequence of feeding frequencies on feed management, nutrient utilization and fish performance. Since the feed cost accounts a proportion of 40 to 60% of the total operating costs in intensive culture systems (Agung, 2004), the economic feasibility of the fish farming operation depends on the feed type and feeding frequency.

Almost all the feeds used by fish farmers in Africa are farm made, moist or dry pelleted feeds (Hecht, 2007; Ponzoni and Nguyen, 2008). Non dried ingredients comprises of poultry innards, minced poultry farm offal, abattoir waste, butchery sweepings, fish market waste (mainly fish leftovers and offal), maggots, termites, earthworms, hotel or restaurant kitchen trashes and live juvenile tilapia (Dale *et al*., 2004). Mostly, these moist ingredients are mixed with milled oilseed cakes (Niger seed, soy, cotton, sunflower, palm kernel) and relatively inexpensive ingredients such

as sweet lupin kernel, maize or wheat or rice bran and brewery by-products (Bureau *et al*., 1995). A concise list of alternative ingredients have been demonstrated as a potential source of farm made feeds in the sub Saharan Africa (Gabriel *et al*., 2007).

Many farmers use moist ingredients such as chicken leftover as a standalone feed and witnessed to achieve Feed Conversion Ratios (FCR) of around 1.3:1 (Ayinla, 2007). There are suggestions that juvenile catfish fed on mixed moist feeds (with moisture content of 34%) have poorer performance indices (weight gain, specific growth rate (SGR), FCR, protein efficiency ratio (PER)) than juveniles fed on a dry feed prepared from the same ingredients (Fagbenro, 1994; Fagbenro and Jauncey,1994; Fagbenro *et al*., 1997). Studies in Uganda also found that fish grown using chicken leftover results inappropriate deposition of fat in their abdomen (Ponzoni and Nguyen, 2008).

The non-conventional ingredients that have been successfully tested as feed ingredients for *C. gariepinus* also include fish silage (Fagbenro and Jauncey, 1994; Fagbenro *et al*., 1997); hydrolyzed feather meal; maggot, termite and toad meal (Fasakin *et al*., 2003; Madu and Ufodike, 2003; Dada and Akinwande, 2004; Ayinla, 2007); dried water fern (*Azolla pinnata*) (Fasakin and Balogun, 1998); cassava leaves and peanut vines (Bureau *et al*., 1995); grasshopper meal (Nnaji and Okoye, 2004); rumen epithelial meal (Sotolu and Adejumoh, 2008); pigeon pea meal (Ogunji *et al*., 2008); winged bean meal (Fagbenro *et al*.,1999) and others such as duckweed, periwinkle meal, sweat potato peel meal, garden snail meal, cassava meal, jackbean seed meal and sweet lupin kernel meal.

However, in most cases the higher cost of manufacturing alternative meals for fish feed greatly limits their use (Ayinla, 2007). It would appear that meat and bone meal cannot be used at the same high levels at which poultry byproduct meal is used to

substitute fishmeal in the diet of *C. gariepinus*. Studies have shown that golden apple snail meal (*Pomacea canaliculata*) replaced 100% of the dietary fishmeal component of commercial feeds in Asia (Phonekhampheng *et al.*, 2009).

Factors influencing the feeding rate in fish culture includes fish size, species and rearing systems (Cho *et al*., 2003) and also the presence of the nutrients in the feed (Mihelakakis *et al*., 2002). Through adjusting the feeding rates and ingredients, it is possible to reduce production cost and maximize growth by managing other factors like variation in individual size and physicochemical and biological characteristics of culture water on which both of them are deemed vital in fish rearing

From a farming perspective, a broad range of feed ingredients of animal and plant origin, including fish, may be considered in formulating fish feeds that will satiate the dietary requirements of a catfish (Schoonbee, 1969; Huisman, 1976; Richter, 1976; Bruton, 1979b; Spataru *et al*., 1987). Its tendency toward a carnivorous feeding habit suggests that *C. gariepinus* has a relatively high dietary protein requirement, from 40 to50 % of crude protein on a dry weight basis. The fact that the African catfish also feeds on plant material reflects its ability to digest and assimilate plant proteins and make use of carbohydrates as a source of energy (Clay, 1979; Uys*et al*., 1987; van Weerd, 1995).

For the first 4 to 6 days after the start of an external food, *C. gariepinus* needs live food in the form of enriched *Artemia* nauplii than dry food only (Hecht 1996; Awaiss and Kestemont, 1998). Studies indicated that periphyton could also be used partially or wholly as an alternative to *Artemia* for rearing *C. gariepinus* larva (Nwachukwa, 1999).

The stomach becomes functional 5 to 6 days after the start of feeding at a temperature of 27 ^{0}C (Verreth *et al*., 1992). Once the digestive system becomes functional and pepsin activity contributes significantly to protein digestion, the larvae of *C. gariepinus* be able to be weaned from livefood to a dry formulated feed (Verreth and van Tongeren, 1989; Verreth *et al*., 1993). Weaning onto a formulated diet takes place progressively from 6 or 7 days following hatching to the end of 10th day, where after the fry is fed exclusively on formulated feed. After 12–14 days, the fry are stocked into nursery ponds at a stocking density between 65 and 2,000/m^{2} (Viveen *et al.*, 1985; Hecht *et al.*, 1998) depending on fish age and the intensity of rearing protocols.

Based on the proximal composition of diets of undomesticated populations, Uys (1989) predicted that *C. gariepinus* would have a relatively high protein demand (>45 %), a lipid demand of around 18.5 % and a dietary energy requirement of around 18 MJ/kg. Common to most species, *C. gariepinus* juveniles (up to approximately 10g body weight) have a high protein demand of around 50% (van Weerd, 1995; Adebayo and Alasoadura, 2001) and the best growth rates and food conversions of pre-starter *C. gariepinus* (fishes of <10g) are achieved with diets containing up to 50% crude protein.

The most essential nutritional requirements during the grow-out phase ranges from 40 to 43% protein, 10 to 12% for lipid and between 15 and 32% for carbohydrate and *C. gariepinus* grow outs cannot make use of dietary carbohydrate levels above 35% carbohydrate (Ali, 2001). Dietary carbohydrate levels of between 26 and 32% had a significant protein sparing effect, promoting the greater use of carbohydrates in catfish diet formulation (Pantazis, 2005). This fish is also capable of digesting

carbohydrates from an early stage and this continues throughout the animal's lifespan. The pancreatic and foregut amylase and the intestinal alpha-amylase activity improved with increasing the levels of dietary carbohydrate (Uys and Hecht, 1987; Uys *et al*., 1987; Uys, 1989; Ali and Jauncey, 2005).

In intensive *C. gariepinus* monoculture, feeding with very well formulated diet, especially adjusting the ratio of protein to energy (P/E) is the basic requirement. The P/E is very much dependent on temperature (Henken *et al*., 1986) and increases markedly from 25.4 mg/kJ at 24 °C to 34.7 mg/kJ at 29°C. But, the body composition in *C. gariepinus* is not influenced by varying dietary P/E ratios (Ali and Jauncey, 2005). The optimum digestible energy is between 14 and 16 MJ/kg and the protein to energy ratio is optimal between 26 and 29 mg/kJ of digestible energy (Pantazis, 2005). Diets formulated from less costly ingredients and the desired feeding frequency have revealed that profitability can be maximized at dietary protein level between 35 and 38% (vanWeerd, 1995).

At a dietary protein content of 40%, the optimal lipid to carbohydrate ratio is around 1:2.5. Increasing dietary lipids can help to reduce the elevated costs of diets by partly sparing protein in the feed. But, problems such as excessive fat accumulation in the liver which can excessively affect fish health and drops the quality of fish in the market (Craig, 2009). Fat deficient diets may result in poor growth performance and physiological mess (De-Silva and Anderson, 1998).

Research findings indicated that when fish oil is used as the only source of lipid, the growth of *C. gariepinus* is negatively affected (Ng *et al*., 2003; 2004). However, the dietary lipid source does not affect the overall body composition or muscle lipid level in *C. gariepinus* (Ng *et al*., 2003). Studies suggested that early juveniles grow better

when at least 10% of the total lipid comprises of fish oil (Subasinghe and Tarlochan, 2001).

Fish practically obtains a considerable proportion of micro nutrient requirements from the environment under pond farming condition. Farmers have found that incorporating a general vitamin and mineral premix of 1% of the diet is more than adequate to fulfill micro nutrient requirements (Wilson and Moreau, 1996). Studies revealed that mineral supplementation for fish feeds containing 27% fishmeal had no beneficial effect on the growth of juvenile *C. gariepinus* and advised not to include a mineral mix into diets containing a high proportion of fishmeal (Ng *et al*., 2001).

2.5.3 Water quality requirements

African catfish has several adaptation mechanisms for low oxygen level due to the arborescent organ enabling the fish to obtain oxygen from the atmosphere (Hecht *et al*., 1996). These organs above gill arches is an accessory air-breathing organs functioning like a lung and render them to be capable of aerial respiration and thus able to get 80–90% of the dissolved oxygen requirements (Moreau, 1988). Dissolved oxygen concentrations greater than 4 mg/l are required for better growth performance of catfishes.

The African catfish is an easily cultivable fish because of its resilience and high tolerance level for environmental conditions below their optimal requirements, e.g., high ammonium and nitrite concentrations (Ip *et al*., 2004; Schram *et al*., 2010). Toxicity tolerable levels of ammonia depend on individual species; however, toxicity level below 0.02 ppm is generally considered as non detrimental (FAO, 1999). An elevated level of ammonia concentrations are mostly manifested in water reuse

systems, where water is continually recirculating. Nevertheless, the intermediate form of ammonia, i.e. nitrite, has been known to take place at toxic levels in fish ponds unless controlled.

Catfishes are an example of a warm water species, with a temperature range for growth between 22 and 30°C. A temperature of 28°C is generally considered as optimum for the growth of catfishes. The acceptable optimum pH range in fish culture is between pH of 6.5 to 9.0.

Chapter 3. Effect of Female Broodstock Size on Spawning, Fertilization, Hatchability and larval Survival on Indigenous African Catfish, *Clarias gariepinus* (Burchell, 1822).

3.1. Introduction

3.1.1. Background

The African catfish, *C. gariepinus* is one of the three commercially important fish taxa in the Lake Tana sub basin, Ethiopia. Compared to *O. niloticus* and *Labeobarbus spp*., this fish has better growth performance. The percentage contribution of *C. gariepinus* in the Lake Tana fisheries was almost the same as the other two commercially important species, *O. niloticus* and *Labeobarbus spp.* (Tesfaye Wudneh, 1998). But the stock biomass of *C. gariepinus* in Lake Tana declined from 22.9 kg/ha in 1991 to 5.1 in 2001 (Alayu Yalew, 2006).

The sun dried *C. gariepinus* meat has been exported to other African countries through Sudan since the last 15 years and generating foreign currency. This fish has a very good market demand in the Eastern parts of Ethiopia, even preferred than Nile

tilapia (*O. niloticus*). The price of a kilo of filleted *C. gariepinus* in 2017 was one USD around Lake Tana and 2-3 USD around Lake Beseka (Metehara area), depending on the season. This fish has also good acceptance and commercial value in other countries like Nigeria (Adewolu and Adoti, 2010).

Clarias gariepinus mostly reproduces once in a year and the breeding season in the wild usually commences with the advent of the rains. *C. gariepinus* reaches maturity after two years in nature (Bromage and Roberts, 1995). In captivity, it reaches the first sexual maturity between 7-10 months of age, in which males mature earlier than females (Legendre *et al*., 1996). The testis is completely developed once they attain a weight of 200 g (Rurangwa *et al*., 2004).

Female *C. gariepinus* has entirely developed ovaries which contain mature eggs the whole year round, if kept in ponds with water temperature over 22^{o}C (De Graaf *et al*., 1995). Unless the environmental factors stimulating to do so is facilitated at the fish farms, catfishes do not spawn in captivity and need to be provoked with inducing compounds. Artificial reproduction with induced breeding of *C. gariepinus* depends largely on the time gap in between injection of the female fish and stripping of eggs, which is commonly known as the latency period (Zonneveld *et al*., 1988) and the water in which the female broodstock is kept once injected with spawning inducers (Crandell *et al*., 1995). Understanding the precise latency period helps to attain best performances in fertilization, hatchability and survival of the larvae (Zonneveld *et al*., 1988).

The ripening of the ovary after injection depends on the type of hormone used to induce the female fish (Crandell *et al*., 1995), feeding (Knox *et al*., 1998; Sule and Adikwu, 1999) and water temperature (FAO, 1996; Sahoo *et al*., 2007). The higher

the temperature, the shorter is the latency period. The amount of hormone or pituitary extract used to inject fish is also important and higher dosage shortens the time of stripping (Hogendoorn and Vismans, 1980). The eggs of a ripe female make up 15 - 20% of the body weight which is equivalent to about 150 - 200 g of ripen eggs for a gravid female of 1 kg (de Graaf *et al*., 1995) and 1g egg sample contains approximately 600 -700 matured eggs (Viveen *et al*, 1985). For large eggs and fry production, female brood stock of 1-1.5kg sized *C. gariepinus* were advised to be used in fish hatchery operation (Hogendoorn and Vismans, 1980; Viveen *et al*., 1985; Bichi *et al*., 2014).

But growing fishes to a bigger size more than 1kg costs more and is difficult to manage as it requires more feed, facility and expertise. In the meantime, the number of fries to be produced from mature and ripe broodstocks having smaller sizes (< 1000g) has not been studied well in Ethiopia. Hence it is more than important to evaluate whether indigenous female *C. gariepinus* broodstocks under 1000g body weight could give viable eggs and surviving larvae.

3.1.2. Objectives

The objectives of this research were;

- to quantify and compare the number of eggs spawned, fertilized and hatched from different broodstocks
- To determine the percentage survival of 15 days old larvae of *C. gariepinus*.

3.2. Materials and Methods

3.2.1 Experimental place and setup

This research was conducted in the Laboratory of Fishery and other Aquatic Life Research Center (FALRC) located at Bahir Dar city ($11^0$36'36" N and $37^0$22'35.5" E). The average air temperature varied between 20 and 27^0C and dissolved oxygen was in between 6 and 10 mg/L during the experimental period in July and August. A total of 15 glass aquarium from FALRC having dimensions of 35cm width, 50cm length and 30cm height each were used. Each aquarium was filled till the height of 28 cm and gave a total volume of 50L. They were placed in a standing rack at a height of 1m from the floor. To keep the room temperature in between 25 to 28 ^{0}C, a central room heater was fixed. In order to supply an optimum temperature and dissolved oxygen for the hatched larvae, thermostat and aerator were fixed to each experimental aquarium.

3.2.2 Experimental design

The experiment was carried out using a total of 15 culture aquariums distributed over five treatment (T) groups randomly. The treatments were the 6 ADPE injected gravid female brooder recipients in each size class. Each treatment was replicated three times (n = 2 per replication). The first group encompasses female parents of size from 500-600g ((T_1) , the second group from 601 to 700g (T_2), the third from 701-800g (T_3), the fourth and fifth group were from 801-1000g (T_4) and 1001-1200g (T_5), respectively. Milt was collected from 3 male broodstocks of 1000 to 1200g size to fertilize eggs from a single parent..

3.2.3 Selection of female broodstock and handling

The experimental female fishes, having a weight of 500-1200g were selected among 300 fishes reared from F1 broodstocks and grown in a concrete pond. The experiment was conducted from July to August 2017 at FALRC. The fish were reared from the same parent fish and had been managed to grow in the research center. The selected experimental parents were stocked in a concrete pond of 100 m^2 area and, placed in an open air. Nets were stretched over the surface to protect the fishes from predators coming from the surrounding of Lake Tana.

Male broodstocks, used as source of milt had a size that ranged from 1000 to 1200g. From the total population (n=300), 90 healthy looking males and 60 females ready for reproduction were recruited. The selection was made by external morphological characteristics using the method of Ayinla *et al* (1994) through examining their genital organ. Among the recruited fishes, 30 female (n=6 per treatment) and 90 male (n = 18 per treatment) broodstocks were selected for the different treatment groups. The female broodstock fishes were selected according to their size and stocked separately in outdoor basins (n = 5) having a volume of 4 m^3 each. But male broodstock were stocked in one concrete pond all together. These fish were fed at a rate of 3% of their weight for a month using formulated feed with CP content of 42.87% (Table 5.1) prepared from local ingredients including fishmeal, bone meal, soy bean, wheat bran, oil cake and premixes.

3.2.4 Pituitary preparation and injection

The pituitary was collected from 1200 - 1500g weighed wild *C. gariepinus* from fish production and marketing Enterprise (FPME) at Bahir Dar. The pituitary was collected from the brain of the fish at the ventral side below the hypothalamus (Annex

4 plate 2). The collection of the pituitary glands was made two months prior to injection. While collecting the glands, they were placed in a falcon tube having an acetone for 12 hours to dehydrate and defatten the glands. The acetone was spilled off and replaced after another12 hours. Once the glands were placed in acetone for a total of 24 hours, the acetone was drained off again and the granules of pituitary glands were dried on a tissue paper. The dried glands were then stored in a glass phial, pressed down with a ball of fine cotton, corked tightly, and sealed by tape.

While using, the ADP glands were crushed with smooth ceramic mortar and dissolved in 2ml physiological salt solution (9gm salt in 1 liter of water) (Ayinla, 1991; Nwadukwe *et al*., 1993). Mature females with well distended abdomen were identified and eggs oozed out freely when their abdomen was gently pressed antero-posteriorly and males with reddish genital papilla at the top were selected for injection.

The selected gravid female and male broodstocks were injected with acetone dried pituitary extract at a dosage of 4mg/kg of body weight (Haniffa and Sridhar, 2002) using a 2ml graduated syringe (baby syringe) intramuscularly at an angle of 45° at the dorsal fin. As the injected fish stays nervous for some time, the broodstock fishes were kept in a bigger circular plastic trough separately according to their weight class. This protects the injected broodstocks from unnecessary damage which might occur due to fighting. The circular plastic troughs had a water volume of $0.1m^3$ (60 cm diameter and 35 cm depth).

3.2.5 Stripping eggs, Milt preparation and Fertilization

The fish were checked one by one for rippenning starting from the 10th hour from injection and the rippnned females were stripped. During stripping, the fish was handled carefully and held firmly at the head and tail region with a towel which was soaked in a warm water. Stripping was performed smoothly towards the genital vent of the fish. Then the abdomen of the female was pressed carefully by thumb to ooze the eggs out from the fish and collected with a dry and clean ceramic bowl. Stripping continued turn by turn for other broodstocks who got ready. The spent female then returned back to their basins and stayed for 15 hrs before they went back to their growing ponds. The whole stripped eggs from a brooder were weighed before being fertilized and a subsample of 2g egg was taken from each replication and counted to determine the total number of eggs stripped from each fish. When stripping gets over, all the aquariums were cleaned thoroughly for placement of fertilized eggs.

To take off the testis, fishes were placed dorsally on a wet towel, held so firmly down. Using a sharp blade, the abdominal cavity of live fish was dissected and opened (Annex 4 plate 4). Both testes were carefully removed, cleaned, lacerated using sharp surgical blades, cut into pieces with a scissor and crushed gently. Unnecessary impurities from the crushed testis were removed using forceps and a clean and ready milt was prepared. For each replication, milt from 6 male parents were pooled together (at male to female ratio of 3:1) and stirred manually before mixed with the eggs.

Milt was mixed with the eggs thoroughly using feather for 5 minutes and activated in a clean water. Wet fertilization was performed by adding 5ml saline water followed by sufficient amount of UV treated water (van der Waal, 1978). Prior to hatching,

immediately after mixing eggs and milt in a replication, 2g sample was taken and incubated in respective aquariums per treatment group (Szabo *et al*., 2002).

3.2.6 Incubation of the eggs

Eggs mixed with milt were spread over the cleaned common reed (*Phragmites australis*) roots and placed in a 20L aquarium per treatment. The root of the emergent macrophyte (*P. australis*) was preferred because of its accessibility and having a very fibrous root that can suspend at the surface of the water and give enough substrate for the fertilized eggs. Substrates from plant materials are also advantageous interms of cost minimization and providing more hatching rates than artificial substrates (Macharia *et al.*, 2005). Ten hours after incubation, the sample was observed to screen and count the number of fertilized (green, transparent and flattened ones) and unfertilized (whitish opaque and thick ones) eggs for each replication in a treatment.

Figure 3. 1. Eggs stacked to common reed root

The fertilized eggs in each replication were incubated for 20 to 30 hrs in glass aquarium at a temperature of 25-26^{o}C with continuous aeration. Starting from the 15th hour of fertilization, each aquarium was inspected every half hour whether hatching has been started from a sample of 2g fertilized eggs. The time that the hatchlings started to appear was recorded for each experimental aquarium.

After the 30 hours of incubation, *P. australis* root used as a substrate for the sample fertilized egg, together with dead and unviable eggs, was separated carefully and took off from the aquarium. The dead and unviable eggs rested at the bottom were

siphoned off using plastic pipette and distributed over green colored plastic plate for enumeration. A hand lens was used during the separation of dead and unviable eggs from the substrate and during counting. To keep the larvae in a clean environment, debris and wastes at the bottom of the aquarium were removed very carefully using long pipette. Once the waste gets rid off from the culture aquarium, treated clean water with similar temperature was filled to refresh the incubation water. The hatchlings were inspected four times a day to look after and count the dead larvae. The larvae provided *Artemia nauplii* twice a day since their 5^{th} day.

3.2.7 Quality of the culture water

The water was pumped from Lake Tana and cleaned with an indoor biofiltration system and treated with UV light installed in the recirculation system established at FALRC wet laboratory. To keep the fry in a clean environment, about 50% of the incubation water was refreshed with treated clean water having similar temperature every day. The water temperature, pH and DO were kept in between 25-26^0C, 7-9 and 5- 6 mg/L, respectively. The physicochemical parameters of culture water (Temperature, DO, pH, salinity, total dissolved solids (TDS) and electrical conductivity (EC) measurements for each aquarium was taken twice a day (at 9:00am and 5:00pm). Ammonia test kit (JBL, Gmbh CO., Germany) was used to check whether its reading went beyond the normal level.

3.2.8 Fertilization, hatching and survival rates estimation

The time taken to stripe the eggs after injecting the fishes with ADPE and required for the fertilized egg to hatch was recorded to estimate the latency and hatching time. The

number of fertilized eggs from 2g sample was extrapolated to estimate the total number of eggs incubated. Fertilization rate was estimated from the surviving embryos 10 hours after fertilization. The percent hatching was the number of hatched fry relative to the fertilized eggs while, the percent survival was the number of surviving fry after 14 days of feeding. Larvae fed *Artemia nauplii* from day 5 till day 10 and then formulated feed till the experiment ends when they are 15 days old.

The fertilization rate (FR), hatching rate (HR) and percentage survival (PS) resulted from a sample of 2g were computed based on standard formulas.

$$\text{FR (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Total no.of eggs incubated}} * 100 \quad \text{(Hogendoorn, 1979).}$$

$$\text{Or } \frac{Totalnumberofeggsspawned - Numberofbadeggscounted}{Totalnumberofeggsspawned} * 100.$$

$$\text{HR (\%)} = \frac{\text{Number of eggs hatched}}{\text{Total no.of fertilized eggs}} * 100 \text{ (Olubiyi, } \textit{et al.}\text{, 2005).}$$

$$\text{PS (\%)} = \frac{\text{No.of hatchlings alive up to larvae stage}}{\text{Total no.of hatchlings}} * 100$$

3.2.9 Data collection and Analysis

All the necessary weight measurements for sampled eggs and larvae were measured using an electronic weighing balance having 0.01g precision. The different water quality parameter readings (Temperature, DO, pH, TDS, EC) were recorded using multi-meter (Model 556 MPS). All the data were recorded in excel prior to analysis. The mean of the five different treatments were compared by one way analysis of variance (ANOVA) to test the significant differences ($p<0.05$) for fertilization, hatchability and survival rates using SPSS package version 20.

3.3. Results and discussion

3.3.1 Water quality of larvae rearing aquariums

The mean value of the physical characteristics of culture water for all aquariums indicated that water used to rear larvae was within the required range for the growth of *C. gariepinus* larvae. The water temperature was maintained in between 25-26^{0}C using thermostats fitted in each aquarium. The value of DO varied between 5 and 5.2mg/L and the pH was between 8.1 to 8.9.

Higher hatching and survival rates were observed at this pH range on the previous studies also (Ariole and Okpokwasili, 2012). The salinity of culture water in all aquariums was 9‰ for the whole experimental period. The TDS reading was between 0.122 to 0.123mg/L and EC varied between 0.189 to 191µS/cm, respectively. But the difference in water quality parameters between the treatments was not significant at 0.05 level (Table 3.1).

Table 3. 1. Water quality of larvae rearing aquariums.

Exp'l groups	***Temperature,*** ^0C	***DO,*** *mg/L*	***pH***	***TDS,*** *mg/L*	***EC,*** *µS/cm*
Trt I	25.3± 0.04^a	5.9±0.28^a	8.1±0.94^a	0.122±0.00^a	0.189±0.00^a
Trt II	25.2± 0.06^a	5.7±0.03^a	8.84±0.14^a	0.122±0.00^a	0.188±0.00^a
Trt III	25.7± 0.07^a	5.9±0.09^a	8.85±0.06^a	0.122±0.00^a	0.191±0.01^a
Trt IV	25.0± 0.02^a	5.7±0.02^a	8.56±0.13^a	0.123±0.00^a	0.19±0.00^a
Trt V	25.2± 0.04^a	5.8±0.22^a	8.86±0.07^a	0.122±0.00^a	0.189±0.00^a
P value	0.063	0.794	0.584	0.617	0.935

3.3.2 Eggs spawned and its proportion to fish body weight

The difference in size between the broodstock fish (treatment groups) was significant at $p < 0.05$ (Table 3.2). The mean number of eggs spawned. More eggs were spawned at T_5. The mean number of eggs spawned were 29.45±0.96, 32.6±1.47,37.23±1.07, 41.24±2.01 and 52.15±2.31 (in thousands) for T_1, T_2, T_3, T_4 and T_5, respectively (Table 3.2). As depicted on Fig 3.2, there existed a positive correlation ($r^2 = 0.98$) between body size and the number of eggs spawned. The result was in agreement with Rideout *et al*. (2005) which indicated that larger females produced more number of eggs.

Table 3. 2. Number of eggs spawned and their proportion with fish body weight.

Treatment group	***Broodstock mean BW, g***	***Spawned eggs ('000)***	***No. of egg/kg fish***	***Egg wt to BW ratio***
T_1 (500-600g)	568.8 ±8.98 [a]	29.45±0.96 [a]	51,776	12.4±0.27[a]
T_2 (601-700g)	662 ±16.48 [b]	32.6±1.47 [ab]	49,245	13.±0.28 [ab]
T_3 (701-800g)	756 ± 15.32 [c]	37.2±1.07 [bc]	49,246	13.7±0.36 [b]
T_4 (801-1000g)	907.2±13.97 [d]	41.24±2.01 [c]	45,458	14.8±0.12 [c]
T_5 (1001-1200g)	1110± 35.54 [e]	52.15±2.31 [d]	46,982	15.2±0.43 [c]
P value	0.024	0.023		0.01

Values(mean ± SE) with the same superscript letters in a column didn't differ significantly ($p < 0.05$).

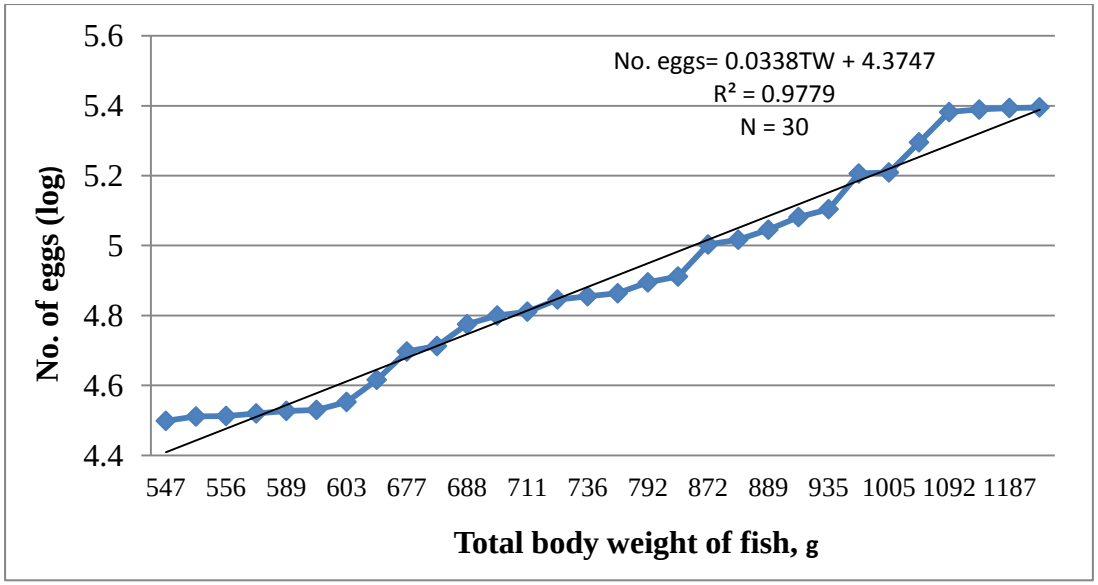

Figure 3. 2. Relationship between number of eggs spawned and Broodstock weight.

The relationship of female weight to weight of egg mass produced was also considered. The proportion of egg weight spawned to body weight of parent fishes was 12.4, 13.02, 13.68, 14.78 and 15.17% for T_1, T_2, T_3, T_4 and T_5, respectively (Table 3.2). The difference was significant ($p < 0.05$) between broodstocks with 500-800g weighed and 801-1200g sized ones (Table 3.2). The proportion of egg to BW was not in agreement and lower compared with the previous results (de Graaf *et al*., 1995) except for T_5 (1001-1200g sized groups). This might be as a result of the lower level of management as the fishes were growing in a pond with feed prepared from local ingredients without considering their nutritional requirement.

As indicated in table 3.2, the number of eggs produced by 500-600g weighed broodstock in T_1 was 51,776/kg BW and 46,982/kg for 1001-1200g weighed treatment groups (T_5). It was due to the egg size difference between broodstocks, as visually observed during the experiment, that the size of egg from 500-600g weighed parent were very small compared to the other treatment groups. Hence, there was a

clear relationship between fish weight and the number of eggs spawned and hatched. The heavier the brooder, the more the eggs.

3.3.3 Latency and incubation periods and their effect on fertilization and hatching

The ripening of the ovaries after injection depends on temperature (de Graaf and Janssen, 1996) and the type of hormone used to induce the female (Crandell *et al*., 1995). The time required for stripping a female after the injection of ADPE preparation was longer for T_1 compared to T_5. The latency period varied nearly between 14 and 16 hrs. But the difference was not statistically significant ($p<0.05$) between the treatment groups except T_1 . Eggs from T_1 *C. gariepinus* groups needed more time to get spawned after the injection of the ADPE (Fig 3.3).

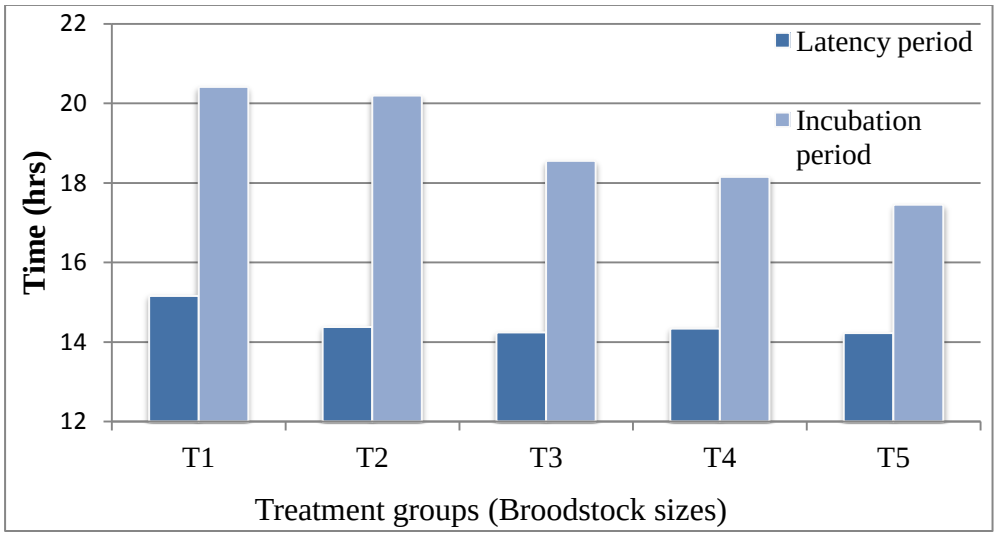

Figure 3. 3. Time required to stripe the eggs after injecting ADPE and the fertilized eggs to be hatched.

Previous studies also indicated that fertilization rate of *C. gariepinus* largely depends on the latency period (Hogendoorn and vismans, 1980; Zonneveld *et al*., 1988). The period needed for a fish to spawn was a bit longer in this experiment compared to

results from previous works (Viveen *et al*., 1985; Legendre ,1986; Sahoo *et al*., 2007) which was 7-11 hrs at 30^0C. This might be due to lower water temperature in which the experimental brood stocks were kept.

The time required for the fertilized eggs to hatch (incubation period) was longer (20:41 hrs) for broodstocks from T_1 compared to those in T_2 and there existed significant difference ($p < 0.05$) between the treatments (Table 3.3). The result clearly indicated that the time needed for the fish to spawn and the fertilized eggs to get hatched related inversely with body size (weight), i.e. the smaller the size, the more was the time required for spawning and hatching (Fig 3.3).

The incubation period was in confirmation with the results of other experiments at an incubation temperature of 27 to 30 ^{0}C (Rao *et al.,* 1994). This result indicated that apart from the type of hormone used, feeding, pH and temperature (Rao *et al.*,1994; Crandell *et al*., 1995; Knox *et al*., 1998; Ariole and Okpokwasili, 2012), broodstock size also affected the ripening time of the ovaries.

Table 3 3. The effect of latency and incubation periods on fertilization and hatching

Treatments	***Latency period (hrs)***	***Fertilized eggs ('000)***	***Incubation period (hrs)***	***Hatched eggs ('000)***	***Survived larvae ('000)***
T_1	15:16±0:09^a	13.79±0.78^a	20:41± 0:09^a	13.24±0.87^a	10.21±0.78^a
T_2	14:38±0:15^b	18.61±1.26^b	20:19± 0:04^b	17.87±1.21^{ab}	14.55±1.02^b
T_3	14:24±0:12^b	24.08±0.75^c	18:55± 0:06^c	23.19±0.72^b	19.49±0.75^c
T_4	14:34±0:04^b	30.22±1.62^d	18:15± 0:00^d	29.69±1.62^c	25.02±1.38^d
T_5	14:22±0:03^b	41.65±2.1^e	17:45± 0:00^e	40.9±2.04^d	31.1±1.4^e
P- value	0.028	0.017	0.01	0.014	0.016

Numbers (mean± SE) with the same superscript letters in a column were not significantly different at $p < 0.05$.

The mean number of larvae hatched from 500-600g weighed (T_1) *C. gariepinus* group were about 13 thousand but more than 30 thousand larvae had been hatched from 800-1000g weighed (T_4) groups and more than 40 thousand larvae from 1001-1200g (T_5) weighed groups. There existed a significant difference ($p < 0.05$) between the treatment groups (Table 3.2).

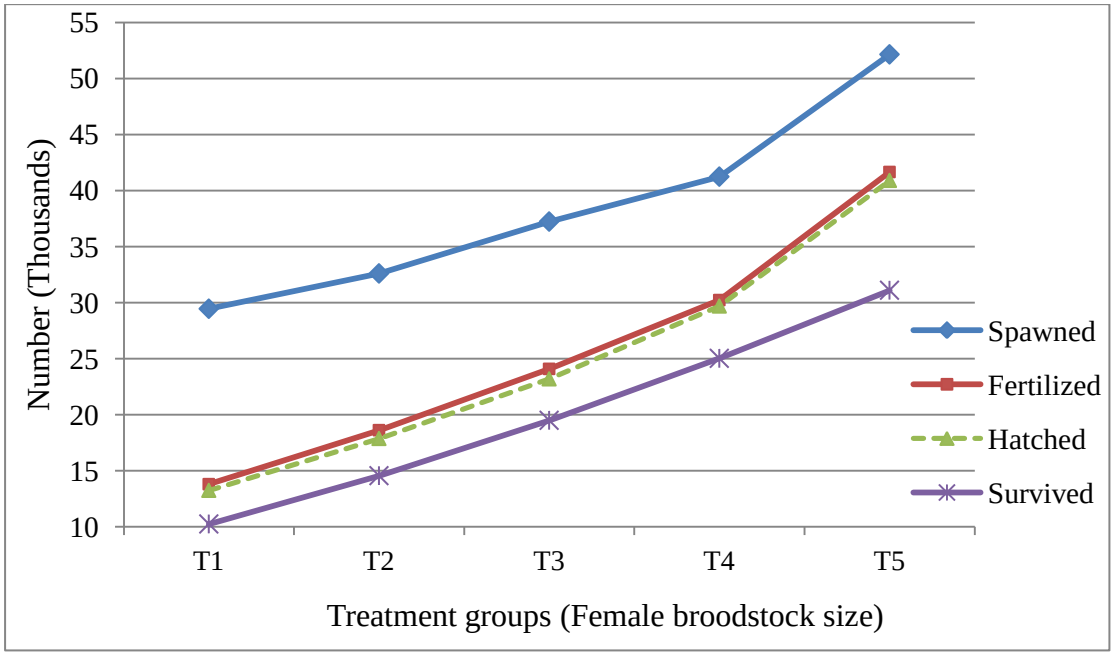

Figure 3. 4. The performance of broodstock sizes on some reproductive parameters.

The number of early larvae that survived during the experimental period increased with size of the broodstock. The mean number of larvae that survived in the first treatment was more than 10 thousand and more than 14, 19, 25 and 31 thousand per fish for T_2, T_3, T_4 and T_5, respectively (Table 3.3). As depicted on Fig 3.4, the number of eggs spawned, fertilized and hatched increased with broodstock size. The same is true for number of larvae that survived.

3.3.4 Fertilization and hatching rates and percentage survival

Latency period affected the rate of fertilization as more fertilized eggs were observed on the groups ripened in a shorter time. The rates of eggs fertilized and the hatchability of the fertilized eggs varied among the treatment groups. Rates of fertilization and hatchability was poor for brooder fish in T_1 and the difference in hatchability between T_1, T_2 and T_3 sized treatment groups (500-800g) was not significant ($p > 0.05$). Hatchability was the same for T_4 and T_5 (> 800g BW). The lower fertilization rate in smaller group (T_1) might be due to the longer time required for these fish to spawn and the quality of the eggs spawned. But there existed a significant difference in hatchability between the groups with body weight less than 800g and greater than 800g fish groups (Table 3.4). Studies indicated that over ripening of the ovaries can result in poor fertilization and hatchability (Ohata *et al.*, 1996; Oyelese, 2006).

The whole egg spawned and fertilized was not hatched in all the treatment groups and hence the hatching rate was less than 100%. But as indicated in Table 3.4, majority of the fertilized eggs were hatched and the rate was more than 95% in all the treatment groups. Apart from over ripening of the ovaries, the age of the broodstock affects fertilization and hatchability (Legendre *et al*., 1996). According to the results of this experiment, rather the size of the female broodstock fish was very important in the reproductive performance of *C. gariepinus* as the hatchability of eggs from T_4 and T_5 was significantly higher ($p < 0.05$) compared to the other treatments (Table 3.4).

Table 3. 4. Rates of fertilization and hatching and percentage survival

Trt groups (Broodstock size)	***Fertilization rate, %***	***Hatching rate, %***	***percentage survival, %***
T_1 (500-600)	46.64±1.24 [a]	95.8±0.9 [a]	76.8±1.4 [a]
T_2 (601-700)	56.82±1.3 [b]	96.04±0.55 [a]	81.4±0.7 [b]
T_3 (701-800)	64.66±0.5 [c]	96.31±0.5 [a]	83.9±0.9 [bc]
T_4 (801-1000)	73.2±0.7 [d]	98.21±0.5 [b]	84.2±0.4 [c]
T_5 (1001-1200)	79.84±0.7 [e]	98.22±0.2 [b]	76.1±0.6 [a]

Note:- Mean values with the same superscript letters in a column are not significantly ($p < 0.05$) different.

More than 75% of the total hatched larvae survived in each treatment group irrespective of the weight of the female broodstocks. The survival rate was 76.8, 81.4, 83.9, 84.2 and 76.1% for T_1, T_2, T_3, T_4 and T_5, respectively. The result indicated lower survival rates in T_1 and T_5 experimental groups and higher in T_3 and T_4 groups. The difference was significant ($p < 0.05$) between the experimental groups (Table 3.4). The lower survival might resulted from the quality of larvae from the smaller groups and the amount of *Artemia nauplii* provided might not sufficient for large larvae as equal amount of feed was given irrespective of their size.

3.4 Conclusion

The aim of this experiment was to compare the different sizes of female *C. gariepinus* broodstock in affecting the different reproductive parameters. The study indicated that the size of broodstock affected the reproductive performance indicators and it is in agreement with results of previous works that recommended to use larger sized ones

to get large sized eggs (Bromage and Cumaranatunga, 1988; Ataguba *et al*., 2012; Bichi *et al*., 2014). But the study also indicated that smaller sized broodstocks could produce more number of eggs per kg body weight than larger ones with the same weight.

The study result had an economic implication as it would minimize cost of production which was expended to keep and manage the broodstock for longer period till attaining bigger body size. The latency period was in between 14 and 15 hrs for all groups and there had never been significant difference in between the different size groups. In addition the survival rate of early larvae from larger sized *C. gariepinus* parents was not more compared to small sized ones, even more survived larvae were obtained from medium sized parents. It is advisable to use *C. gariepinus* fishes of 500-600g and not necessary to keep broodstocks till attaining 1kg of body weight. The growth performance of larvae and fingerlings from different sizes of broodstocks need to be compared.

Chapter 4. Production of indigenous zooplanktons and effect of feeding frequency on the growth and survival of early larvae of the African Catfish, *C. gariepinus*.

4.1 Introduction

4.1.1 Background

Compared to other fish species, after hatching and resorption of the yolk sac, *C. gariepinus* fry cannot rely on formulated feed directly (Verreth *et al*., 1992; Tocher, 2010). During the onset of external feed, *C. gariepinus* larvae require live food as their digestive system is not complete to assimilate the formulated dry feed at this early stage (Kolkvoski, 2001). Feeding live zooplankton from nearby fresh water fish ponds seem to be the most reliable technique for Ethiopia since importation of Artemia cyst is very costly. Rather, water bodies in the tropics are rich in diversity of important zooplanktons used as live food (Abaho *et al*., 2016) and easy to multiply. Furthermore, microcrustacean zooplankton in Lake Tana (mainly of freshwater species) constitutes a major component of the food chain on which the copepods and cladocerans contributed the majority of the crustaceans (Eshete Dejen, 2003; Ayalew Wondie, 2006; Imoobe and Akoma, 2008).

Live food like small *Daphnia*, *Moina* or other zooplankton of suitable size are good sources of freshwater live food for the first 4 to 6 days after the start of exogenous feeding (Bryant and Matty, 1980; Arrhenius and Hanson,1993; Haylor, 1993; Hecht, 1996; Awaiss and Kestemont, 1998; Olojo *et al.*, 2003) and then the larvae can be weaned from live food to a dry feed (Verreth *et al*., 1993) 5-7 days after the start of

taking live food (Verreth *et al*., 1992; Segner *et al*., 1993) as the stomach starts to digest feed.

Zooplankton can multiply in short time (Polo *et al*., 1992; Sipauba-Tavares and Bachion, 2002; Piasecki *et al*., 2004; Hecht, 2013), economically viable (Fernando, 1994; Watanabe and Kiron, 1994; Hecht, 2013) and important source of protein, lipids, fatty acids, minerals, enzymes like amylase, protease, exonulease and esterase (Munilla-Moran *et al.,* 1990; Mims *et al.,*1991; Olojo *et al.,* 2003) and carotene (Spenelli, 1979). Zooplankton can satisfy the nutritional requirements (Yurkowski and Tabachek, 1979) with high ratio of unsaturated fatty acids to saturated fatty acid (Lokman, 1994) and good quantity of amino acids, mainly Lysine and methionine (Dabrowski and Rusiecki, 1983).

Copepods have superior feed quality and resulted in high growth and survival rates with lower incidence of malformations (Hamre *et al*., 2008; Van der Meeren *et al*., 2008). Due to their better nutritional, morphological and behavioral characteristics and tolerance to lower and higher temperature ranges, rotifers are potential substitutes for *Artemia* (Watanabe *et al*.,1983; Koven *et al*., 1990; Ludwig, 2000; Lubzens *et al*., 2001; Stelzer, 2012).

Zooplanktons can be fed for 7-30 days depending on fin fish species at a rate of 3 to 5 rotifers/ml at a stocking density of 10 to 20 larvae/L for a day (Treece, 1995). A partially bigger mouth in *C. gariepinus* larvae (Yilmaz *et al*., 2006) enables them to predate zooplankton with sizes greater than 200 μm.

Time and frequency of feeding have been reported to have an effect on intake of feed and growth performance in different Clariids (Noeske- Hallin *et al*., 1985). Feeding

frequency has direct impact on the performance of fish mainly survival rate and growth of *Clarias* larvae *(*Verreth *et al*., 1987*)*. Feeding frequency in *C. gariepinus* varies with species, size and age of fish, management and fish husbandry, quality of feed and environmental factors (Goddard, 1995). The *Artemia nauplii* feeding frequency of 3 times/day is adopted by Nigerians to avoid water fouling and ease of feed provision and other management aspects (Abaho *et al*., 2016).

Imported *Artemia* cyst have been used and nauplii were hatched in the laboratory to feed an early larvae at the research centres in Ethiopia (personal experience and observation). However, the cost of *Artemia* is not affordable and necessary to look for alternative livefood sources. In addition, the practical use of zooplankton harvested from fertilized ponds as a live food source and its feeding frequency for *C. gariepinus* larvae not tested and determined in Ethiopia.

4.1.2 Objectives

The main objective of this research was to multiply zooplankton groups as live food and determine the appropriate feeding frequency for larvae of *C. gariepinus*.

Specific objectives:

- To identify the zooplankton taxa harvested from fertilized ponds to the lowest possible level.
- To determine the density and other quantitative indices for cultured zooplankton groups.
- To test the growth and survival rates of *C. gariepinus* fed diverse zooplankton groups at different feeding frequencies.

4.2 Methodology

4.2.1 Experimental setup

The source of zooplankton taxa cultured in concrete ponds and (Annex 4 plate 5) used as live food were indigenous species available in naturally in Lake Tana. The different zooplankton groups were inoculated in three culture ponds. Each multiplication pond has an area of 100 m^2. To multiply the zooplanktons in a big mass, the pond water was fertilized, 2 weeks before zooplanktons were inoculated, using commercial fertilizer (Di-Ammonium Phosphate - DAP) at a rate of 5g/m^2 **(**Hepher,1963).

The experiment was conducted at the laboratory of FALRC in Bahir Dar using 12 glass aquariums of 50L capacity arranged in a rack in the larval rearing room (Annex 4 plate 8). Experimental *C. gariepinus* larvae were stocked at a lower rate (2 fish/L) compared to other studies (Treece, 1995) as the biomass of zooplankton multiplied in ponds might not accommodate more population. The temperature of the culture room was kept between 24 - 27^0C using room heater. The culture water of each aquarium was refreshed daily using the recirculating water in the hatchery. The recirculation facility has biological filtration system (with sand and bids) and UV light to treat the culture water before it was used in the rearing aquaria.

All the experimental aquariums were cleaned thoroughly using non toxic detergents, antibacterial and antifungal chemicals. There were four treatment groups (four different feeding frequencies) and each treatment was triplicated. All the treatment groups were fed with indigenous zooplankton species harvested daily from plankton multiplication ponds. Feeding started at late 8:00 am in the morning and stopped at 5:00 pm.

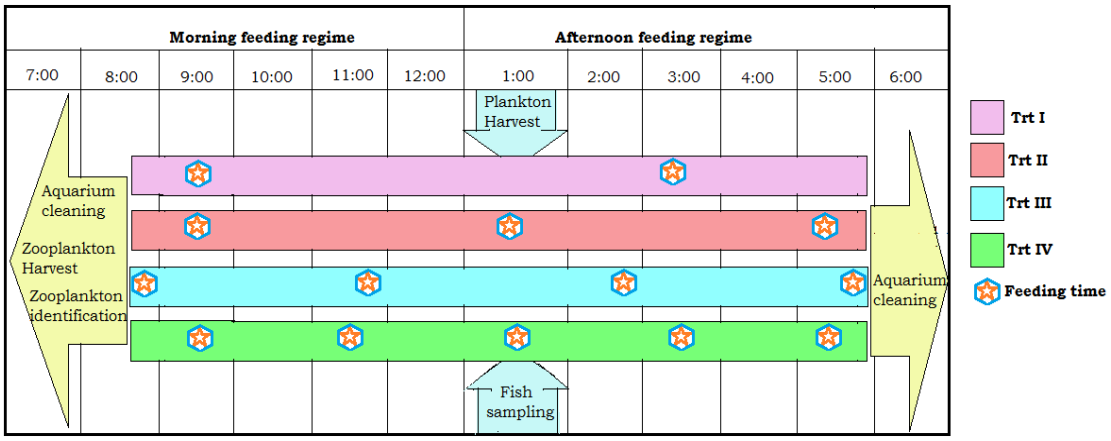

Figure 4. 1. Schematic representation of daily routines during the experimental period.

The larvae in the first treatment (T_1) were fed with zooplankton mass every 6 hrs (twice a day). Treatment two (T_2) received every 4 hrs (3 times a day), the third (T_3) and fourth (T_4)) groups were provided with harvested zooplankton mass every 3hr (4 times a day) and 2hrs (5 times a day), respectively. A protocol used during feeding and other routine activities in a hatchery was prepared as indicated on Fig 4.1.

4.2.2 Zooplankton collection

Zooplankton groups used as live food were locally available ones from Lake Tana inoculated in three multiplication ponds. The plankton were collected from eutrophic ponds using 150µm mesh sized plankton net having 200ml collection cup.

The net had a mouth opening of 50 cm diameter and 100cm length and zooplankton was collected through pulling the net horizontally. Every day, zooplankton were harvested from three ponds and each pond was seined 20 times, 10 times early in the morning and 10 times in early afternoon. The harvested zooplankton mass from each pond was filtered through 125 µm sieve in order to remove bigger sized zooplankton from the collection. The filtered and concentrated zooplankton mass was washed

again and again using 40micron zooplankton sieve to clean the dirt water brought from ponds as suggested by Mack *et al* (2012).

The concentrated zooplankton mass was immediately poured in a 2liter bucket with treated hatchery water. The cleaned zooplankton mass (2L in volume) harvested from each pond was pooled together, mixed and stocked in a 10L total capacity aquarium with gentle aeration. The zooplankton mass harvested at a time (6L) served only for half day and provided to larvae portion by portion according to the feeding regime indicated on Fig 4.1 for the whole experimental period of 7 days.

4.2.3 Sampling the harvested zooplankton

Immediately after cleaning the concentrated mass, before mixing zooplanktons from all ponds, a subsample of 1 m1 was taken from an aliquot sample 5ml from each pond using pipette and fixed with 4% formalin for identification. Zooplankton identification was based on standard methods (Fernando, 2002). From the sample aliquot of 5ml, another subsample of volume 0.5ml was taken with micro pipette and poured into the gridded glass counting chamber to determine the number of individuals (Lind, 1979). Identification and enumeration was assisted using binocular compound microscope (Olympus CH-2) at different magnifications (Fig 4.2). The total number of zooplankton counted from the subsample were then extrapolated to the total mass of the aliquot harvested in a day.

Figure 4. 2. Zooplankton identification and enumeration

Density, frequency of occurrence, species richness index, species diversity index (Shannon-Wiener diversity index), relative abundance and species evenness indexes were calculated to determine the species composition and their diversity across the different harvesting ponds. The different quantitative measures were calculated using different standard formula:

- Density of zooplanktons; $(\text{Number of individuals/L}) = \frac{AC}{L}$. Where; A = Average number of individual per ml; C = Volume of concentrated sample in ml; L = Volume of filtered water in Liter.
- Frequency of occurrence; $F = \frac{Ts}{TS} * 100$. Where "Ts" is the number of samples in which the taxon (species) is present, and "TS" is the total number of samples. If the calculated value was > 70%, a species occurrence is said to be much Frequent, if 70% - 40% it is Frequent, 40% - 10% - Less Frequent and < 10% the species occurrence was judged as infrequent or sporadic.
- Diversity of zooplankton is the distribution of the number of individuals among the zooplankton communities. Commonly the Shannon and Wiener diversity index is calculated to determine the diversity of species. Shannon-Wiener diversity index; $H' = -\sum_{\infty}^{s} Pi\ (\ln Pi)$ (Shannon and Wiener, 1949). Where H' is Shannon-Weiner index of species diversity, Pi is the proportion of species (Pi = S/NS) , S is the number of species and N is total number of all individuals in the sample.
- Species richness (d) is the total number of different organisms present, not the proportion and distribution of each species, within the local aquatic community. The Margalef's species richness index (Margalef, 1968),

represented as by d is taken as a measure of richness. And, $d=\frac{(S-1)}{\ln N}$ where S is the number of taxa, and N is the total number of individuals in the sample.

- Relative abundance (Ra) is expressed with the formula; $Ra = \frac{N}{Ns} * 100$. Where N is the number of organisms of each taxon (species) in the sample; and Ns is the total number of all organisms in the sample.

  The result in percentage (%) was then used to prepare a semi-quantitative approach. If the percentage value is > 70%, a species abundance was called Dominant, values between 70% to 40% are said to be Abundant, 40% to 10% is less Abundant and it is called rare if it is < 10%.

- Species evenness index (e) expresses how evenly the individuals in a community are distributed among the different species and calculated as ,$e = \frac{H\prime}{\ln S}$ (Pielou, 1966). When there were similar proportions of all species, then evenness value is very close to one, but when the abundance are very dissimilar (some rare and some dominance species) then the value decreases.

4.2.4 Feeding the larvae

Each aquarium has been cleaned twice a day (early in the morning and late evening) using siphoning tube. Thermostat and aerator were fixed to each aquarium to keep the temperature and oxygen level of the culture water at its desired range for larval rearing. The culture water spilled during cleaning was replaced, in the meantime refreshed, using cleaned water. Before taking the zooplankton for feeding, the mass was mixed thoroughly through applying higher aeration for 2 minutes. Each aquarium received an average of one liter of aliquot of zooplankton mass in a day and the mass of zooplankton was equally divided in portions according to their respective feeding

frequency. Once the number of zooplankton taxa harvested in one ml was enumerated and adjusted, larvae were fed at a minimum rate of 5 individuals/ml for fishes stocked at a density of 20 larvae/L as indicated in other studies (Treece, 1995; Ut *et al*., 2013).

4.2.5 Experimental fishes

The source of gravid broodstocks (both male and female) used to hatch the larvae were F_1 (first filial generation) from FALRC at Bahir Dar grown in a concrete pond. Larvae were hatched from these gravid parents induced with acetone dried catfish pituitary extract. On the third day after hatching, 12,000 early larvae were selected for the experiment. On the fourth day, 1200 healthy looking larvae were selected from the total population. The recruited experimental larvae were distributed over 12 aquariums and each aquarium received 100 *C. gariepinus* larvae. Feeding started at 4^{th} day and the experiment was conducted for a period of 7 days.

Every day, at 1:00 pm, a sub sample of 10 *C. gariepinus* larvae was taken randomly from each aquarium and weighed. Regular inspections to record the dead had been done every morning and afternoon. The total counts of live experimental *C. gariepinus* larvae in an aquarium was taken during the start and at the end of the experiment and checked with the daily mortality record to confirm the number of larvae died during the experimental time.

At the 7^{th} day, all the experimental larvae in each aquarium were collected, counted and their weight measured using electronic sensitive balance. Mean weight gains, percentage weight gain (%/day), specific growth rate (mg/day) and survival rate (%) of fishes were estimated using formulas;

- $\text{Mean Weight gain (MWG)} = \text{Wf} - \text{Wi}$

- Mean Daily gain (MWG) $= \frac{(\mathrm{Wf} - \mathrm{Wi})}{\mathrm{T}}$
- Specific growth rate (SGR) $= \frac{(\mathrm{Ln\ Wf} - \mathrm{LnWi})}{\mathrm{T}}$
- Survival rate (SR) $= \frac{\text{No.of hatchlings that survived}}{\text{Total no.of hatchlings stocked}} \mathrm{x}100$; where W_i is the initial weight in mg, W_f is the final weight in mg, and T is time in days.

4.2.6 Data analysis

Basic statistical measurements were used to describe the mean and standard error. Zooplankton in an aliquot was identified and enumerated to analyze the variations among the different feeding dates. The difference among the means on growth performances and survival of *C. gariepinus* larvae grown in four different feeding frequencies were compared. Data were analyzed using one way ANOVA (Steel and Torrie,1960). Where there existed difference between treatment means, post hoc multiple comparison was made. The statistical data analysis was carried out using SPSS version 20 software with the aid of computer.

4.3 Results and discussion

4.3.1 Zooplankton groups identified

4.3.1.1 Zooplankton species composition and diversity

The multiplication ponds showed diverse zooplankton community composition with good species richness from different families that comprised a total of 17 species (Table 4.1). This did not include the unidentified nauplii and other invertebrates which escaped the screen. The diversity of harvested zooplankton from these

fertilized concrete ponds was better compared to previous studies on Lake Tana (Tesfaye Wudneh, 1998; Ayalew Wondie, 2006) and Lake Awassa (Seyoum Mengistou, 1989), and similar with other studies in terms of the number of copepods (Eshete Dejen, 2003). But the diversity was lower compared to the zooplankton community studied by Imoobe and Akoma (2008).

Cladocers and Rotifers were composed of 7 species each and copepod group comprised only three species (17.6%). The contribution of Cladocers, which are rich in essential nutrients, from the total production (harvest) was higher (35%) compared to rotifers and copepods (Fig 4.3). The number of Cladocers continued to grow from day to day as the condition in the pond was suitable for them to multiply.

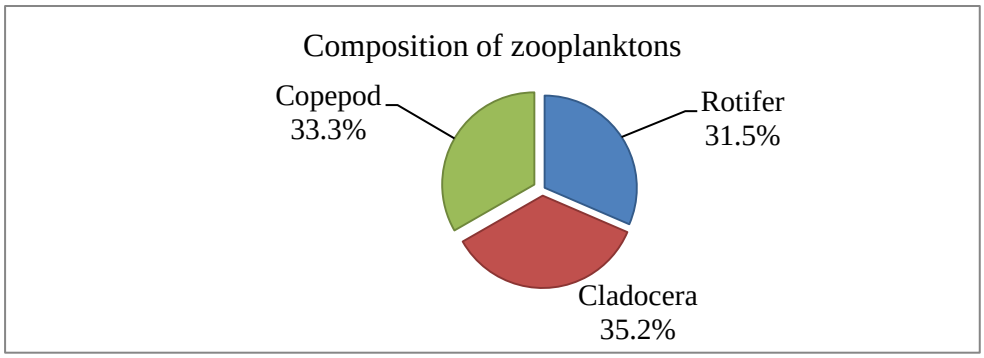

Figure 4. 3. Contribution of each taxa for total zooplankton.

The higher contribution of Cladocerans in these multiplication ponds might be due to their ample morphological and ecological plasticity (Martinez-Jeronimo *et al*., 2007), adapting to an eutrophic water condition (Wang *et al*., 2007) and quick resistance to changes in oxygen concentrations due to their ability to synthesize hemoglobin (Rottmann *et al*., 2003). Studies indicated that large and medium sized zooplankton, mainly Cladocerans dominated zooplankton multiplication ponds when there is no fish predation(Havens and Beaver, 2011).

Table 4. 1. List of zooplankton taxa identified from the harvest.

Taxa	**Family**	**Species**	**Recent records in Lake Tana**
Rotifera	Brachionidae	*Keratella crassa*; Ahlstrom, 1943	Tesfaye Wudneh, 1998
		Keratella tropica; Apstein, 1907	Imoobe and Akoma, 2008
		Keratella cochlearis; Gosse, 1851	Imoobe and Akoma, 2008
	Filinidae	*Filinia longiseta;*Ehrenberg, 1834	Imoobe and Akoma, 2008
	Lecanidae	*Lecane bulla*; Gosse, 1886	Imoobe and Akoma, 2008
	Trichoceridae	*Trichocercasimilis;* Wierzejski, 1893	Imoobe and Akoma, 2008
		Trichocerca longiseta; Schrank,1802	Imoobe and Akoma, 2008
Cladocera	Bosminidae	*Bosminia longrostris*; Muller, 1785	TesfayeWudneh, 1998; Eshete Dejen, 2003; Imoobe and Akoma, 2008
	Chydoridae	*Alona quadrangularis*; Muller, 1785	Imoobe and Akoma, 2008;
	Daphniidae	*Ceriodaphnia cornuta;* Sars, 1885	Tesfaye Wudneh, 1998; Eshete Dejen, 2003;Imoobe& Akoma, 2008
	Moinidae	*Moina micrura;* Kurz, 1874	Eshete Dejen 2003; Imoobe and Akoma, 2008
	Sidiidae	*Diaphanosoma exisum* ; Sars, 1885	Eshete Dejen 2003; Imoobe and Akoma, 2008
		Diaphanosoma sarsi; Richard,1894	Eshete Dejen 2003; Imoobe and Akoma, 2008
	Daphnidae	*Daphnia lumoholtzi* ; Sars, 1885	Eshete Dejen 2003; Imoobe and Akoma, 2008
Copepoda	Cyclopidae	*Thermocyclop galebi* ; Defaye, 1988	Eshete Dejen, 2003;Ayalew Wondie, 2006; Imoobe & Akoma, 2008
		T. ethiopiensis; Defaye, 1988	Eshete Dejen, 2003; Imoobe and Akoma, 2008
		Mesocyclop aequatorialis; Kiefer, 1929	Eshete Dejen, 2003; Ayalew Wondie, 2006; Imoobe and Akoma, 2008

Being situated side by side and having the same water source (L. Tana), all three zooplankton multiplication concrete ponds did have similar type of species (Annex 1). As indicated on Fig 4.3, more number of zooplankton were harvested from the first pond and that was due to the exposure of the pond to direct sunlight for longer hours of a day compared to the other two ponds. There were variations in production and distribution of zooplankton groups over the different harvesting dates and the difference in daily production was significant at $p < 0,05$ level between zooplankton taxa (Table 4.2 and Annex 2).

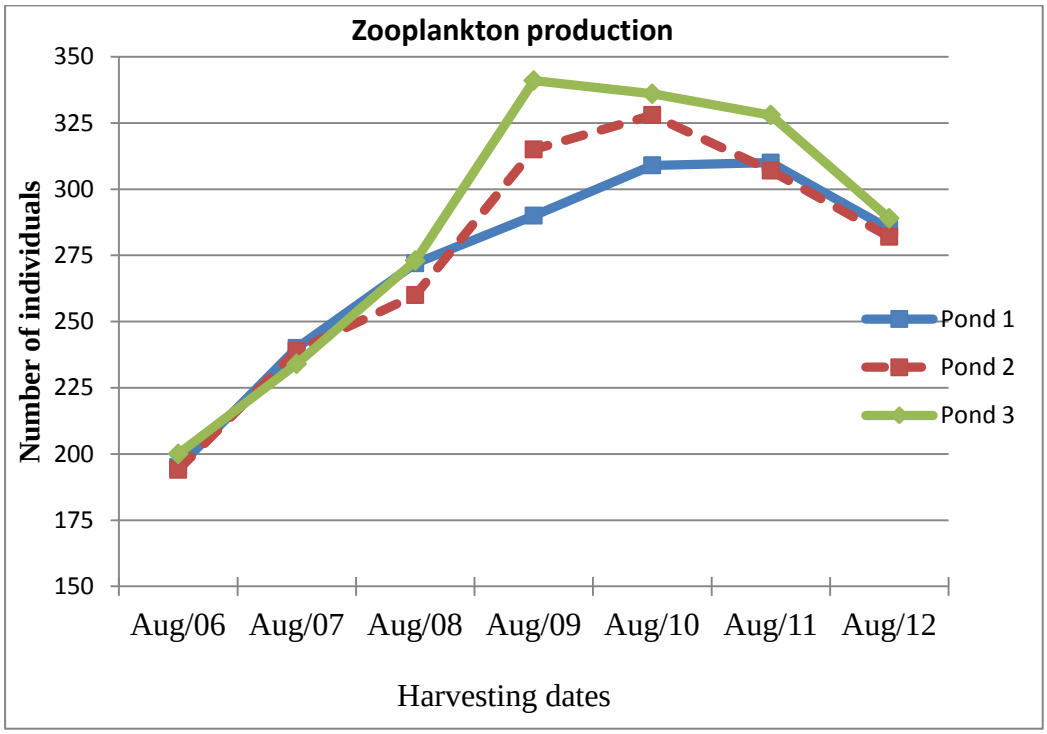

Figure 4. 4. Pattern of zooplankton harvested from each pond over time.

As depicted on Fig 4.4, the overall production was lower during the beginning, and reached its maximum at the third and fourth day of harvesting. Their number started to decline at the fifth and sixth day of the experiment after successive harvests were

made. This could be due to the lower multiplication rates of zooplankton groups that resulted as a consequence of over exploitation and depletion of primary producers.

The scarcity of phytoplankton might occurred due to the depletion of nutrients and continues removal as a result of repeated seining while harvesting zooplankton. Studies also confirmed that overexploitation of resources can result in reduced multiplication of individuals or increased mortality and thereby provides an upper limit to population size (Hairston *et al*., 1960; Arcese and Smith 1988).

Table 4. 2. Zooplanktons harvested (Mean +SD) daily from all concrete ponds

Harvesting dates	**Zooplankton mass from aliquot of one liter**			
	Rotifers	**Cladocers**	**Copepods**	**All Taxa**
August 6/2017	66,000±3,464[a]	50,667±6,028[b]	79,667±3,215[c]	196,333±3,215
August 7/2017	77,667±3,055[a]	73,333±2,517[a]	86,667±4,163[b]	237,667±3,215
August 8/2017	88,333±6,658[a]	91,000±4,359[a]	89,000±3,000[a]	268,333±7,234
August 9/2017	108,333±7,095[a]	108,333±13,051[a]	98,667±7,572[a]	315,333±25,502
August 10/2017	102,333±2,309[a]	118,667±9,504[b]	103,333±4,509[a]	324,333±13,868
August 11/2017	87,000±2,000[a]	125,333±6,351[b]	102,667±7,024[c]	315,000±11,358
August 12/2017	81,667±4,041[a]	117,000±2,646[b]	86,667±4,163[a]	285,333±3,512
Daily average	87,333±2,091[a]	97,762±3,176[a]	92,381± 2,366[a]	277,476±7,633

Values are means ± SD from triplicate zooplankton ponds where the means in each row with different letters are significantly different ($P< 0.05$).

Rotifer production started to drop earlier than the Cladocers and Copepods (Table 4.2). This is because at higher population densities, rotifers undergo sexual reproduction mechanism triggered by secretion of chemical substances and hence

undergo slow multiplication process (Stelzer, 2012). Comparably, the concentration and abundance of zooplankton species in a taxa was better at the end of the experiment compared to the beginning. However, the mean daily number of zooplankton harvested from culture ponds was more than enough to feed the *C. gariepinus* larvae during the 7 days experimental period (Table 4.2).

4.3.1.2 Density and abundance of Zooplankton species

The density (number of individuals/L) of plankton varied between the different zooplankton taxon and also within the zooplankton taxa. Furthermore, the density of rotifers, cladocers and copepods was by far more, compared to the previous studies in Lake Tana (Tesfaye Wudneh, 1998; Eshete Dejen, 2003). The number of *Keratella crassa* (Ahlstrom, 1943), *Diaphnosoma sarsi* (Richard, 1894) and *Thermodiaptomus galebi* (Defaye, 1988) from Rotifera, Cladocera and Copepoda taxa, respectively dominated the zooplankton community (as indicated on Fig 4.5; Annex 2).

The number of *Thermocyclop galebi* from the daily harvest was higher from copepods and also from the other species of all zooplankton taxa (Table 4.3; Fig. 4.5) and similar result was reported by other studies from Lake Tana (Eshete Dejen, 2003; Ayalew Wondie, 2006; Imoobe and Akoma, 2008). The abundance of Cladocers were also higher (35%) as compared with the other zooplankton groups (Fig 4.3) and the result is in agreement with the previous works from Lake Tana (Eshete Dejen, 2003, Ayalew Wondie, 2006).

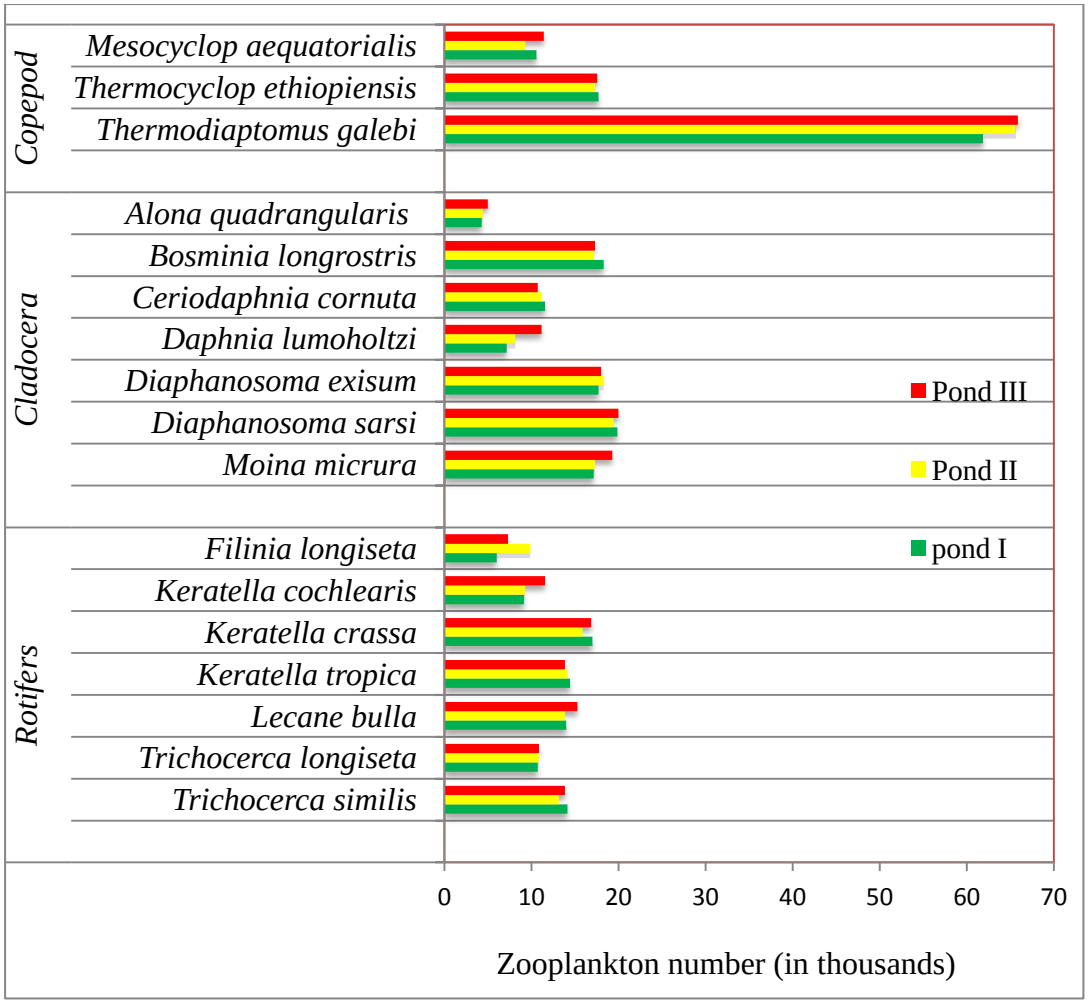

Figure 4. 5. Average number of species harvested from each pond per day.

The overall mean (±SD) standing number of zooplankton from the aliquot was 277,476±7,633 per liter which was very high compared to the density of zooplankton found from Lake Tana (Tesfaye Wudneh, 1998; Eshete Dejen , 2003; Ayalew Wondie, 2006). The highest diversity and mass of zooplankton from ponds might be the suitable conditions created for primary producers, which are sources of food for zooplankton through the application of fertilizer on pond water.

Table 4. 3. Frequency of occurrence (%), Relative abundance (%) and Density (Number of individuals/ml) of each species

Zooplankton taxa	***Frequency of occurrence,%***	***Relative abundance***	***Density(mean ± SD)***
Rotifer groups			
Trichocerca longiseta	100	3.90	10.81±3.86
Lecane bulla	100	5.18	14.38±5.63
Keratella crassa	100	5.97	16.57±5.79
Keratella tropica	100	5.10	14.14±4.36
Trichocerca similis	100	4.94	13.71±4.21
Filinia longiseta	67	2.78	7.71±8.40
Keratella cochlearis	76	3.60	10.00±8.17
Cladocer groups			
Alona quadrangularis	52	1.65	4.57±6.27
Diaphanosoma sarsi	100	7.12	19.76±3.42
Moina micrura	100	6.45	17.90±4.67
Bosminia longrostris	100	6.33	17.57±5.91
Diaphanosoma exisum	100	6.49	18.00±5.04
Daphnia lumoholtzi	67	3.17	8.81±9.39
Ceriodaphnia cornuta	76	4.02	11.14±9.54
Copepod group			
Thermodiaptomus galebi	100	23.22	64.43±8.41
Mesocyclop aequatorialis	76	3.76	10.43±8.81
Thermocyclop ethiopiensis	100	6.32	17.52±4.90

4.3.1.3 Quantitative indices of zooplankton community

The frequency of occurrence of most (65%) of zooplankton species (except *Filinia longiseta, Keratella cochlearis, Alona quadrangularis, Daphnia lumoholtzi, Ceriodaphnia cornuta and Mesocyclop aequatorialis*) was 100%, meaning majority of the species occurred every day while harvesting (Table 4.3; Annex 1). Therefore 16 of the species occurred much frequently except *A. quadrangularis*, which was categorized as "frequently occurred". The three zooplankton community fed to larvae of *C. gariepinus* varied in different indices. The abundance of all zooplankton taxa was less compared to previous studies on Lake Tana (Ayalew Wondie, 2006) but the Cladocerans dominated the community of zooplanktons in the culture ponds.

The diversity of species also varied between the different groups. The diversity index value of rotifers was close to one, compared to the others. As depicted in table 4.4, copepods had an index value of 0.46 which implies very low diversity than rotifers and cladocers. As the species diversity is a more reliable measure of biodiversity, the probability that two randomly selected individuals will belong to the same species in the copepod community is higher compared to rotifers and cladocerans (Table 4.4).

From the live food harvested from ponds and fed to fish larvae, species richness and evenness values also varied. Species richness was measured using the Menhinick's index and rotifers did have higher evenness value indicating its richness in species. Evenness is a measure of how similar the abundance of different species is and it uses species richness and the diversity index into consideration.

As there were an evenness value closer to one, similar proportions of all species were abundant (species were equally present) in rotifers. As the evenness value was very

low, species abundance in copepods was very dissimilar (some rare and some dominant species) and showed poor evenness. Generally, Rotifers did have higher indices value compared to the other groups (Table 4.4). Evaluating the different indices helped to evaluate the diversity and contribution of each taxon on larval feeding.

Table 4. 4. Quantitative analysis of zooplankton samples

Zooplankton community	**Mean ± SD density (# of individuals/L)**	**Ra, (%)**	**H'**	**d**	**e**
Rotifers	87,333±2,091[a]	31.5	0.85	0.75	0.99
Cladocers	97,762±3,176 [a]	35.2	0.84	0.56	0.96
Copepods	92,381± 2,366 [a]	33.3	0.46	0.31	0.42
All taxa	277,476±7,633	100			

Note:- Ra= Relative abundance, H' = Diversity index, d= species richness and e = evenness.

4.3.2 Water quality parameters of larval rearing aquarium

The source of the culture water was cleaned and treated water from an indoor hatchery system. Mean temperature of the culture water from the aquarium was 24.97, 25.02, 25.15 and 25.29 ^{0}C for treatments T_1, T_2, T_3 and T_4, respectively indicating warmer temperature on T_4. Water salinity was maintained at 0.09ppt for all the treatments throughout the experimental period. The DO level varied between 5.67 and 5.83 mg/l. But the difference in water quality between the treatments was not statistically significant at $p < 0.05$ level (Table 4.5).

Table 4. 5. Mean (±SE) of water quality parameters for different treatments

Parameters	T_1	T_2	T_3	T_4	p-value
Temp , ^{0}C	24.97±0.32[a]	25.02±0.37 [a]	25.15±0.27 [a]	25.29 ±0.56 [a]	0.757
DO, mg/L	5.83±0.11 [a]	5.75±0.06 [a]	5.72±0.16 [a]	5.7±0.19 [a]	0.712
pH	8.66±0.06 [a]	8.76±0.07 [a]	8.68±0.04 [a]	8.14±0.73 [a]	0.216
Salinity, ppt	0.09±0.0 [a]	0.09±0.0 [a]	0.09±0.0 [a]	0.09±0.0 [a]	0.794
TDS, ppm	0.122±0.002 [a]	0.123±0.001 [a]	0.121±0.0 [a]	0.123±0.001 [a]	0.263
EC , (µs/cm)	0.188±0.004 [a]	0.190±0.004 [a]	0.187±0.001 [a]	0.191±0.002 [a]	0.395

Note:- Values are means from triplicate group of aquarium.

The mean values of temperature, DO, pH, salinity, TDS and EC for each treatment was within the desired range in *C. gariepinus* larviculture indicated by other studies (Bruton, 1979a; Britz and Hecht, 1998). This was due to the regulation of temperature using room heater and thermostats fitted in each culture aquarium.

4.3.3 Growth performance of C. gariepinus larvae fed zooplankton at different frequency

4.3.3.1 Weight gain and growth rates

Larvae of *C. gariepinus* fed with local zooplankton groups harvested from multiplication ponds showed >100% growth (weight increment) in one week time. The final weight of the experimental fish in a week time was 134.7, 138.5, 152.8 and 136.1mg for treatments T_1, T_2, T_3 and T_4, respectively.

Clarias gariepinus early larvae fed with zooplankton mass four times a day (T_3) showed significantly higher final weight compared to the others (Table 4.6). Mean

daily weight gain was 15.95±1.63 for T_1, 16.51±0.32, 18.91±0.38 and15.88±1.24mg/L for T_2, T_3 and T_4, respectively. This indicated that treatment groups fed zooplankton mass every 3 hr showed higher weight gains and the difference was significant (p <0.05) compared to the other treatments (Table 4.6).

There was a difference in specific growth rate (SGR) between the treatments. The SGR of T_3 was higher compared to the other treatments and the difference was significant (at $p < 0.05$). Treatment groups fed zooplankton mass 2 times a day and 5 times a day showed lowest weight gain and growth rate compared to the other treatment groups (Table 4.6; Fig 4.6). The slower weight gains and growth rates recorded on those larvae fed at 5times a day might be due to the energy lost while competing for a prey for the whole day as the number of zooplankton provided at a time was small. The larvae might use the live food rather for maintenance instead of growth and tissue development.

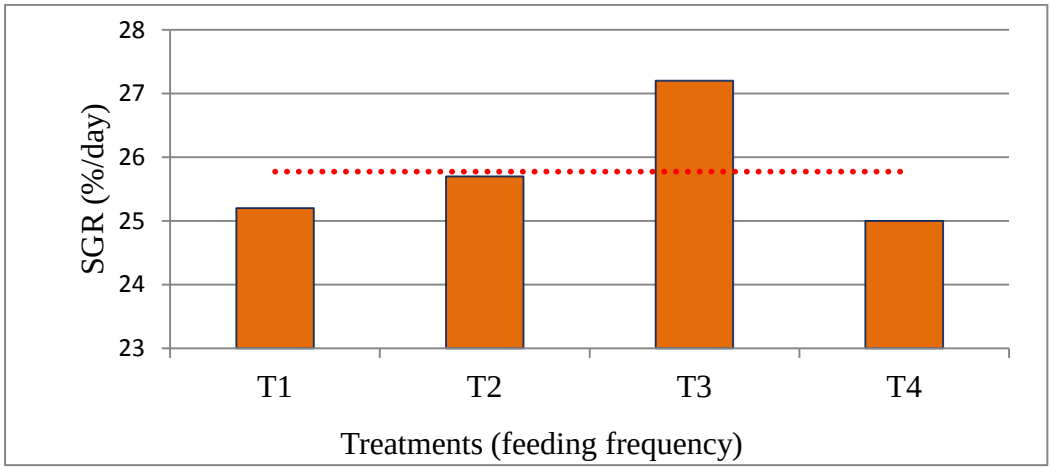

Figure 4. 6. Mean specific growth rate of early larvae fed on zooplankton.

Note:- Dotted lines indicate the overall geometric mean per treatment.

The higher growth performance in T_3 might resulted from the availability of zooplankton at the exact time interval at which larvae required them. This could help the larvae to conserve energy which was lost in search of livefood.

Table 4. 6. Growth indices (Ẍ±SE) and percentage survival rate of *Clarias gariepinus* early larvae fed live food at different frequency.

Groups	***Initial weight, mg***	***Final weight, mg***	***Weight gain, mg***	***Daily weight gain, mg/day***	***SGR, %/day***
T_1	23±0.00 [a]	134.67±4.54 [a]	111.67±4.54[a]	15.95±1.63 [a]	25.2±0.5 [a]
T_2	23±0.00 [a]	138.6±2.23 [a]	115.6±2.23 [a]	16.51 ±0.32 [a]	25.7±0.2 [a]
T_3	23.27 ±0.25 [a]	155.67±2.91 [b]	132.4±2.67[b]	18.91±0.38[b]	27.2±0.1[b]
T_4	23.27±0. 15 [a]	134.4±8.85[a]	111.13±8.7 [a]	15.88±1.24 [a]	25±0.8 [a]

Means with the same superscripts do not have significant differences at $p < 0.05$.

The lower growth of larvae fed twice a day might be due to higher concentration of zooplankton beyond the threshold level. Larvae were observed resting for long time on the bottom of the aquarium with a very big belly after feeding. Studies confirmed that *C. gariepinus* stomach at an early stage gets stuffed and tempted to digest all the ingest (Verreth and Segner, 1992).

4.3.3.2 Effect of feeding frequency on larvae survival

Mortality was higher during the first two days of the experiment and observed in all the treatment groups. The number of deaths decreased through time in all the treatments and stopped at the fourth day except T_1 (Fig 4.7). The total number of early larvae that died during the experimental period were higher on T_1 (74) and T_4 (39) compared to T_2 and T_3 which was 19 and 14, respectively (Table 4.7).

The death in T_1 might be due to the inability of the larvae to digest the ingested zooplankton. This was confirmed by clearly visible big belly observed while they were alive and the larva were moving very slowly. This situation might expose the larvae to be attacked by predating zooplankton. Very high mortality observed on T_1 might also be due to the higher mass of zooplankton added at a time which could create unfavorable condition for larvae.

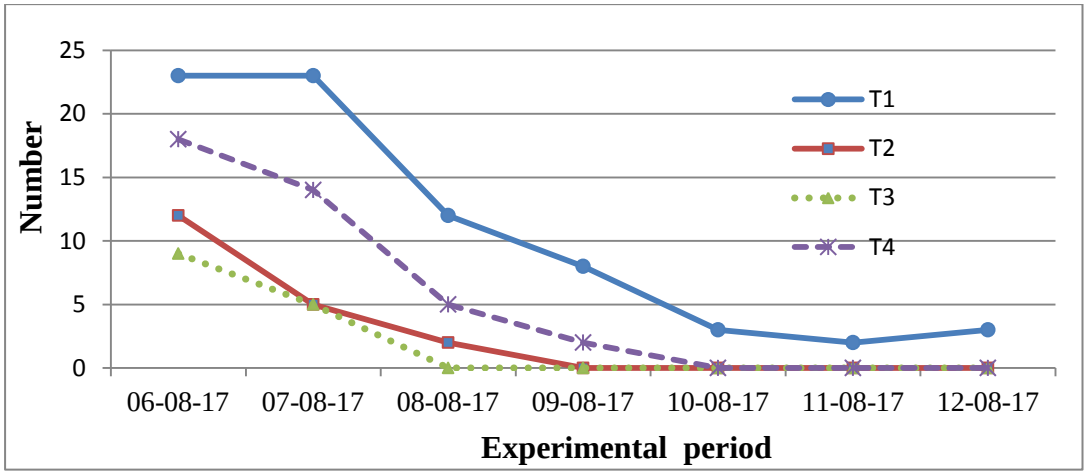

Figure 4. 7. Observed number of larvae died during the experimental period.

At the end of the 7th day, the mean number of survived larvae were 75.33, 93.67, 95.33 and 87 for treatments T_1, T_2, T_3 and T_4, respectively (Table 4.7). The proportion of larvae that died during the experimental period in T_1 was higher (nearly 25%). Higher survival rates were observed in T_2, T_3 and T_4 compared to T_1 and there existed a significant difference ($p < 0.05$) as indicated in Table 4.7. The larvae of *C. gariepinus* fed with zooplankton mass every 3 hour (T_3) showed better growth performance and survival rate compared to those received over and under this feeding frequency (Fig 4.7).

Survival rate increased with feeding frequency to a certain level and decreased at a "very frequent" feeding level. The lower survival of early larvae in the treatment

group that received live food very frequently might be due to scarcity of live food as the number of zooplanktons provided at each feeding time was very low.

Survival rate increased with feeding frequency. The lower survival of early larvae in the treatment group that received live food very frequently might be due to scarcity of livefood as the number of zooplankton provided at each feeding interval was very low. The higher mortality of *C. gariepinus* larvae in T_1 might be due to the higher load of zooplankton added during feeding which might attack fish larvae. Studies confirmed that some cyclopoids are micropredators of fish larvae, especially at early stages (Piasecki *et al*, 2004).

Fish larvae are attacked by adult copepods and more advanced copepodid stages resulting in serious lesions of blood vessels and different body parts particularly the gills (Hartig *et al*, 1982). Piasecki (2000) also reported that mortality rates of larvae depends on the cyclopoid density and the availability of alternative food for copepods.

Table 4. 7. Number of *Clarias gariepinus* larvae died and their survival

Counts and rates	T_1	T_2	T_3	T_4
Larvae stocked (Total)	300	300	300	300
Larvae died (Total)	74	19	14	39
Survived (Ẍ ± SD)	75.33 ± 5.51^{b}	93.67 ± 4.93^{a}	95.33 ± 4.62^{a}	87 ± 4.58^{a}
PS (Ẍ± SD)	75.33 ± 5.51^{b}	93.67 ± 4.93^{a}	95.33 ± 4.62^{a}	87 ± 4.58^{a}

Note:- Values (Mean ± SD) with the same superscript letter in a row was not significantly different at 0.05 level.

4.4 Conclusion

Results of the experiment indicated that multiplying zooplanktons indigenous to lake Tana through fertilization of pond water resulted in good diversity of species. Feeding of early larvae *C. gariepinus* with live food sources resulted in 25 and more percentage daily growth rate (SGR), more than 100% final weight gain and more than 75% survival. Hence, local zooplankton species could be used as replacement of an imported and expensive Artemia cyst. Feeding frequency has an effect on the growth and survival of *Clarias gariepinus* larvae. Better growth and survival were achieved through feeding *C. gariepinus* larvae four times a day. But dividing the total volume of zooplankton mass into a very small portion and feed them very frequently resulted in lower survived and emaciated larvae. Hence, it is commendable to provide *C. gariepinus* larvae growing in an indoor hatchery with local zooplankton mass four times a day. It seems very necessary to screen zooplankton species that can easily be multiplied and result in better larval growth and survival.

Chapter 5. Evaluation of different stocking densities on the Growth Performance and Percentage Survival of the African Catfish, *Clarias gariepinus* in three culture practices.

5.1 Introduction

5.1.1 Background

Stocking density varies with the production system and the level of technology employed. Production systems vary from farm to farm and from place to place, no farm is organized exactly the same as any other. Fishes in extensive culture practices can be stocked at a very low density, mostly 1fish/m^2. The first objective of extensive fish farming is to ensure food security and livelihood improvement of a household through producing fish for own consumption and local market (Adewolu, 2008).

Semi - intensive culture is practiced using fertilized earthen ponds and fish is supplemented with well formulated feed (Phonekhampheng *et al*., 2009) but no aeration and frequent exchange of water. The stocking density can vary from 1-5 fish/m^2. This production system is practiced using cheaper feeds and application of locally available water fertilizing inputs for the multiplication of phytoplanktons (Sevilleja *et al.,* 2001) and hence increase the biomass of zooplankton and benthic organisms (Jha *et al*., 2004). Intensive production can be practiced in cages, ponds, raceways and tanks using small amount of water as the same water is re-used. The recirculation aquaculture system (RAS) minimizes frequent water refreshment, clearing waste, and overcome water and land availability limitation (Martins *et al*., 2010; Zhang *et al*., 2011). Fish stocked at a density of 60-70 fishes/$m^2$and sustained on feed formulated from locally available ingredients (Hecht *et al*., 1996).

According to Dale *et al*., 2004, the nutrient composition of tilapia meal from its offal is 54.8% CP and 23.7 % DE. Earlier studies (Tucker and Robinson, 1990; Wellborn and Cichra, 1995) suggested that up to 60% of the protein in catfish fry diets should consist of fish meal and fishmeal-free diet reduced the performance of catfish fingerling (Mohsen and Lovell, 1990; FAO, 2018). Following oil extraction, Niger seed (locally Noug), *Guizotia abyssinica* cake can be stored and used as feed ingredient (Getinet Assefa and Sharma, 1996). Niger or Noug seed (*G. abyssinica*) Cake contains 32.7% CP, 4.4% Ether Extract (EE), 27% carbohydrate, 17.6% Crude fat, 18kJ/g gross energy and 12% crude fat (Tadele Dessie and Ogle,1997).

Meat and bone meal is high in protein and good source of mineral in diets of fishes, but the inclusion rate should not exceed 5% (Wang *et al*., 2007). Wheat bran prevent constipation because of its swelling and water holding capacity due to its fiber and starch free carbohydrate content. According to Tekeba Eshete (2005) the CP content of wheat bran is 16.4%, 4.2% lipid and 17 KJ/g Gross energy.

The African catfish is suitable for aquaculture as it tolerates high concentrations of ammonia (NH_3), nitrite (NO_2), and resist also low oxygen concentrations (de Graaf and Janssen, 1996; Dada and Wonah, 2003). The optimum range of water temperature is between 22 to 32ºC; salinity between 0.1 to 2.5 ppt and pH from 6 -11 (Pouomogne, 2008). The oxygen concentration threshold in RAS is 50% (4.13 mg/l at 25 °C) (Mongirdas and Kusta, 2006). Catfish is valuable species due to its high growth rates, tolerance to a wide range of temperature and DO levels, good palatability and high fecundity (Hecht *et al*., 1996; Rad *et al*., 2003; Soltan and Tharwat, 2006; Amisah *et al*., 2009). Furthermore, African catfish can be reared with

low cost using unconventional feed ingredients and makes it suitable to run effective and feasible aquaculture in Ethiopia.

Although African catfish is considered as valuable candidate for farming, its performance has never been tested in any of the production systems in Ethiopia. Hence, verifying the performance of this fish is more than important, as the country planned to promote aquaculture production during the GTP II (2016 - 2020) period through the establishment of fish hatcheries and farms (MoA, 2015).

5.1.2 Major Objectives

The main objective was to evaluate different stocking densities on the growth performance of *C. gariepinus* in extensive and semi intensive pond and intensive tank cultures.

Specific objectives

- To determine the stocking density of *C. gariepinus* that produced more yield for a culture system.
- To evaluate the potentials of impounded water for production of *C. gariepinus* as an alternative fisheries.

5.2 Materials and Methods

5.2.1 Experimental area

Three production systems (Extensive, Semi-intensive and Intensive) were evaluated from July, 2016 to October, 2017. The experiments were conducted at the Blue Nile basin area mainly around Lake Tana sub basin, Ethiopia. Extensive farming was

tested around Fogera plain land (Fig 5.1) using the flooded water retained for several months in impoundments. Semi-intensive farming experiments were conducted at three different districts around Lake Tana sub basin area namely; Dangila Achefer and Bahir Dar Woredas (districts) situated at an altitude of 1878m, 1898m and 1808m asl, respectively (Fig 5.1). The experiment was conducted from February to September, 2017.

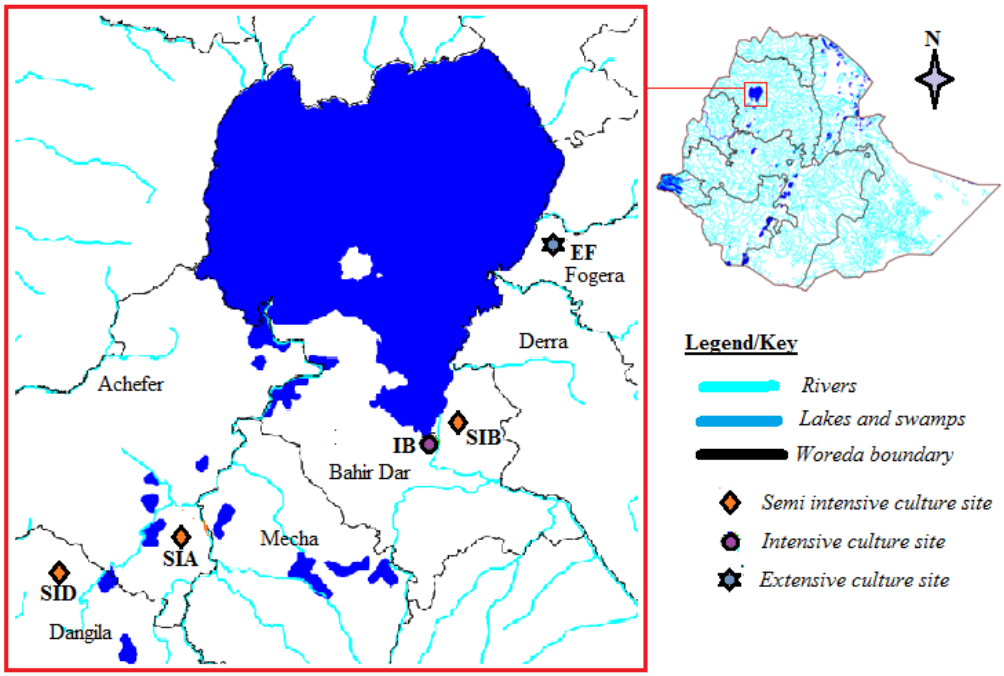

Figure 5. 1. Map of part of Lake Tana sub basin indicating experimental sites.

Note:- EF represents extensive farming at Fogera, IB is for Intensive farming at Bahir Dar, SIA, SIB and SID for Semi intensive farms at Achefer, Bahir Dar and Dangila, respectively.

Intensive farming trial was conducted at the FALRC (Fig 5.1 and 5.2 at site IB) using an indoor recirculation system. The recirculation facility was established during this experiment for the first time using fish tanks made of fiber glass.

5.2.2 Experimental set up

For extensive fish farming experiment, three adjoining impounded waters 5km far from Woreta town of Fogera district were selected. While flooded, the impounding water rises 105cm above the ground surface. Each impoundment had three experimental ponds each with $25m^2$ (5mx5m) area. Hence, there were a total of 9 ponds for this experiment. These ponds were prepared on April before the comencement of the rain, at a depth of 50 cm from the normal ground level and used as a basket to shelter the fish while the flood recedes during from February to March. Each pond in impoundment was fenced with 100 mm^2 meshed galvanized wire net at 2m height to protect the experimental fishes from escape and entrance of alien fishes. The three ponds in a site were treatments received three different stocking densities and the three sites were replicates of each treatment. The experimental fish were harvested totally when the impoundments dried and the water level dropped to the pond surface level.

The semi-intensive culture was conducted using farmers' backyard ponds situated at different sites, i.e. districts (Fig 5.1). The water source for each district (site) was surface water from a river, the management practice was the same (applied the same amount of fertlizer and provide supplemental feed for experinmental fish at the same rate) and there was no water quality difference between the sites as indicated on table 5.2. There were three different ponds in each district which received experimental fishes at different stocking densities. The different stocking densities were treatment groups and each site was considered as replicates of the different treatment. The area of the ponds varied in size between 64-100 m^2 but their depth was 100cm on the inlet

and 125cm in the outlet. The bottom and walls of culture ponds were covered with lining material (geo-membrane) to protect the culture water from seapage.

The intensive culture experiment was conducted at the simple recirculation facility established at BFALRC.The recirculation system has 12 fish tanks (Annex 4 plate 15) each with a dimension of 2mx1m & 65cm depth. Each fish tank was filled to a depth of 0.5m, length 1.44m and 0.7m width that gave a volume of $0.5m^3$ and a surface area of $1m^2$. The surface of each fish tank was covered with dark wooven plastic mat to minimize cannibalism and protect the fishes from jumping.

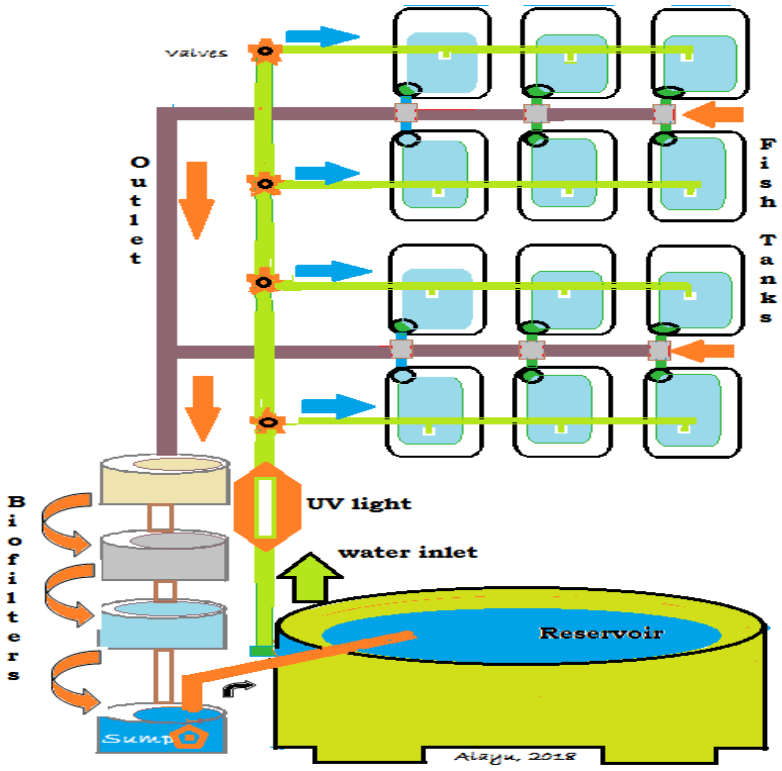

Figure 5. 2. Schematic arrangement and water flow for the recirculation system

In Intensive culture, there were 4 treatment groups replicated three times. Water was pumped from Lake Tana and stored in the reservoir until ditstributed to each tank. The water returning from each culture tank was filterred using consecutive biofilters and pumped back to the reservoir. The water from the reservoir was then treated with UV light before entering into the culture tanks.

Schematically, the RAS facility was prepared as shown in Fig 5.2. Fish tanks for each treatment had a gate valve that controlled the flow rate. Each fish tank had aeration through connecting air tubes to a central air pump. There was a central room heater to warmth the atmospheric air and thermostats fitted to each tank for an optimum culture water temperature.

5.2.3 Collection and Stocking of Experimental Fish

Fingerlings of *C. gariepinus* collected from the natural breeding ground at the Dibankie impoundment in Bahir Dar city were managed in FALRC culture ponds. Dibankie is an impounding water filled with runoff water every year during the main rainy season and dries up during the dry season, mainly from end of February to the beginning of April. The impoundment has a direct connection with Lake Tana through a big canal which helps the brood fish to migrate from Lake Tana for spawning while the two waters got connected.

Stocker sized fishes collected from the previous year batches were used for extensive farming experiment and had a size of 120-150g (average 143g). These stocker sized fishes were injected with digital microchips for identification before stocked in experimental ponds at the impoundments. The total number of fishes used for the culture experiments were 4954 (n =264, 4180 and 510) for extensive, semi intensive and intensive culture, respectively. Fingerlings collected from natural breeding areas (n = 6,000) for intensive and semi-intensive culture were the same batches hatched during the main rainy season in 2016.

The fingerlings collected from the natural breeding area (Dibankie) using 10m long 4mm meshed seining net, transported with plastic tanker and stocked in a pre-

prepared concrete pond of 100m^2 at BFALRC. These *C. gariepinus* fingerlings were kept for two months in a pond to recover themselves from stress, acclimatize the new environment and exposed them for artificial feed. These fingerlings had a weight of 32 - 40g (average 37) and used for semi intensive and intensive farming experiments.

In extensive and semi intensive farming system experiments, three different stocking densities were compared. But for intensive culture system, four different stocking densities were compared. Experimental fishes in extensive farming were stocked at a density of 1 fish/2m^2, 1 fish/m^2 and 2fishes/m^2 for treatments (T) 1, 2 and 3, respectively.

The stocking density in semi intensive was 5 fishes/m^2 for T_1, 8 fishes and 10 fishes/m^2 for T_2 and T_3, respectively. For intensive farming experiment in a RAS, fingerlings were stocked in a fish tank at a density of 70 fishes/m^3 for T_1, 80 fishes/ m^3 for T_2, 90 for T_3 and 100 fishes/m^3 for T_4. Beyond the indicated stocking densities in a culture practice, fish can be suffered chronically to a stressful condition (Hecht *et al*., 1996; Sugunan and Katiha, 2004; Dasuki *et al*., 2013).

5.2.4 Pond fertilization and fish feed and feeding

Ponds for extensive farming trial at the impounding areas around Fogera plain area were not fertilized. But semi-intensive ponds were regularly fertilized with cow dung at a rate of 0.5kg/ m^2/week as in Tacon (1988) or 0.4kg/m^3 /week (Jha *et al*, 2004) to stimulate the growth and multiplication of zooplanktons, insects and benthic organisms. These organisms could be used as main food sources for the growth of experimental animal in a pond.

Artificial feed was formulated from fishmeal (Tilapia), bone meal, Soybean (*Glycinemax*), wheat flour and bran (*T. aestivum*), Niger (Noug) seed cake (*G. abyssinica*), a premix containing vitamins, salt and fish oil (Table 5.1). Each feed ingredient was ground individually to a fine powder by using hammer mill machine, then individually weighed and properly mixed together with adequate water to ensure smooth pelleting. A dough was made to make the strands of varied thicknesses using meat mincer. The strands were cut into short pieces (Annex 4 plate 14) and dried in a shade for 3 days to remove moisture (Eyo, 1994).

Table 5. 1. Inclusion level of the ingredients and nutritional composition of formulated feed fed for *C. gariepinus* in intensive and semi-intensive culture.

Ingredients used in the formulated feed	***Inclusion level, %***	***Chemical composition of the feed***
Fishmeal	55	Dry matter (DM) ..... 91.8% Crude protein ..... 42.87% Digestible Energy ..... 18.78% Crude fat ..... 12.78% Ash 17.37%
Bone and blood meal	4	
Soy bean meal	15	
Wheat flour	5	
Wheat bran	15	
Noug seed cake meal	5	
Premix (mineral, vitamin, salt)	1	
All ingredients	100	

Laboratory analysis result of the formulated feed (91.8% dry matter) indicated a chemical composition of 42.87% CP, 18.78% DE, 12.78% Crude fat and the rest was ash (Table 5.1). Feed was provided for fish as powdered form at the beginning and pelleted form once the experimental fishes attained 70g and more for fishes in

intensive and semi-intensive farming experiments. Feed was provided in powdered form so that the feed can float for long and stay been consumed by the fingerlings. The pelleted feed was then packed in water impermeable bags (plastic bags) and kept in freezer until been used (Gabriel *et al.*, 2007; Amisah *et al.*, 2009).

The experimental fishes in intensive farming were fed with formulated feed at a rate of 5% of their live body weight (Li and Robinson, 2008) until they attained 300 g and then reduced to 2% afterwards as indicated by other reports (Hogendoorn *et al*., 1983; Hecht *et al*., 1988). They were fed twice a day, at 9:00 am in the morning and 4:00 pm in the afternoon. Experimental fishes in semi-intensive culture were supplemented at a rate of 2% of their BW. The supplemental feed was provided once in a day between 11 and12:00 am in the morning. The total experimental period was 8 months for both intensive, semi-intensive and extensive farming systems. The extensive culture experiment was conducted from July 2016 to February 2017 while semi-intensive and intensive culture was conducted from January to September, 2017.

Weight of experimental fishes was measured using an electronic weighing balance with 0.01g precision and recorded in a recording sheet. Fish were weighed every month for intensive and semi intensive experiments. All the experimental fishes in each treatment group were weighed at the start (stocking) and end (harvest) of the experiment. A sample of atleast 10% of the total experimental fish population was taken and weighed every month from semi intensive production experiments. But, in intensive tank culture, each experimental fish in a treatment was measured every two weeks for their growth and determine the amount of feed to be provided. The fish in extensive culture were measured only during the beginning and end of the experiment while harvested.

5.2.5 Characteristicss of the culture water

Water quality parameters, such as dissolved oxygen, pH, salinity and temperature of the culture water were monitored regularly. The level of ammonia and other water quality parameters were monitored every day for intensive culture in RAS. But for semi-intensive and extensive farming experiments, the water quality parameters were recorded every month. Multi-meter (Model 556 MPS) was used to measure the physico chemical parameters of culture water. The Ammonium/ ammonia concentration was checked using an ammonia testing kit (JBL, Gmbh CO. Germany).

5.2.6 Growth performance and Feed utilization evaluation

Different fish growth parameters and feed conversion efficiency were evaluated based on the following formulas:

- Survival rate $= \frac{\text{No.of fish that survived}}{\text{Total no.of fishes stocked}} \text{x}100.$
- Weight gain $(\text{WG}) =$ Wf – Wi; W_f and W_i = Final and Initial mean weights(g)
- Daily Weight gain $(\text{DWG}) = \frac{(\text{Wf} - \text{Wi})}{\text{T}}$;where T = Rearing period (days).
- Percentage Weight Gain, $(\%WG) = (\frac{\text{Wf}-\text{Wi}}{\text{Wi}}) * 100.$
- Specific Growth Rate (%/day), $\text{SGR} = (\frac{ln\,\text{Wf}-ln\text{Wi}}{\text{T}}) * 100$; where "$ln$" represents natural logarithm.
- Productivity or yield =($\frac{\text{NW}}{\text{SA}}$); where N = average number of fish produced, W = mean weight of fish and SA = surface area of pond/tank in m^2.
- Food Conversion Ratio, $\text{FCR} = \frac{\text{Feed offerred for fish (g)}}{\text{Weight gained by fish (g)}}$

5.2.7 Statistical Analysis

Data from water quality measurement, fish growth and provided feed was subjected to one-way analysis of variance (ANOVA) and the means from the various treatments were compared for significant differences ($p< 0.05$). Computer assisted SPSS program was used for data analysis.

5.3 Results and discussion

5.3.1 Water quality of culture water

The water temperature for all types of production systems was within in the optimum range for *C. gariepinus* (Pouomogne, 2008; Njieassam, 2016). The nitrogen and phosphorous content of the culture water in semi intensive tank culture, in terms of NO_3 and PO_4 forms, varied between treatments but the difference was not statistically significant ($p < 0.05$). Except the difference in temperature, there was no statistically significant difference in other water quality parameters in extensive pond culture during the experimental period. The lower temperature might be resulted from site difference as there was a vegetation cover difference between the sites. The temperature was lower compared to the other studies (Datta, 2012). This was mainly due to the colder weather during autumn which caused a drop in the temperature of culture water.

In intensive culture experiment, the water quality parameters did not show significant difference ($p < 0.05$) between the treatments as a result of the regulation of the different water quality parameters in a RAS at its optimum range except TDS which had lower value at lower stocking density (Table 5.2).

Table 5. 2. The physicochemical characteristics of culture water

Treatments	DO, mg/L	pH	Salinity, ‰	TDS, ppm	EC, µS/cm	Temperature, ^{0}C	NO_3	PO_4
A. Semi-intensive								
I	7.64^a ±.35	7.33^a±0.1	0.105^a±0.001	0.197^a±0.001	0.28^a±0.003	24.7 ^{a}±0.34	2.18 ^{a}±0.13	0.09 ^{a}± 0.07
II	7.67^a±0.39	7.33 ^{a}±0.13	0.102 ^{a}±0.001	0.195 ^{a}±0.002	0.28 ^{a}±0.004	24.45 ^{a}±0.36	3.05 ^{a}±0.48	0.19 ^{a}±0.03
III	7.69 ^{a}±0.59	7.5 ^{a}±0.36	0.106 ^{a}±0.003	0.198 ^{a}±0.002	0.28 ^{a}±0.006	24.83 ^{a}±0.05	3.06 ^{a}±1.16	0.17 ^{a}±0.12
B. Intensive								
I	6.88 ^{a}±0.09	6.99 ^{a}±0.06	0.09 ^{a}±0.00	0.184 ^{a}±0.006	0.286^a±0.009	25.97 ^{a}±0.035	0.44^a ±0.01	0.01^a±0.0
II	6.73 ^{a}±0.08	6.85 ^{a}±0.05	0.09 ^{a}±0.00	0.187 ^{ab}±0.007	0.287^a±0.012	26.11 ^{a}±0.074	0.46^a ±0.02	0.02^a±0.0
III	6.71 ^{a}±0.24	6.88 ^{a}±0.13	0.09 ^{a}±0.00	0.195^b±0.002	0.30^a±0.005	26.1 ^{a}±0.17	0.45^a ±0.01	0.01^a ±0.0
IV	6.79 ^{a}±0.03	6.86 ^{a}±0.06	0.09 ^{a}±0.00	0.19 ^{b}±0.003	0.30^a±0.004	26.06 ^{a}±0.11	0.45^a±0.01	0.01^a ±0.0
C. Extensive								
I	7.67 ^{a}±0.18	7.72 ^{a}±0.41	0.142 ^{a}±0.003	0.20 ^{a}±0.001	0.29 ^{a}±0.006	22.52^a±0.36	Invisible	0
II	7.65 ^{a}±0.11	7.61 ^{a}±0.3	0.142 ^{a}±0.003	0.2 ^{a}±0.007	0.29 ^{a}±0.003	22.05^b±0.07	0	Invisible
III	7.67 ^{a}±0.12	7.78 ^{a}±0.12	0.142 ^{a}±0.003	0.202 ^{a}±0.002	0.29 ^{a}±.006	22.99^c±0.062	0	Invisible

Note:- Mean values in the same column with different superscript are significantly different ($p < 0.05$)

Fish performance evaluation at different stocking densities

5.3.2 Extensive fish production

The percentage survival rate (PS) of fishes varied from treatment to treatment between 83 and 97% (Table 5.3). More fish survived in the treatments with lower stocking density compared to higher ones and the difference was significant ($p < 0.05$). This might be due to the abundance of enough food and space that enabled the experimental fish to perform better stocked at lower density. In addition, leeches (*Hirudo medicinalis*) were found infesting a pond stocked with experimental fish at higher stocking density which was not observed in the other treatment groups adjacent to it. Leeches (*H. medicinalis*) were stacked on the body and observed biting the fish (Annex 4 plate 23).

The abundance of leeches might be due to the availability of sufficient food for their growth and multiplication on more populated stock. Studies also confirmed that declining abundances of *H. medicinalis* population are results of lower energy available for growth, reflecting leeches feeding predominantly on amphibian blood of lower energetic value than mammalian blood (Davies and McLoughlin, 1996). Leeches are reported to be one of the most important ectoparasites of *C. gariepinus* fishes in tanks and ponds (Morrison *et al*., 1993).

The experimental fishes in all treatments showed growth increment (weight gain) with time. Treatments with lower stocking density (one fish/$2m^2$ and 1fish/m^2) showed more than threefold increment. Whereas, a fish in more populace treatment increased only by 2.75 folds only (Fig 5.3).

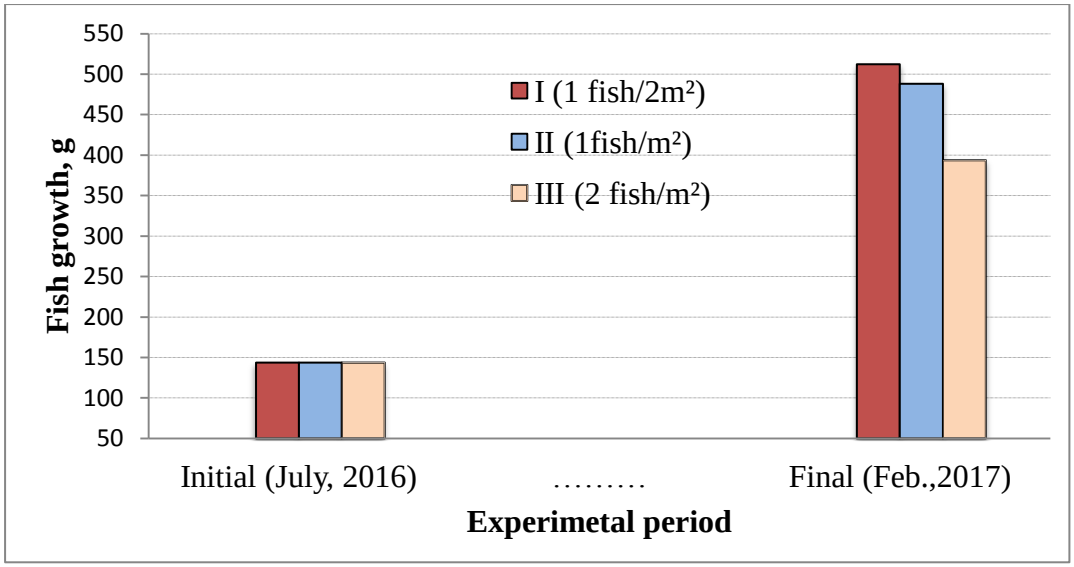

Figure 5. 3. Growth of stocker sized fishes at different stocking densities.

The weight gained by a fish was 368.3, 344.6 and 249.9g for T_1, T_2 and T_3, respectively. The daily weight gain varied between treatments and inversely related with the stocking rate. The weight gained by an individual fish and SGR was better for treatment groups with lower stocking density and there existed a significant difference ($p < 0.05$) between the different stocking densities (Table 5.3). Better performed experimental fishes were observed on treatments with lower stocking density and that might be due to the availability of natural food enough for them to grow. The availability of food has a limiting effect on the carrying capacity hence fish survival, growth and physical condition may be affected. Studies also confirmed that the availability of food resources and water quality conditions limit the rate of growth in fish (Diana, 1997).

Table 5. 3. Fish survival and growth in extensive culture system

Parameters	***T_1(1fish/2m2)***	***T_2(1fish/m^2)***	***T_3 (2fish/m^2)***
Survival rate (SR)	97.4^{a} ±4.4	93.3^{a} ±4.6	83.3^{b} ±3.1
Initial Weight ($W_{i)}$, g	143.8^{a} ±0.14	143.5^{a} ±0.08	143.7^{a} ±0.12
Final weight (W_f), g	512.1^{a} ±7.8	488.1^{b} ±5.5	393.6^{c} ±15.6
Wt gained (WG), g	368.3^{a} ±7.6	344.6^{a} ±5.4	249.9^{b} ±15.6
Daily weight gain (DWG), g/day	1.64^{a}±0.03	1.54^{b}±0.02	1.12^{c}±0.07
Percentage weight gain, %	256.2^{a} ±5	240^{b} ±3.7	174^{c} ±10.9
Specific growth rate (SGR), %/day	0.57^{a} ±0.01	0.55^{a} ±0.01	0.45^{b} ±0.02
Yield, kg/m^2	0.26^{a} ±0.02	0.46^{b} ±0.03	0.66^{c} ±0.05

Note:- Treatment (Trt) groups were the different stocking densities. Values (mean± SD) with different superscripts within a row differed significantly at 0.05 level.

But, as indicated in table 5. 3, more yield was harvested from densely stocked treatment group (T_3) as the biomass was more in this treatment group. The mean values were 0.26±0.015, 0.46±0.03 and 0.66±0.05 kg/m^2 for T_1, T_2 and T_3, respectively and the difference was statistically significant ($p < 0.05$). This production systems indicated the possibility of producing fish in impounding areas. But, the yield harvested in this experiment was lower compared to other results reported by Dasgupta *et al* (2007), which was 0.8kg/m^2. This might be effected by different causes mainly scarcity of food during the rainy season while the area was regularly flooded, lower temperature that occurred during November and December and external parasites observed during the dry season.

5.3.3 Semi-intensive Culture

5.3.3.1 Fish growth and harvest

Fish growth increased from month to month for all treatments and there existed a direct relationship with time (Fig 5.4). The percentage weight gain was higher and *C. gariepinus* fingerlings gained more than thirteen fold from their initial weight in all the treatment groups (stocking densities). Those stocked at a rate of 5fishes/m^2 (T_1) showed better weight gain (526.66±3.49g). The other treatments with a stocking density of 8 fishes/m^2 (T_2) and 10fishes/m^2 (T_3) gained 502.94±20.75 and 493.8±27.11g in 8 months time (Table 5.4). But the difference in fish growth and weight gained between the different stocking densities (treatments) were not significant at $p<0.05$ level.

Studies also indicated that fish welfare and growth significantly improved with increasing stocking density, as the sign of aggression stops and feed consumption and food conversion ratio gets improved (Hecht and Uys, 1997; van de Niewegiessen *et al*., 2009).

The weight attained by individual fishes was lower compared with other results (Hecht *et al*., 1996) which was in between 700 and 800g in the same culture period at a stocking density of 10fishes/m^2. This might be due to the smaller sized experimental ponds which might affect the movement and performance of the experimental fishes and scarcity of natural food.

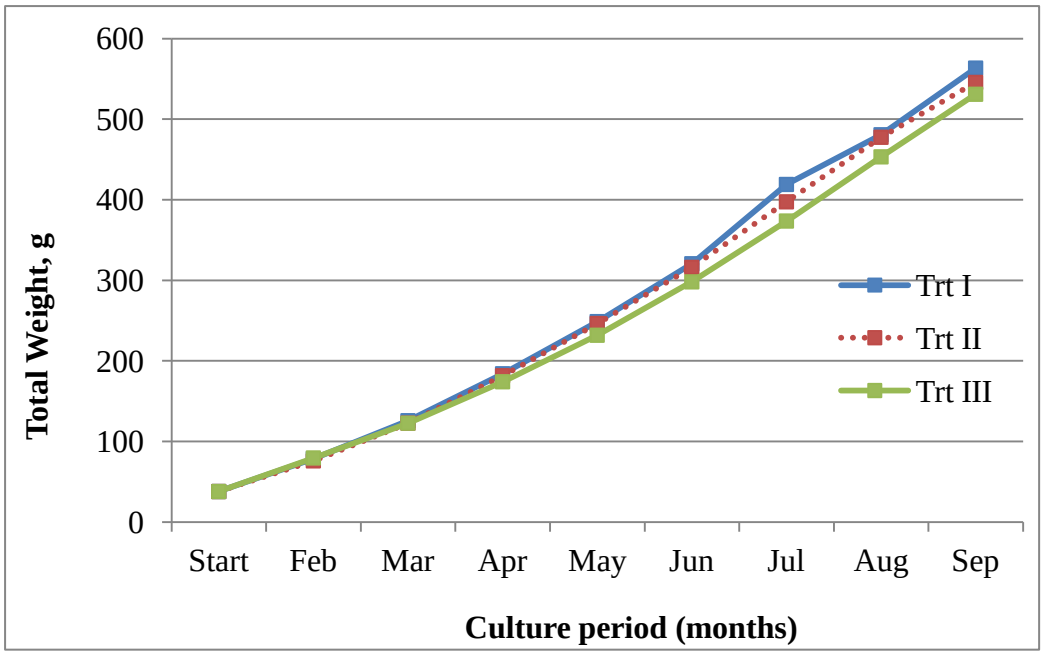

Figure 5. 4. Growth performance of *C. gariepinus* with time in semi-intensive culture.

Fish product harvested from the experiment varied between the treatments. The mean production was 2.65±0.1, 3.91±0.32 and 4.77±0.55 kg/m^2 for T_1, T_2 and T_3, respectively and increased with stocking densities. The difference in mean harvest (yield) was significant ($p<0.05$) between the treatments and more product was harvested from ponds with higher stocking density (Table 5. 4). This might be due to the higher number of individuals contribution to the final harvest.

The amount of fish harvested at a stocking density of 10 fishes/m^2 in this experiment was better compared with the other reports which was reported as 3.38 kg/m^2 (Dasgupta *et al.*, 2007; Gyalog *et al.*, 2015) and similar with reports of Hecht *et al.*, 1996. This might be due to the suitability of the environment in which the culture ponds were situated and the management activities (fertilization and water refreshment) practiced for the culture ponds.

Table 5. 4. Mean weight gain and growth attained by experimental fishes and the final fish harvested

Growth parameters	**Experimental groups (stocking densities)**			**p**
	T1 (5fishes/m^2)	***T_2 (8fishes/m^2)***	***T_3 (10fishes/m^2)***	
Mean initial weight, g	37.91^{a} ±0.1	37.9 ^{a} ±0.1	37.89 ^{a} ±0.1	
Mean final weight, g	564.6 ^{a} ±3.5	540.8 ^{a} ±20.8	531.7 ^{a} ±27.1	
Mean weight gained, g	526.7 ^{a} ±3.5	502.9 ^{a} ±20.8	493.8 ^{a} ±27.1	
DWG, g/day	2.35 ^{a} ±0.02	2.24 ^{a} ±0.09	2.2 ^{a} ±0.12	
Percentage weight gain	1389.2 ^{a} ±8.8	1327.2 ^{a} ±51.9	1302.7 ^{a} ±70.5	
Yield, kg/m^2	2.65^{a}±0.1	3.91^{b}±0.32	4.77^{c}±0.55	

Note:-Mean values in the same column with different superscript(s) were significantly different ($p < 0.05$).

5.3.3.2 Fish survival, food conversion and specific growth rates

The experimental fishes mean survival rate was 93.8, 90.2 and 89.7% for T_1, T_2 and T_3, respectively. More fish survived in experimental ponds stocked with *C. gariepinus* at lower stocking density but the difference was not significant ($p < 0.05$). The higher death rate that occurred in a more populated experimental group might be due to confinement and the scarcity of resources in the culture water mainly natural food.

As depicted on Table 5.5, mean food conversion ratio and specific growth rate values varied between treatments though not statistically significant ($p < 0.05$). The food conversion ratio values were higher and showed lower efficiency compared to other results (Hecht *et al.*,1996; Robinson and Li, 2015; FAO, 2018) for the same sized fishes. More than 9 kg (average of 9.68kg) feed was provided to produce an average

of 545g *C. gariepinus* fish in 32 weeks culture period. The higher FCR value (lower efficiency of feed) might be due to the quality of the ingredients as the feed was formulated from local farm products and industrial (by) products.

Table 5. 5. Percentage Survival and specific growth and food conversion rates of *C. gariepinus*

Trt	**PS,%**	**SGR, %/day**	**FCR**	**Feed offered, kg/fish**
I	93.8 [a] ±3.02	1.21 [a] ±0.003	1.85 [a] ±0.04	9.736[a] ±0.187
II	90.2 [a] ±4	1.19 [a] ±0.016	1.95 [a] ±0.11	9.807 [a] ±0.181
III	89.7 [a] ±5.92	1.18 [a] ±0.022	1.93 [a] ±0.18	9.498 [a] ±0.373

Mean values in the same column with different superscript are significantly different ($p < 0.05$).

5.3.4 Intensive tank culture experiment

Four different stocking densities were the treatments compared in this experiment. The experiment was conducted in a recirculation system facility at FALRC using 12 fish tanks with a volume of $0.5m^3$culture water ($1.44m * 0.7m*0.5m$) each. The fish were fed with similar feed formulated from locally available ingredients. The survival rate of experimental fishes was more than 90% and varied between treatments. The difference in survival rate was significant ($p <0.05$) and more fishes survived in treatment III stocked at a rate of 90 fishes/m^3 (Table 5.6). This might be due to the lower aggression behavior of *C. gariepinus* at higher stocking density.

Table 5. 6. Survival and growth of fishes in intensive culture system

Parameters	***T_1 (70fishes/m^3)***	***T_2 (80fishes/m^3)***	***T_3 (90fishes/m^3)***	***T_4 (100fishes/m^3)***
SR, %	$90.48^{a} \pm 1.65$	$90.83^{a} \pm 1.44$	$97.04^{b} \pm 1.28$	$94^{b} \pm 2$
W_i, g	$37.27^{a} \pm 0.11$	$37.22^{a} \pm 0.26$	$37.37^{a} \pm 0.31$	$37.34^{a} \pm 0.06$
W_f, g	$579.54^{a} \pm 0.1$	$576.7^{b} \pm 0.07$	$586.49^{c} \pm 0.07$	$574.78^{d} \pm 0.06$
WG, g	$542.27^{a} \pm 0.19$	$539.48^{b} \pm 0.19$	$549.12^{c} \pm 0.38$	$537.43^{d} \pm 0.1$
DWG, g	$2.42^{a} \pm 0.000$	$2.41^{b} \pm 0.001$	$2.45^{c} \pm 0.002$	$2.4^{d} \pm 0.001$
% WG	$1454.9^{ab} \pm 4.9$	$1449.4^{a} \pm 10.7$	$1469.5^{b} \pm 13.3$	$1439.2^{a} \pm 2.4$
SGR, %/day	$1.225^{ab} \pm 0.001$	$1.223^{a} \pm 0.003$	$1.229^{b} \pm 0.004$	$1.221^{a} \pm 0.001$
FCR	$1.996^{a} \pm 0.000$	$1.998^{a} \pm 0.01$	$1.981^{a} \pm 0.04$	$1.992^{a} \pm 0.01$
Yield, kg/m^2	$9.18^{a} \pm 0.17$	$10.48^{b} \pm 0.17$	$12.8^{c} \pm 0.17$ d	$13.51^{d} \pm 0.29$

Note:- Mean values in the same row with different superscript are significantly different ($p < 0.05$)

Fishes stocked at lower density (70 fishes/m^3) attained the best mean final weight (579g) within a culture period of 32 weeks. The daily weight gained in this system was 2.42g/day for T_1, 2.41, 2.45, and 2.4g/day for T_2 , T_3, and T_4, respectively . More yield (13.51kg/m^2) was obtained from fish tanks stocked with more number of *C. gariepinus* fishes and there existed a significant difference between the treatments at 0.05 level (Table 5.6). But, the total production with highest stocking density was lower compared to previous studies and reports (Hecht *et al.*, 1996; Dasgupta *et al.*, 2007; Gyalog *et al*., 2015). This might be due to the lower temperature compared to these studies which was reported between 29 and 30 ^{0}C.

The FCR in this experiment varied between 1.98 and 1.99 but there was no significant difference between the treatments (Table 5.6). The FCR value was higher, The efficiency was lower compared with the other study for intensive *C. gariepinus* culture system (Hecht *et al*., 1996). This was due to the frequent temperature drop due to electric interruption which led fishes to refuse feed provided to them in addition to the difference in feed quality.

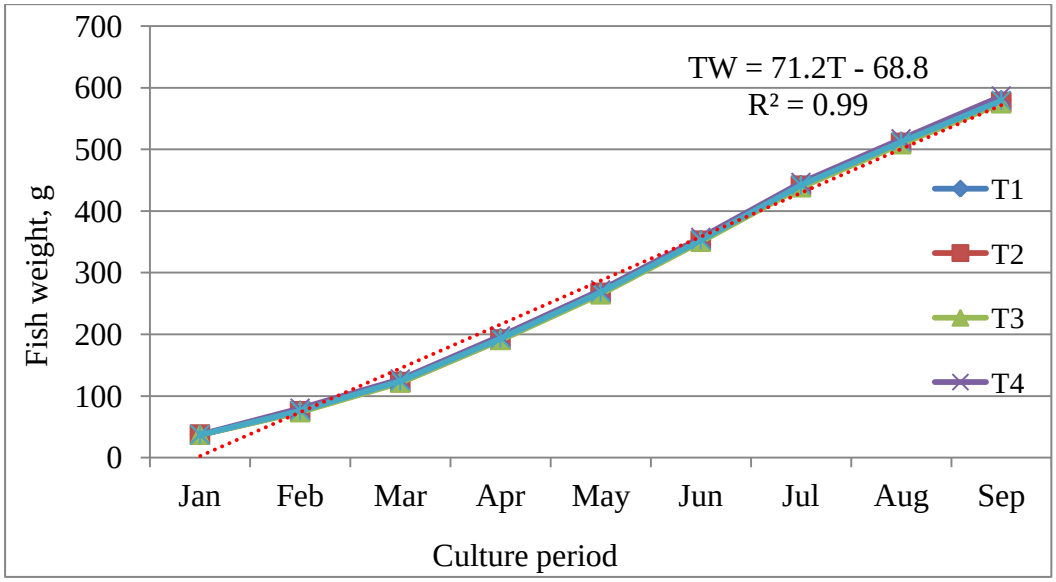

Figure 5. 5. Fish growth curve with time (T) in intensive culture system.

As indicated in Table 5.7, experimental fish growth in total weight (TW) had a direct relation ($R^2 \geq 0.95$) with time in all the treatments and their overall mean ($R^2 = 0.99$). This could be due to the provision of the same management practice and to the experimental fishes were growing in a controlled environment. The growth pattern was straight forward starting from the 4th month of the culture period (Fig 5.5) and this might be due to the regulation of the experimental fishes with the existing culture condition and feeding level adjustments from 5% to 3% of their body weight.

Table 5. 7. Mean experimental fish growth (g) in time (month)

Culture period	***T_1 (70fishes/m^3)***	***T_2 (80fishes/m^3)***	***T_3 (90fishes/m^3)***	***T_4 (100fishes/m^3)***	**p**
Jan	37.27^{a}±0.11	37.22^{a} ±0.26	37.34^{a} ±0.06	37.37^{a} ±0.31	0.44
Feb	76.2^{a} ±0.33	74.91^{b} ±0.89	73.66^{c} ±0.42	79.8^{d} ±0.25	0.01
Mar	124.7^{a} ±0.25	122.3^{b} ±0.64	121.9^{b} ±0.44	127.4^{c} ±0.25	0.04
Apr	193.51^{a} ±0.14	191.36^{b} ±0.26	190.79^{c} ±0.06	197.34^{d} ±0.07	0.02
May	268.35^{a} ±0.15	267.1^{b} ±0.02	264.78^{c} ±1.25	271.9^{d} ±0.11	0.02
Jun	352.62^{a} ±0.08	351.6^{b} ±0.17	350.2^{c} ±0.05	357.1^{d} ±0.22	0.00
Jul	441.81^{a} ±0.07	440.87^{b} ±0.17	438.5^{c} ±0.06	446.3^{d} ±0.09	0.00
Aug	512.29^{a} ±0.14	510.38^{b} ±0.07	508.4^{c} ±0.04	517.1^{d} ±0.07	0.00
Sep	579.54^{a} ±0.1	576.7^{b} ±0.07	574.8^{c} ±0.06	586.5^{d} ±0.07	0.00

Mean values in the same row with different superscript are significantly different ($p < 0.05$).

5.4 Conclusion

The overall average mortality of the experimental fishes was less than 20% and this might be due to the prior acclimatization and adjustment of the fingerlings and stockers for pond conditions for a longer time and their uniformity in size while stocked. The experimental fishes gained weight through time and more gain was recorded on fishes stocked with lower stocking density in each culture system. The most preferred stocking density in terms of weight gain and better survival was 1fish/m^2 for extensive, 5 fishes/m^2 for semi- intensive and 90 fishes/m^3 for intensive production.

Difference in stocking density led both individual weight gain and total harvest to vary between the treatments in ponds and tanks. The total harvest or yield at the end was higher in ponds and tanks with higher stocking density in each culture system. Hence, in order to get more harvestable product, fish should be stocked at a rate of 2 fishes/m^2 in extensive, 10 fishes/m^2 in semi-intensive pond culture and 100 fishes/m^3 in intensive farming systems.

The result indicated that an average of 4.6 tons of fish could be produced in 32 weeks time, in one ha of impounded waters stocked with 143g stockers of *C. gariepinus* at a density of 2 fishes/m^2. As the surface of impounded waters cover vast area of land around the Lake Tana sub basin, stocking big sized *C. gariepinus* fingerlings could boost fish production and hence used as an alternative means to reduce fishing pressure on the overfished and endangered fish species. The total production in a 100 m^2 semi intensive pond using *C. gariepinus* fish was more (0.477 ton) compared to Tilapia spp. which was reported as 0.02 ton (Alayu Yalew, 2011). To improve the production and productivity of African catfish farming, better adapting and growing fingerlings, that can withstand the existing environmental conditions and perform better in poor management practice, should be raised.

Chapter 6. Comparison of production performance and economic efficiency among three production practices of African catfish (*Clarias gariepinus*).

6.1 Introduction

6.1.1 Background

Aquaculture in the African continent has been stagnated almost entirely at farm level for subsistence, with little more production being sold in local market. Aquaculture in Ethiopia is even in its lowest level and under developed compared to many African countries. In the African continent, economic analysis in fish farming business is a relatively recent practice and its social and economic contributions had not been reported like the other agricultural sectors (Egna and Boyd, 1997).

In the selection of an appropriate aquaculture production system and technology, the economic issues to be considered include the potential for its income returns, economic efficiency, and eventually the farmers access to investment capital and appropriate technology (Hebicha *et al*., 1994). There are no much more studies on the economic evaluation of aquaculture production of the African catfish compared to that of tilapia production (Omondi *et al.*, 2001; Veverica *et al.*, 2001).

Extensive system is characterized by use of very limited input and hence lower cost, little control over the environment and farmers do not provide feed for fishes. Since food used to fish is limited on the natural environment for fish production, stocking densities are much lower than in semi intensive systems and must be adjusted to food production. Slower fish growth, lower production and harvest yields are typical characteristics of extensive system compared with intensive systems. Due to the

limited application of food and other inputs (including water management), the cost of production in extensive system is much lower than (semi) intensive systems.

Semi-intensive system on the other hand is characterized by higher stocking density and involves supplemental feed provision though much of the animal's nutrition comes from natural production. Farmer has some mechanisms to control the environment, usually in the form of water exchange and aeration. Improved environmental conditions and adding feeds usually improve growth rates, survival and harvest yields. Environmental control (i.e. refreshing culture water and aeration), use of formulated feeds and pond fertilization all increase production cost compared to extensive systems.

Intensive culture system is characterized by high stocking density, substantial environmental control and the fish is exclusively fed on prepared formulated feeds. Recirculating aquaculture systems (RAS) is typical intensive culture system utilizing a series of tanks, and water treatment processes. In RAS, water quality is maintained by recirculating the culture water continuously throughout several technological components (mechanical filtration, bio-filtration, disinfection, gas management and protein skimming) prior to re-entering the culture tanks. Ideally, a recirculating aquaculture system allows the operator to maintain optimal water quality parameters for the species being cultured, although in reality it can often be a management challenge (Badiola *et al*., 2012). Reports (Martins *et al.,* 2010) indicated that the financial cost associated with a RAS is one of the major constraints and limits for further implementation because high capital costs and energy inputs are required for construction and daily operations. Several studies suggest that energy use in a RAS is

the most significant environmental concern associated with these production systems (Bostock *et al.,* 2010*;* Martins *et al*., 2010; Dalsgaard *et al*., 2013).

Due to the heavy reliance on pumping and water treatment technologies, the principal operational cost associated with RAS is energy. Energy consumption in RAS has been studied extensively (Aubin *et al,* 2009; Ayer and Tyedmers, 2009; Jerbi *et al.,* 2012) and the findings of these studies indicate that RAS are much more energy-intensive than other aquaculture production systems. For example, RAS has energy requirements of 17.6 to 22.6 kWh/kg fish as compared to traditional flow through systems (FTS) that requires between 9.75 and 13.4 kWh/kg fish (Ayer and Tyedmers, 2009; Roque d´Orbcastel *et al*., 2009*).*

All culture systems cannot give the same economic benefit. There are also conditions that might not permit to do all kinds of farming systems in an area due to scarcity of land, power, labor or all. So choosing the best alternative farming system might become necessary. The effectiveness of a given culture system is measured by its production performance and the economic benefit it delivered.

6.1.2 Objectives

- To compare the performance and productivity of the three culture systems in African catfish farming,
- To evaluate and compare the costs expended and the economic return contributed by each culture system., and
- To recommend the best *C. gariepinus* production system in Ethiopia, particularly around the Lake Tana sub basin.

6.2 Materials and Methods

6.2.1 Data collection and values

Primary data and information on specific aspects of experimental fishes in each production practice were recorded using different data recording sheets indicated on Annex 3. The price of some inputs was taken from the local market. Information was also derived from personal observations and experiences.

6.2.2 Culture systems considered for comparison

The three experimental pond and tank culture systems (extensive and semi-intensive pond, and intensive tank culture) were used for this comparison. Catfish fingerlings with a mean weight of 37.9 g from semi-intensive pond culture and 37.3g from intensive culture in a tank were selected purposely. For extensive farming stockers with a mean weight of 143.7g were used (Table 5.3).

Within a culture system, the treatment which delivered significantly higher productivity or yield per unit area from chapter 5 (indicated on Tables 5.3, 5.4 and 5.6) were selected for this comparison. Hence and therefore, productions of fish at a stocking density of 2fish/m^2, 10fish/m^2 and 100 fish/m^3 were selected for the comparison of extensive, semi-intensive and intensive culture systems, respectively.

6.2.3 Fish performance evaluation

The daily weight gain and specific growth rate of fish in each culture system was compared. In addition, the performance of experimental fish in each culture system

was evaluated using performance index of fish (PIF) calculated with a formula adapted from Adewolu *et al*. (2008);

$$\text{FPI} = \frac{\text{Survival rate} * (\text{Final mean weight (g)} - \text{Initial mean weight(g)})}{\text{Rearing duration in days}}.$$

The fish harvested from each culture system after a culture period of 8 months was evaluated to compare the production potential or yield of each culture system.

6.2.4 Economic indicator

Cost–benefit analysis (CBA), sometimes called benefit costs analysis (BCA), is a systematic approach to estimate the strengths and weaknesses of alternative culture systems. It is used to determine options that provide the best culture system to achieve benefits while farming. The main costs that vary during the production cycle between the three culture systems were expenses to manage the fish (labor cost), costs to purchase inputs (fingerlings and feed ingredients), transportation and power (costs of fuel or electricity). All the costs were timely listed in a record sheet (Annex 3) during 8 months culture period and summarized for each culture system (Table 6.2). The major investment cost for each culture system was costs related with extensive and semi intensive culture ponds and intensive tank culture and the necessary facilities associated with it. There was no significant variation in investment costs between the culture systems and hence not considered.

The average size by weight and the estimated total biomass of the catfish population in the pond were the basis for determining the total daily ration requirement in semi intensive pond culture. In an intensive tank culture all the experimental fishes were counted and weighed regularly to determine the daily ration. The cost expended for

feed to grow a fish was calculated from the feed provided to the catfish and the price of a unit of feed.

The daily feeding (DF) was calculated using the formula modified from Janssen (1987); $DF = F * (\frac{NW}{1000})$kg. Where: F = Feeding ratio; N = total number of catfish in the pond or tank and W= average body weight of catfish(g). Further details on the running costs expended during the production period, their quantities, values or assumptions and costs were indicated on table 6.1. and 6.2.

The source of an income or revenue for each culture system was only from the sale of African catfish harvested at the end of the experiment. The cost of production was calculated and subtracted from the revenue at the end of the production cycle and the result was net income of the culture system. Considering all the costs expended and an income generated, profit index was estimated to assess the economic benefit of the three culture systems. The income return from each culture system was calculated using profit index (PI) formula; PI = $\left(\frac{\text{Value of fish } (ETB)}{\text{Cost of production } (ETB)}\right)$.

6.2.5 Statistical analysis

Microsoft Excel 2007 was used to record the different field data, calculate the different costs expended and income generated for each culture system, and made comparison graphs. To compare the different means and make an ANOVA to test the significance, IBM SPSS statistics version 20 was employed.

Table 6. 1. Characteristics and values considered

Characteristics	***Extensive (100 m^2 pond)***	***Semi-intensive (100 m^2 pond)***	***Intensive (100 m^3 tank)***
Pond or tank	Earthen pond	Geo-membrane lined	Fiber glass fish tanks
Average no. of fishes stocked	200 stockers	1,000 fingerlings	10,000 fingerlings
Average no. of fishes harvested in a pond or tank	167	896	9300
Mean initial weight/head,g	143.6867	37.89675	37.342
Mean final or harvest weight/head,g	393.58	530.9858	574.7759
Survival rate	83.3%	89.6%	93%
Culture duration	32 weeks	32 weeks	32 weeks
Total production, kg	65.65748	477.045	5345.423
Biomass, No. of fish/m^2	1.67	8.96	93
Energy (power) required for	No need of energy	Feed grinding, pelleting, pumping water during harvest	Feed grinding, pelleting, pumping, aeration, heating
Price of catfish seed	10.00 ETB/stocker	1.00 ETB/fingerling	1.00 ETB/fingerling
Total feed offered, kg	No feeding	35.67005	227.0006
Price of feed, ETB/kg	-	25.38	25.38
Price of harvested fish	20.00 ETB/head	25.00 ETB/head	10.00 ETB/head
Labor required	Stocking, water fertilization, water sampling and harvesting.	Stocking, feed and feeding, water fertilization, fish/ water sampling and harvesting.	Stocking, feed and feeding, cleaning tanks, sampling and harvesting.

Table 6. 2. Variable costs considered for a 100m^2 sized pond and 100m^2 fish tank during the production period of 32 weeks.

Cost titles	**Values or assumptions used in each culture system**		
	Extensive pond culture	*Semi -intensive pond culture*	*Intensive tank culture*
1. Labor costs			
♦Count and stock fishes	2 PDs paid at100 ETB	6 PDs paid at 100ETB	10 PDs paid at 100 ETB
♦Ration formulation (mixing, pelleting, drying, sealing	No feed formulated	1 PDs paid at 75 ETB/MN for 8MNs	2 PDs paid at 150ETB/MN for 8 MNs
♦ Daily feeding of fishes in a pond/tank	No feeding	1 person day paid at 5 ETB/D for 224 days	5 person hour paid at 70 ETB/D for 224 days
♦ Pond fertilization	5 PH at 25 ETB/WK for 20WK	5 PH at 25 ETB/ WK for 32 WK	No fertilization
♦Water quality monitoring	Not needed	2 person at 50ETB/MN for 8 MNs	2 person hour paid at 20ETB for 224D
♦Harvesting fish	2 PDs paid at 150ETB	2 PDs paid at 150ETB	1 PDs paid at 150 ETB
2. Cost of catfish seed	200 stockers at 10ETB/hd	1000 fingerlings paid at 1ETB/hd	10,000 fingerlings paid at 1ETB/hd
3. Feed cost	No feed cost	35.67kg feed paid at 25.38ETB/kg	227kg feed paid at 25.38 ETB/kg
4. Energy/power for facilities	No cost	2 L/MN for 8 MN at 16.36 ETB/L	5L/day for 224d paid at 16.36 ETB/L.
5. Transport cost (feed, fish)	2 rounds at 500 ETB each	3 rounds paid at 500 ETB/round	One day paid at 500 ETB

Note:-PH= person hour, PD =person day, D= day, WK= weeks, MN= Month, hd=head, ETB=Ethiopian Birr

6.3 Results and Discussions

6.3.1 Fish performance and growth comparison between systems

The growth of fish in terms of daily weight gain and SGR varied between culture systems and the variation was significantly higher ($p < 0.01$) between extensive and the other two culture systems. The observed SGR of fishes between semi-intensive and intensive culture system was 1.18 and 1.22 but the difference was not statistically significant at $p < 0.01$ level.

Performance indicator for experimental fish (PIF), which considers the adaptability of fishes to a culture environment and their growth and development, confirmed the existence of differences in fish performance between the three culture systems. The PIF value of *C. gariepinus* (mean ± SD) was 0.98±0.09, 1.98±0.24 and 2.26±0.05 in extensive, semi intensive and intensive culture, respectively (Table 6.3).

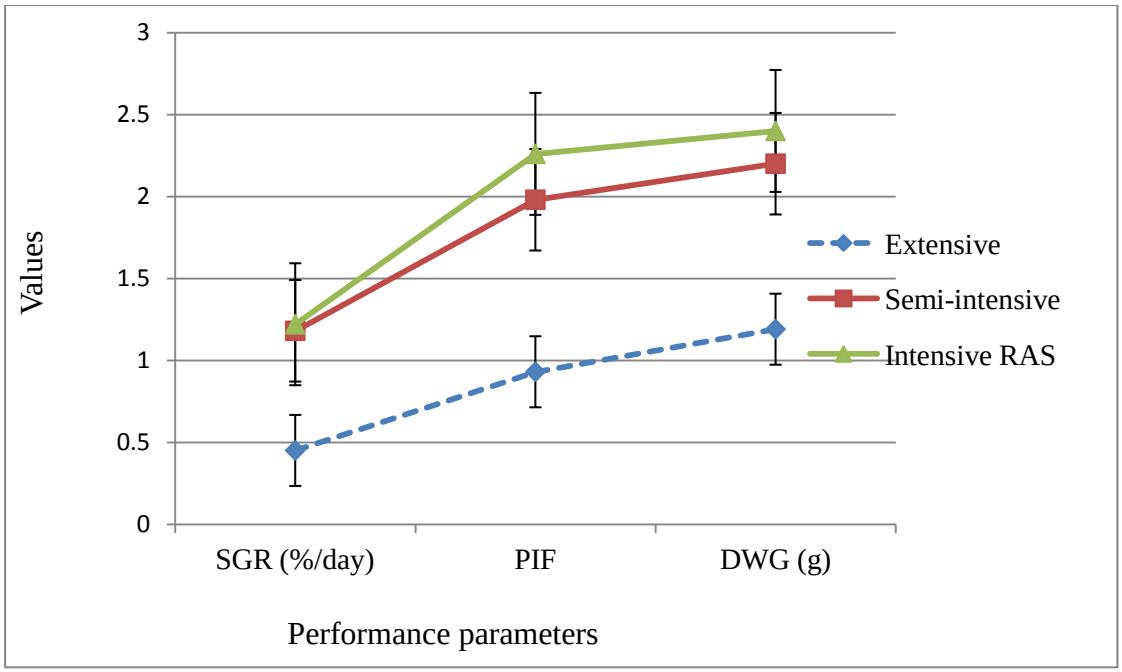

Figure 6. 1. Performance of fishes in different culture systems.

The PIF value of extensive system was significantly lower ($p < 0.01$) compared to the other two systems, but the difference was not significant between semi-intensive and intensive culture systems (Table 6.3). The observed higher PIF value for semi-intensive and intensive culture indicated that these culture systems provide best performing fishes than extensive culture (Table 6.3; Fig 6.1). At higher PIF value, the production of *C. gariepinus* was higher and that was due to the higher number of fish harvested and their individual weight increment shown at the end of the culture period (Fig 6.1: Table 6.3).

6.3.2 Fish Production and productivity

The total fish harvested from intensive RAS culture was 5.34 tons in $100m^2$ fish tank within 32 weeks time. The total fish harvested from $100m^2$ semi intensive and extensive pond was only 0.477ton and 0.066 ton, respectively in 8 months culture period. The productivity of intensive tank culture in a recirculation system was 11 times more than semi-intensive pond culture. The difference was statistically different ($p < 0.01$) between the three culture systems (Table 6.3).

The highest productivity in intensive RAS system compared to the others might be due to the management of experimental fishes in a controlled environment which enabled them to use the feed effectively for growth and development. Albeit fishes kept in extensive culture ponds changed in size, they could not perform well like those in RAS and semi intensive culture. This might be due to the exposures of the fishes for different environmental changes, absence of enough food and interferences from different organisms. These challenges and calamities might reduce their potential and performance to use the energy for growth and development.

Table 6. 3. Comparison between culture systems for performance and productivity of *C. gariepinus*.

Production system	***DWG,g***	***SGR,%/day***	***PIF***	***Yield, kg/m^2***
Extensive	1.19 [a] ±0.07	0.45 [a] ±0.02	0.93 [a] ±0.09	0.66[a]±0.05
Semi-intensive	2.2 [b] ±0.12	1.18 [b] ±0.02	1.98[b] ±0.24	4.77[b] ±0.55
Intensive RAS	2.4 [b] ±0.0004	1.22 [b] ±0.001	2.26[b] ±0.05	53.45[c] ±0.29

Mean values in a column with different superscript are significantly different ($p < 0.01$)

6.3.3 Cost benefit analysis of the culture systems

Costs that varied between the culture systems (variable costs) were considered to make this comparison. The total cost(TC)of production for extensive and semi intensive pond culture in a 100m^2 pond was 4,000 ETB and 8,500 ETB, respectively (Table 6.4). The major cost in extensive culture was seed cost as the system requires large sized *C. gariepinus* fishes (stockers) and hence the TC expended for seed contributed 50% of the total cost. The highest TC cost in semi-intensive culture was labor cost which accounted 57% (Table 6.4; Fig 6.2). Labor was required for daily feeding and guarding, to fertilize the pond weekly, formulate feed and sampling water and experimental fishes monthly, and managing the culture water (Table 6.2). The water management activities which were performed regularly on semi intensive ponds include replacing the water lost due to evaporation, cleaning the culture water mainly removing algae when bloomed.

The TC of production for a 100 m^2 intensive tank culture was 53,014.55 ETB. From this TC, labor cost and cost of energy covered the highest proportion, which was

nearly 35% each. Because RAS is labor and power intensive system so as to control the quality of water and manage the system, recirculate the water and keep the water temperature and oxygen at its optimum range. The requirement of higher energy in intensive RAS culture is reported to result in higher cost of production (Martins *et al.*, 2010) and environmental concerns (Bostock *et al.*, 2010; Martins *et al.*, 2010; Dalsgaard *et al.*, 2013). Cost of feed was only 10.8% which is very low compared to other studies (Agung, 2004) as the feed was formulated from locally available and cheaper ingredients.

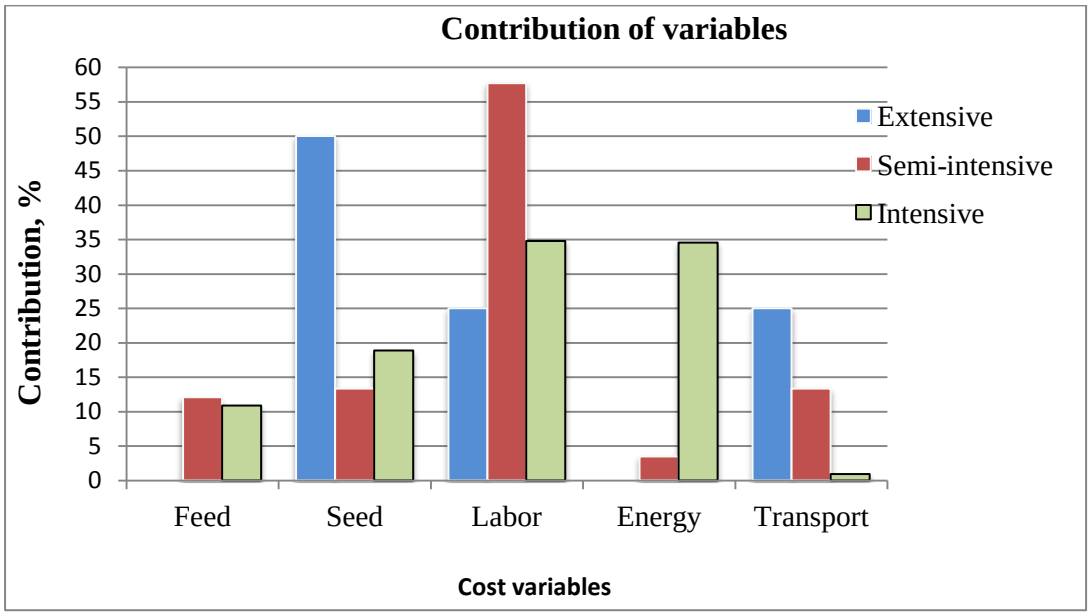

Figure 6. 2. Costs required for production of *C. gariepinus* in different culture systems.

An income source in each culture system was only from sales of harvested whole fish. Catfish is commonly sold as whole fish in the local market around Bahir Dar. The market price was estimated by experienced fish sellers at the open fish market in Bahir Dar found at Kebele 13 (Shimbit). The sale price of *C. gariepinus* varied both with its size and color as it was not filleted. Fish from semi -intensive culture were larger in size with normal dark color and sold at a better price (25 ETB/kg). The

market price of fish produced from intensive RAS culture was lower (10ETB/kg) as the color of the fish changed from its normal dark to white. The other fishes from extensive culture were smaller in size at harvest and sold at lower price (20 ETB/kg) compared to semi-intensive culture.

All costs were calculated and subtracted from the return and gave a NI contribution of ETB 4,439.06 for semi-intensive and ETB 439.68 for intensive culture. The NI was 2,686.85 below zero for extensive (Table 6.4). As depicted in Fig 6.3 (log transformed values for all TC, TR, NI and PI, The TC exceeded over the TR and led the NI value below zero in extensive culture. The TC was by far below the TR point in semi intensive culture and hence resulted in higher NI value. Hence Semi-intensive culture resulted10 times more return than intensive farming and by far greater return than extensive systems.

Profit generated from *C. gariepinus* farming for each culture system was evaluated and compared. Accordingly, the PI was higher for semi-intensive culture and confirmed that semi intensive pond culture was the preferred culture system for *C. gariepinus* farming in Ethiopia.

Table 6. 4. Comparison of costs of production and income generated among three *Clarias gariepinus* culture systems.

List of variables	**Values (ETB) for each culture system**		
	Extensive ($100m^2$ pond)	*Semi-intensive ($100m^2$ pond)*	*Intensive ($100m^3$ tank)*
1. Labor cost for a pond or tank	***1,000 (25%)***	***4,820 (56.8%)***	***18,630 (35%)***
• Stocking fishes	200.00	600.00	1,000.00
• Ration formulation	0.00	1,400.00	1,800.00
• Daily feeding of fishes	0.00	1,120.00	11,200.00
• Weekly pond fertilization	500.00	800.00	0.00
• Harvesting fish	300.00	500.00	150.00
• Water quality monitoring	0.00	400.00	4,480.00
2. Cost of stockers or fingerlings	***2,000 (50%)***	***1,000 (11.8%)***	***10,000 (18.8%)***
3. Feed cost	***Not fed***	***905.3 (10.6%)***	***761.25 (10.8%)***
4. Cost of Energy (fuel/electricity)	***Not required***	***261.76 (3.1%)***	***18,323.3 (34.4%)***
5. Transport cost	***1,000 (25%)***	***1,500 (17.7%)***	***500 (1%)***
Total cost of production (TC), No. 1 to 5	*4,000.00*	*8,487.06*	*53,214.55*
Total return (TR) - sale of fish	*1,313.15*	*11,926.13*	*53,454.23*
Net income	**(-) 2,686.85***	**3,439.07**	**239.68**
Profit index	**(-) 0.671**	**1.405**	**1.004**

* Net income is below zero and hence is PI. Numbers in bracket are percentage contribution of the variables.

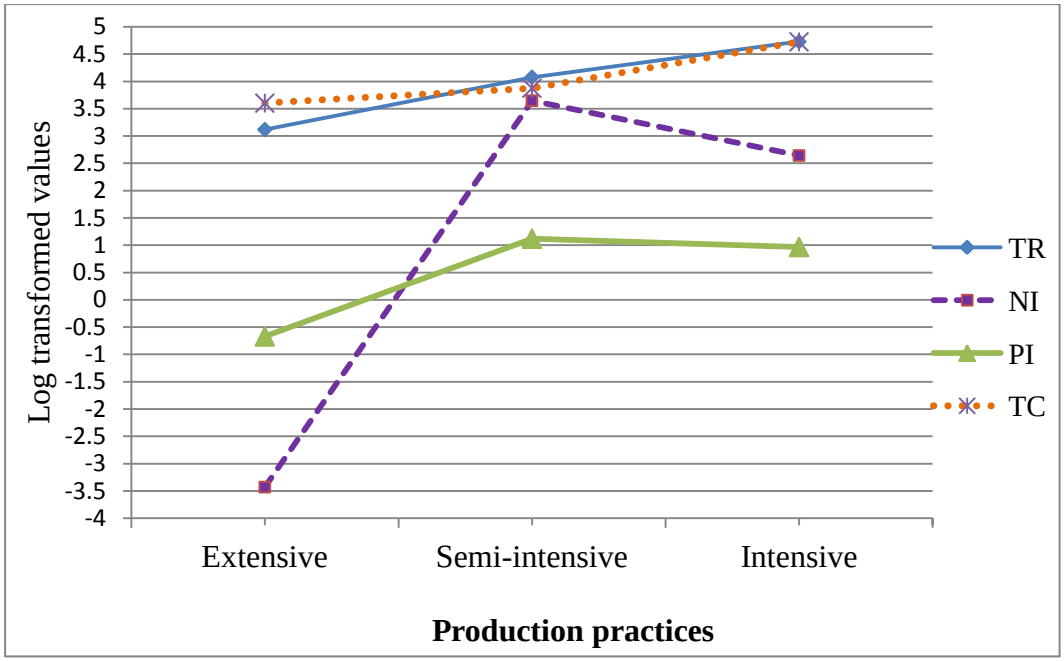

Figure 6. 3.Comparisons between culture systems for economic indicators.

Note:-TC= Total cost, TR = Total return, NI=Net income and PI = Profit index. All values were log transformed except PI for extensive production practice as the value was below 0 (- 0.067).

The income return from extensive pond culture system was lower compared to semi-intensive pond and intensive tank culture system as the price of the stocker sized fingerlings were 10 times more than the others (Table 6.2).

6.4 Conclusion

The African catfishes performed well in all the three culture systems interms of growth and production, but fish in intensive RAS performed better than semi intensive and extensive pond culture systems. The total production was higher in intensive culture, and hence resulted in more gross income. Although resulted in good performing fishes and produced more yield, intensive tank culture required more cost

to produce *C. gariepinus* as compared to extensive and semi intensive pond culture systems. Hence the net income was far below semi intensive culture system. The major variable costs for intensive culture system were labor and energy or power required to run the recirculation system. Mostly the source of power was fuel as the interruption of electricity was so frequent and delayed for half and more hours in a day, even for the whole day.

The CBA that considered only variable costs and fish sale, indicated that semi intensive culture resulted nearly 15 times more net return than intensive culture. The net return for extensive culture was below zero as the cost of stockers and transport them was higher. The profit index value was also higher for semi intensive culture than intensive culture. Therefore, in order to run a better and profitable business, it is commendable to culture *C. gariepinus* in a semi intensive pond through the supplementation of formulated feed. If needed to produce *C. gariepinus* in an intensive tank culture in a RAS, alternative energy sources and means to run RAS should be sought. To enhance the water bodies and make a harvestable produce from extensive pond culture or employing impounded waters, there should be a hatchery nearby which can provide stocker sized *C. gariepinus* at a reasonable price.

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Annexes

Annex 1. Zooplankton species checklist and their distribution over the sampling dates

Zoo taxa	Species	Harvesting dates and ponds																					Occurred
		06/08/17			07/08/17			08/08/17			09/08/17			10/08/17			11/08/17			12/08/17			
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
Rotifers	*Trichocerca longiseta*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	21
	Lecane bulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	21
	Keratella crassa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	21
	Keratella tropica	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	21
	Trichocerca similis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	21
	Filinia longiseta	0	+	0	0	+	0	0	0	0	+	+	+	+	+	+	+	+	+	+	+	+	14
	Keratella cochlearis	0	0	0	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16
Cladocers	*Alona quadrangularis*	0	0	0	0	0	0	0	0	+	0	0	+	+	+	+	+	+	+	+	+	+	11
	Diaphanosoma sarsi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	21
	Moina micrura	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	21
	Bosminia longrostris	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	21
	Diaphanosoma exisum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	21
	Daphnia lumoholtzi	0	0	0	0	0	+	0	+	+	0	+	+	+	+	+	+	+	+	+	+	+	14
	Ceriodaphnia cornuta	0	0	0	+	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16
Copepods	*Thermodiaptomus galebi*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	21
	Thermocyclop ethiopiensis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	21
	Mesocyclop aequatorialis	0	+	0	0	0	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	16

Note: ("0" represents absence, and "+" is presence of a species; 1-3 represents the harvesting ponds and OC= occurrence

Annex 2. Live zooplankton mass harvested from 3 ponds and daily counts (mean ± SD) from an aliquot of one ml sub sample.

No.	***Species***	***August 06/2017***	***August 07/2017***	***August 08/2017***	***August 09/2017***	***August 10/2017***	***August 11/2017***	***August 12/2017***	***Daily Average***
1	*Trichocerca longiseta*	8±1.0	12.3±2.3	15±2.7	14.3±3.2	12.3±1.5	8.3±1.5	5.33±1.5	10.8±3.9
2	*Lecane bulla*	19±2.65	19.7±1.5	20.3±3.1	15.7±1.5	11.3±0.6	7.7±1.2	7±1	14.4±5.6
3	*Keratella crassa*	21±1.0	21±1.7	20.7±2.1	20.3±2.1	16.3±2.1	9.7±1.2	7±1.7	16.6±5.8
4	*Keratella tropica*	9.7±1.5	11.7±0.6	13.3±2.5	22.3±1.5	17.7±1.2	13.3±1.2	11±1	14.1±4.4
5	*Trichocerca similis*	8±2	12.3±1.5	14.3±2.5	19.7±2.5	18.3±1.2	12.3±1.2	11±2	13.7±4.2
6	*Filinia longiseta*	0.3±0.9	0.3±0.6	0	4.3±1.5	12±4.4	17.3±4	19.7±4.2	7.7±8.4
7	*Keratella cochlearis*	0	0.3±0.6	4.7±2.1	11.7±2.1	14.3±2.5	18.3±3.1	20.7±1.5	10±8.2
8	*Alona quadrangularis*	0	0	0.3±0.6	0.7±1.2	3.33±1.2	11.7±0.6	16±1.7	4.6±6.3
9	*Diaphanosoma sarsi*	13.7±2.5	18.3±1.2	19.7±2.5	20.7±2.1	23.3±1.2	23±0.0	19.7±1.5	19.8±3.4
10	*Moina micrura*	16.7±2.5	21.7±2.5	22±3	22±4	17.7±0.6	14.7±3.1	10.7±1.5	17.9±4.7
11	*Bosminia longrostris*	9.3±1.5	15±1	23±1	24.7±2.1	21.7±3.1	17.7±4	11.7±2.5	17.6±5.9
12	*Diaphanosoma exisum*	11±2	17.7±1.2	20.7±1.5	23.7±2.5	23.7±2.3	16.7±2.5	12.7±1.5	18±5
13	*Daphnia lumoholtzi*	0	0.3±0.6	1.7±2.1	5±5	12±5	19.3±2.5	23.3±1.2	8.8±9.4
14	*Ceriodaphnia cornuta*	0	0.3±0.6	3.7±2.1	11.7±0.6	17±1.7	22.3±1.5	23±1.7	11.2±9.5
15	*Thermodiaptomus galebi*	70±1.7	74±3	68.7±2.5	65±8.2	63.7±5.8	58±7.9	51.7±2.5	64.4±8.4
16	*Mesocyclop aequatorialis*	0.3±0.6	0.3±0.6	3.3±3.1	13±2.6	18±1	22.7±0.6	15.3±4.5	10.4±8.8
17	*Thermocyclop ethiopiensis*	9.3±1.5	12.3±1.2	17±2	20.7±1.5	21.7±2.5	22±1.7	19.7±1.2	17.5±4.9
	Harvested daily	196.3±3.2	237.7±3.2	268.3±7.2	315.3±25.5	324.3±13.9	315±11.4	285.3±3.5	277.5±45.9

Annex 3. Different data recording sheets

3.1. Culture water daily physico chemical parameter recording sheet

Physico chemical parameter _____

Treatment	***Aquaria***	**Recording time and date**																																			
A	I																																				
	II																																				
	III																																				
B	I																																			
	II																																				
	III																																				
C	I																																			
	II																																				
	III																																				
D	I																																			
	II																																				
	III																																				

3.2. Fish growth (in weight, W, and length, L) recording sheet

Treatment ---------- Replication----------

Fish No. or code	***Recording Date and/or Time***																							
	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W

3.3. Reproduction performance recording sheet

Date _____-

Treatment	*Spawning Tank*	*Average Wt. of parent fish, kg*	*Hormone dose injected, mg*	*Time injected*	*Time stripped*	*Total Wt. of eggs spawned, g*
A	I					
	II					
	III					
B	I					
	II					
	III					
C	I					
	II					
	III					
D	I					
	II					
	III					

3.4. Fertilized and hatched eggs recording sheet from 1g sample egg.

Date ----------

Treatment	Aquaria	Total No. of eggs	Time fertilized	No. of non fertilized eggs	Time hatched	Total No. of hatchlings	Total number of day old larvae
A	I						
	II						
	III						
B	I						
	II						
	III						
C	I						
	II						
	III						
D	I						
	II						
	III						

3.5. Variable and investment cost expenses recording sheet

Date	**Expense title**	**Extensive system**		**Semi-intensive system**		**Intensive system**		**Remark**
		Unit cost	Total cost	Unit cost	Total cost	Unit cost	Total cost	

Annex 4. Illustrations

Plate 1. Checking for broodstock maturation

Plate 2. Acetone dried African catfish pituitary glands

Plate 3. Egg striped from *C. gariepinus* broodstock


Plate 4. Testis from *C. gariepinus* broodstock

Plate 5. Fertilized concrete ponds for zooplankton multiplication

Plate 6. Parent zooplanktons collection from Lake Tana for inoculation

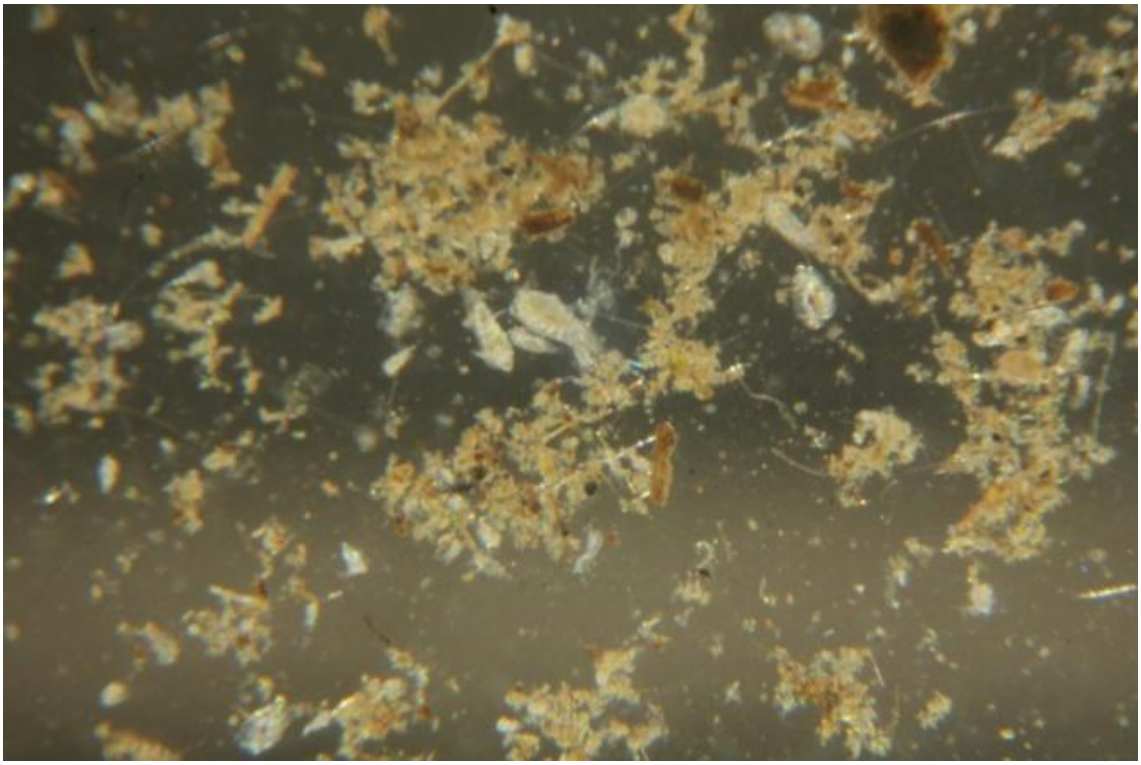

Plate 7. Zooplankton groups identified from multiplication ponds

Plate 8. Larvae culture aquariums (zooplakton feeding frequency experimental setup)

Plate 9. Early larvae of African catfish, *Clarias gariepinus* in glass aquarium

Plate 10. African catfish, *Clarias gariepinus* larvae start feeding formulated feed

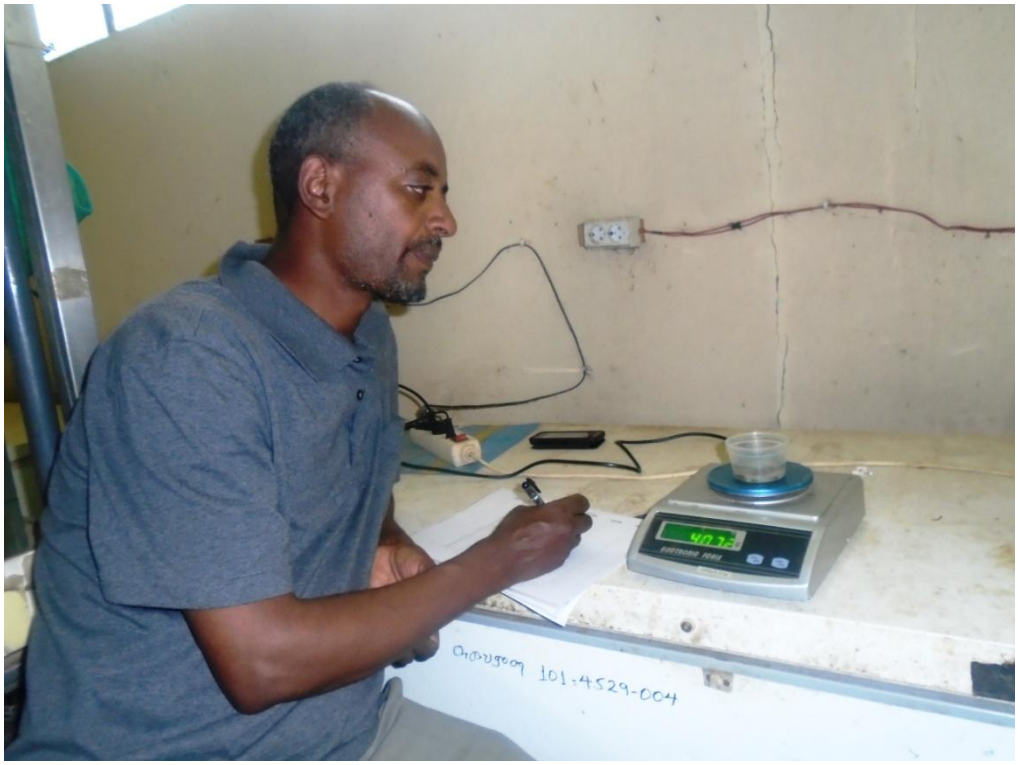

Plate 11. Data recording

Plate 12. *Clarias gariepinus* fingerlings in acclimatization

Plate 13. Fingerlings recruited for semi intensive and intensive production

Plate 14. Formulated feed from local ingredients

Plate 15. Intensive tank culture (recirculation system) setup

c

Plate 16. Experimental fishes in a tank during feeding

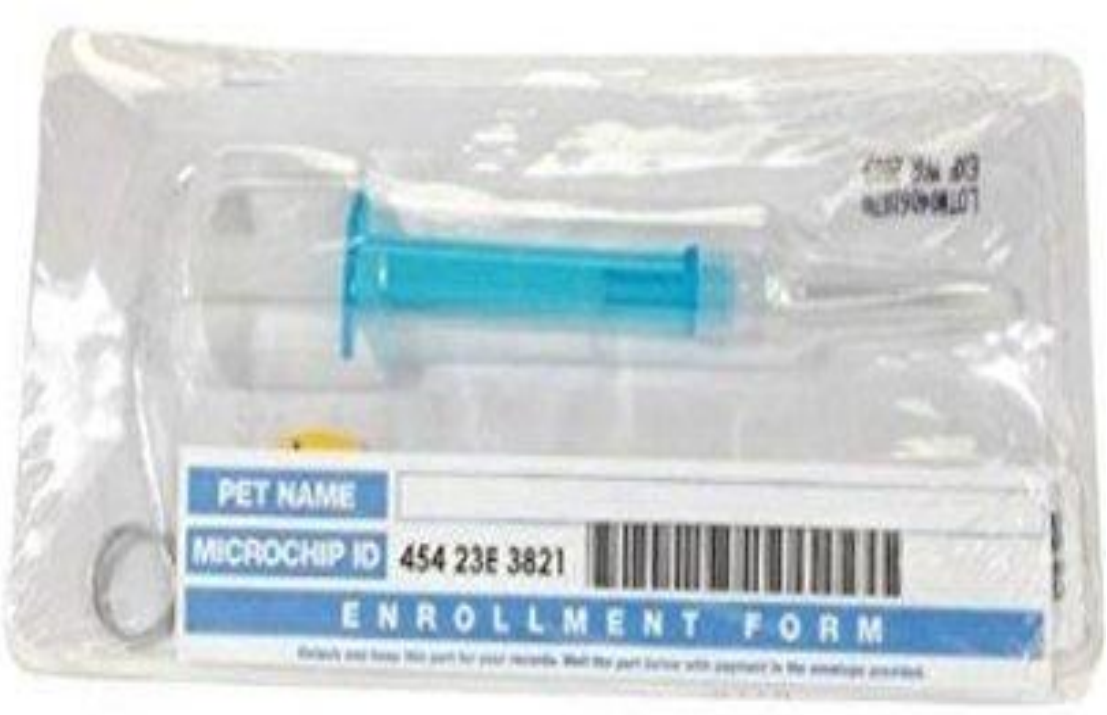

Plate 17. Microchips used for fish tagging

Plate 18. Impounded water (experimental sites) at Fogera, during August and January

Plate 19. One of the semi intensive experimental pond

Plate 20. Water sample from semi intensive culture ponds

Plate 21. Fish produced from intensive tank culture system

Plate 22. Fish farmer with filleted *C. gariepinus* fish from experimental pond

Plate 23. Leech (*Hirudo spp.*) found stacked to the body of experimental fishes

Plate 24. Identification and counting zooplankton taxa under microscope