Growth performance and survival rate of African catfish larvae *Clarias gariepinus* (Burchell 1822) fed on different types of live and formulated feeds.

Master of Science Thesis

By: Tarekegn Arage Ayele

Supervisors

Ao.Univ.Prof. Dr.Phil. Herwig Waidbacher	(Boku)
Adamneh Dagne	PhD (Sebeta, Ethiopian)
Paul Meulenbroek	MSc (Boku)
Esayas Alemayehu	MSc (Sebeta,Ethiopian)

This thesis is submitted in partial fulfillment of the requirements for the joint academic degree of Master of Science in Limnology and Wetland Management jointly awarded by the University of Natural Resources and Life Science (BOKU), Vienna, Austria, UNESCO-IHE Institute for Water Education, Delft, the Netherlands and Egerton University, Njoro, Kenya.

Examination committee

Ao.Univ.Prof. Dr.Phil. Herwig Waidbacher

Ass. Prof. Dr. Wolfram Graf

Ao. Univ. Prof. Dipl.-Ing. Dr. nat. techn. Werner Zollitsch

MSc research host institution symposium

University of Natural Resources and Life Science (BOKU)

2015

Acknowledgment

Firstly, I am grateful to express my sincere gratitude to my advisors Ao.Univ.Prof. Dr.phil. Herwig Waidbacher, Adamneh Dagne (PhD), Paul Meulenbroek (MSc) and Esayas Alemayehu (MSc) for the continuous support of my master's study and research with their patience, motivation and enthusiasm.

Besides my advisors, I would like to thank the IPGL-course coordinator Mr. Gerold Winkler for his understanding and full support on providing equipments and funds in every needed situation. Additionally, I would like to thank Austrian government for providing me with the generous funding that ensures me the opportunity to follow my MSc studies.

My sincere gratitude also goes to Dipl.-Ing.(FH) Hanna Michele Hentschel for her assistance on guiding assistance. Further appreciation goes to Miss Nina Haslinger for her support and help with my thesis writing.

My special thanks for Mr. Esayas Alemayehu for his patient support during my experiment conducting stage. He was involved with every detail from equipment scouting to data collecting. I am lucky to have you as an advisor and friend. I wish all the best in your life. I thank my colleague, Miss Roman and Mr.Seifu who assisted me in the whole research period and to all the staff of NFLARC who were by my side.

Last but not the least; I would like to thank my parents Sayish Asefa and Arage Ayele supporting me spiritually throughout my life.

Abstract

Catfish (*Clarias gariepinus*) is one of the best candidate aquaculture species. Feed plays the major role on culturing fish, especially at early stages. Various studies have found low survival rate and poor growth performance as a major obstacle in meeting the demand of fry, particularly in developing countries like Ethiopia. In thirty two days of the experimental period, this study investigated growth and survival rates of *Clarias gariepinus* larvae by feeding *Daphnia magna,* Ostracoda (*Heterocypris incongruens*), commercial feed, fish meal mix and soybean meal mix. The trials were conducted in 25 tanks, with 150 fish per tank, 5 treatments and 5 replicas. Water flow with the help of oxygen aerator was allowed, and a feeding frequency of three times per day was applied.

Water quality was in an accepted range of a mean temperature 26.3 ± 1^{O}C, Oxygen 5.8 ± 0.35mg/L (94.3 ± 4.5 %), pH 7.7 ± 0.1 and ammonia concentrations was less than 0.2mg/l in all treatments. Before weaning strategy was practiced, *Artemia* nauplii were used as starter feeds that gave an overall average growth of 6.03 mm TL and 2.25 mg TW. Among the five treatments, larvae fed on *Daphnia* demonstrated the highest growth performance in terms of mean monthly weight gain (79.75 mg) and change in length (14.77 mm). The next on the ranking is commercial feed (57.75mg and 12.87mm), followed by *Ostracoda* (53.75mg and 12.07mm), then fish meal mix (23.75mg and 8.67mm) and lastly, soybean meal mix (21.75mg and 8.27). There was a significant difference recorded between treatments ($P<0.05$). Best survival rate of 84.4±6.6% was documented on commercial feed. Whereas 76.3 ± 7%, 70.9±6.4%, 59.5 ± 4.9%, 56.3± 2.5 was recorded retrospectively on the groups *Ostracoda, Daphnia*, fish meal mix, soybean meal mix. Significant difference between each treatments ($P <0.05$) was also recorded. The lowest result was due to low digestibility and palatability, this drive for more study on locally formulated feed. More studies on *Ostracoda* will be needed as it was one of the best feeds among the treatments. The data were analysed to determine the significant difference and homogeneity among treatments by one-way ANOVA.

Key word: *Clarias.gariepinus*, feeds, *Daphnia magna, Ostracoda*, commercial feed, fish meal mix and soybean meal mix, growth, Crude protein, survival rate.

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Abbreviations

CP	crude protein
DO	Dissolved oxygen
F.meal	fish meal
S.meal	soybean meal
SD	standard deviation
R&D	Research and development
C.gariepinus	clarias gariepinus

1 Introduction

The global aquaculture fish production constituted a record 42.2 % of global fish production (total 158 million tonnes in 2012) from capture fisheries and aquaculture (FAO, 2014). Sub Saharan Africa contributed only 0.68% of the total global aquaculture production of eatable fish in 2012 (FAO, 2014). These insignificant aquaculture productions observed in aforementioned region can be attributed to issues that include poor aquaculture development policies; economic restrains; inappropriate technologies/approaches; lack of fish seed; unavailability of feed; weak extension services and limited coordination between research/development sectors (Machena and Moeh, 2001; Hecht, 2005). However, several countries in the Sub Saharan Africa have the physical and socio-economic situations that make them very suitable for the sector to flourish (Hecht, 2005).

Ethiopia has a huge potential for aquaculture. The country has vast water resources; agro-ecology enriched land along with labor and presence of suitable fish species. (Dejen and Mintesnot, 2012). The scarcity of seed can sustain a commercial production, and unavailability of commercial feeds had been cited as the most pressing constraints for aquaculture development in Ethiopia (Rothuis et al., 2012). These two crucial restraints cannot be viewed individually as they act in correlation.

Nile tilapia (*Oreochromis niloticus*) and the African catfish (*Clarias gariepinus*) are among the candidate fish species for aquaculture production in Ethiopia. In the last two decades *C. gariepinus* has developed into one of the most successfully cultured species in Africa next to tilapias. The suitability of the species for aquaculture arise from its fast growth rate; tolerance of high stocking density and poor water quality; acceptance of both artificial and non specialized feeds and high market demand (Ponzoni and Nguyen, 2007).

The development of a successful aquaculture program requires a reliable and consistent supply of seeds. The major concern of any seed production in the hatchery system is to produce the maximum number of quality fingerlings from the available brood stock. Since the African catfish do not reproduce naturally under captivity, artificial reproduction methods had been developed.

The most reliable method developed so far that ascertained reliable and mass production of seed through induced spawning of catfish in indoor hatchery (Haylor and Muir, 1998).

The hatchery seed production includes use of hormones (natural or synthetic) to induce spawning, artificial fertilization, incubation of fertilized eggs and subsequently feeding live feed followed by dry feed (Hogendoorn, 1983). The success of this method heavily depends on larval rearing (Haylor and Muir, 1998). For many fish species, the larval period is considered critical in their life history. Successful larval rearing depends mainly on the availability of suitable diets that are readily consumed, efficiently digested and that provide the required nutrients to support good growth and health (Giri et al., 2003).

Larvae, especially first-feeding larvae, often depend on live food. In general fish species like catfish, carps, salmon, trout and some others have been reared successfully in aquaculture at larvae period with fully digestive system on starting time of feeding. Independent of their nutritional value, live foods are easily detected and captured, due to their swimming movements in the water column, and highly digestible, given their lower nutrient concentration (water content >80%) (Conceição, 2010).

The most widely used live food in aquaculture is the brine shrimp *Artemia salina*. It is popular for mass production for its ease of transport, due to its ability to form cysts and viability over longer periods of time. The simplicity of cysts hatching establishes its market demand as the most convenient live food used in larvae culture (Conceição et al., 2010). *Artemia* is also very nutritious to the larvae (>56% crude protein, 17% lipid and 3% carbohydrate) (Garcia et al., 1998). Despite all those advantages of *Artemia* it also has disadvantages: the price remains expensive due to contrast between high demand and low/unreliable natural resources. Such price points made *Artemia* feed unattractive and unavailable to the sub Saharan Africa farmers.

The other live food currently being used in Africa and other parts of the world are zooplanktons. They are valuable sources of crude protein, amino acids, lipids, fatty acids, minerals, enzymes and carotene (Pillay, 1990). They improve flavor, color and texture of fish that fed on them. Among zooplankton *Daphnia*, *Moina* and rotifers have been used most extensively as live foods for rearing fish larvae and fry (Leger et al., 1986). However, absolute use of live foods as diet for larval rearing encountered issues associated with high cost and time consuming production techniques (Gonzalez et al., 2008) which can be slightly alleviated by weaning with dry feeds.

Suitable dry feeds for larval and fry rearing should satisfy the nutritional requirements of the species and should be readily accepted. A fundamental aquaculture species considers dietary protein essential since adequate dietary protein significantly influence growth, fish survival and feed cost (Giri et al., 2003). The utilization of dietary protein for growth depends on the quantity as well as quality of feed proteins. The main source of protein in aqua feeds remains to be fishmeal, as it contains a profile of high quality protein with balanced amino acid. The high demand for it, along with supply fluctuation made fishmeal expensive. As a result, relentless efforts have been made to substitute fish meal with other cheaper sources of protein. Several protein sources of both animal and plant origins are being tested as fishmeal replacements. Blood meal, soybean, wheat bran, maize and other formulated feed are in use by different aquaculture farms (Munguti et al., 2012).

Different locally available feed stuffs for fish feed have been identified, and their proximate nutrient composition has been analyzed (Assamnew et al., 2012). However, these feed stuffs have not been formulated into commercial or local fish feeds. Agriculture in Ethiopia being the main source of economy, can be a cheap source of locally available fish feed ingredients. However, no research has been conducted to formulate feed for different stages of *C. gariepinus* from locally available materials so far. For the aquaculture sector to develop in Ethiopia, feed from locally available ingredients has to be produced to available quality fish fry.

The present study is designed to formulate feed from locally available sources for the early stages of *C. gariepinus* and to compare the growth performance and survival rate of larvae fed on local and commercial feeds. Furthermore, the potential of *Daphnia* and *Ostracoda* for larval and fry as live foods were studied.

2 Objectives

2.1 General Objective.

The overall objective is to evaluate survival and growth performance of *Clarias gariepinus* fry under different feed types.

2.2 Specific Objectives

- To compare the effect of different live feed and dry feeds on the growth of *Clarias gariepinus* larvae.
- To compare the effect of different live feed and dry feeds on the survival rate of *C. gariepinus* larvae.

3 Research question

- Is there any significant difference in growth performance among *Clarias gariepinus* larvae fed with live and formulated feed?
- Is there any significant difference in survival rate among C. gariepinus larvae fed with live and formulated feed?
- What will be the effect of feeding different feeds to *C. gariepinus* larvae on the water quality of the culture system?

4 Hypothesis

- There will be no significant difference in growth performance of *Clarias gariepinus* larvae that fed on soya bean meal, fish meal, commercial feed, *Daphnia magna* and *Ostracod* spp.
- There will be no significant difference in survival rate of *C. gariepinus* larvae that fed on different feeds.
- Water quality will not be affected by the different feed types.

5 Literature review

5.1 Aquaculture

Aquaculture is a science, technology and business to produce live organisms in limited aquatic system (Pillay, 1993). It has long history with the start of commercial fish farming in China in the 12th century B.C. Then it extends throughout the world (Ling, 1977, Silva, 2012). In the past decade due to its fast development, aquaculture accounts 76% of global fresh water finfish production (El-Sayed, 2006; FAO, 2008). From world aquaculture production Asia accounted for 89 percent by volume in 2010 (FAO, 2012).

5.2 Aquaculture in Africa

Aquaculture was introduced into many countries of Africa to serve as a source of protein and avoid total dependence on crops in the 1950s. From 1960 to 1990s development of the sector was facilitated by the help of funds from FAO and other governments and nongovernmental organizations. Around $500million was raised by multilateral and bilateral donors to fund 300 projects throughout the continent (Brummett and Williams, 2000). Increased technical assistance was also observed during this time. Hecht (2005) divided the development of aquaculture into three distinct phases:

***Phase 1*:** 1950–1970. The introductory phase: during which the sector was popularized but with limited knowledge and understanding. Most government stations were built during this era.

***Phase 2*:** 1970–1995. The expansion phase: significant donor support, active R&D, government involvement in seed supply and extension. Commercialization of the industry in some African countries (e.g. Nigeria, Madagascar, Côte d'Ivoire, Zambia, and South Africa) also took place.

***Phase 3*:** 1995 to present. The adjustment phase: reduced donor support, re-orientation of public support towards facilitation, emergence of the commercial sector.

Africa's contribution to world aquaculture in 2012 amounts to 1,485,367 tones (18 times as much as produced in 1990) which is 2.23% of the global production (FAO, 2014). Sub-Saharan Africa contributed only 0.68% of the total production, with Egyptian production of 1million

tones for the first time (FAO, 2014). However, the African aquaculture showed the fastest growth in the world at a rate of 11.7% since the turn of the millennium (FAO, 2014). The aquaculture sector in Africa employs more than 290,000 by 2012 accounting for 10% of the world fish farmers (FAO, 2014).

Egypt, Nigeria, Uganda, Madagascar and Zambia are major aquaculture producers in Africa (Bhujel, 2014). Nile tilapia (*Oreochromis niloticus*), Flathead grey mullet (*Mugil cephalus*), and the African catfishes (*Clarias gariepinus*) are the species produced in the highest quantity in the continent (FAO, 2012). Farming system employed includes ponds, raceways, pens, cages and recirculation systems. Ponds range from $500m^2$ to 2.5ha with production levels of 3–10 tones/ha/year with inorganic or animal manure. Raceways are used mainly for trout and tilapia. Pens and cages (square and round) range from $15m^3$ to $1{,}600m^3$ and are used for farming of tilapia, trout, clariid and bagrid catfish and high-density water recirculation systems are used for fingerling and Table fish production of African catfish in Nigeria and South Africa (Brummett and Williams, 2000, Hecht, 2005).

At 2.23% Africa stands as the continent with the lowest aquaculture production. Aquaculture development in Africa started at about the same time as Asia, but lags far behind in terms of production volume and revenue (Buhjel, 2014). Interplay of institutional, bio-technical and economical factors have been ascribed to the slower development of the sector in the continent. These factors include: lack of clear aquaculture development policy, poor governmental support, weak research and extension as well as research and development linkages, inappropriate and inflexible technical support, heavy dependence on donor support, unavailability of credit, inadequate seed and feed supply both in quality and quantity, lack of farmer participation in extension systems, poor and often unreliable data collection system (Machena and Moehl, 2001; Hecht, 2007).

The declining capture fisheries of the region, the high population growth rate in Sub-Sahara Africa and the current shortfall of fish all emphasize the need for rapid aquaculture sector growth. Aquaculture in Africa need to be promoted to be able to meet the projected demand of 3 million metric ton annually, for which aquaculture production has to grow by 10% annually for the next five years until 2020 based on its current level of production of 1 million metric ton per

year. 30 million hectors of land was estimated to be suitable for aquaculture in Africa. An additional 12 million hectors of floodplains would also be suitable for fish production. There is also a potential for cage culture, given the availability of water bodies throughout the region (Machaena and Mohel, 2001).

The future for aquaculture in Africa is promising. A rapid decline in wild catch, along with an increase in public awareness and priority given by the government indicate that aquaculture may take off very soon (Buhjel, 2014, Munguti et al., 2014). Exponential growths that were seen in Egypt and Nigeria can be replicated in other parts of the continent. The following points have been suggested as possible directions for the development of the sector in the continent: careful planning is necessary to guide future aquaculture development and ensure that available resources are well used. A strategy for aquaculture development should be developed in these countries. Conducive policy frame work has to be created for the strategy to be implemented. Furthermore, strong ad appropriate research and extension system has to be fashioned. Readily flow of information between farmers and professionals and effective training and extension services have to be developed (Brummett & Williams, 2000, Atta-mills et al., 2004).

5.3 Aquaculture in Ethiopia

As in most African countries, aquaculture in Ethiopia is underdeveloped. It was introduced in 1974 by the establishment of Sebeta Fish Breeding Center (now the National Fisheries and Other Aquatic Life Research Center) by the help of the Japanese Oversees cooperation (JICA) (Rothuis, et al., 2012). The center breeds different species (mainly tilapia and common carp), also stocked and enhanced several lakes and reservoirs. Several ponds were constructed in different parts of the country over the years since 1974. One project that introduced cage culture system in temporary water bodies was carried out in 2006-2009. Several research works have been conducted to study the effect of stocking densities and different feed types on the growths of Nile tilapia in the cage systems (Gibtan et al., 2008).

But the development of the sector did not live up to its expectations. No commercial farms have been established yet. There are extensive aquaculture operations in small rural based fish ponds ranging in size from 100 to 300m^2 (Rothuis, et al., 2012). Many of the factors mentioned in the above section can be attributed to the underdevelopment of the sector in the country. The most

pressing issues that facing Ethiopian aquaculture is facing are the scarcity of seed and feed in both quantity and quality. Locally available feed ingredients have been identified and their proximate composition were analyzed (Assamnew et al 2012).

Market, water and land are available for the development of aquaculture in Ethiopia. A total area of 15,158 km2 of land is highly suitable for aquaculture production (Dejen and Mintesnot, 2012). The presence of a policy document for the sector, the National Aquaculture development Strategy of Ethiopia (2009) can be viewed as an important step for the sector's flourishment. However, the lack of value chains for sustainable development of the sector, absence of fish feeds, scarcity of seed (absence of mono sex fingerlings of tilapia), unreliable source of fish meal can all be mentioned as the draw backs for the aquaculture development in Ethiopia (Rothuis, et al., 2012).

Government support is essential for the sector to be developed mature enough to meet the fish demand in Ethiopia. The establishment of a large-scale intensive commercial fish production or semi-intensive small-scale commercial fish production is recommended to be able to jumpstart the industry (Rothuis, et al., 2012).

5.4 The African catfish

Clarias gariepinus, indigenous fish species of Ethiopia, can be defined as having an elongated cylindrical body with dorsal and anal fins being extremely long. The head is flattened, highly ossified, and the body is covered with a smooth scale-less skin. It has four distinctive pairs of unbranched barbels (Graaf and Janssen, 1996). The major function of the barbels is prey detection. A supra-branchial or accessory respiratory organ, composed of a paired pear-shaped air-chamber containing two arborescent structures is generally present. The accessory air breathing organ allows the fish to survive for many hours out of the water or for many weeks in muddy marshes (Haylor and Muir, 1998).

C. gariepinus is a widespread freshwater benthic species, found from Turkey, the Middle East, and throughout Africa (Spataru *et al*., 1987).

It inhabits natural lakes, impoundments, fish ponds, streams, and natural ponds in both shallow and deep waters. Even though some of these habitats are subject to seasonal drying, the species is

capable of living there due to the presence of the accessory breathing organs (Graaf and Janssen, 1996).

Bruton (1979) suggested that *C. gariepinus* is a euryphagy, an organism feeding on a wide variety of organisms according to their availability. *C. gariepinus* has a remarkable array of anatomical adaptations that made it capable of euryphagy. These adaptations allowed the species to feed on a wide variety of diet and size ranges, from a minute zooplankton to a fish half its own size (Bruton, 1979). The diet of the species included small crustaceans, insects, mollusks, oligochaetes and other fish (Bruton, 1978 and 1979; Wudneh, 1998, Dadebo, 2009). Fish, particularly tilapia, have been found to be important prey of African catfish in some waters (Dadebo, 2009 and 2014)). *C. gariepinus* is a slow foraging predator, with very small eyes, using their four pairs of barbels to feel their way around in the dark and find food detected by the array of sensitive taste buds covering the barbels and head. Approximately 70 percent of feeding activity takes place at night (FAO, 2014).

C. gariepinus shows a seasonal gonadal maturation which is usually associated with the rainy season. The maturation processes are influenced by annual changes in water temperature and photoperiodicity and the final triggering of spawning is caused by a raise in water level due to rainfall (Graaf et al., 1995). Spawning usually takes place at night in the shallow inundated areas of the rivers lakes and streams. Courtship is preceded by highly aggressive encounters between males. Courtship and mating takes place in shallow waters between isolated pairs of males and females. A batch of milt and eggs is released followed by a vigorous swish of the female's tail to distribute the eggs over a wide area (Bruton, 1979). There is no parental care for ensuring the survival of the catfish offspring except by the careful choice of a suitable site.

5.4.1 Feeding behavior of catfish larvae

Good nutrition is a factor for proper growth of fish and is more pronounced with fish in enclosure as they need adequate nutrition (Omoruwou and Edem 2011). *Clarias gariepinus* is an opportunist feeder fish. Many factors are known to influence larval prey selection within the restrictions imposed by ontogenetic development (Ghan and Sprules, 1993). These include prey characteristics such as size, density and motion (Yilmaz et al., 2006) and also larval characteristics related with trophic level such as sensory capabilities, previous experience, mouth

dimensions, mouth gape and body size and species type (Truemper andLauer, 2005, Sánchez-Hernández and Cobo, 2015).

Most first-feeding fish larvae are dependent upon vision for prey detection, although non-visual senses have also been implicated in prey detection by selective planktivorous fish larvae (Lee et al., 2014). Many studies concentrate on the relationship between prey size and mouth size as the primary determinant of prey selection (Dabrowski, 1984; Riley et al., 2012). In addition, some water-soluble chemical compounds, i.e. L-amino acids and betaine have influenced the food intake of the larvae as an attractant by stimulating non-visual senses (Yilmaz et al., 2006).

5.5 Clarias gariepinus in aquaculture

Production of the African catfish has risen tremendously from a mere 5,013 tons in 1992 to 181,601 tons in 2012 (FAO, 2014). Development of seed production and growth technologies, the species ability to withstand high densities, its high growth rate, its ability to feed on a wide array of feed and its high demand in the market can be ascribed to its increased production in the world (Ponzoni, 2008). Nigeria is the leading producer of *C. gariepinus* in the world with a production of 89,193 tons in 2009 (FAO, 2011). The involvement of the private sector in the seed production and formulation of feed paved the way for this growth in production (Adewumi and Olaleye, 2011).

Seed production is the critical aspect of any aquaculture practice. The major bottleneck in the culture of *C. gariepinus* is seed production, as the species is relatively difficult to reproduce. Larval rearing is still a major problem, with average survival rate of 30% from larvae to fingerling stages, despite many years of research that has established a relatively simple and effective method for induced breeding (Biosciences, 2009). Cannibalism is a fundamental issue and requires an investment of hundreds of man hours to sort the fish into different sizes (Baras and Jobling, 2002, Marimuthu et al., 2011,and Musa et al., 2012). The other obstacle in the seed production of the *C. gariepinus* is its requirement of high protein feed which are generally expensive (Harpath, 2008).

Different methods are employed for the seed production of *C. gariepinus*. The most widely used method in recent times is induced spawning followed by larval and fry rearing and producing fingerlings in a hatchery to supply the market. Different systems use different stocking densities,

water circulation rates and also obtain different survival rates. In most African countries the methods followed for seed production of *C. gariepinus* is induced spawning followed by larval rearing by the use of live feed (*Artemia, Moina, Brachionus* and *Daphnia*), then sub sequentially fry rearing with dry feed, sorting by size and stocking in nursery ponds (Martins et al., 2005; Yong et al., 2006; Matsiko and Mwanja, 2008). However, Reticulating Aquaculture System (RAS) has been shown to produce higher number of seeds and ascertained higher stocking densities (Nieuwegiessen et al., 2008). Up to 3 million fingerlings per year have been reported in intensive RAS in Nigeria (Williams, et al., 2008).

5.6 Feed and nutrition in *Clarias gariepinus* larval rearing

In animal production system good nutrition is essential for economical production of a healthy and high quality production. Nutrition is critical in fish farming because feed represents 40-50% of the production costs (Ninawe and Khedkar, 2009, Ogello, 2013).

In aquaculture, feeding rate and nutrition are important factor that affect the growths of fish. Hence determining the optimal feeding rate is important to the success of any aquaculture operation(Marimuthu et al., 2011, Omoruwou and Edema, 2011). Several factors influence the feeding rate in culture systems. Such as fish size, species and rearing systems (Almazán et al., 2004). Feeding rate is also influenced by the feeds nutrient content (Almazán et al., 2004, Marimuthu et al., 2011). For many fish species, the larval period is considered critical in their life history (Trushenski et al., 2006). Successful larval rearing depends mainly on the availability of suitable diets that are readily consumed, efficiently digested and that provide the required nutrients to support good growth and health (Giri et al., 2002 and Wang et al., 2005). Larvae, especially first-feeding larvae generally depend on live foods. Nutrients which take most of the proportion in both natural and formulated fish feeds are proteins, lipids and carbohydrates. Macronutrients, vitamins and minerals as micronutrients are required in minor quantity. When the fish obtain sufficient supplies of certain essential nutrients body functions and growth process will be effective (Malcolm, 2010).

In general fish species like catfish, salmon, trout and some others have been reared successfully in aquaculture at larvae period with fully digestive system on starting time of feeding. African catfish (*Clarias gariepinus*) which efficiently utilizes commercial feeds and grows rapidly, is

increasingly becoming an important commercial species in Africa, Europe and part of Asia (FAO, 2010). However, its highly cannibalistic nature discourages its rearing in culture systems.

Fish larvae still relies on live foods during the early life stages. Independent of their nutritional value, live foods are easily detected and captured, due to their swimming movements in the water column and are highly digestible, given their lower nutrient concentration (water content > 80%) (Conceição et al., 2010).

C. gariepinus passes through distinct stages in its life time. The larval stage, which is the initial stage, depends on factors such as temperature and nutrition, takes 14-42 days to complete. The fry stage, which is the second stage, is characterized by the redundant movement of the fish to the surface of the water. This movement also signifies that the fish can be stocked into ponds. The fingerling stage is reached when the fins are fully developed and most organs have been formed. It ends with the start of gametogenesis (Hailor and Muir, 1998; Truemper andLauer, 2005, and Sánchez-Hernández and Cobo, 2015).

Several researches in the past three decades had tried to come up with solutions for problems associated with larval rearing and nutrition of *C. gariepinus*. The nutritional requirements of specific stages of the species have been determined (Hailor and Muir, 1998). The optimal environmental conditions and feeding behavior during the early life stages have been understood (Verreth, 1994, Yilmaz et al., 2006). Husbandry and feeding technologies that encompass specific and changing practices have been developed (Potongkam, 2006, Musa et al., 2012).

Nutritional aspects are apparent key factors for success in larval rearing. Larval diets may be selected according to different sets of criteria depending upon the viewpoint of the farm manager or of the biological requirements set by the growing larva (Léger *et al.* 1987). In recent times simplicity and versatility are advocated to cut both direct and indirect costs associated with feed preparation. Survival rate is given more importance than growth rate as fingerlings are sold based on numbers rather than weight (Vereth, 1994). Constant availability of the diet is also emphasized upon. Palatability and digestibility are most important criteria for the choice of feed. These factors are in turn affected by size of the diet in relation to the size of the fish (Osman, et al., 2008). In general, the food size should be around 2-3% of the larval length (Uys and Hecht, 1985). Given a certain size, the most important feature of a larval diet is its nutritional quality. Its protein content and dietary levels of essential fatty acids (n-3/n-6) and amino acids play a crucial

role (Vereth, 1994). The nutritional requirement of *C. gariepinus* differs slightly at different stages of growth. At larval stage protein requirement of 55% was determined (Uys and Hecht, 1985), while 50 and 40-42% were determined for nursery and grow out phases, respectively. Regarding amino acid requirements, the only requirements for methionine (2.5%) and Lysine 57g/kg were determined (Uys and Hecht, 1985; Fagbenro, et al., 1998). Similarly, the fatty acid requirements are unknown, except that a 1:1 ratio of n3 and n6 fatty acids appears to be optimal for growth and body condition (Uys and Hecht, 1985). Crude lipid content of 9% and carbohydrates content as high as 21 percent of the diet was reported (Uys and Hecht, 1985).

At the start of exogenous feeding, larvae of *C. gariepinus* are introduced to live feed. These live feed could be rotifers (*Brachionus spp*.), *Artemia* spp. or cladoceran zooplankton (*Moina* spp. and *Daphnia* spp.). However, enrichment of these feeds has been widely used as they have insufficient n-3 HUFA with a technique called bio-encapsulation (Leger et al., 1987)

Formulated dry feed have been used as a first feed in *C. gariepinus* larval rearing with the aim of avoiding the dependence on expensive and time consuming live feed (Uys and Hecht 1985; Appelbaum.,1988). All the successful diets share the common feature that their major component (50-70%) consists of Single Cell Protein (SCP) (yeast cells belonging to the genera *Candida, Torula* and/or *Kluyveromyces).* However, reported growth rates are usually below the ones which are obtained with live food and hence, supplemental feeding with live food remains necessary (Hecht *et al.* 1988). Supplemented feed on the ingredient used on the formulation.

With recognition of early and advanced larval stages, different techniques for extensive and intensive fingerling production of *C. gariepinus* have been developed (Hecht et al., 1988, Verreth et al., 1993). During the early larval stage, live food is necessary for the first 10-14 days. The dependence of the larvae up on the live food decreases as of the 6^{th} day and additional dry feed is administered. The progressive reduction of live food with increasing amount of dry feed is called weaning. Weaning has been used as a compromise between using only live food or dry feed (Dabrowski 1984, Osman et al., 2008).

Formulated feeds can be use as a diet for fresh water species as early as mouth opening (Cahu and Infante, 2001). However, attaining feeds which assure the nutritional needs of larvae is difficult since nutritional requirements, means of absorption and digestion all change during larval development (Dabrowski, 1984, Olurin and Oluwo, 2010). Although inert diets are well

ingested at the early stage, larvae can die with full guts, telling that they are not able to digest compound diets (Cahu and Infante, 2001). Allowing early co-feeding period to prepare the gut for accepting and processing inert diets is better for growth performances than when weaning starts at the end of the larval stage (Conceição et al., 2010).

Feed formulation is a process of mixing different ingredients which can meet animal nutrition requirement (Refstie et al., 2000, Siddiqui et al, 2014). Feeds Formulared with high Level of plant protein such as soybean meal and other economic plant protein source may be deficient of methionine (Siddiqui et al., 2014). Findings on replacement of fish meal mix by plant protein sources usually are based on growth, feed utilisation and survival of fish in response to the substitution level (Kikuchi, 1999, Nguyen et al., 2009, Wuet al., 2015). Plant protein sources are not easily digested, due to anti-nutritional factors and an imbalanced amino acid profile, possibly leading to loss of nutrients either in egests or metabolic excretion (Ahmad et al, 2012, Siddiqui et al., 2014). Inclusion of soybean meal of protein in growth and conversion efficiencies of the fish at higher levels causes' methionine insufficiency causing slower growth reduced feed conversion efficiency and less protein utilization (Siddiqui et al., 2014).

C. gariepinus larvae are reared from hatching to fingerling size, either completely in extensive pond culture systems (Graaf *et al.* 1995), solely in the hatchery (Verreth *et al.* 1993, Haylor 1993) or for a 10 - 16 day period in the hatchery, followed by a nursery phase in ponds (Polling *et al.* 1988, Hecht 1988,). Three protocols are widely used for larval rearing of *C. gariepinus*. In the first protocol larvae will be fed with live food and dry single cell protein feed until the 6^{th} day and then only the dry feed will continue until the 14^{th} day. Larvae will then be transferred to nursery ponds where they will be fed 40% protein grow out feed (Hecht et al., 1988). In the second protocol larvae are fed with only live feed for the first 15 days and then will be transferred to single ingredient dry feed. In the third protocol larvae are fed with live food for the first 9 days after which they will be weaned onto dry feed until the 14^{th} day. Catfish fry pellet will be administered until the 30^{th} day (Verreth et al., 1993).

5.7 Water quality and hatchery management

Water quality is the important factors to have successful fish fingerlings grow out. Temperature and pH plays major role in determining the effectiveness of digestive enzymes as a whole. Good

water quality management follows preventing accumulation of organic debris and nitrogenous wastes limiting ammonia build up, maintaining appropriate pH and temperature depend on cultured species (Gabriel and Akinrotimi, 2011).

Ammonia which is an end product of the breakdown of organic matter heterotrophic bacteria and excreted with the droppings of aquatic animals as it is highly soluble when fish is cultured in intensive culture system. It is important to monitor and check the culture systems regularly before it reaches critical level (concentration > 0.5mg/l) (Buttle et al., 1995; Gabriel and Akinrotimi, 2011)

Table 1: Water quality parameters for catfish grow out

Parameters	**Eggs early fry**	**Larvae (tolerance)**	**Fry fingerlings/adult**
Oxygen	80-100%	3-5 ppm	>3 ppm
Temperature	Opt-30^{o}c	Opt-30 ^{o}c	Opt 26-28 ^{o}c
NH_3-N		0.1 ppm	
NO_2-N		0.5 ppm	
NO_3-N		100 ppm	
pH		6-9	
CO_2-C		6 ppm (10-15 ppm)	
Salinity		10 ppt (15-16 ppt)	

Source: (FAO 2006b, Gabriel and Akinrotimi, 2011).

6 Materials and Methods

6.1 Description of the study area

The study was conducted at the National Fishery and other Aquatic Life Research Center (NFALRC). The center (8°55′N 38°37′E) is located at Sebeta 24 km South-West of Addis Ababa at an altitude of 2200 m.a.s.l. The experiment was conducted in the center's experimental indoor hatchery. The hatchery is equipped with heating tanks with a thermostat, brood fish holding tanks, hatching basins (for catfish) and larval rearing basins. For *Daphnia* and Ostracoda mass culture, plastic tanks (3000 l capacity) and several Aquaria (30 cm by 60 cm) were used. The water source for the center is from borehole and the springs.

Brood catfish and zooplankton (Ostracoda) was collected from koka reservoir (low land) and Daphnia was from Lake Hashenge (highland lake)

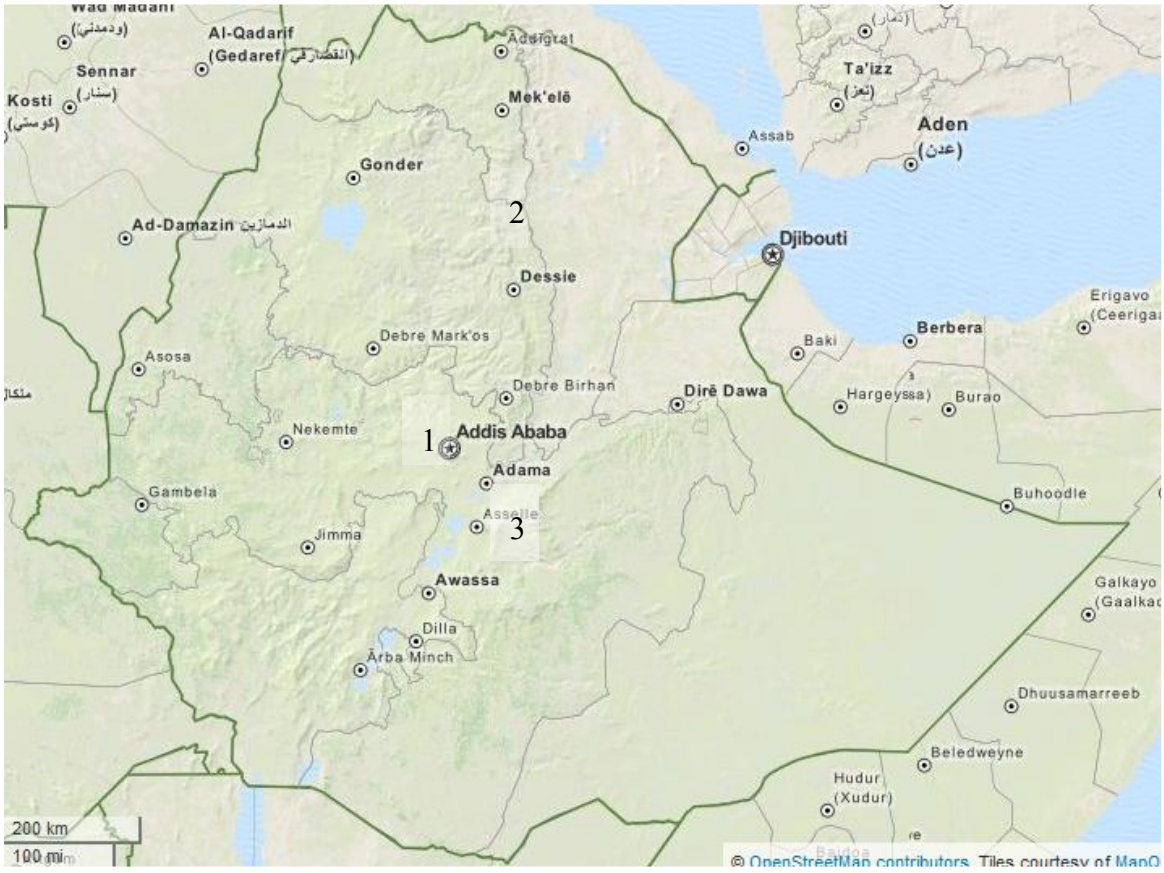

Figure 1: Map of Ethiopia: Sebeta (1), Lake Hashenge (2), Koka Reservoir (3).

Figure 2: Indoor hatchery

6.2 Preparation of experimental units (obtaining catfish larvae)

To acquire larvae of African catfish, we found it necessary to carryout artificial reproduction of catfish at NFALRC hatchery and apply hypophysation technique (Olurin and Oluwo, 2010). Brood stocks were selected from centrally located parent stock holding ponds. Males and females were conditioned in separate concrete ponds. The brood stocks were fed with 29%CP formulated feed, acclimatized for three weeks, then transferred into the hatchery holding tanks. During the experiment, the temperature of water in the tanks was maintained between 24 to 27 ^{o}C to stimulate ovulation. One female catfish (1.3 kg weighted at the experiment) was treated with carp pituitary collected from the wild (Koka reservoir).

The ovulation control started within 12 hours after pituitary stimulator injection. Fish were checked for ovulation by gently pressing the abdomen (Freund et al., 1995, Legendre et al., 1996). In order to avoid further damages and casualties, we prefer continuous follow up to control early egg ovulation instead of stitching the genital papilla. The eggs obtained from striped female were fertilized in a bowl with milt obtained from one sacrificed male catfish. The

fertilized eggs were placed in incubating basins where continuous flow of water was allowed until hatching is finished in 26 hours (After 22 hours, fertilized embryos start to hatch) in warm water of 25-27 ^{0}C.

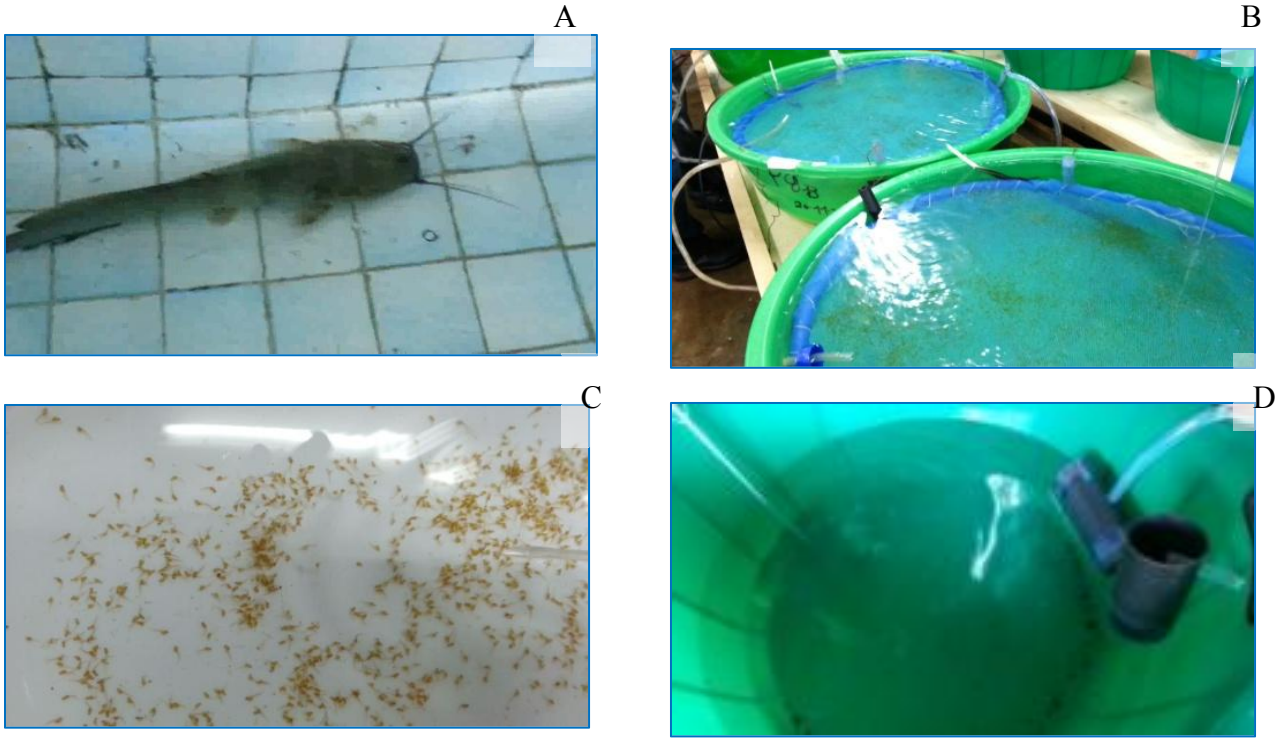

Figure 3: Conditioning broods (A), Incubation (B), Collection of hatched larvae (C) and Place the larvae into the experimental containers (D).

Equipments used for incubation and fertilization:

- Mosquito net (well cleaned)
- Plastic jar
- Feathers (for mixing eggs and sperm)
- Bowl
- Plastic tubes
- Salt solution (9%)
- Scissor and forceps
- Scup net(for fish handling)
- Syringe (3cc)

- Pituitary gland (common carp)
- Measuring balance
- Thermometer and probes
- Thermostat

6.3 Formulation and Preparation of feeds

6.3.1 Live feed

Daphnia were collected from Lakes Hashenge, and brought to the NFALRC and Koka reservoir were a source for Ostracoda. They were cultured in several aquaria and tank which can hold up to 3000L of water. They were fed on algae which were dominated by *Scenedesmus* spp. followed by *Peridinium* spp., *Chlamydomonas* spp., *Tetraedron* spp. and *Trachelomonas* spp. The cultured species were harvested by siphoning and using 100µm sieves. Only small sized *Daphnia* are given to the larvae while the large ones are returned back to the tanks.

Culturing zooplanktons were done in outdoor system. Due to this reason the temperature varies between 16^{o}C to 31.5^{o}C from night to day in aquariums and 16-24^{o}C in the big tanks. There was monoculture as well polyculture of both Daphnia (*Daphnia magna longispina*) and Ostracoda (*Heterocypris incongruens).*

Preparation and harvesting process

- Collecting the live feeds from lake Hashenge and reservoir Koka
- Identification of species for monoculture as well polyculture .
- Placing them in a plastic containers and aquariums.
- Collection and sieving of phytoplankton by using 40µm sieves.
- Add the sieved plankton to the tanks in every 2 -3 days, depend on the condition.
- Follow up the population number (production) until they are enough to harvest.
- Harvest with nets and siphon materials.
- Grade the Daphnia and Ostracoda based on sizes. The smaller sizes that will fit the size of fish larvae remain.
- Return the bigger sized Daphnia and Ostracoda back to the tank for more production.

Zooplankton production and collecting procedure

Figure 4: production system of zooplanktons, Collecting (A, B), Culturing (C, D), Green algae (E), Harvesting and filtering, (F) Ostracoda (G) and Daphnia (H), Returning the remainder to culturing tanks and aquarium (I) and / or place *Daphnia magna* in ice box for later use (J).

Artemia was used as a first exogenous feed. Cone shaped incubators were used to hatch the cyst, to which aquarium aerators and 60W light bulb were installed (3 gm Artemia cyst per liter of 35ppt water with a temperature of 28-30 ^{O}C was used). The hatching process took 24 hours and the hatched nauplii are harvested, separated from the egg shells and unhatched cysts, rinsed with water and given to the larvae in the required quantity (Figure. 5). The larvae fed Artemia for the first 12 days in different concentration. Artemia cyst was donated from Belgium; the steps used for production are listed below

- Installing jar holders and adjusting the system for the production. Local materials were used, i.e. fiber glass, aerator, light bulb, and heater.
- Measuring the cyst, warm water (28-30^{o}c) and salt with the ratio of 1gm: 1L: 30gm. Place all in jar.
- Add bicarbonate to increase pH (8-8.5).
- Install aerator from the bottom of the jar to aerate, mix and to avoid settling.
- Wait 24 hours for harvesting.
- Collect the hatched nauplii and remove or re incubate un-hatched cysts.
- Put fresh water to the nauplii and feed the larvae.

Incubation Hatched To the larvae

Figure 5: Way of Artemia production and feeding to larvae

6.3.2 Dry feed preparation and formulation

Locally available ingredients were used to formulate dry feeds. The ingredients were wheat bran, brewery waste, fish carcass and soybean. Fish carcass and soybean were the highest inclusions. During formulation, pre-mix and methionine was also added. Supplementation of methionine was introduced (0.025%) due to the shortage of plant source origin feeds in this Amino acid (Peres, Lim and Klesius, 2003).

Fish carcass was chosen for protein potency, which will help to compare local formulated feeds with foreign commercial feed (COPPENS catfish feed 45%CP), as well as live feeds. Soybean has been tested to replace animal source proteins (Kikuchi, 1999; Robinson et al., 2006). It was heated to 30 ^{o}C to avoid potential anti- nutritional factors (Peres et al., 2003).

This study also has an aim to replace 40% the fish carcass feed with soybean. Commercial premix (Deutsche Vilomix GmbH) that contains minerals and vitamins summarizes the composition of this premix (Benkendorff, 2004). According to the suggested amounts of vitamins and minerals from Chapman (1992) and Soltan and Laithy (2008), a diet with additives was calculated to contain 2% of the premix. The additives are important in improving and maintaining nutritional value, palatability, wholesomeness and flavor. They also impart desired color.

Each experimental diet was offered daily to the fish at a rate of 10% of their body weight. Frequency of feeding was three times per day for a month in powder form with crumble size of (0.2- 0.8 mm) for different stage of larvae (Table-5). The amount of the feed was adjusted based on the body weight of the fish on sampling day.

Steps of preparing the feed stuff before feeding the larvae

- Literature review.
- Search for the diets in nearby
- Dry brewery waste and fish left over and remove unwanted stuffs
- grind and heat soybean to 30 ^{o}C
- Sieve with 0.25-0.3 mm sized mesh
- Calculate for formulation
- Mix ingredients
- Store in a dry place

Proximate analysis for each ingredient was done in nutritional laboratory.

Table 2: nutrition analysis

Ingredient	**Moisture (%)**	**Crude protein (%)**
Soya bean	4.08	38.34
Brewery waste	6.22	31.67
Fish left over's	5.34	57.4
Wheat bran	8.06	14.37

Table 3: Percent of experimental feed ingredients in the larval diet

Feed ingredients	Soya bean meal based		Fish meal mix based	
	KG inclusion	Total CP%	KG inclusion	Total CP%
Soya bean	45	17.25	0	0
Brewery waste	8	2.59	12	3.8
Fish Caracas	40	22.96	68	39.03
Wheat bran	5	0.72	18	2.59
Premix	2	0	2	0
Methionine	0.025	0	0.025	0
Total	100.025	43.47	100.025	45.42

Figure 6 feed formulation

6.4 Commercial starter feed

The COPPENS® Catco CRUMBLE EXCELLENT is used as the experimental feed. It is specially formulated to meet the nutritional requirements of catfish fry after the yolk sack stage. This feed contains high quality ingredients and an immune stimulant (β-glucans) for natural disease resistance. Its protein content is 45% and the size starts from 0.2-0.3mm. The protocol recommended by the company was followed.

6.5 Experimental set up

The experiment was conducted in five treatments each with five replicates each (Table 4). Circular basin with a volume of 35 L was used to run the experiment. Treatments were assigned randomly and 150 larvae were added into each replicate.

Table 4: Experiment set up. CP (crude protein)

Treatments						**Total**
Feed type	Fish meal mix 45.42% CP	Daphnia magna 45% CP	Soybean meal mix 43.47% CP	Ostracoda (Heterocypris incongruens) 30% CP	Commercial feed 45% CP	
Replica	5	5	5	5	5	25
No of larvae's /container	150*5 =750	150*5 =750	150*5 =750	150*5 =750	150*5 =750	=3750

Figure 7: End of the experiment, experimental plastic containers set up and trainers

Feeding procedure and follow up

6.5.1 Feeding larvae

Depend on the size of larvae, live foods percentage (Artemia) and dry feeds were calculated for a period of five months based on the COPPEN's recommendations. Live food (Artemia) was the sole food source for the larvae during their first week. After the week, the Artemia declined progressively in quantity and would be replaced by dry feeds. Fish larvae were fed 10 % of their body weight per day. Due to the harvest of live feeds from the polyculture tanker, 2-3% of Daphnia and Ostracoda mix would be given to the larvae once a week. The amount of Live feeds were estimated, based on the amount of Artemia is needed for individual larvae per day. According to Graham (1998), larvae with weight of 4.32mg need to be fed by 50-70 individuals Artemia nauplii per day. The amount will increase in relation to the increase of larvae weight and age. Based on this finding, the amount of Daphnia and Ostracoda was estimated. Weight differences between them were taken in to consideration.

Table 5: Feeding procedure and the size of feeds adopted from Coppens fish Food Company

Day	Feeding procedure mm of dry feed /crumble size or live feed (volume)	Amount of dry feed
1-5	Artemia	10 % of their body weight.
6	90%Artemia + 10% 0.2-0.3	
7	75% Artemia + 25% 0.2-0.4	
8	50% Artemia + 50% 0.2-0.5	
9	25%Artemia + 75% 0.2-0.6	
10	10% Artemia + 90% 0.2-0.7	
11	5% Artemia + 95% 0.2-0.8	
12	75%0.2-0.3 + 25%0.3-0.5	
13	50%0.2-0.3 + 50%0.3-0.5	
14	25%0.2-0.3 + 75%0.3-.5	
15--23	0.3-0.5	
24-37	0.5-0.8	
37-60	0.8-1.2	
60~	1.2-1.5/1.5	

6.5.2 Monitoring water quality parameters

Continuous flow of water was allowed for 12 hours per day. Feed left over and waste excreta were removed twice a day by siphoning with minimal disturbance. Water quality parameters (Temperature, pH, DO) were measured in every three days and ammonia levels were measured once every two weeks.

Figure 8: water quality monitoring

One of the critical issues in feeding experiment is water quality monitoring since the feeds not utilized will change the physico-chemical features of the water. Digital electronic probe (Multi meter) was used to measure different water quality parameters such as water temperature and dissolved oxygen (DO). Besides the physico-chemical parameters, nitrogen compounds, ammonia levels were determined in the laboratory with the aid of a Spectrophotometer. Due to the toxic effect when produced in the culture system (Bhatnagar and Devi 2013).

The sampling time for ammonia occurred in the mornings for both periods. We expect the highest ammonia concentration to be at early morning since there was no water flow for the whole night except of two water changes. In the study around 80% of tank water was replaced by fresh water (27^oC) two times during the night shift to avoid temperature to possibly drop below 24^oC with the support of aerator.

NH_3-N was determined by using the Indo-Phenol blue method (Khosravi et al., 2012). A stock solution of 1g L^{-1} NH_3-N was prepared by dissolving 3.819 g NH_4Cl in 1000 ml of distilled water. Intermediate solution of 10 mg L^{-1} was prepared by diluting 10 ml of stock solution to 1000 ml of distilled water. Working solution of 250 µg $L^{-1}$was prepared by diluting 25 ml of intermediate solution into 1000 ml of distilled water. A sample with volume of 25 ml was used to determine the amount of NH_3-N in the sample and then extrapolated into each treatment.

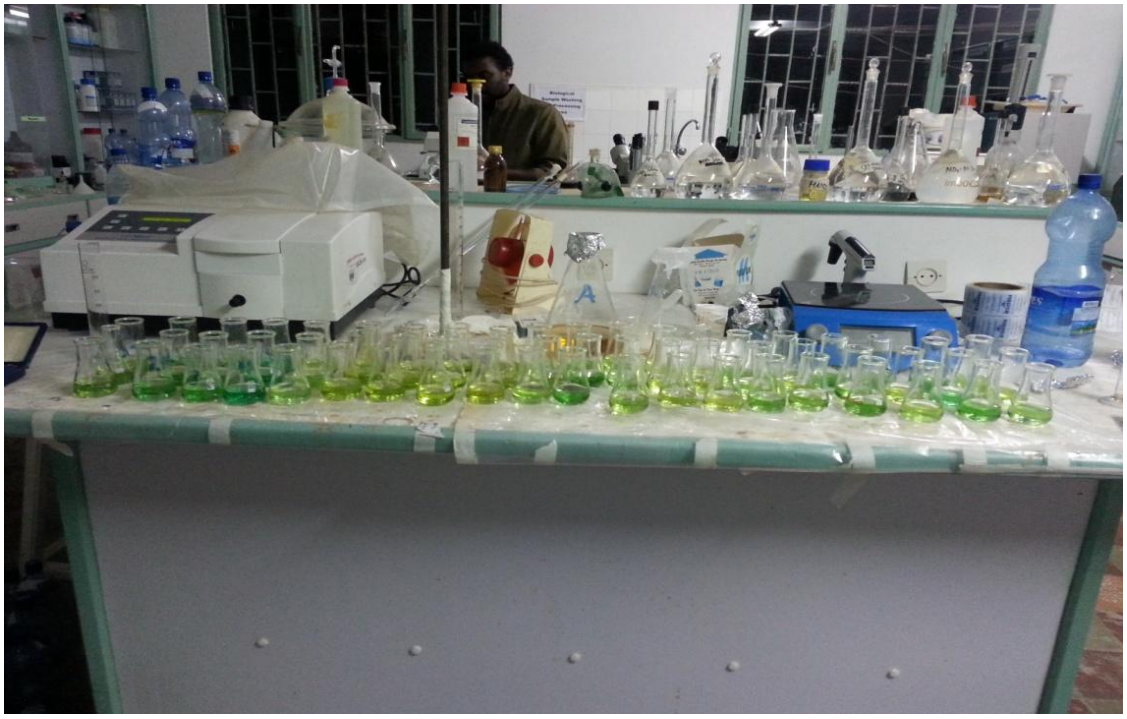

Figure 9: Ammonia analysis

Reagents

A) Sodium salicylate solution: 130g Sodium-Salicylate and 130g of Trisodiumcitrat-Dihydrat was dissolved in 800 ml of distilled water, then 0.97 g of sodium nitropruside was added and the volume made to 1L.

B) Hypochlorid solution: 32g of NaOH was dissolved in 1000ml of distilled water just before use. Then 0.2g Sodium dichloroisocyanurat was dissolved in 100ml of the base (reagent B). 2.5ml of reagent A were added to 25ml of filtered sample and standard series. After shaking, 2.5ml of reagent B were immediately added. Eventually the samples were stored in the dark for 1.5h at 25ºC for colour development, and then the absorbance was measured at 655nm.

6.5.3 Measurement of Growth and survival rate of larvae

Each of the 25 experimental basins contains 150 larvae. They were subjected to live feeds (Artemia) at the 3rd day after hatching. The larvae were fed 10% their body weight/day. Weight measurements were conducted four times in the experimental period. The first larvae weight measurement was taken before they were fed with Artemia, the second was taken before the introduction of artificial feed. The third measurement was taken during the midst of the

experiment, and the fourth measurement took place at the end of the experiment. Approximately 20 % of the larvae were taken under the first three measurements and 35% on the last one. During each sampling, fish measurements were fairly uniformed within treatments. They were starved for an average six hours prior to measurement in order to avoid misleading values.

Wet weight of larvae was measured to the accuracy 0.0001g sensitive balance and graded ruler. They were placed on nylon net and the adhering water was removed with a paper towel applied from below for a few seconds.

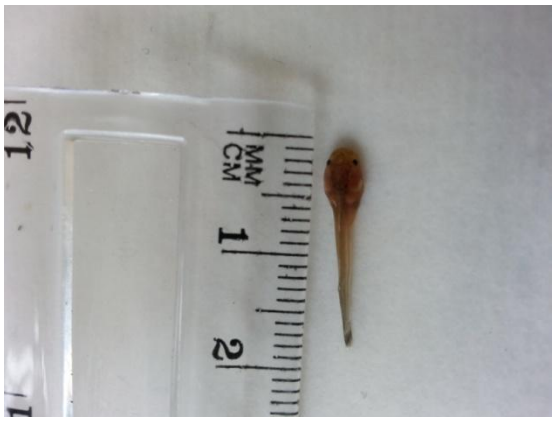

Figure 10: Length measurement using graded ruler (mm/cm)

Weaning with different feed types was carried out according to the COPPENS® protocol. Dead larvae were removed daily and then replaced from reserve stocks. Data was recorded daily. At the end of the experiment, the numbers of surviving fish was first recorded, and then used for survival rates and cannibalism calculation.

Growth rates were calculated according to (Florence and Harrison, 2012)

$$[1]\text{Weight} - \text{Growth rate per day} = \frac{(\ln \text{final weight}) - (\ln \text{initial weight}) * 100}{\text{Number of day's} * \text{initial weight}}$$

$$[2]\text{Lentgh} - \text{Growth rate per day} = \frac{(\ln \text{final length}) - (\ln \text{initial length}) * 100}{\text{Number of days} * \text{initial length}}$$

$$[3]\text{Specific weight} - \text{growth rate (SwGR} = \frac{(\ln \text{final weight}) - (\ln \text{initial weight}) * 100}{\text{Number of days}}$$

$$[4]\text{Specific length} - \text{growth rate (SlGR)} = \frac{(\ln \text{final length}) - (\ln \text{initial length}) * 100}{\text{Number of days}}$$

$$[5]\ \text{Survival rate} = \frac{100\text{X no. of survivals}}{\text{No. of initial fish}}$$

Condition factor (k) was calculated according to Arimoro, (2007) and Davies et al. (2013)

$$[6]\ \text{K} = \frac{\text{W} * 100}{\text{L\^3}}$$

According to Khan and Abidi (2007) Protein efficiency ratio was calculated as:

$$[7]\ \text{Protein efficiency ratio} = \frac{\text{Weight gain}}{\text{protein fed}}$$

6.6 Statistical analysis

Data management was done in Excel. One way ANOVA was used to test for significance differences and POST HOC (Tukey) analysis was done to verify homogeneity between treatments. Pearson's two tailed correlation was used to see the effects (relationship) of ammonia concentration on the survival rate of fish larvae. Differences were considered significant at $P<0.05$. All data were analyzed using the SPSS-18 and Excel 2007.

7 Result

The result explanation starts by showing how was the water quality in the experiment period; then how was the growth response of larvae's we try to focus on the difference and similarities between treatments as well replicas and finally it mainly focus on survival rate and it relation with water quality (ammonia).

7.1 Water quality

Water quality characteristics in the treatments commercial feed, Daphnia, soybean meal mix; fish meal mix and Ostracoda during the 33-days experiment are summarized in (Table 6).

Table 6: Mean value of water quality throughout the experiment period

Parameters	**Mean ± SD**
Dissolved Oxygen (mg/l)	5.8 ± 0.35
Dissolved Oxygen concentration (%)	94.3 ± 4.5
Temperature (^{o}C)	26.3 ± 1.1
pH	7.7 ± 0.1
Conductivity (µs/cm)	276.3 ± 4.9

Water quality was measured twice a week .Water flow and temperature was regulated by the same approach for each treatment. Oxygen was in the range of (5.29-7mg/L) with a concentration (85.2 -103.2 %) and temperature ranges between (23.9-29.3 ^{o}c). whereas a range of 7.52-7.93 pH and 270-290 µs/cm conductivity was obtained.

Ammonia

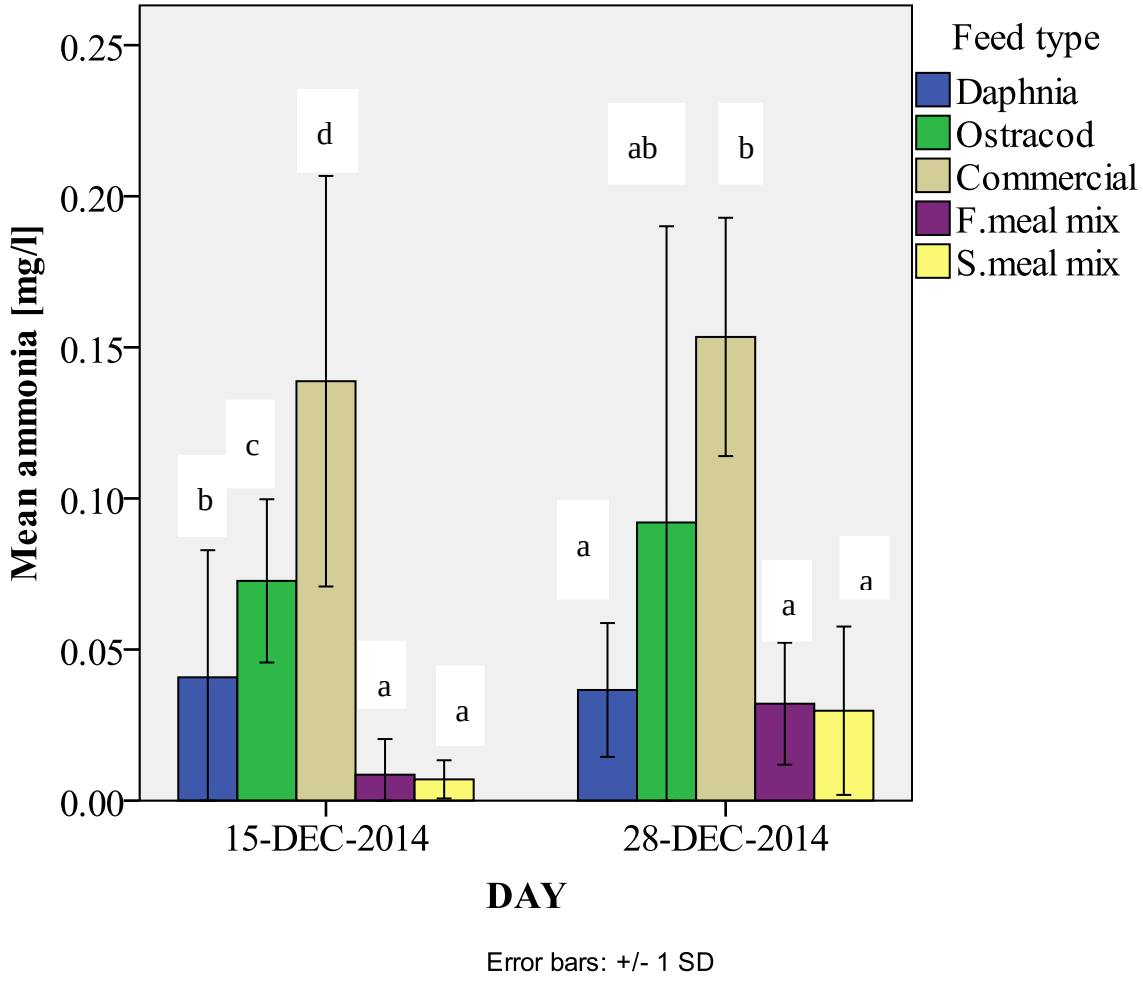

Figure 11: Ammonia concentration on mid and end of the experiment period

Ammonia concentration at the mid of the experiment (15-dec) was significantly different among treatments ($P < 0.05$). Homogeneity between treatment was observed on larvae fed on fish meal mix (8.5 ±11.8 µg/l) and soybean meal mix (7±6.3 µg/l), a, ($P > 0.05$(p=0.99). Highest concentration was at commercial feed (138.7 ± 67.9µg/l) followed by Ostracoda (70.7 ± 29.2 µg/l).

Ammonia concentration at the end of the experiment (28-dec) was significantly different among treatments ($P < 0.05$). Homogeneity between treatment was observed on larvae fed on Daphnia (34.6 ± 24.8 µg/l), fish meal mix (32.1 ± 20.2 µg/l) and soybean meal mix (29.7 ± 27.8 µg/l), (a),

(P > 0.05(p=0.99). Highest concentration was at commercial feed (153.4 ± 39.4 µg /l) followed by Ostracoda (70.7 ± 29.2 µg/l) and Daphnia (92 ±43.8 µg/l)

In both measuring period the sample was taken at morning those concentration values are the maxim expectation due to water flow at day time.

7.2 Response of *Clarias gariepinus* for different feed treatments

Weight and length growths rate of *Clarias gariepinus* for each treatment are shown in Table 10. The final weight, growth rate, and specific growth rate were significantly affected by diet, with the maximum values obtained in larvae fed on *Daphnia* (298 mg and 36 mm) While the lowest with recorded on fish and soy meal mix (14.4mg and 13 mm) (Table-10).

Table 7: Growth parameters Specific Growth Rate (SGR), Daily Growth Rate (DGR) on different dietary treatments

Feed type	**Parameters**				
	Weight specific growth rate mg/day (SGR)	Weight gain mg/month	Length specific growth rate.mm/day (SGR)	Change in Length mm/ month	Weight gain/protein fed
Daphnia	2.49	79.75	0.46	14.77	1.8
Ostracoda	1.75	53.75	0.38	12.07	1.6
Commercial	1.81	57.75	0.40	12.87	1.3
Fish meal mix	0.70	23.75	0.27	8.67	0.57
Soybean meal mix	0.68	21.75	0.26	8.27	0.55

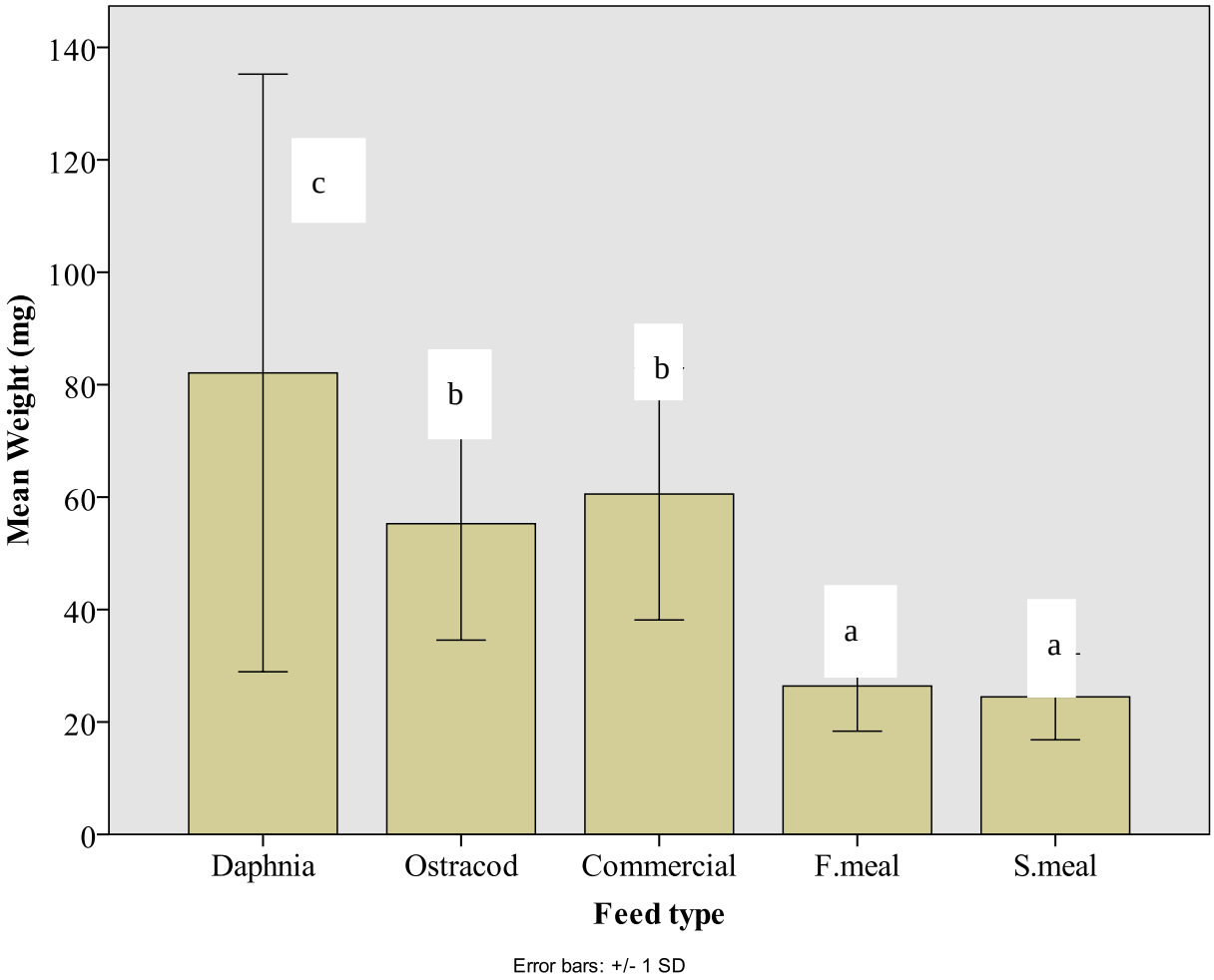

Figure 12: Final weight of larvae (mean ± SD) a, b and c shows homogeneity and heterogeneity of treatments

There was significant difference of growth performance among treatments (Figure. 12) and (Table 11). Larvae fed on *Daphnia* had the highest weight (82.1 ± 53.15) and it was significantly different from the other treatments ($P < 0.05$, $p=0.00$). Larvae fed on Commercial feed (60.55 ± 22.4) and *Ostracod* (55.3±20.71) showed homogeneity (Figure. 12. (a, b, c)) but were significantly different from other treatments. Larvae fed on fish meal (26.4 ± 8.04) and soybean meal (24 ± 7.64) showed homogeneity (Table 11, 12) but was significantly different from others and recorded the least growth performance (Figure. 12, Table 11).

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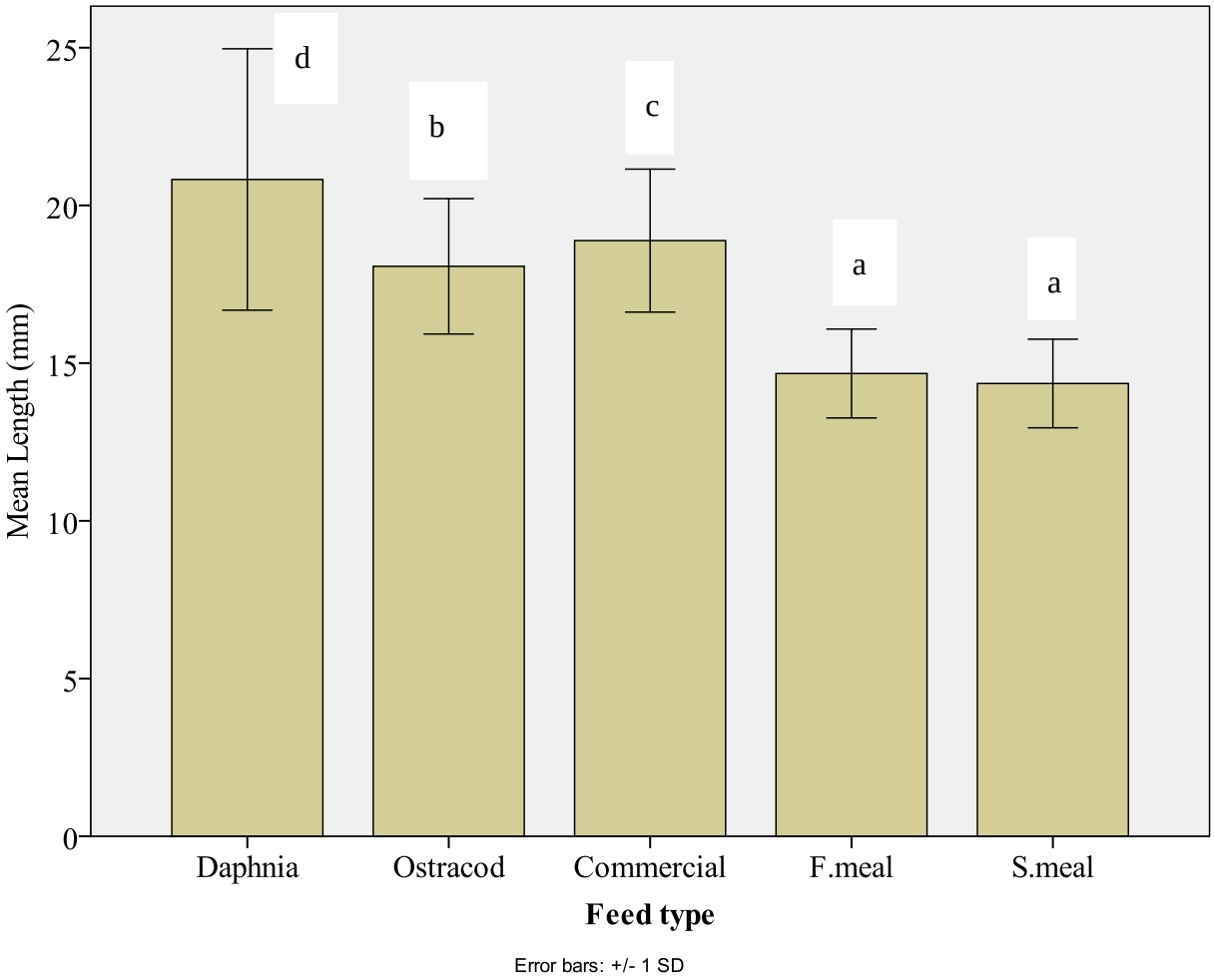

Figure 13: Final mean Length (mean ± SD) b, c and d shows homogeneity and heterogeneity of treatments

Mean length was significantly different among treatments (Figure. 13) and (Table 10). Larvae fed with *Daphnia* had the highest length (20.8 ± 4.14) and it was significantly different from the other treatments ($p < 0.05$, $p=0.00$). Larvae fed with commercial feed (18.9 ± 2.5) and *Ostracod* (18.1 ± 2.14) also showed differences (Figure 13. A, b, c, d). Larvae fed with fish meal (14.7 ± 1.41) and soybean meal (14.3 ± 1.4) showed homogeneity but significantly different from others and poor growth performance was record on both treatments (Figure. 13, Table-12).

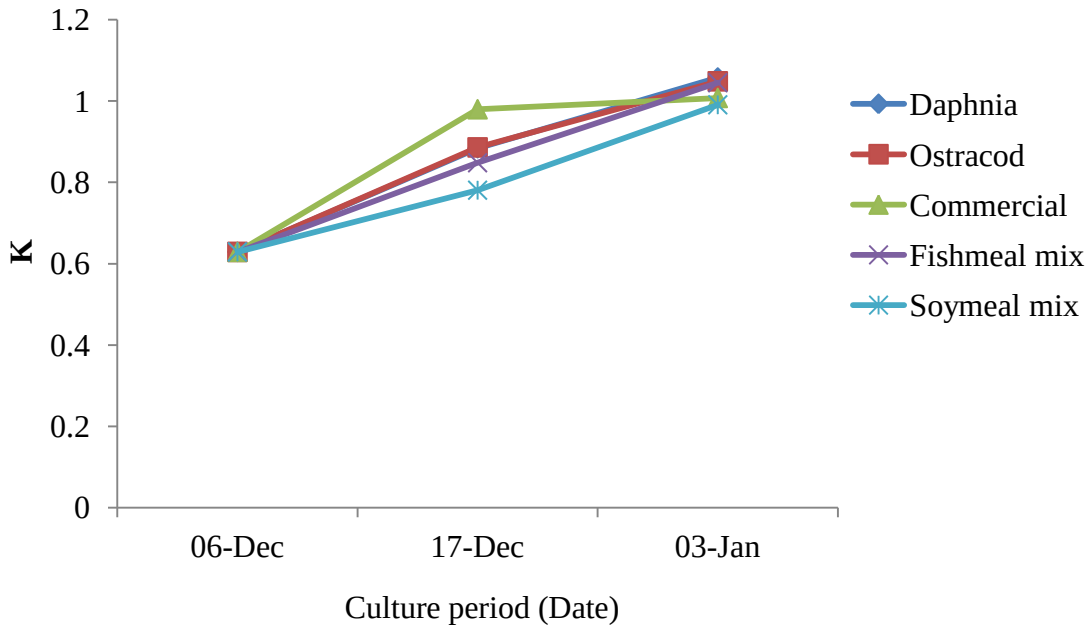

Figure 14: Fluctuation of mean condition factor (k) of Clarias gariepinus larvae fed different types of feed through a month culture period.

The K value for all treatments shows an increasing starting from introduction of artificial feed. in the first two weeks larvae fed on Commercial feed shows a higher length weight relation value changes from 0.63 to 0.98 and the change in the next weeks were by 0.02.Where as the highest K value was on *Daphnia* and *Ostracoda* treatments in the thirty two days of experiment both shows a same trend 0.63 to 0.88 first two weeks where as 1.06 and 1.05 on last fifteen days respectively. And also fish meal mix treatment shows relatively similar trend of K value 0.63-0.85 and to 1.04. Whereas the lowest K value was record on soybean meal mix that first weeks 0.63- 0.78 and final of 0.99 on the three records times.

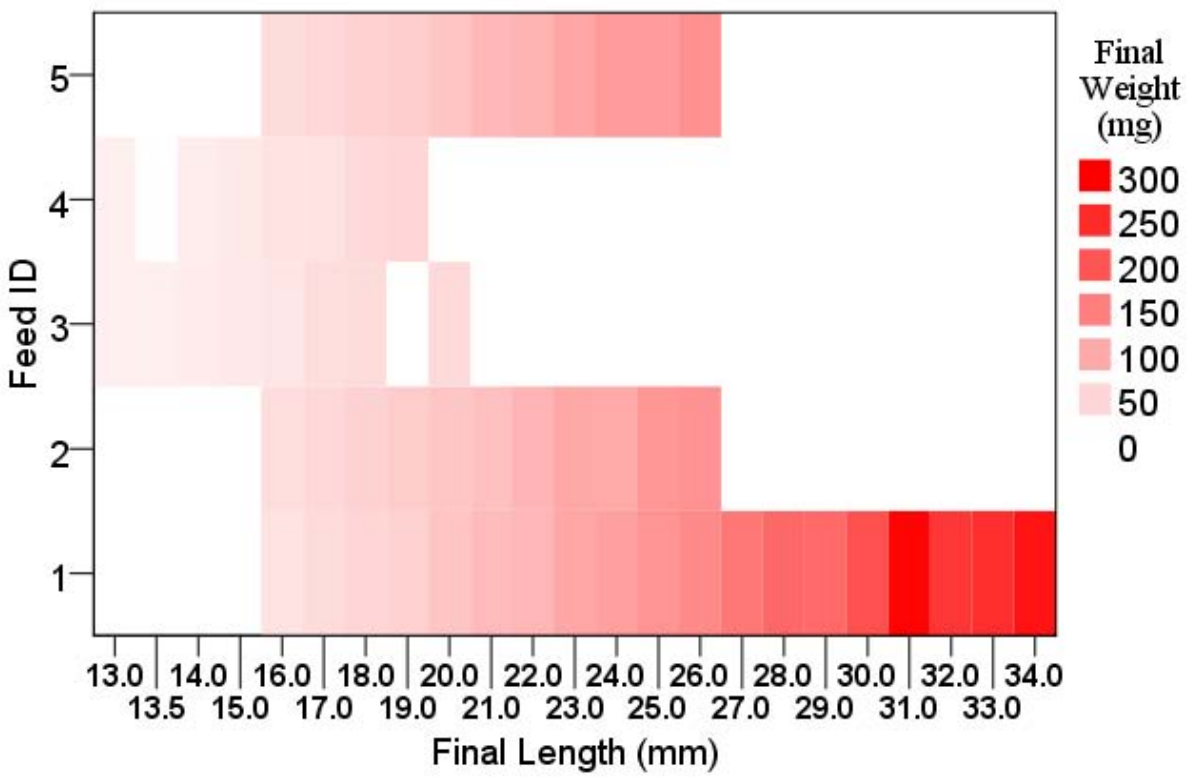

Figure 15: Final Length weight distribution of larvae after a 32 days feeding on Daphnia (1), Commercial feed (2), Fish meal mix (3), Soybean meal mix (4) and Ostracoda (5). The color difference range between light red to dark red shows weight range in a treatment.

Almost all treatments have gain different weight and length and there was a high difference between the minimum and the maximum value. Larvae fed on Daphnia shows the highest record on both weight and length and highest variation between the least and the maximum (19.6-295.5 mg and 16-34 mm) was recorded. Larvae fed on Ostracoda (26.5-130.5 mg and 16-26 mm) and commercial feed (19.5-129.9 mg and 16-26 mm) were the second highest range recorded. The last groups were fish meal mix (14.9-55mg and 13-20mm) and soybean meal mix (14.4-53.3mg and 13-19mm).

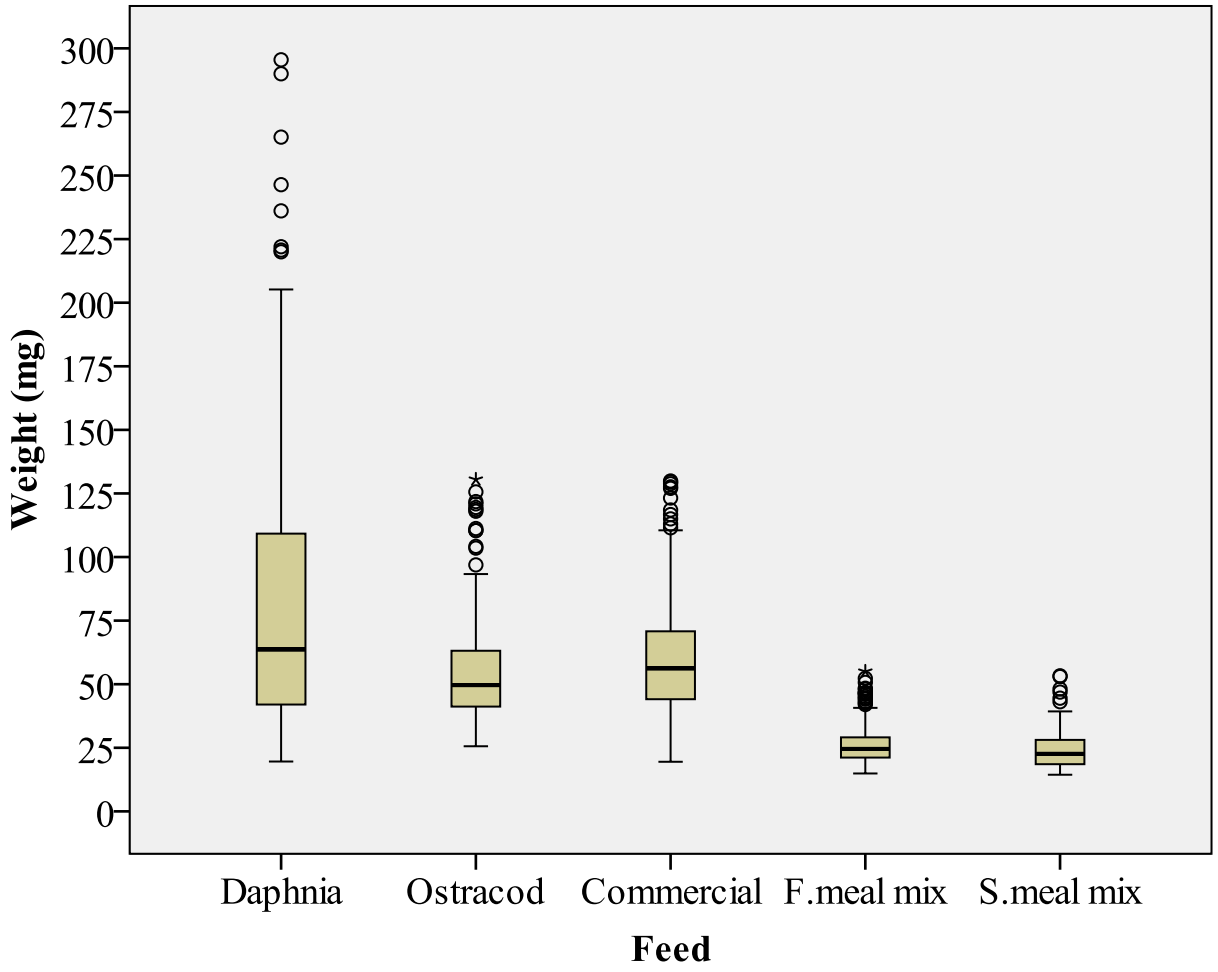

Figure 16 Weight gain distribution of larvae for different feed types. O and * shows outliers

The final weight of *C. gariepinus* in the different treatments was in between different ranges (Figure 15, 16 and 18), and there was significant difference among three treatments pools (Daphnia, Ostracoda/Commercial, F.meal/S.meal) ($P < 0.05$). The highest variation of weight gain was in larvae fed on *Daphnia* (19.5-295.5 mg) followed by larvae fed on commercial feed (19.5 - 129.9 mg), *Ostracod* (25.6 - 130.5 mg), fish meal (14.9 - 55 mg) and soybean meal (14.4 - 53.3 mg). Outliers were observed in each treatment. Most of the mean values were above the median for larvae fed on *Daphnia* and *Ostracod*. There was outliers on each treatment.

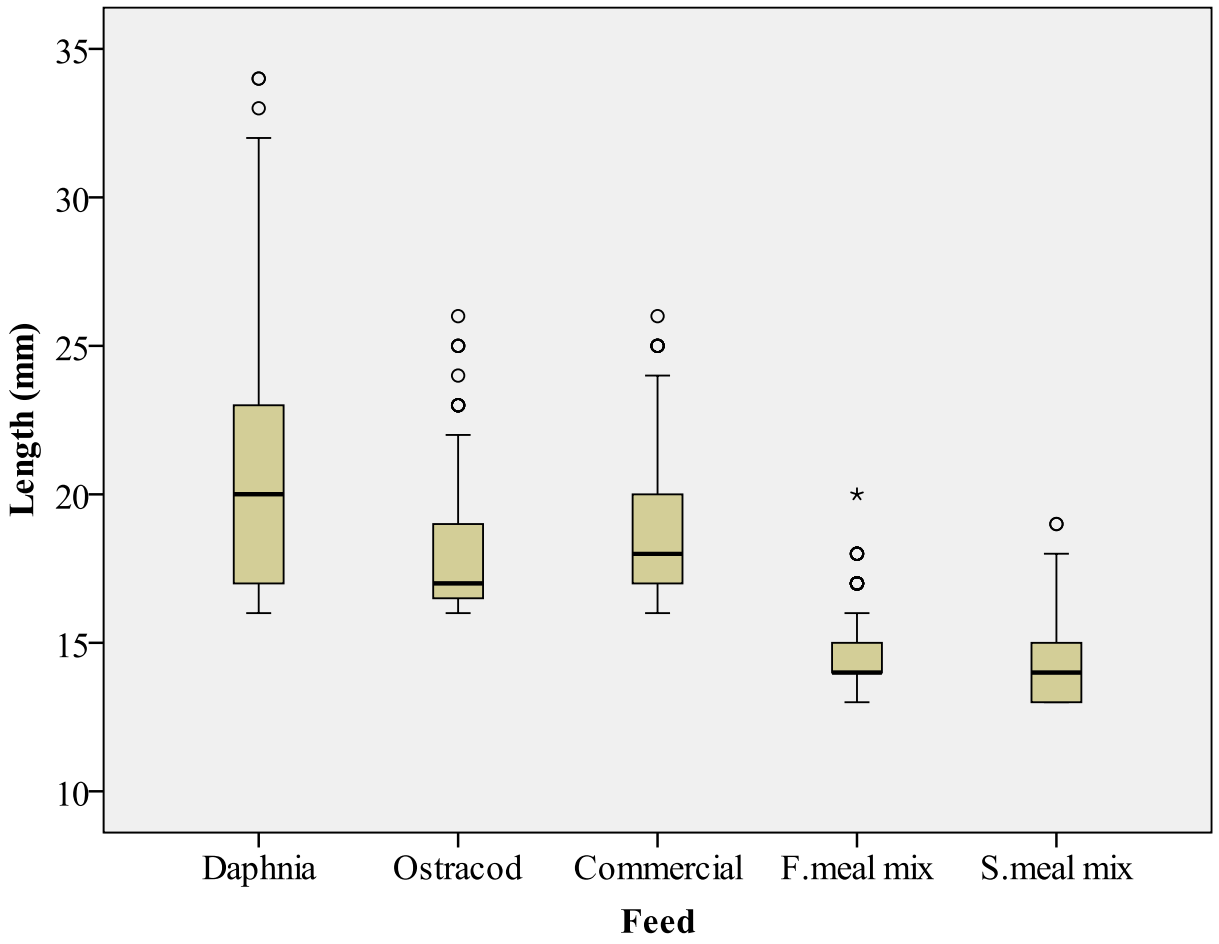

Figure 17: Final length distribution of larvae for different feed types after 33 days of the experiment day. o and * shows out lairs

Length distribution of *C.gariepinus* after thirty three days for different treatments was in between different ranges (figure 15, 17 and 18) and there was significant difference among four treatments pools (Daphnia, Ostracoda, Commercial, F.meal/S.meal ($P < 0.05$). The highest range of length was larvae fed on Daphnia (16 - 34mm) followed by larvae fed on commercial feed (16 - 26 mm), Ostracoda (16 - 26 mm), fish meal mix (13 - 20 mm) and soybean meal mix (13 - 19 mm). Mean length of larvae fed on commercial feed and Ostracoda most of the values were higher than the median. There was out lairs on each treatment.

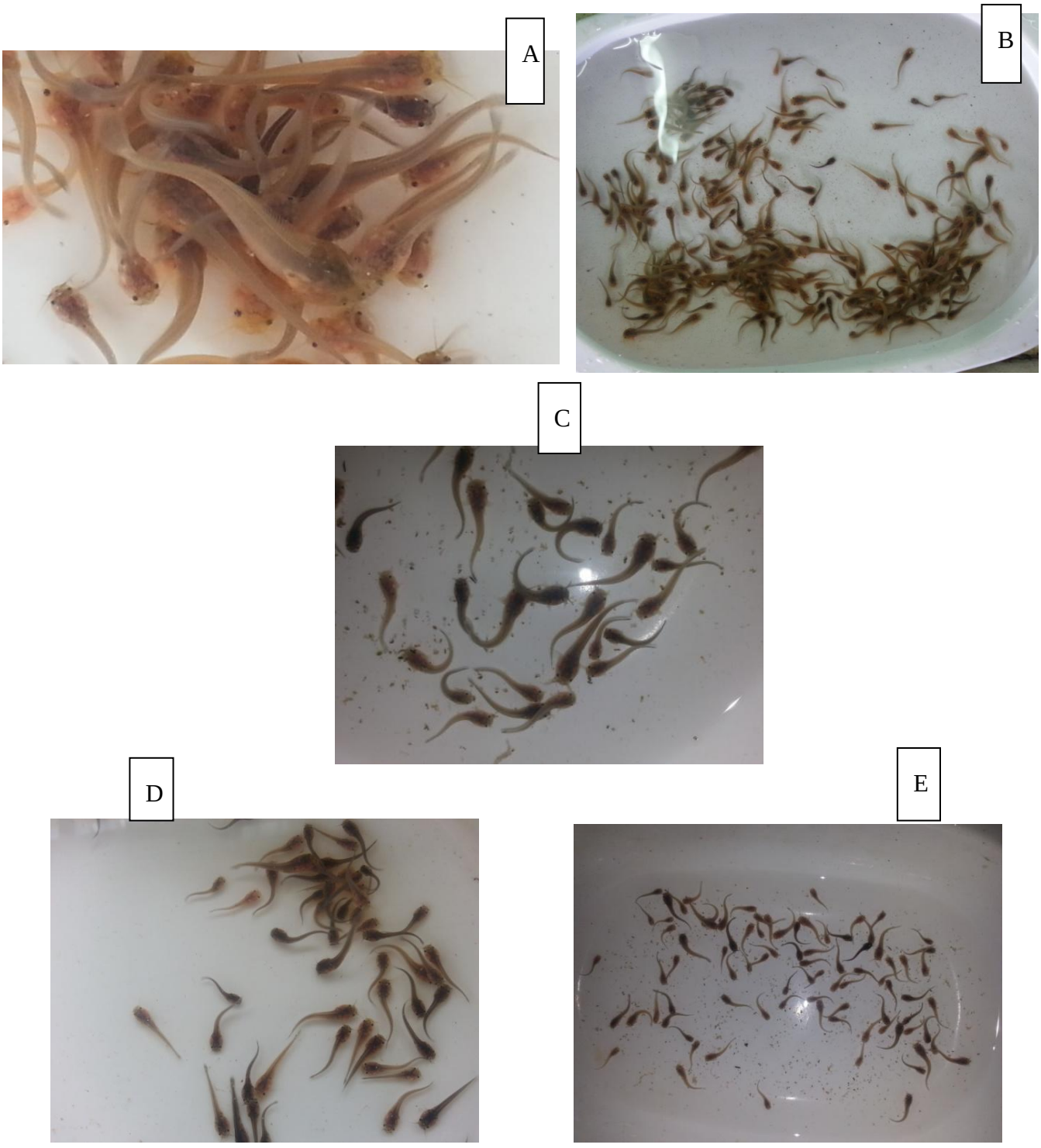

Figure 18 Larvae fed on, commercial feed (A), shows homogeneity size, Daphnia (B),shows heterogeneity size, Ostracoda (C) slight difference, fish meal mix (D), homogeneity, soybean meal mix (E) homogeneity

7.3 Growth trend

7.3.1 Weight gain trend

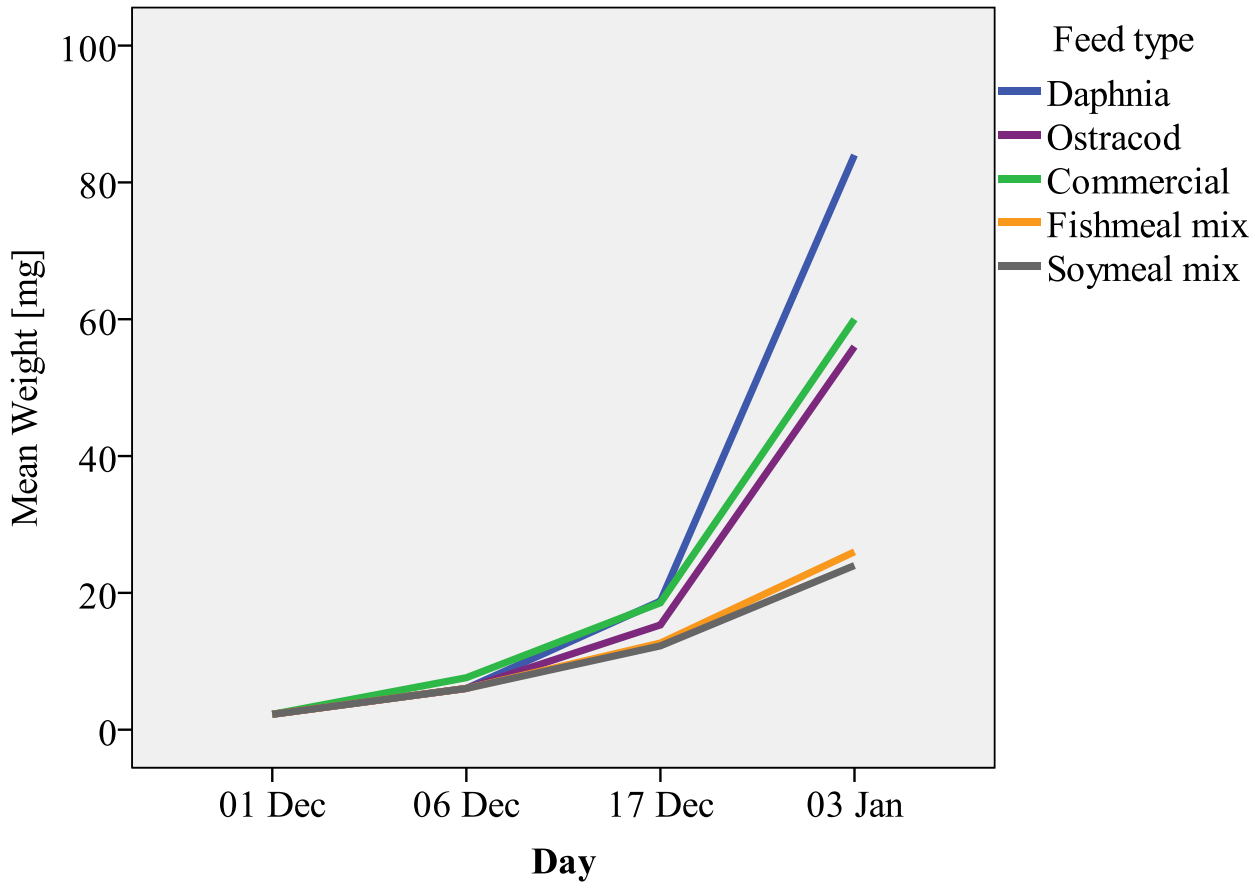

Figure 19: Effect of different feed types on the growth (weight) trend of Clarias gariepinus Larvae.

The growth trend shows varies after the introduction of different feeds on 6th of December. Artemia nauplii used as starter feeds that gave an overall average growth of 6.03 mm and 2.25 mg as TL and TW before other feeding types were introduced. The weight gain after two weeks was not highly differing comparatively to the weight gain after a month. Growth response after two weeks was significant different between treatments. Two homogeneity groups were created the one is that Soybean meal mix (12.7 ± 3.1), Fish meal mix (12.6 ± 2.8) and Ostracoda (15.3± 4.7) (P>0.05,P=0.25) The others were Ostracoda, Daphnia (18.8± 7.3) and Commercial feed (18.5± 5.7), (P>0.05,P=0. 61).

Daphnia treatment shot on the last weeks and shows highest growth and also larvae fed on commercial feed and Ostracoda shows the same trend and puts them as mid rank on weight gain trend. Comparatively too the others treatments fish meal mix and soybean meal mix have shown the lowest growth trend. The growth trend starts to differ at the introduction of new feed types at 6th of December. After two weeks the growth of the larvae on different feeds shows a different growth performance (figure 19).

7.3.2 Length gain trend

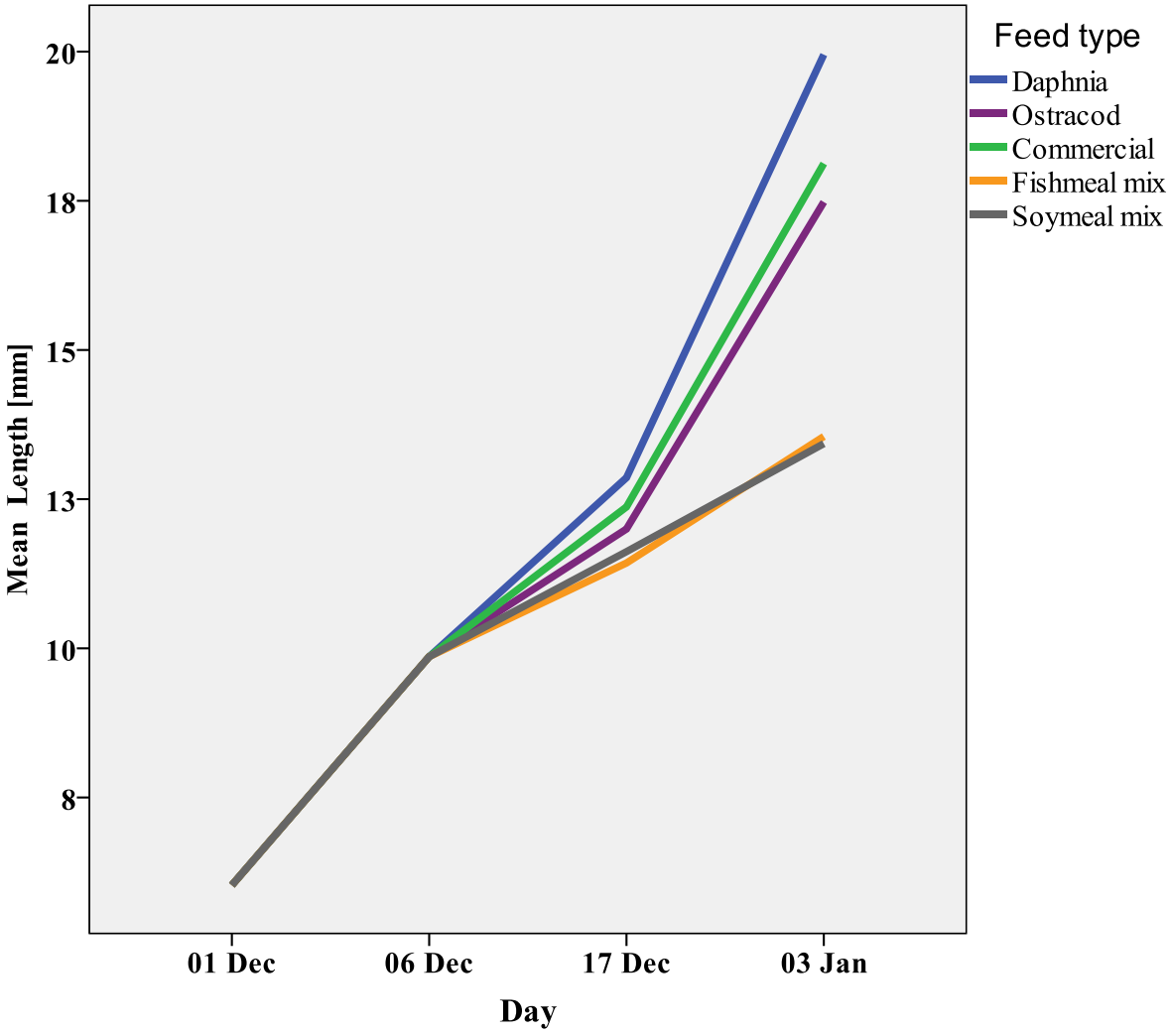

Figure 20 Effect of different feed types on the growth (Length) trend of C.gariepinus larvae

The length gain trend showed significant variations ($p<0.05$) among treatments after the introduction of different feeds after the second week of the experiment. The length gain trend

followed the same pattern as the weight gain trend. Differences in length were observed at the introduction of the experimental feeds. Three homogeneity groups were observed, Soy meal (11.4 ± 0.9), Fish meal (11.6 ± 0.7), *Ostracod* (12 ± 1.3) *Daphnia* (12.86 ± 1.5) and Commercial feed (12.4 ± 1.2), ($P<0.05$). Larvae fed on *Daphnia* showed the highest length gain trend, where as larvae fed on Commercial feed and *Ostracod* showed similar trend and puts them as mid rank (Figure-20). Larvae fed on fish meal and soy meal showed the least growth starting from the second week of feeding strategy.

7.4 Survival rate

Survival was significantly affected by different diet. Commercial feed treatment shows the highest and the lowest was recorded on soybean meal mix. Mortality within the thirty two days of the experiment was variable for different diets and there was significant different and homogeneity among treatments. Commercial feed(c) and Ostracoda (cb) treatments have no significance difference (figure 21 c, cb), (p>0.05, p=0.21) and also larvae fed on Daphnia (b) and Ostracoda (cb) did not show significant different, (P > 0.05, P= 0.594). Homogeneity of survival rate was observe on fish meal mix (a) and soybean meal mix (a), (P > 0.05, P=0.9).

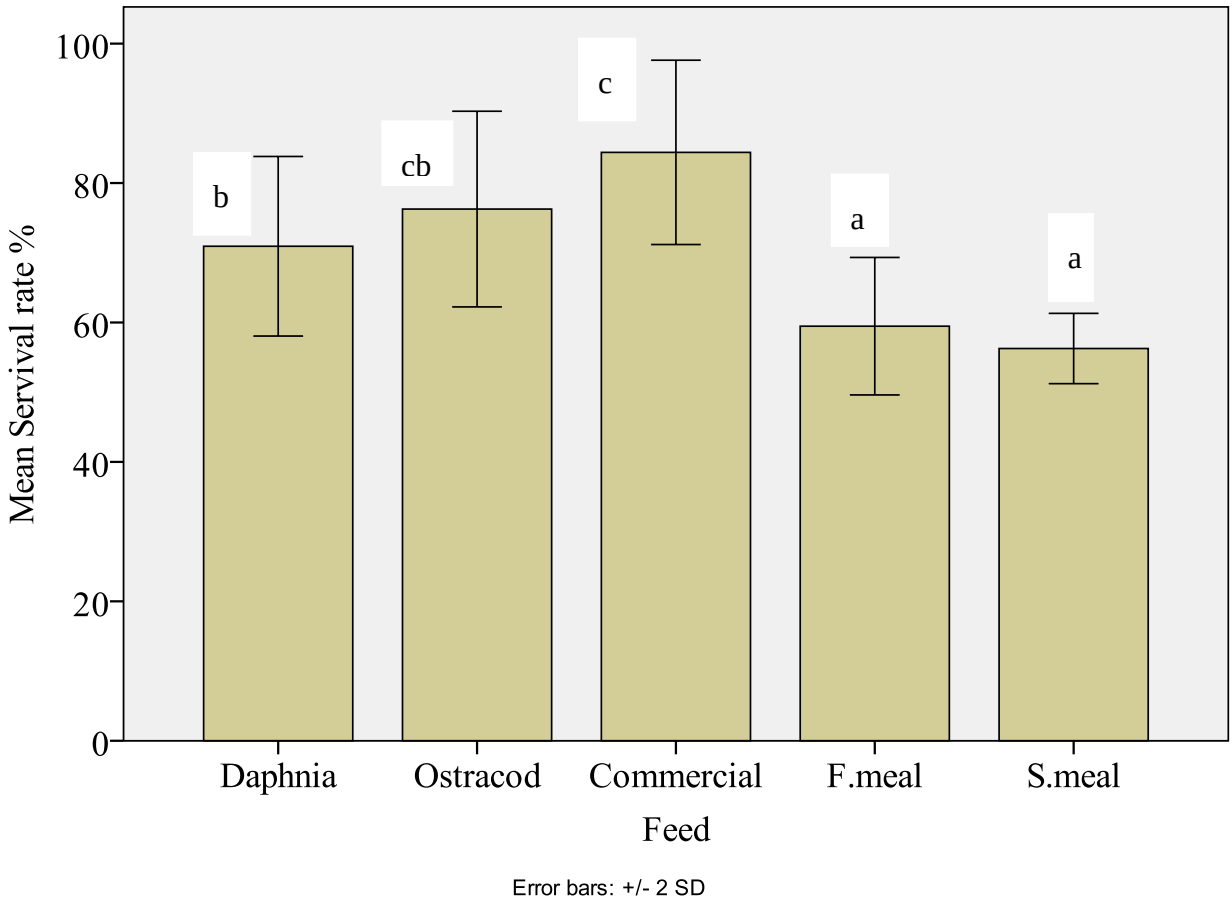

Figure 21: Survival rate (mean %) of larvae's with in the period of the experiment a, b, c and cb shows homogeneity and difference between treatments.

The overall survival of Clarias gariepinus larvae reared was 69.5 ± 11.9% and was significantly different among treatments (P < 0.05) and it was not similar within all tanks. The highest

survival rate occurred on fish fed high Commercial feed (84.4±6.6%), followed by those fed Ostracoda (76.3 ± 7.02%) and Daphnia (70.9±6.4%). The lowest survival was observed in fish fed fish meal mix (59.5 ± 4.9%) and those that were fed soybean meal mix (56.3± 2.5%).

7.5 Correlation ammonia concentration and survival rate

Correlation between feed type and ammonia concentration in relation to survival rate was positive only on fish larvae fed on Ostracoda and commercial feed (r=0.41, 2.37) respectively but on larvae fed on Daphnia, fish meal mix and soybean meal mix were negative correlation(r=-0.67,-0.73,-0.75) respectively

Table 8: Pearson correlation of ammonia concentration and survival rate

Feed			**Survival**
Commercial	ammonia	Pearson Correlation	0.24
		Sig. (2-tailed)	0.70
Daphnia	ammonia	Pearson Correlation	-0.68
		Sig. (2-tailed)	0.21
Fish meal	ammonia	Pearson Correlation	-0.73
		Sig. (2-tailed)	0.16
Ostracoda	ammonia	Pearson Correlation	0.41
		Sig. (2-tailed)	0.49
Soybean meal	ammonia	Pearson Correlation	-0.75
		Sig (2-tailed)	0.14

8 Discussion

The overall study aims to explore and compare the effect of five different feeds which are categorized as live feeds, commercial feed and locally formulated feeds. The main goal was to develop suitable diets for an improved and sustainable *C.gariepinus* larvae production for Ethiopian domestic implementation. We attempted to focus on factors that may improve or inhibit the growth and survival rate of larvae. Specifically on the feed quality that concerns palatability, digestibility, anti-nutrition factors and water quality parameters. The results of this study were also compared to other related studies.

8.1 Water quality in relation with growth of larvae

In the present study, fish feeding leads to changes in water quality parameters like dissolved oxygen, pH and ammonia. The parameters were not affected by different feed type application during the four weeks feeding trial. However ammonia levels vary significantly with different feed type. Those parameters were within the appropriate ranges of the catfish culture and have no apparent influences on catfish growth. Other studies also found and recommend that every growth rate is related to the water quality (Marimuthu et al., 2011, Bhatnagar and Devi, 2013). This study result shows the larvae during first feeding days have good condition, and applying different type of feeds doesn't influence water quality.

Temperature and pH plays a main role in determining the effectiveness of digestive enzymes in the fish larvae as a whole (Murashita et al., 2014). A study by Haylor and Mollah (1995), shows temperature influences fish growth, specifically on the sensitive early stage. According to present study of the physico-chemical parameter range, pH (7.52-7.93, mean 7.7 ± 0.1) and temperature of (23.9-29.3 ^{o}c) were considered suitable according to Haylor and Mollah (1995), Bhatnagar and Devi (2013). A fair amount of studies do not concern conductivity and salinity, usually if the water source is from ground water which have more consistent water quality compared to surface water, and is less likely to be contaminated by pathogens and other pollutants (Bhatnagar and Devi, 2013).

Oxygen varies with altitude (atmospheric pressure) and temperature, which have an inverse relationship (Bhatnagar and Devi, 2013). Several studies accept and recommend (Appelbaum and Kamler, 2000; Akbary et al., 2010; Faruque, 2010) oxygen greater than 5mg/l and a concentration between 80-100% is enough for a healthy growth for *C.gariepinus* larvae.

In this study the results fall in the range of good water quality, with collected data between (5.29-7 mg/l, mean of 5.8 ± 0.35) and concentration (85.2 -103.2 %, mean 94.3 ± 4.5) but lower relatively to other studies (Abdulraheem et al., 2012). Additionally, in the study by (Davies et al., 2013) the data of 9.7 ± 0.43 mg/l of oxygen was obtained at a temperature of 26.75 ±0.63^{O}C. This difference might be the high altitude at the experiment location of Sebeta highland.

Ammonia is toxic to fish, if its concentration exceeds more than 0.3mg/l, it will result in stress, retarded growth and even death (Abdelwahed et al., 2011, Bhatnagar and Devi, 2013,). In our study, the amount of ammonia retained in reasonable quality, however varies significantly between treatments (Figure 11). In terms of both sampling time and highest ammonia concentration, commercial feed (0.15 ± 0.04 mg/l), with bigger data collection, results as last in terms of suitability ranking. Meanwhile, the other four treatments all has than 0.1mg/l in ammonia concentration rate, which makes water quality passable, as proved by prior studies. (Musa et al., 2012, Bhatnagar and Devi, 2013).

8.2 Water quality in relation with survival rate

Except for ammonia, there were no significant differences among treatments on physico chemical parameters such as oxygen, pH, temperature and conductivity. The ranges were in the safe side for all treatments according to other studies and recommendations (Bhatnagar and Devi, 2013). In a *C.gariepinus* larvae study by Olurin and Oluwo (2010), water quality parameters was recorded: temperature 25-28^{o}c, mean 25.4^{o}c, pH 7.0-7.2, mean of 7.0, ammonia 0.0mg/l. Three types of feeds were applied and the results follow: the survival rate with decapsulated Artemia feed is 80.7±3.1%; the survival rate with *Daphnia* specie is 77.2±2.6%; the survival rate with commercial diet is 62.9±1.3%.

During the experiment, at both sample times, the groups that feed on fish meal mix and soybean meal mix diets have the lowest ammonia concentration. Such data do not correlates (Table-8)

with the data of survival rate (r^2=-0.73 and -0.75, P > 0.05) respectively. Nevertheless, the lowest survival rate was recorded on these two feeding options (59% for fish meal mix and 56% for soybean mix) raised by other studies shows that mortality can occur due to factors of over feeding, easy bacterial decomposition and the increase ammonia concentration (Stone and Thomforde, 2004, Bhatnagar and Devi, 2013). Despite those reasons, the larvae fed on commercial feed have the highest ammonia concentration (still within an accepted range) in comparison to other treatments. Surprisingly the commercial feed group has the highest survival rate of (84%) among the treatments.

Most of the factors that contribute to water quality deterioration do not impose as cause for mortality. The study results demonstrate the candidate feeds for the experiment might not raise concern if water flow is incorporated along with reasonable stocking density (five larvae per one litter) similar results were observed by study of Hengsawat et al, (1997). In the same study, fifty larvae per cage results in high weight gain in comparison to its higher stocking density counterparts. Mortality was not affected by stocking density, and different feeding ratios at 12% and 8% do not affect mortality and cannibalism. (For the thesis experiment, we implied a feeding ratio of 10%).

Overall, the water quality results do not impact the survival rates among different treatment groups. Other factors that could be included in the cause can be nutritional component and skeleton development i.e. phospholipids concentration (Cahu et al., 2003, Lall and Lewis-McCrea, 2007).

8.3 Growth performance of Clarias gariepinus larvae

Stated by Graham and James (1998) and Nyina-Wamwiza et al, (2007), at the onset of exogenous feeding, larvae of the African catfish are able to eat, digest, absorb, and metabolize nutrients with their sizeable mouth and digestive system. In this study *Clarias gariepinus* larvae readily consumed only Artemia for six days after yolk absorption. On those days the larvae reached an overall average growth of 6.03 mm TL and 2.25 mg TW before other feeding types were introduced. This result is within the standard average recommended for larvae in limited period of time by Graham (1998).

One finding in this study was, that locally formulated diets resulted in the least growth when used as weaning feed. Artemia was offered for 12 days in reduced amounts as other works investigated (Hecht, et al., 1988; Janssen 1985a, 1985b; Hogendoorn, 1980; Verreth et al., 1993). In *C.gariepinus*, the earliest weaning time from Artemia to crumbles of a commercial trout diet is between 1.8 to 4.1 days, depending on the temperature (Olurin and Oluwo, 2010). Such data were confirmed by COPPEN's fish food company (Catco excellent catfish feed) with a recommended water temperature of 27-29 °C.

Diets that are readily consumed efficiently digested to provide the required nutrition's. Protein quality and quantity determine growths of larvae (Giri et al., 2003). If there is a big difference of crude protein content between feeds, the larvae also shows different growth performances as the study examples indicate (Furuya et al., 2004, Owodeinde and Ndimele, 2011).

This study tried to minimize the gaps of protein amount between treatments (45-40%CP). Our results are: fish meal mix (45.42%), soybean meal mix (43.47), commercial feed (45%), Daphnia (45% ± 4) and Ostracoda (30%). The protein content do not impose as an obstacle, but the feed palatability might be (Ahmad et al., 2012). Since there is a significantly high growth difference between treatments.

The local formulated feeds palatability and digestibility were not analysed. The lowest weight record of monthly growth: 26.4 ± 8.04 mg for fish meal mix, and 24 ± 7.64 mg for soybean meal mix. Low protein digestibility (Refstie et al., 1998) might be the cause. An expectation for a healthy growth by Graham (1998), for a period of 14-40 days fed on decapsulated Artemia cysts lays between 2.5 and 50 mg. This result might support that *C. gariepinus* cannot metabolize co-dried fish silage protein as efficiently as fish meal mix protein when used as the sole dietary protein (Faruque, 2010). Diets with those composed primarily of plant based ingredients and high amount of animal protein are more digestible (Musa et al., 2012). The present study doesn't show a significant difference on both diets.

Improvement of protein digestibility possibly attribute to the reduction/elimination of different anti nutrients during the pretreatment process, notably phytic acid and tannins which are known to interact with protein to form complexes. This can be also related to higher efficiency of the thermal treatment, reducing trypsin and chymotrypsin inhibitory activities (Alonso et al., 2000, Peres et al., 2003). According to those proofs we had also conducted thermal treatments on

soybean. However, larvae fed on soybean meal mix had a poor performance in growth, falls behind larvae that fed on other diets throughout the culture period (Figure 19 and 20).

Several factors can trigger the poor larvae growth performance. As the weight frequency chart (Figure 22) shows most of the larvae weights between 10 and 30 mg being fed on soy meal mix, whereas the larvae with fish meal mix weights in between 20 and 30 mg.

Low palatability and digestibility are considered possible causes for poor growth performance. Numbers of findings suggested that palatability issues for the fish crop initials when soybean meal was introduced to the diets. This effect is more important for the first period of fish feeding (Ahmad et al. , 2012). However, soybean meal could be increased up to 20% and still be acceptable (Barros et al., 2002). In the present study, such increase can be allowed up to 40%, such methods could be potentially problematic. There are no significance differences between fish crops that were fed on soybean mix and fish meal mix. There are difference levels of maximum dosages of soybean meal given to different species (Bhosale et al., 2010, Refstie et al., 2000).

Besides locally formulated feeds, the treatment groups with other diets turned out to have healthy growths. While commercially formulated fed larvae shows a good growth performance, behind the outcome of the group that were fed on *Daphnia magna.*

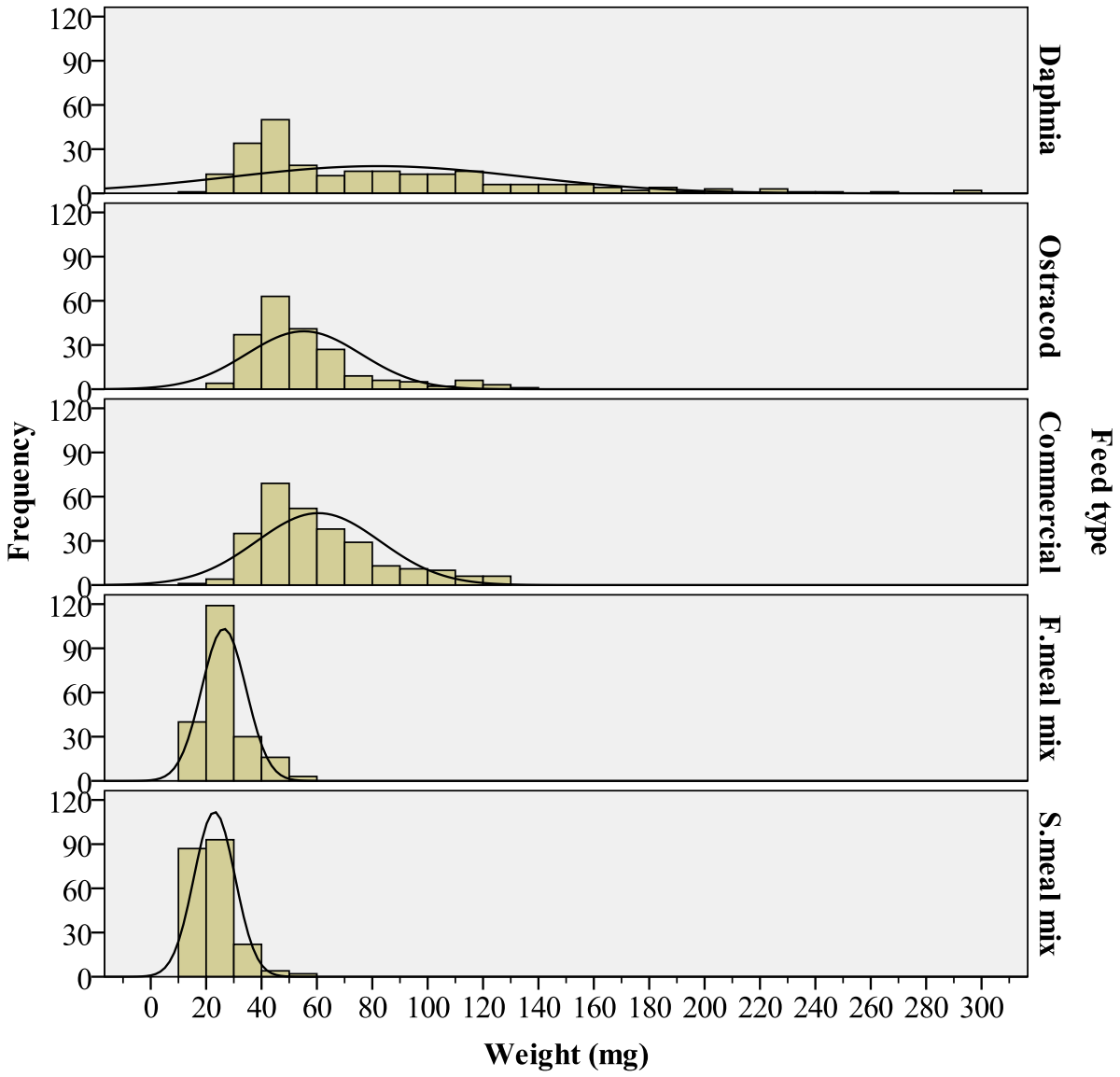

Figure 22: Final Larvae weight frequency for different feed types

Zooplanktons are preferred by prey due to their good nutrition, flavour, texture, digestibility, palatability and attraction for prey (Refstie et al., 1998; Conceição et al., 2010). Crustacians (Daphnia), copepods, rotifers and Artemia are known live feeds (Conceição et al., 2010, Shaheen, 2013). A study by Abdulraheem et al. (2012) on *C.gariepinus* obtained the highest growth on *Moina dubia* and mixed zooplankton in comparison to larvae fed on commercial feed. The present study also demonstrated highest weight from zooplankton fed larvae, where Daphnia

with a mean of 82.1 ± 53.15 mg, with highest individual weight of 295.5 mg. The weight frequency divers in different ranges (Figure 22).

Compare to the study by Olurin and Oluwo (2010), larvae fed on Daphnia recorded a mean body weight of 18.6 ± 3.1 mg within seven days. Within our own study, the record yields less within the same time period - a result of possibly under feed larvae. Ostracoda, the less well known live feed, also shows a good result with an average weight of 55.3 ± 20.71 mg and the highest weight of 150 mg.

The effect of Ostracoda feed in comparison to Daphnia feed is less suitable based on to its several characteristics. Its body is covered by shell; the texture could be hard to digest for the larvae. Movement of Ostracoda is not centered at the water column; instead they are positioned and attached at corners. Such measure made it difficult for larvae to prey. The energy spent on feed hunting lead to the low weight grain rather than somatic growth (Veras et al., 2013), poor nutrient enrichment could also possibly occur.

The condition factors (K) showed both positive and negative outcomes in terms of allometric fish growth (Davies et al., 2013). The result of this study (Figure 14) is similar with a previous study by Arimoro (2007), larvae fed on mixed zooplanktons and rotifers receives a K value between 06-1.8 and it is higher than the values 0.65-0.70 documented by Anyanwu et al (2007). According to this, *C.gariepinus* juveniles were reared in recirculation system at a feeding ratio of 3% body weight in adjacent to a ratio of 5% weight per day.

The present higher K values might be attributed to feeding the fishes at the station. Further on, the higher K values also indicated that the *C.gariepinus* juveniles are in good condition of health in the plastic tank. According to Davies et al (2013), several criteria would affect the condition factor of fishes. Such criteria range from feeding, food nutrient composition and fat accumulation. The trend of the condition factor shows an increase but in different scales. Ostracoda and Daphnia showed uniformed rate of increase, while commercial feed decreased after two weeks while the crumble size shifts to 0.3-0.5 mm.

8.4 Survival rate

Survival rate was significantly affected by different types of diet. Most of larvae that were fed on commercial feed, soybean meal mix and fish meal mix fall in a short lengths range (Figure 22). The homogeneous growth performance in each individual larvae resulted minimized or prevent cannibalism (Abdulraheem et al., 2012). Despite their size homogeneity; soybean meal mix and fish meal mix record lowest survival rate and the highest survival rate was recorded on larvae fed on commercial feed. This study shows that larvae mortality is mostly related to the digestibility and palatability of the locally formulated feed. Meanwhile, larvae fed on live feeds ended up with a high length/weight range and distribution. Their survival rate is also surprisingly higher.

From the studies of Olurin and Oluwo (2010) and Marimuthu et al. (2011) commercial catfish feed (38% CP) on different application rate does not alter either the survival rate or cannibalism. The result is similar to our study, the same feed with 45% of protein was not affected by feed with 10% application ratio, but different on the same feed type. According to a study by Abdulraheem et al, (2012) *C.gariepinus* had less survival rate when comparing to larvae fed on *Moina dubia* and mixed zooplankton.

Survival rate and growth (length and weight gain) have a strong codependent relationship. If environmental variables and husbandry necessities in the hatchery remain constant (Musa et al., 2012), then the survival rate and the growth are directly proportional to each other. In this experiment, the survival rates for commercially fed fish were generally high.

Such results are similar with in other study records even with less protein amount involved. Slight differences might occur probably due to different techniques employed within husbandry. When working with catfish fry, major mortalities occurred during the early nursing phase period (Musa et al., 2012). In this study, mortality remained constant from the first day where the larvae was introduced to new feeds (except larvae fed on Daphnia, which shows slight increase in cannibalism due to high size differences).

The reduced survival rates of larvae fed with soybean meal mix and fish meal mix probably could not be attributed to diet composition, it could be a result of starvation. The fish appeared unconscious and weak due to under feeding and high nutrient leaching. The latter act is the main

constraint in suitable diet production (Olurin and Oluwo, 2010), as the larvae might not have sufficient enzymes to thrive compound feeds.

Larvae fed on live prey that provide exogenous enzyme. Such processes are necessary for early stage of fish development, either autolysis or zymogens are capable to activate the larval endogenous digestive enzymes. The same processes also contain gut neuropeptides and nutritional 'growth' factors which boost digestion (Kolkovski, 2001; Cahu and Infante, 2001).

Larvae fed on Daphnia and Ostracoda had shown a better survival rate (70.9 ± 6.4% for Daphnia and 76.3 ± 7.02% for Ostracoda).Within the study by Olurin and Oluwo (2010), there is a 77% of survival rate of catfish larvae that were fed on Daphnia for seven days. The present study had shown fairly good results despite the different experiment periods.

Nevertheless, cannibalism among *Clarias gariepinus* larvae is primarily caused by uneven fish growths in a population that is lacking of feeds (Marimuthu et al., 2011, Musa et al., 2012). Fish sizes were fairly uniformed within each treatment, before each sampling they were starved for an average six hours for proper measurements. Cannibalism was not observed in the four treatments except Daphnia, the cause of such phenomenon could be high growth variations between individuals (Figure 15 and 22). Range and frequency of similar-sized larvae are very high in the treatments except for the larvae fed on *Daphnia*. However, food digestibility showed the greatest effect on fish survival. The quantity and frequency of feeding were not factors of cannibalism. The fish crop was fed with a food ratio of 10%, applied within three times per day.

Cannibalism was highly expected among locally formulated fed larvae. However that is not the case, since the larvae did not utilize the feed and were too weak to cannibalize. Such result agrees with Hecht (2000), who found that weak fish were not capable to command cannibalizing.

The result for the lowest weight and length gain (Figure- 23, 24) in one container for daphnia and commercial feed among replicas might be the position in the experiment setup .since two containers were exposed for light due to nearest position to the window. According to Pablo et al (2004) Fish spent more time swimming under continuous light than under dark .i.e. Furthermore; the time spent on swimming was higher at high light intensity.

8.5 Comparison between live and formulated feed

Numerous studies have been done on formulated feeds and live feeds for fish larvae rearing. Generally in most studies, live feed had a record for better growth as well as a higher survival rate for *C.gariepinus* (Arimoro, 2007; Olurin and Oluwo, 2010; Conceição et al., 2010 and Faruque, 2010). Formulated feed for early stage fishery has become more practiced, and it has been used as an alternative diet due to its budget friendliness (Cahu and Infante, 2001; Bulletin, 2006 and Hamre et al., 2013). A combination of live and formulated feed have been used as a weaning, the results are better compare to formulated feeds direct use (Armando et al., 1996). In the present studies, we use weaning strategies and two kinds of results have been found. Generally the larvae fed on live feeds shows better growth performances than the one with formulated diets.

9 Conclusion and check of Thesis-Hypothesis

It has been established that the potential of Daphnia mass cultivation is high in aquaculture. From the catfish larvae that we studied, the growth performances and survival rates were best when fed with these organisms. The results of this study showed that diet types play important roles in the feed utilization, growth and survival of *C. gariepinus* larvae. Additionally, by applying different type of feeds to *C. gariepinus* larvae, water quality would not deteriorate if water flow is allowed and oxygen aerator is used.

Generally the fingerlings fed with *Daphnia* have high feed intake and utilization. In result this group demonstrates outstanding weight gain that significantly separates themselves apart from the others. The highest survival rate of larvae was recorded within the group that was on commercial feed, followed by Ostracoda. Newly formulated feeds were not readily consumed due to low digestibility and palatability. They did not give any strength in growth performances or survival rate.

The result shows larvae do not require high amounts of feed. However, the older the fish, the higher the quantities of feed needed. It is recommended not to use the locally formulated feed for the larval stages.

Larvae fed on Daphnia shows relatively lower survival rate due to cannibalism caused by high size variation. Grading in an early stage of aquaculture is necessary. Part of the study result shows similarity with other studies, but it needs a fair amount of further supporting research in the future. The important factors that should be considered are

- To conduct supporting experiments on the palatability and digestibility of locally formulated feeds with different ingredients, feeding rates and feeding frequencies.
- To develop estimation methods for the amount of live feeds.
- More future work needs to be done for Ostracoda mass culturing and production since it has shown a promising results.
- Locally available and sourced materials are the cheapest and easiest way of starting a hatchery for fish production.

Check of hypothesis

- There will be no significant difference in growth performance of *Clarias gariepinus* larvae that fed on soya bean meal, fish meal, commercial feed, *Daphnia magna* and *Ostracod* spp.

Above hypothesis rejected

- There will be no significant difference in survival rate of *C. gariepinus* larvae that fed on different feeds.

Above hypotheses rejected

- Water quality will not be affected by the different feed types.

Above hypothesis rejected for ammonia content

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11 Appendices

Table 9: Descriptive statistics for water quality

	N	Minimum	Maximum	Mean	Std. Deviation
Mg/L	121	5.16	7.0	5.85	.34
%	121	85.2	103.2	94.35	4.3
TEMP	121	23.9	29.3	26.33	1.14
pH	45	7.52	7.93	7.74	.10
Cond	45	266.0	290.0	276.32	4.95
Valid N (listwise)	45				

Table 10: Descriptive statistics of final Weight and Length measurement. Daphnia (1), Commercial feed(2), fish meal (3), soyabean meal mix (4), and Ostracoda (5).

		Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
length (mm)	1	20.8	4.1	.26	20.3	21.3	16.0	34.0
	2	18.9	2.3	.13	18.6	19.2	16.0	26.0
	3	14.7	1.4	.09	14.5	14.9	13.0	20.0
	4	14.4	1.4	.11	14.1	14.6	13.0	19.0
	5	18.1	2.1	.15	17.8	18.4	16.0	26.0
	Total	17.7	3.5	.11	17.5	17.9	13.0	34.0
weight (mg)	1	82.1	53.2	3.4	75.4	88.8	19.6	295.5
	2	60.5	22.4	1.4	57.9	63.2	19.5	129.9
	3	26.4	8.0	.6	25.3	27.5	14.9	55.0
	4	24.5	7.6	.6	23.3	25.6	14.4	53.3
	5	55.3	20.7	1.5	52.4	58.1	25.6	130.5
	Total	52.5	36.3	1.1	50.4	54.7	14.4	295.5

Table 11: Test of ANOVA on the homogeneity final mean weight(A) and length (B). Daphnia (1), Commercial feed(2), fish meal (3), soyabean meal mix (4), and Ostracoda (5)

A weight (mg) Tukey HSDa,b

feed ID	Subset for alpha = 0.05		
	1	2	3
4	24.465		
3	26.4		
5		55.3	
2		60.55	
1			82.1
sig	0.91	0.34	1.00

B Length (mm)

feed ID	N	Subset for alpha = 0.05			
		1	2	3	4
4	164	14.354			
3	208	14.671			
5	204		18.069		
2	274			18.883	
1	246				20.821
Sig.		.712	1.000	1.000	1.000

Table 12: Test of Homogeneity of Variances on final weight of treatments

Feed	Levene Statistic	df1	df2	Sig.
commercial	6.877	4	269	.000
Daphnia	3.057	4	241	.018
Fish meal mix	4.407	4	203	.002
Ostracoda	2.094	4	199	.083
Soybean meal mix	1.421	4	159	.229

Table 13: Regression analysis of survival rate with ammonia concentration. (a) Predictors: (Constant), ammonia. Dependent Variable: mortality

feed	Model		Sum of Squares	df	Mean Square	F	Sig.
Commercial	1	Regression	9.850	1	9.850	.179	.701[a]
		Residual	164.906	3	54.97		
		Total	174.756	4			
Daphnia	1	Regression	75.6	1	75.6	2.512	.211[a]
		Residual	90.3	3	30.1		
		Total	165.9	4			
F.meal	1	Regression	51.8	1	51.8	3.414	.162[a]
		Residual	45.5	3	15.2		
		Total	97.2	4			
Ostracoda	1	Regression	33.1	1	33.1	.607	.493[a]
		Residual	163.84	3	54.6		
		Total	196.97	4			
S.meal	1	Regression	14.4	1	14.4	3.905	.143[a]
		Residual	11.05	3	3.7		
		Total	25.4	4			

Table 14: Homogeneity of larvae fed on commercial feed between replicas

container	**Subset for alpha = 0.05**	
	1	2
2	45.911	
4		60.368
19		66.723
3		68.761
17		71.117
Sig.	1.000	.052

Table 15: Homogeneity of larvae fed on Daphnia feed between replicas

Container	Subset for alpha = 0.05	
	1	2
1	66.570	
10	81.717	81.717
8	85.002	85.002
18	89.398	89.398

9		94.498
Sig.		.074

Table 16: Means for fed larvae on fish meal mix groups in homogeneous subsets.

container	**Subset for alpha = 0.05**		
	1	2	3
21	23.647		
20	24.127	24.127	
16	26.079	26.079	26.079
14		28.878	28.878
6			29.238
Sig.	.631	.054	.369

Clustered analysis weight gain of replicas of all treatment

There was a significant difference among replicas of larvae final weight in some of the treatments, and some of them show some homogeny with in a treatment (Figure 23) and (Table 7). Larvae fed on soy meal mix shows consistency and there was no significant difference among replicas (P>0.5, P=0.229). Although larvae fed on Ostracoda shows no significant difference and but slight consistency of weight within replicas (P > 0.05, P=0.083) .Other treatments have shown significant difference between replicas (P < 0.05).

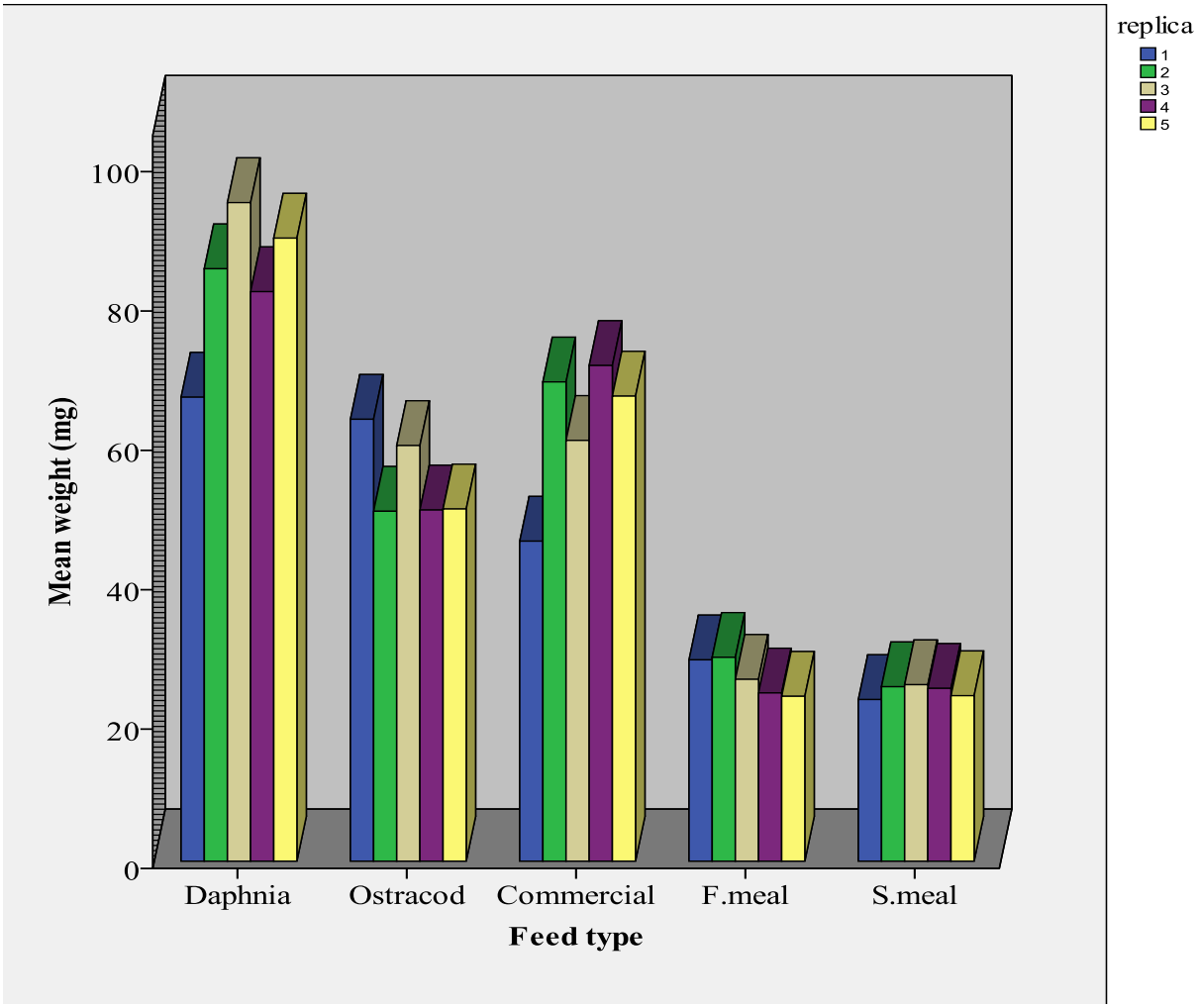

Figure 23 Clustered analysis weight gain of replica of all treatment

Larvae fed on commercial feed Container (2) (Figure 22 and Table 9) was significantly different from others replicas and it was the lowest weight (45.9±13.6) recorded among the other four (68± 23.7, 60.17.4, 71± 24.8, 66.7± 22.2).There was also significant difference among replicas of larvae fed on daphnia the weight gain. Container (1), (66.5 ± 42.5 mg) was significantly different from container (9), (94.5 ± 64.2 mg) and it was the lowest weight gain among the replicas. But it still shows homogeneity with other replicas it shows homogeneity (P >0.05) as shown by (Table 10). Similarly the replicas that larvae fed on fish meal have significant difference within each other (Table 11).

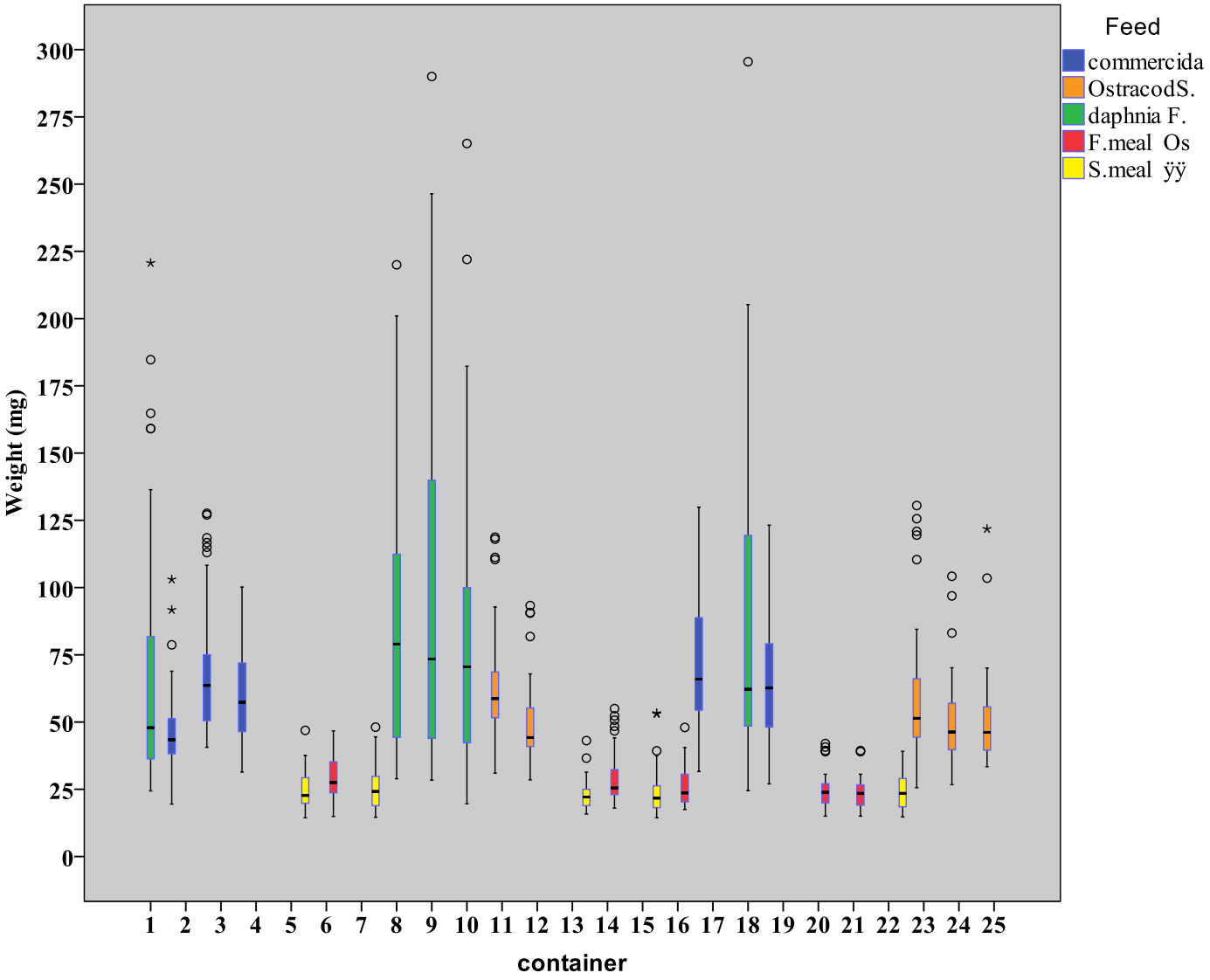

Figure 24: Clustered analysis weight gain of replica of all treatment

Table 17 some part of data from final individual's weight and length of clarias gariepinus larvae.

Feed ID	Feed type	Length (mm)	Weight (mg)	feed ID	Feed ID	Feed type	Length (mm)
1	*Daphnia*	23	118.7	2	commercial	18	71.8
1	*Daphnia*	19	60	2	commercial	18	59.5
1	*Daphnia*	18	40	2	commercial	17	39.3
1	*Daphnia*	19	78.3	2	commercial	20	58.6
1	*Daphnia*	18	44.3	2	commercial	23	90.6
1	*Daphnia*	17	43.9	2	commercial	17	45.1
1	*Daphnia*	23	112.9	2	commercial	22	82.2
1	*Daphnia*	20	117	2	commercial	22	84
1	*Daphnia*	16	33.2	2	commercial	21	94
1	*Daphnia*	20	47.2	2	commercial	25	128.8
1	*Daphnia*	18	70	2	commercial	25	129.9
1	*Daphnia*	20	46.1	2	commercial	17	43.1
1	*Daphnia*	17	44.2	2	commercial	20	79.8
1	*Daphnia*	21	77.2	2	commercial	17	54.6
1	*Daphnia*	22	90.6	2	commercial	18	55.8
1	*Daphnia*	19	79	2	commercial	19	57.4
1	*Daphnia*	22	84.3	2	commercial	18	67.4
1	*Daphnia*	21	95.3	2	commercial	17	62.9
1	*Daphnia*	23	112.3	2	commercial	18	57.6
1	*Daphnia*	30	220	2	commercial	23	129.5
1	*Daphnia*	23	118.4	2	commercial	18	51
1	*Daphnia*	25	97	2	commercial	19	74.1
1	*Daphnia*	21	93.9	2	commercial	17	54.2
1	*Daphnia*	25	147.5	2	commercial	19	63.1
1	*Daphnia*	17	38	2	commercial	22	91.8
1	*Daphnia*	17	41	2	commercial	22	98.7
1	*Daphnia*	16	41.6	2	commercial	21	91.6
1	*Daphnia*	20	86.7	2	commercial	17	50.7
1	*Daphnia*	16	44.8	2	commercial	20	75
1	*Daphnia*	28	184.4	2	commercial	17	42.9
1	*Daphnia*	26	118	2	commercial	16	37.7
1	*Daphnia*	30	201	2	commercial	17	44.1
1	*Daphnia*	25	165.4	2	commercial	22	95.4
1	*Daphnia*	24	112.4	2	commercial	24	104.4
1	*Daphnia*	24	120.2	2	commercial	19	70.4
1	*Daphnia*	21	102.5	2	commercial	23	101.5
1	*Daphnia*	22	87.3	2	commercial	20	78.6
1	*Daphnia*	32	236.1	2	commercial	19	66.6

1	*Daphnia*	33	246.4	2	commercial	17	44.1
1	*Daphnia*	21	80.6	2	commercial	16	31.6
1	*Daphnia*	30	204.9	2	commercial	20	70.7
1	*Daphnia*	17	35.5	2	commercial	21	78.4
1	*Daphnia*	28	179.6	2	commercial	19	69.5
1	*Daphnia*	18	40.7	2	commercial	17	40.1
1	*Daphnia*	26	139.6	2	commercial	18	58.2
1	*Daphnia*	27	155.7	2	commercial	18	53.2
1	*Daphnia*	28	165.5	2	commercial	25	110.5
1	*Daphnia*	25	132.2	2	commercial	21	81.4
1	*Daphnia*	24	106.2	2	commercial	19	58.4
1	*Daphnia*	19	57.5	2	commercial	17	41.4
1	*Daphnia*	21	74	2	commercial	19	66.6
1	*Daphnia*	20	59.7	2	commercial	21	81.4
1	*Daphnia*	18	42.7	2	commercial	21	64.6
1	*Daphnia*	22	72.9	2	commercial	16	27
1	*Daphnia*	18	48.8	2	commercial	21	70.7
1	*Daphnia*	18	47.5	2	commercial	17	54.7
1	*Daphnia*	19	43.9	2	commercial	21	72.9
1	*Daphnia*	17	37	2	commercial	20	70.5
1	*Daphnia*	24	110	2	commercial	23	108.7
1	*Daphnia*	34	290	2	commercial	19	57
1	*Daphnia*	27	143.5	2	commercial	17	48.3
1	*Daphnia*	27	182.1	2	commercial	21	78.1
1	*Daphnia*	26	141.1	2	commercial	17	47.7
1	*Daphnia*	27	155.8	2	commercial	20	48.2