MAKERERE UNIVERSITY

USE OF ROTIFER (*Brachionus calyciflorus*) AS AN ALTERNATIVE STARTER LIVE FEED TO *ARTEMIA* IN THE FEEDING OF AFRICAN CATFISH (*Clarias gariepinus*) LARVAE.

BY

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DECLARATION

I Abaho Ivan, hereby declare that this work is entirely original and has never been submitted to any University or Institution for any academic award. All sources of information quoted from previous work, have been fully acknowledged. All photos are by Abaho Ivan.

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DEDICATION

This work is dedicated to my wife Leticia, our daughter Teresa and my parents who were a continuous motivation towards the completion of this study.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ACRONYMS

AA	Arachidonic acid
ANOVA	Analysis of variance
DAP/UREA	Diammonium phosphate/Urea
DHA	Docosahexaenoic acid
DO	Dissolved Oxygen
EFA	Essential Fatty Acid
EPA	Eicosapentaenoic acid
HUFA	Highly unsaturated fatty acids
LA	Linoleic acid
LNA	Linolenic acid
MUFAs	Monounsaturated fatty acids
OA	Oleic acid
pH	Potential of Hydrogen ions
PUFAs	Polyunsaturated fatty acids
SFAs	Saturated fatty acids
SD	Standard Deviation
TL	Total Length
USA	United States of America
µ	Micron

ABSTRACT

Imported *Artemia* cysts (the Brine Shrimp) are currently the readily available live starter feed to African catfish breeders in Uganda but are highly priced. In this study, the potential use of locally grown rotifers (*Brachionus calyciflorus*) as an alternative starter live feed to *Artemia* in the feeding of African catfish (*Clarias gariepinus*, Burchell, 1822) larvae was explored. African catfish larvae were cultured in experimental tanks under ambient hatchery conditions and tested on three experimental live starter diets; freshly decapsulated *Artemia* cysts, rotifer *B. calyciflorus* and a combination of the two on the third, fourth and fifth days to meet their exclusive requirement for live starter feeds during the first three days. A short period (3 days) of diet experimentation used in this study may have limited the full understanding of growth performance with diet in larval African catfish, but it was nonetheless based on the practice by all commercial fish farmers in Uganda to provide a live starter diet for only two to three days following commencement of exogenous feeding of the African catfish larvae. Change in Total Length (TL) measurements of larvae was used as a measure of growth. Fatty acid profiles of six-day old larvae were determined using Gas chromatography-mass spectrometry (GC-MS) method and cost effectiveness of utilizing either *Artemia* or Rotifer starter diets computed. To explain whether different *Artemia* strains used as live starter feeds for African catfish larvae may impart different growth performance, three commonly used *Artemia* (USA - *Artemia franciscana*, Asia - *Artemia salina* and China -*Artemia* strain) were tested on two days old African catfish larvae. Feeding African catfish larvae on different *Artemia* strains yielded no significant differences in growth ($F= 0.875$, $P=0.42$) and survival ($F=1.485$; $P=0.299$). However, overall growth of Rotifer-fed African catfish larvae was significantly better than *Artemia* – fed larvae ($F= 47.605$, $P=0.000$). Noteworthy, was the fact that catfish larvae fed on a mixture of Rotifers and *Artemia* grew faster (10.04 ± 0.45 mm, $P=0.000$) than those fed on either rotifers or *Artemia* (Rotifer-

9.04±0.58 mm, *Artemia* -8.78±0.54 mm, P=0.147). Significantly higher composition of Arachidonic acid (AA), Docosahexaenoic acid (DHA) and proportions of DHA/EPA and AA/EPA were recorded for Rotifer -fed larvae than for *Artemia*-fed larvae (AA, F=22.292, P=0.016 and DHA, F=28.740, P=0.011, respectively). These essential fatty acids play a significant role in the structural, physiological and functional development of larval fish and may explain the better growth recorded in this study. The combination of rotifers with *Artemia* was probably of an added advantage due to the large-sized *Artemia* that makes catchability easy. On the other hand, partial or total replacement of *Artemia* with Rotifer was more cost effective than using only *Artemia*. The results of this study recommend *B. calyciflorus* rotifers as alternative to *Artemia* in hatchery in raising African catfish larvae because they compete favourably with *Artemia* in fish larvae growth performance and are cost effective. However, utilization a combined diet of rotifers and *Artemia* as live starter feeds in African catfish hatcheries would provide added advantage in achieving better growth results of the fish.

CHAPTER ONE

1.0 Introduction

1.1 Background to the study

The main objective of any fish hatchery system is to produce maximum number of high quality fish seed from the available brood stock (Marimuthu and Hanifa, 2007). However, the bottleneck in most inland freshwater aquaculture is obtaining adequate number of seed from the hatcheries, due to their high mortality at early life stages. This has been attributed to several factors, among which is inadequate supply of good quality and quantity food at early larval stages (Arimoro, 2006). Because most larval fish are very small and/or possess a digestive system that is not fully developed at the onset of exogenous feeding, they require a starter feed that is small-self digesting starter feed. Live feeds such as rotifers, copepods and *Artemia* therefore, offer an appropriate size ingestible by a wide range of larval fish species and are rich carriers of digestive enzymes (Kolkvoski, 2001). Additionally, the high nutritional quality (presence of nutrients such as lipids) of these live starter feeds meets the demand for high growth and development rates that occurs at this stage of fish (Olurin *et al.*, 2012). Lipids are particularly important in larval fish nutrition not only for supplying calorific energy but also for providing the essential polyunsaturated fatty acids (PUFA) that allow optimal physiological performance in the growth process including visual development, optimal pigmentation and immunity, maintenance of cell membrane fluidity that is expressed in better growth and survival of fish larvae (Kainz *et al.*, 2004; Das 2006; Tocher, 2010).

The most important live food organisms currently used for most cultured fish species include micro algae, rotifers, copepods, cladocerans and the brine shrimp (*Artemia* species) (Yilmaz *et al.*, 2006) The size selectivity of these feed organisms and duration of requirement to utilize

them are species specific. Yilmaz *et al.*, (2006) report a partially bigger mouth in African catfish larvae than most cyprinid larvae that permit newly born larval *Clarias gariepinus* to consume rotifers with sizes greater than 200 µm. (Watanabe *et al.*, (1983) and Lubzens *et al.*, (1989) note that rotifers are valuable live food for the culture of larvae of many fish species because of their very small size, their ability to stay suspended in the water column, their high reproductive rate, ability to function normally at high density cultures in addition to their relatively slow swimming motion that makes them useful prey for active larvae. The rotifers of the genus *Brachionus* are broadly used as live food for both marine (Lubzens *et al.*, 1989; Sarma, 1991) and freshwater (Arimoro, 2006) fish larvae. On the other hand, brine shrimp (*Artemia*) is often included as a reference diet in catfish larval nutrition. Verreth *et al.*, (1987) and Pector *et al.*, (1994) report on the successful use of decapsulated *Artemia* cysts for larval rearing of *Clarias gariepinus.* It is however documented that not all strains of *Artemia* guarantee equal culture success in aquaculture hatcheries (Leger and Sorgeloos, 1984). The nutritional quality of *Artemia* may vary considerably according to the geographical strain, processing batch and development stage (Leger *et al.*, 1986) as observed by Wickins (1972) while culturing Sole larvae (*Soleasolea* L.) on two different strains of *Artemia* nauplii. The high cost and occasional scarcity of *Artemia* also make it us suitable for commercial aquaculture (Herath and Atapaththu 2012).

In Uganda, the current low survival rate (15%) in hatchery-based catfish seed production (has been attributed to mainly poor larval nutrition. The present practice among fish farmers range from use of crushed manufactured feeds, crushed chicken egg yolk and decapsulated cysts of different *Artemia* strains for three days following commencement of exogenous feeding. On the fourth day, the fish are weaned progressively to different sizes of manufactured feeds until they

attain the fingerling stage. There is a need therefore to explore alternative live feeds to the currently used *Artemia* to counteract this low survival rate.

Rotifers are currently not utilised because of lack of culture and economic knowledge concerning the cost-effectiveness of rotifer application compared to other commonly used feed items such as the brine shrimp *Artemia*. However, these rotifers especially *Brachionus calyciflorus* rotifers are viewed as potential substitutes for *Artemia* as a live starter feed in African catfish larvae rearing because of their good morphological, behavioural and nutritional characteristics (Lubzens *et al.*, (1989, Lubzens *et al.*, 2001, Koven *et al.*, 1990). This study therefore compared the effect of utilizing of *B. calyciflorus* and *Artemia* on the performance of African catfish larvae and a cost-effectiveness analysis was also made on these two alternative live feeds (*B. calyciflorus* rotifers and *Artemia*) in larval African catfish rearing with an overall goal of enhancing the production of African catfish larvae in Uganda. The study formed part of an ongoing research project on the utilization of locally grown zooplankton as live starter feeds in hatchery based fish farming bringing together research teams at the Department of Biological Sciences, Makerere University and two collaborating commercial fish farmers (Ssenya Fish Farm in Masaka District and Kireeka Fish Farm in Wakiso district). In study, a cost-effectiveness analysis is made on two alternative live feed items (rotifers and *Artemia*) in larval African catfish rearing.

1.2 Problem statement

The production cost of fish seed for propagation of African catfish as practiced in most hatcheries in Uganda remains high as farmers continue to rely on the expensive imported brine shrimp (*Artemia*) as a starter feed for larvae once the yolk sac is used up. *Artemia* is currently the only available live starter feed available to fish hatchery operators in Uganda. The costs of

importation and unpredictable market supply, is reflected in its current high shelf price of US $ 70-100 for every 500g tin. This high cost of starter food results in increased cost of fish seed production that leads to low profit margins. Secondly, Reports from global trends indicate declining yield of *Artemia* cysts from the main wild source, the Great Salt Lake in Utah, USA, (Lavens and Sorgeloos, 2000). This trend, combined with possible climatic change impacts and increasing demand for aquaculture, implies that the demand for *Artemia* cysts will soon be exceeded by what the wild can offer. The low larval survival rates (15%) currently recorded for hatchery-based catfish in Uganda, to some extent may imply that *Artemia* does not meet all the basic nutritional requirements of African catfish larvae or results from a possibility of utilizing low quality *Artemia* strain since there are different geographical *Artemia* strains on the market. Despite these above outlined challenges posed by dependency on *Artemia* as the main starter feed, utilization of potential alternative starter feeds is limited by lack of researched information on their nutritional suitability and their cost effectiveness for use in catfish seed production. Therefore, this study is proposed to bridge the existing knowledge gaps towards utilization of rotifer *B. calyciflorus* as a possible alternative to *Artemia*.

1.3 Objectives

1.3.1 Overall objective

The overall objective of this study was to evaluate the suitability of utilizing rotifer *B. calyciflorus* as an alternative starter feed to *Artemia* in the African catfish seed production.

1.3.2 Specific objectives

1. To compare growth performance and fatty acid profiles of African catfish larvae grown on *Artemia* and Rotifer *B. calyciflorus*.

2. To determine the cost effectiveness of utilizing *B. calyciflorus* as an alternative to *Artemia* (Chinese strain) as a live starter feed for African catfish larvae.

1.4 Hypotheses

1. There is no difference in the growth performance and fatty acid profiles of African catfish larvae fed on *B. calyciflorus* and *Artemia*
2. There is no difference in cost of utilizing of *B. calyciflorus* and *Artemia* as starter feeds for African catfish larvae

1.5 Justification

Production of nutritionally adequate and cost effective live starter foods is a bench mark for successful fish seed production of freshwater fish species. This study will provide verified information on nutritional suitability and cost effectiveness of utilizing *B. calyciflorus* as an alternative to *Artemia* in fish larviculture. This information will thus address a critical milestone towards mass production of African catfish by possibly providing a better cheaper alternative live starter feed to the current commonly used *Artemia.* In addition, this study will form a basis towards addressing the existing need for a much smaller live starter feed than *Artemia* needed for the domestication of fish species, such as Nile Perch whose larvae require much smaller live starter feeds.

CHAPTER TWO

2.0 Literature review

2.1 Live feed in the larviculture industry

Feed management plays a critical role in the success of fish culture. The current trend in fish culture is towards increased intensification with the culture of more fish species and thus the provision of quantity and quality feeds becoming necessary to cater for this increase. The success depends significantly on the continuous availability of well-balanced nutritionally complete and cost effective compounded feeds. Micro algae and zooplankton such as rotifers, copepods and Brine shrimp play a vital role in the hatchery phase in aquaculture as feed for larval and juvenile crustaceans and fish (Murugesan *et al.,* 2010). The production of fingerlings, however, remains a primary bottleneck in the introduction of new fish species to the industry due to lack of appropriate starter diet (Takeuchi, 2001; Zambonino and Cahu, 2001).

Live zooplankton contains enzymes (amylase, protease, exonuclease and esterase), which play an important role in larvae nutrition (Fluchter and Pembold, 1986) and are easily digestible. These live food organisms have a high food value as a protein source of fish (Yamamoto *et al.,* 1994), are of a small size and their movements in the water column elicit a feeding response (Lubzens, 1989) making them suitable diet candidates in larviculture. On the other hand, the small mouth gape of the fish larvae limits the size of food it can consume and prevents the initial use of larger food organisms. Similarly, the incomplete morphological and physiological growth status of fish larvae on commencement of external feeding limits their ability to easily sight and capture required prey. As such, fish larvae have evolved to feed on natural congregations of zooplankton whose movements produce a stimuli needed by many fish larvae to elicit a feeding response (Olurin *et al.,* 2012). Larval mouth gape and feeding response to various live feeds are species

specific that require sufficient research in order to establish the appropriate zooplankton for culture as starter live foods (Cortney *et al.*, 2009).

2.2 Use of rotifers in larviculture

Many fishes produce tiny larvae following hatching and so necessitate the availability of appropriately sized prey to fit in their developing mouth and gut. Studies have showed that prey preference is mainly dependant on the size of live feed species rather than their nutritional composition, (Grageda *et al.*, 2008), as most fish larvae are thought to be gape-limited predators, and the presence of suitably sized prey at the appropriate time in the foraging environment is a key factor for their growth and survival. Successful culture of most fish fry requires the presence of the smallest zooplankton, mainly rotifers, as first food (Arimoro and Ofojekwu, 2003). Rotifers of the genus *Brachionus* possess several characteristics that favour their use as live feed for these tiny larval fish. Such characteristics include nutritional quality, body size and relatively slow motility (Snell *et al.*, 1984; Lubzens *et al.*, 2001). *B. calyciflorus* rotifers for example can be favoured at the initial stage of feeding because of small size and thus the first live food for the small fish larvae (Lubzens *et al.*, (2001). In addition rotifers remain suspended in the water column, have high reproduction rate and yield high density cultures (Lubzens *et al.*, 2001). Ludwig (1993) demonstrates that rotifers tolerate temperatures between 15 – 31 °C and an optimal pH of 6-8. Suitable size, slow mobility, good nutritional quality, a wide water quality tolerance and high and stable production rate make rotifers ideal food for the high energy-demanding but small-mouthed and slow swimming fish larvae. Rotifers (*Brachionus sp.*) are the most commonly used live feeds in mariculture due to the existence of standardized cost-effective protocols for their mass production. It has also been demonstrated that rotifers yield healthier larvae growth of most marine fish species (Wang *et al.*, 2005). According to Ajah (1998), the

choice of rotifers for early fish larvae has the advantage of providing a low cost, highly predictable and reliable production system. Rotifers have the additional advantage of being maintainable in a stable culture by feeding them recycled diets in a feedback culture system (Hirata and Yamasaki, 1983; Hirata *et al.*, 1983).

Lim and Wong (2004) demonstrated, that the use of rotifers can enable freshwater larviculture to improve larval performance, increase yield, and facilitate breeding of new fish species with small larvae. In order to use rotifers as larval food in hatcheries, they must be continuously available in sufficient quantities so as not to disrupt hatchery production. Due to the increased demand to expand on the domestication of freshwater fish species, the culture of freshwater rotifers recently draws much attention.

2.3 Culture techniques of *B. calyciflorus*

Several techniques have been adopted for the culture of the freshwater rotifer, *B. calyciflorus* (Lubzens *et al.*, 1989; Hoff and Snell, 1997). Both cultures require constant care, precise growing conditions and specialized equipment for isolation to avoid contamination (Arimoro, 2006). Axenic pure cultures are obtained by a series of chemical treatments with diazinon (Basudine) to establish the safe concentration for rotifers. At a concentration of 1.2 mgl^{-1} (Agbon *et al.*, 2002) crustaceans (cladocerans and copepods), aquatic insects including mosquito larvae fail to survive thereby allowing the rotifers to multiply.

Batch culture system has been used to culture *B. calyciflorus*. An inoculum with a relatively low individual density is seeded in a dense microalgae suspension and the rotifer population grows for several days until the exhaustion of the algal cells. The whole production is harvested at the

end of the exponential phase and used. This is a very common system in hatcheries, (Lubzens 1987; Dhert *et al.* 2001). In the semi-continuous system, harvesting and media renovation are frequent, and account for a notable part of the total volume. When the renovation frequency is every 24 h or less and the renovated volume is constant, the culture can be associated with a continuous culture (Schluter *et al.* 1987). The semi-continuous culture is widely used in larviculture and is usually combined with batch culture (Hirayama and Nakamura, 1976; Suantika *et al.*, 2000; Lubzens and Zmora 2003). In addition, a modified method for continuous culture of the rotifers has been employed by Hirata and Yamasaki (1983).

2.4 Physico-chemical culture conditions for Rotifers

Brachionus calyciflorus has been cultured in reconstituted hard water medium (EPA) at a temperature of 25°C, Oxygen level of 4mgl^{-1}, <1mgl^{-1} Ammonia and at a pH of 7.5 (Lucia-Pavon *et al.*, 2001). At these settings, mean peak population density of 1227±83 individuals per ml, was achieved, using *Chlorella* at a density of 4.5x10^{6} cells ml^{-1} as a food source. Hirayama and Kusano (1972), McVey and Moore (1983), Lim and Wong (1997), have all reported an optimum temperature of 25°C for culture of *B. calyciflorus* rotifers, while Mostary et al., (2007), reported an optimum culture temperature of 20°C lowest and 24.5°C highest for *B. angularis.* Moretti (1999) demonstrated the effect of temperature on reproductive activity of *B. plicatilis* where increasing temperature impacted positively on maturation period. Hoff and Snell (1989) reported optimum pH for *B. calyciflorus* to be 6.0 – 9.0, with upper and lower limits at 9.5 and 4.5 respectively. Rotifers have also been reported to tolerate a wide range of temperatures and pH; 15 - 31°C and 6-8, respectively (Ludwig, 1993). Furthermore, Mitchell and Joubert (1986) demonstrated the control of a predatory species, *Asplanchina sp* in *B. calyciflorus* culture

systems by raising pH to 10 for a short period daily. Lavens and Sorgeloos (1996) reports that high levels of un-ionized ammonia are toxic to rotifers, but concentrations below 1mg/l are safe.

2.5 Use of *Artemia* in larviculture

Decapsulated *Artemia* cysts have been used successfully as a larval diet for several crustacean species, and are used as a reference diet for the rearing of African catfish larvae (Ribeiro and Jones, 1998, Lim *et al.*, 2002). Bardocz *et al.*, (1999), Hung *et al.*, (2002) and Lim *et al.*, (2002, 2003) have proved that decapsulated *Artemia* cysts are a good alternative to *Artemia* naupli as a starter diet for African catfish larvae and some ornamental fish, respectively. The decapsulated *Artemia* cysts provide numerous benefits, including disinfection, improved hatchability and easy storage, and are less expensive and labour intensive than regular hatching (Van Stappen, 1996). Even low-quality cysts can be utilized once decysted (Ribeiro and Jones, 1998). Additionally, decapsulated cysts preserve higher energy content than freshly hatched nauplii (Vanhaecke *et al.*, 1983).

Although *Artemia* is still the most preferred and reliable live food in rearing fish and crustacean larvae (Dhont *et al.*, 1993), it is well documented that not all strains of *Artemia* guarantee equal culture success in aquaculture hatcheries (Leger and sorgeloos, 1984). *Artemia* nauplii from cyst material from different sources are recorded to have different biometrical, biochemical and food-value characteristics (Sorgeloos, 1980). Variation in growth performance of fish when fed on different Artemia strains can be as a result of variability in size, caloric content, nutritional composition, and contaminants that occur among geographical strains as explained by (Phillip *et al.*, 1987). A comparative study with eight strains of *Artemia* spp. using *Pseudopleuronectes americanus*; as predator test species confirmed the nutritional variation among Artemia sources

(Klein-MacPhee *et al.*, 1980, 1982) while the study with eight strains of *Artemia* spp. using crab and mysid shrimp as predator test species also confirmed the nutritional variation among *Artemia* sources (Johns *et al.*, 1980, 1981; Leger and Sorgeloos, 1984). Seidel *et al.*, (1982) provided information on variability in the biochemical composition and food value between different geographical sources of *Artemia*. Differences were found in fatty acid composition, total lipid content and chlorinated hydrocarbon contamination levels. The most noteworthy differences between the *Artemia* sources were the higher level of 18:3 n3 in the Canadian and French strains and the high level of 20:5n3 in the Chinese strain. The capability of some Chinese *Artemia* strains to reach high DHA levels during enrichment (Dhert *et al.*, 1993) and to maintain them during subsequent starvation (Evjemo *et al.*, 1997) offers new perspectives for providing higher dietary DHA levels and DHA/EPA ratios to fish and crustacean larvae. This is different from *Artemia franciscana* whose enrichment with DHA is difficult because of the inherent catabolism of this fatty acid after enrichment. DHA/EPA ratios obtained immediately after enrichment with DHA-rich emulsions is considerably lower (Dhert *et al.*, (1993) and Triantaphyllidis *et al.*, 1995). Despite differences in the amounts of a few important polyunsaturated fatty acids (18:3 ω3, 20:5 ω3), as well as in the chlorinated hydrocarbon content, there was little variation in the survival and development rates of *Rhithropanopeus harrisii* (mud crab) larvae fed on *Artemia* from different geographical sources (Seidel *et al.*, 1982)

Nevertheless, the nutritional value of correctly fed *Artemia* is higher than that of manufactured commercial starter food (Segner *et al.*, 1993; Kim *et al.*, 1996; Girri *et al.*, 2002). In some instances, *Artemia* products have been used to supplement dry diets with recorded improved survival and growth in crayfish (Gonzalez *et al.*, 2008). *Artemia* is widely used in aquaculture because so far it is the most convenient, least labour intensive live food to prepare for

aquaculture (Faruque *et al.*, 2010). The *Artemia* cysts can be stored in cans for long periods and cans are readily obtainable off the shelf. Subsequently, decysting for use requires only 24 h of incubation. Secondly, *Artemia* has proved to have sufficient nutritional value and its mobility in water and short production cycle make it an excellent prey suitable for most fish larvae (Moretti *et al.*, 1999).

Studies on African catfish and, to a lesser extent on Asian catfish (Pector *et al.*, 1994; Bardocz *et al.*, 1999; Garcia-Ortega *et al.*, 2000; Hung *et al.*, 2002), cyprinids (Vanhaecke *et al.*, 1990; Harzevili *et al.*, 2003; Kaiser *et al.*, 2003) have shown decapsulated *Artemia* cysts to be a good alternative to nauplii. Feeding African catfish larvae on decapsulated *Artemia* also gave the best growth performance (Verreth and DenBiema, 1987; Verreth *et al.*, 1987; Olurin and Oluwo, 2010). The Decapsulated *Artemia* cysts were reported as a good starter diet for both freshwater and marine fish (Pector *et al.*, 1994; Lavens and Sorgeloos, 2000; Lim *et al.*, 2002; and Harzevilli *et al.*, 2004), because of its balanced nutritional composition. They are also easy to store for future use, less expensive and labour intensive than regular hatching (Bruggeman *et al.*, 1980; Van Stappen, 1996), are disinfected and separated from the cyst shells during the decapsulation process (Sorgeloos *et al.*, 1977), and have the ability to preserve a higher energy content than freshly hatched nauplii (Vanhaecke *et al.*, 1983).

However, the sustainable supply of *Artemia* on market is currently challenged by declining wild harvests from the Great Salt Lake in Utah, USA, that contributes approximately 90% of the world *Artemia* cyst thus posing a potential crisis in future (Lavens and Sorgeloos, 2000). It has also been predicted that climatic changes may generate unforeseen lack of live food inducing economic inflation; the high costs of commercial cysts are expected to considerably affect the aquaculture industry (Lavens and Sorgeloos, 2000). In order to overcome the potential crisis

and limitations posed by *Artemia* to the aquaculture industry in Africa, other live feeds, that are nutritionally stable and cost effective have to be made available.

2.6 African catfish larvae rearing

African catfish (*Clarias gariepinus*, Burchell 1822) is an important fish species in aquaculture industry globally. Its advantages of good taste, fast growth, resistance to low oxygen and considerable ease in farming (Van de Nieuwegiessen *et al.*, 2009) make it an important species for aquaculture both within and outside its natural range of tropical and subtropical environments (Adewolu *et al.*, 2008). However, the availability of a readily available and suitable feed in large quantities is still a considerable challenge in the larval nutrition of this fish. During early stages of growth, larvae fish rely on the yolk sac for nutritional requirements (Kolkvoski, 2001). In most cultured fish species, the transition from an endogenous to an exogenous food supply which marks the onset of the larval stage of fish, is one of the most critical phases of the life cycle and is the period when much of the mortality of hatchery reared stock occurs (De Silva and Anderson, 1995). At the onset of exogenous feeding, live feeds such as brine shrimp (*Artemia* nauplii), yeast, zooplankton and unicellular algae are more appropriate because the fish have difficulty assimilating dry prepared diets because of incomplete development of the digestive system (Kolkvoski, 2001). Wang *et al.*, 2005 showed that it is better for the first feeding of most fish species with zooplankton since they result into healthier larvae growth. Awaiss (1991) for example found a high survival of gudgeon (G. gobio) larvae that received *B. calyciflorus* rotifer cultured on algae indicating the nutritional superiority of *B. calyciflorus* rotifer in fish larval nutrition. *Artemia* currently forms the basis for African catfish larval nutrition in Uganda. Nonetheless, the associated shelf costs of utilizing *Artemia* and consequent reports of low survival of larvae necessitate a search for alternative live starter diets.

2.7 Fatty acids in fresh water fish

Little is known about the Essential Fatty Acid requirements of early life stages of freshwater fish species. This is because it is assumed that feeding newly hatched larvae or fry is not normally a problem with freshwater fish as they are generally large enough to accept formulated feeds whose composition can be defined to ensure maximal growth and survival (Tocher, 2010). However this is not the case with African catfish (*Clarias gariepinus*, Burchell 1822) larvae which during early stages of growth, the larvae fish rely on the yolk sac for nutritional requirements (Kolkvoski, 2001). At the onset of exogenous feeding, live feeds such as brine shrimp (*Artemia* nauplii/ cyst), yeast, zooplanktons and unicellular algae are more appropriate because the fish have difficulty assimilating dry prepared diets because of incomplete development of the digestive system (Kolkvoski, 2001). Live feed diets are ideal since they contain exogenous substances such as gut neuropeptides, enzymes and nutritional growth factors that minimize digestive efficiencies (Kolkvoski, 2001).

The high growth and development rates of the fish larvae demands for optimal nutrition to enhance larval growth and development and improve survival. Lipids are particularly important in fish nutrition not only for supplying calorific energy but also for providing the essential polyunsaturated fatty acids (PUFA) (Tan *et al.*, 2009). These polyunsaturated fatty acids (PUFAs) are essential for optimal physiological performance like somatic growth (Ballantyne *et al.*, 2003), reproduction, optimal pigmentation and immunity, and required for maintaining cell membrane fluidity (Kainz *et al.*, 2004; Das 2006; Tocher, 2010).

Highly unsaturated fatty acids are required in the diets of fish larvae for normal growth and survival, and also for cellular structure and function, including the maintenance of membranes

and eicosanoid metabolism in freshwater fish species (Tocher, 2010). The fatty acids are obtained from their diets especially zooplankton. The amount of fatty acid in rotifer is thought to control both survival and growth rate of fish larvae (Koven *et al.*, 1990). DHA is critical in neural and visual development of larvae, it has important structural and functional roles in all membranes as well, (Sargent et al., 1999; Feller 2008; Wassell and Stillwell 2008; Tocher, 2010). DHA deficiency is known to impair visual acuity (Bell et al., 1995; Shields et al., 1999). Given the extremely important function of DHA, its nutritional deficiencies have major effects on larval growth and development of neural and visual systems; with major impacts on the ability of larvae to capture their prey (McEvoy *et al.*, 1999). This could explain why, DHA is more efficient than EPA for improving growth and survival (Watanabe and Kiron, 1994).

With the distinguished function of fatty acids, all vertebrates require both n-6 and n-3 PUFA as well as their biologically active forms; the C20 and C22 metabolites of 18:2n-6 and 18:3n-3, specifically 20:4n-6 [Arachidonic acid (AA)], 20:5n-3 [Eicosapentaenoic acid (EPA)] and 22:6n-3 [Docosahexaenoic acid (DHA)], which in aquaculture are often termed highly unsaturated fatty acids (HUFA) (Tocher, 2010). These fatty acids are essential since, fish like other vertebrates, cannot synthesize de novo polyunsaturated fatty acids (PUFAs), given that fish do not possess the $\Delta 12$ and $\Delta 15$ desaturase enzymes necessary to produce 18:2n−6 (linoleic acid, LA) and 18:3n−3 (linolenic acid, LNA), respectively from 18:1n−9 (oleic acid, OA). Consequently fish larvae require a dietary supply of these essential fatty acids (Sargent *et al.*, 2002).

CHAPTER THREE

3.0 Materials and methods

3.1 Study area

This research project utilised the facilities of the wet laboratory of the Department of Zoology, Entomology and Fisheries Sciences, Makerere University for plankton culture and two Commercial Fish Farms for nutritional and growth performance experiments of African catfish larvae raised in indoor hatcheries, namely: Kireka Fish Farm (Temperature: 11-33.3°c, Rainfall: 1,320-2,200 mm/year and Altitude: 900-1340m above sea level) and Ssenya – Nalubowa Estates Fish farm (Temperature: 10-30°c, Rainfall: 1,100-1,200 mm/ and Average Altitude: 1,150m above sea level), in Wakiso and Masaka districts respectively.

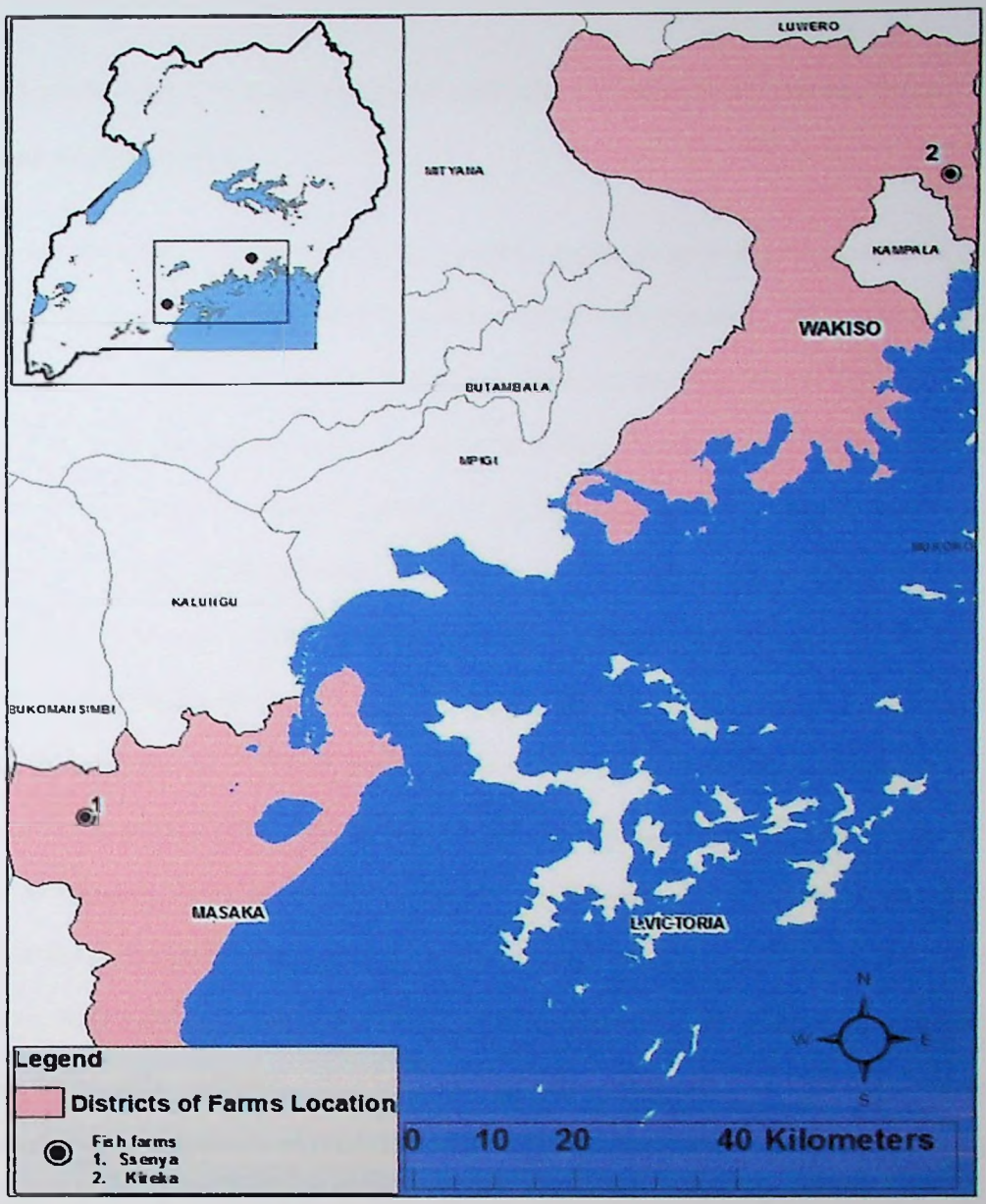

Figure 3.1: Map of Uganda indicating the study area (fish farms) where the experiments were conducted.

3.2 Growth performance (Total length) and survival of African catfish larvae fed on different *Artemia* strains

In order to test for effect of utilizing different *Artemia* strains on growth performance of African catfish larvae, three *Artemia* strains namely: USA - *Artemia franciscana*, Asia - *Artemia salina* and China -*Artemia* strain were used as live starter feeds on three-day old catfish larvae of the same batch for three consecutive days at Kireka Fish Farm hatchery unit. The catfish larvae were obtained on the farm by induced breeding of adult African catfish and subsequent hatching of eggs following routine catfish hatchery procedures. A total of nine experimental plastic tanks each of 30 litre capacity were utilized, set in triplicate and fitted in a hatchery unit flow through system. To each of these tanks, 500 newly hatched African Catfish larvae of a uniform initial mean Total Length (TL) of 8.07 mm were randomly introduced and maintained under ambient hatchery conditions on day three following loss of larval yolk sac. These larvae appeared healthy and active and had no signs of disease. Exogenous feeding was then commenced with, three commonly utilized *Artemia* strains in larval catfish feeding in Uganda namely; USA - *Artemia franciscana*, Asia - *Artemia salina* and China -*Artemia* strain that were identified and recorded through on farm visits. Artemia cysts used in this experiment were decapsulated following standard decapsulation procedures (Sorgeloos *et al.*, 1977). A feeding rate of 400 decapsulated *Artemia* cysts per larva per day was adopted. Water temperature, dissolved oxygen levels, pH and ammonia levels were monitored and maintained regularly after every 1 hour to appropriate catfish hatchery conditions (Temperature: 24-28°C, Dissolved Oxygen: 5-8 mgl^{-1}, pH: 6-8, and Ammonia: less than $0.1 mgl^{-1}$) during the experiment.

The fish larvae were fed for three days on starter feeds following commencement of exogenous feeding (day three) as is the practice of fish farmers in Uganda. The larvae were fed five times a

day in intervals of two hours and feed provided slightly above required estimates to allow feeding to satiation. Starting on day four to the sixth day, 30 larvae were randomly picked from each experimental basin one hour after the first feeding (9.00am) for measurement of total length (TL) as an indicator of growth since it was easier to measure, unlike wet weight measurement which was not feasible since the larvae were too small and fragile. Each of the 30 larvae were placed on filter paper to allow absorption of excess water and create a situation of inactivity before taking length measurements using a Vernier caliper to the nearest 0.05 mm. The mortalities of the fish larvae in each treatment were also recorded on a daily basis.

3.3 Growth performance (Total Length) and fatty acid profiles of African catfish larvae fed on *B. calyciflorus* and *Artemia*

3.3.1 Culture of algae (*Chlorella* spp.)

Chlorella sp. was cultured to act as a source of food for *B. calyciflorus*. 25 litres rectangular glass tanks were used for this culture experiment with continuous supply of aeration supplied through perforated air stones, to keep the *Chlorella* cells in constant circulation prevent settling and facilitate maximum exposure to light. 10g of each of Diammonium Phosphate and Urea were supplied to the *Chlorella* culture as a source of nutrients. The cultures were also supplied with 24 hour constant lighting using a single 40W (daylight) fluorescent tube (equivalent 1000Lux). Water quality parameters; ammonia and pH were monitored daily for the entire period of the experiment and always regulated to fit in the suitable parameters for *Chlorella* growth (Arinaitwe, 2012) by refreshing the cultures with chlorine free water. Counts of cells/ml were taken using a magnification of x200 on an inverted microscope (WILOVERT®) and a *Sedgwick*-Rafter Cell counting chamber to ensure that enough food for the rotifers is available before initiating their culture.

3.3.2 Acquisition of rotifers

Seed rotifers were collected by selective netting with 200μ, 100μ and 50μ zooplankton nets from "green" pond (eutrophic) water at the botanical gardens in Makerere University. To achieve a culture of only rotifers, 'Basudine' an organophosphoric acid ester was applied at a rate of $1.2mgl^{-1}$, following Agbon *et al.*, (2002). This chemical, at the set concentration, knocks off copepods, cladocerans, and mosquito larvae but does not harm rotifers, thereby allowing a clean rotifer population to flourish (Arimoro, 2007). Inoculum water containing the rotifers was completely renewed with 7.5mg/l Furazolidone (disinfectant). This aimed at achieving a population of pure rotifers. Disinfection consisted of killing free – swimming rotifers but not eggs using a cocktail of antibiotics (Erythromycin 10mg/l, Chlolamphenicol 10mg/l, Sodium oxolinate 10mg/l, penicillin 100mg/l, streptomycin 20mg/l). The eggs were separated from the dead rotifers using a 50 micron net and incubated for hatching. The offspring were then used for starting a stock culture.

3.3.3 Identification of *B. calyciflorus*

Identification of rotifers relied on morphological characteristics of the lorica, corona, and mastax (trophi) of dead animals based and behavioural characteristics of live animals. *Brachionus calyciflorus* is a slow swimming rotifer with 4 distinct anterior and posterior spines on the lorica, a feature that is known to be exclusive to the species. Reference to zooplankton identification keys was done to differentiate and isolate *B. calyciflorus* from other rotifers.

3.3.4 Isolation and inoculation

Isolation of *B. calyciflorus* was achieved by using sucker pipettes (Huawei® PIPETTE H100) of 10-30 micron (μ) aperture, coupled with a HUND WETZLER® binocular inverted microscope to

pick out individuals with at least one egg sac, placing them in 25ml EPA solution in a 100ml Erlenmeyer's flask, where *Chlorella* was introduced to allow growth and multiplication.

3.3.5 Population growth and enumeration of *B. calyciflorus*

Experiments were carried out in 10 and 20 litres plastic jerry cans. Batch and semi continuous culture techniques (Granvil and Allen, 2000) were used to culture sufficient numbers of *B. calyciflorus*. Initial culture enumeration was carried out by introducing *B. calyciflorus* rotifers into 8 litres of the treatment food (*Chlorella* sp.) suspensions. *Brachionus calyciflorus* were counted at x20 magnification and transferred into fresh algal suspensions (initial algal densities, x 10^4 cells/ml). Once a day, a 1ml sample was taken from which the number of individuals/ml were counted and recorded. All experiments were carried out at room temperature 25°C with a 12:12h light: dark cycle. To minimize sedimentation, the experimental tanks were gently bubbled with air from a compressor. At day seven of this culture, an adequate number of rotifers (2,700,000 individuals) for the fish larvae was attained. These *B. calyciflorus* rotifers were then transported from culture units at Department of Biological Sciences, Makerere University to Ssenya commercial fish farm in 20 litres plastic jerry cans.

3.3.6 Growth performance (Total length) of African catfish larvae

Commercial fish hatchery unit located in Ssenya fish farm, Masaka district, Uganda was utilized for this feeding experiment. This fish farm hatchery was utilized for testing performance of African catfish larvae fed on Chinese *Artemia* strain (most commonly utilized *Artemia* strain in Uganda), Rotifer *B. calyciflorus* and mixture of Chinese *Artemia* strain and Rotifer *B. calyciflorus* as live starter feeds. All water supplied to the hatchery was sieved through a 50 μm mesh to eliminate zooplankton contamination from the water supply ponds. Experimental plastic

basins (30 L) were used as culture tanks in triplicate of the three feed experiments and modified to fit in the flow – through system of the hatchery unit.

The African Catfish larvae were obtained following induced breeding of adult African catfish and subsequent hatching of eggs following routine procedures used at the farm. To each of the experimental tanks (30 litres capacity), five hundred (500) larvae of a uniform initial mean Total Length (TL) of 7.54 mm were randomly distributed and maintained under ambient hatchery conditions. These larvae appeared healthy and active and had no signs of disease. Water temperature, dissolved oxygen levels, pH and ammonia levels were monitored and maintained regularly after every 1 hour to appropriate catfish hatchery conditions (Temperature: 24-28°C, Dissolved Oxygen: 5-8 mgl^{-1}, pH: 6-8, and Ammonia: less than $0.1 mgl^{-1}$) during the experiment. African catfish larvae were fed for three days on the starter feeds (*Artemia, B. calyciflorus* and mixture of the 2 diets) following commencement of exogenous feeding (day three) as is the practice of fish farmers in Uganda. This experiment was conducted using the China- *Artemia* strain since most catfish hatcheries (more than 90% of famers) in Uganda use this strain as it was discovered while addressing objective 1 of this study. Feeding rate of 400 rotifers per larvae per day (Adigun, 2005) was applied. Decapsulation of *Artemia* cysts followed standard decapsulation procedures (Sorgeloos *et al.*, 1977) and a similar feeding rate of the fish larvae as for rotifers adopted. Fish larvae were fed five times a day at an interval of two hours and feed provided slightly above required estimates to allow feeding to satiation.

Starting on fourth day to the sixth day, 30 larvae were randomly picked from each experimental basin one hour after the first feeding (9.00am) for measurement of Total Length (TL) as an indicator of growth since it was easier to measure, unlike wet weight measurement which was

not feasible since the larvae were too small and fragile. Each of the 30 larvae were placed on filter paper to allow absorption of excess water and create a situation of inactivity before taking length measurements using a Vernier caliper to the nearest 0.05 mm.

3.3.7 Fatty acid profiles of African catfish larvae

On the sixth day of the feeding experiment, a sub sample of 30 larvae was randomly collected from each of the experimental tanks of each treatment, dried with filter paper and wrapped in aluminium foil taking special care to eliminate any contamination. Each sub sample was then labelled based on the treatment, stored under ice and immediately transported to the Department of Chemistry, Makerere University for fatty acid profiling.

3.3.7.1 Fatty Acid Analysis and Estimation

Fish larvae samples were dried in a hot air oven at a constant temperature of 60°C. The dried samples were extracted using Folch method (Folch *et al.*, 1956). Fatty acids in the extracted lipid were saponified and methylated using 2% NaOH in methanol, 14% BF /methanol and heptane. The fatty acid methyl esters (FAME) were determined on a Hewlett Packard HP 5890 gas chromatograph equipped with a flame ionization detector. The sample were injected at 190 °C onto a J and W Scientific DB23 fused silica capillary column (30 m x 0.25 mm i.d., 0.25-µm film thicknesses) with hydrogen as the carrier gas. The column was operated isothermally at an oven temperature of 180 °C and a detector temperature of 210 °C. Fatty acids were identified by comparing with authentic standards (Vengadeshperumal *et al.*, 2010).

3.4 Cost effectiveness of utilizing Rotifer *B. calyciflorus* as live starter feeds at a Commercial fish farm

In order to estimate cost effectiveness of utilizing *B. calyciflorus* as a live starter feed for African catfish larvae, sufficient numbers (at least 32,400,000 individuals) of rotifers were raised by mass culture using batch and semi-continuous culture systems at the rotifer culture Unit in the Department of Biological Sciences, Makerere University. *Chlorella* sp. were grown using the procedure described in 3.3.1 above and daily counts taken for a period of 13 days. The growth trend indicated the day at which maximum number of *Chlorella* spp. cells and maximum concentration per ml were obtained. These acted as a basis for estimating the period and thus labour and other inputs including tanks, electricity tubes, air tubes and stones that would be required to grow enough food needed by the *B. calyciflorus* rotifers (32,400,000 individuals). *B. calyciflorus* rotifers were reared on these obtained algae for a period of 8 days and similarly a growth trend obtained. The date with the highest population of *B. calyciflorus* and maximum concentration per ml was recorded and this was similarly used to estimate the required period, labour and other inputs including inputs air tubes and stones, jericans required to produce the needed *B. calyciflorus* rotifers.

B. calyciflorus rotifers were similarly transported to Ssenya Commercial Fish Farm in 20 litres plastic jericans and used as a starter feed for three days on a batch of 27,000 catfish larvae that were obtained by induced breeding of adult African catfish and stocked in triplicate at a density of 15 larvae/l in 600 litres capacity tanks following yolk absorption. This number of the fish larvae was determined based on the stocking rate used by the farmer in his hatchery tanks. 32,400,000 rotifers were required to sufficiently feed this batch for a period of three days following commencement of exogenous feeding. This was based on the reported daily demand

of 400 rotifers per catfish larvae (Adigun, 2005). Decapsulation of *Artemia* cysts followed standard decapsulation procedures (Sorgeloos *et al.*, 1977) and a similar feeding rate of the fish larvae as for rotifers adopted. On fourth day of exogenous feeding, post hatch, size (Total length) was estimated on a subsample of 30 larvae from each of the six hatchery tanks (3-*B. calyciflorus* tanks and 3- *Artemia* tanks). The cost of raising and feeding 27,000 catfish larvae using *B. calyciflorus* was compared to that of using *Artemia* (Chinese strain). Fixed and variable costs of raising the required numbers of *B. calyciflorus* relative to the growth performance by day four as compared to cost of shelf *Artemia* and relative growth of the catfish larvae in the same period of time were computed [cost in Uganda Shillings (UGX)].

3.5 Data analysis

Growth performance of *B. calyciflorus and Artemia* strain-China- fed larvae, and the larvae fed on the 3 *Artemia* strains was determined based on mean daily growth in larval total length and Specific Growth Rate (SGR). SGR was calculated as: SGR (%) = (ln L_f – ln L_i x 100) / t, where, ln L_f = the natural logarithm of the mean final Length (mm), ln L_i = the natural logarithm of the mean initial length (mm), t = time (days) between ln L_f and ln L_i (Ricker, 1975). Survival was calculated as *[Nt / No]*100,* where *No* is the initial total number of the larvae, *Nt* is the total number of larvae at the end of the feeding experiment. The data obtained were tested for normality and homogeneity of variances and later compared in the feeding trials (treatments) using one-way Analysis of Variance (ANOVA) after the collected data conformed to all the ANOVA assumptions. Fatty acid profiles (nutrition status indicators) of the three settings were determined. Comparisons were also made to estimate the most cost effective starter larval feed while raising African catfish larvae by the initial starter feeding using *B. calyciflorus* and *Artemia*.

CHAPTER FOUR

4.0 Results

4.1 Comparison of growth and survival of African catfish larvae fed on different starter live food (*Artemia* strain-China, *Artemia salina*-Asia *and Artemia franciscana*-USA)

Figure 4.1 shows the trend of catfish larvae growth fed on three different diets from the first day of exogenous feeding to the third day. One way Analysis of Variance (ANOVA) showed no significant differences (F= 0.875, P=0.42) in African catfish larvae growth in TL among the 3 treatments. Figure 4.2 shows the Specific Growth Rate (SGR) of the larvae for the 3 treatments. Similarly, one way ANOVA indicated no significant difference in the SGR of the larvae across the 3 treatments (F=0.882, P=0.418).

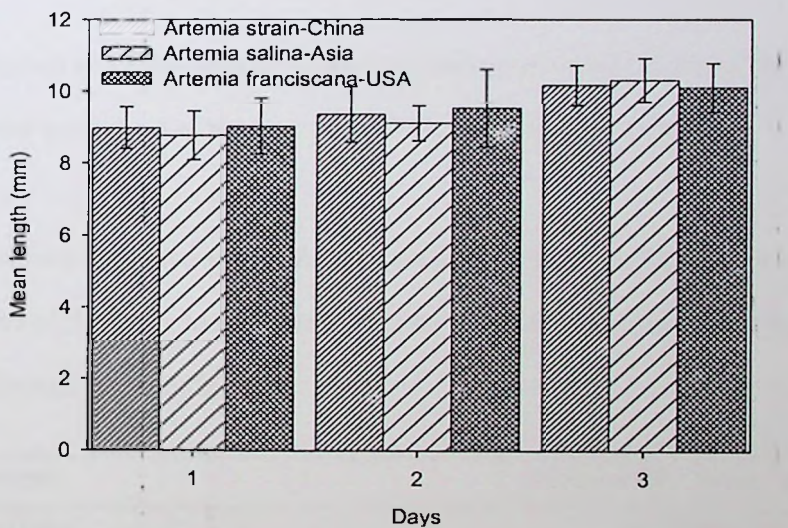

Figure 4.1: Mean length (mm) ± SD of African catfish larvae fed on three Artemia strains-experimental diets (Artemia strain -China, Artemia salina-Asia and Artemia franciscana -USA) from day 1 to day 3 following commencement of exogenous feeding

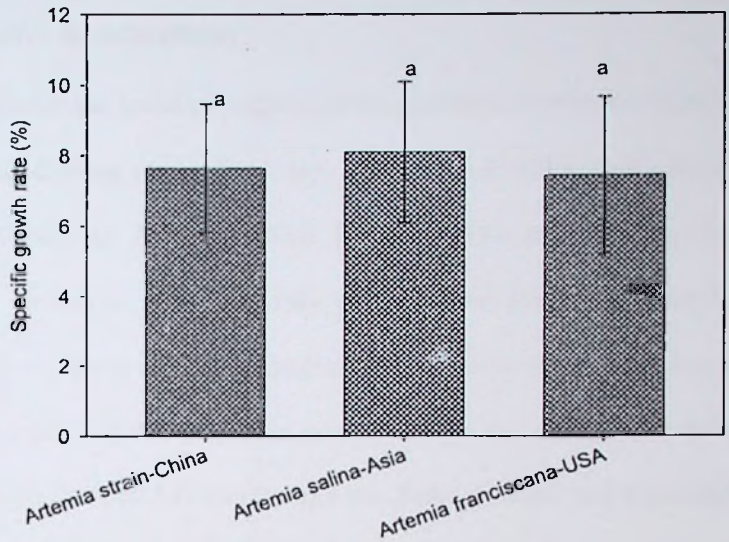

Figure 4.2: Specific Growth Rate (SGR % ± SD) of African catfish (*Clarias gariepinus*) larvae fed on three different *Artemia* strains- experimental diets (*Artemia* strain-China, *Artemia salina*-Asia and *Artemia franciscana* -USA) [Superscripts on the bars indicate no significant difference (p>0.05) in SGR)]

Mortalities occurred in all the treatment tanks and there was no significant difference in survival rates of the larvae in all the 3 treatments (Table 4.1; F=1.485; P=0.299).

Table 4.1: Mean survival rates (%) of African catfish larvae fed 3 different *Artemia* strains during the period of the study in a hatchery [Similar superscripts on the values indicate no significant difference (p>0.05) in SGR]

Feeding treatment	**Mean ± SD %age survival**
Artemia strain-China	98.93±0.50[a]
Artemia salina-Asia	99.70±0.35[a]
Artemia franciscana-USA	99.07±0.81[a]

4.2 Comparison of growth of catfish larvae fed on decapsulated *Artemia* (Chinese strain) and Rotifer *B. calyciflorus*

Figure 4.3 shows the trend of catfish larvae growth fed on three different diets from the first day of exogenous feeding to the third day. One way ANOVA revealed significant differences (F= 47.605, P=0.000) in African catfish larvae growth measured as Total Length (TL) among treatments. However, Turkey's HSD test indicated Rotifer-*Artemia*-fed African Catfish larvae to have a significantly (P=0.000) higher TL (10.04±0.45) mm at the end of the experiment than other treatments. Rotifer-*Artemia* was followed by Rotifer-fed larvae (9.04±0.58) mm and finally *Artemia* (8.78±0.54) mm though the Turkey's HSD test indicated no significant difference (P=0.14) between the 2 treatments. Similarly, one way ANOVA indicated significant differences in Specific Growth Rate (SGR) of catfish larvae among the 3 diets F=46.162, P = 0.000). Turkey's HSD test however indicated that Rotifer-*Artemia* treatment had a significantly high SGR (Figure. 4.4; P=0.000) followed by Rotifer-fed larvae fed and finally *Artemia*- fed larvae with no significant difference in the Rotifer and Artemia treatments (P=0133).

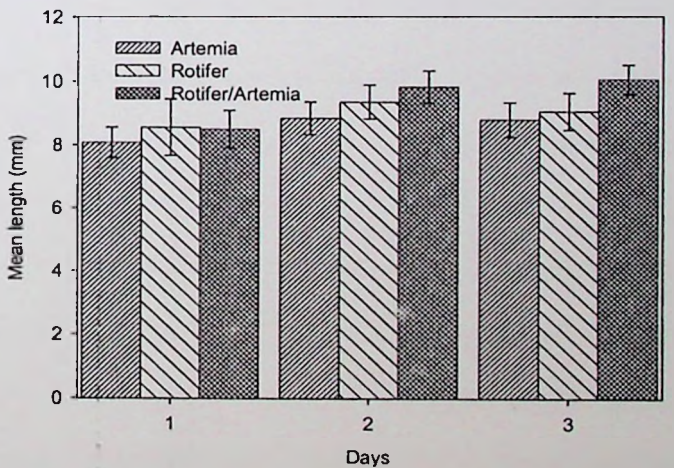

Figure 4.3: Mean length (mm) ± SD of African catfish larvae fed on three experimental diets (*Artemia*, rotifer, and a mixture of *Artemia* and rotifer) from day 1 to day 3 following commencement of exogenous feeding

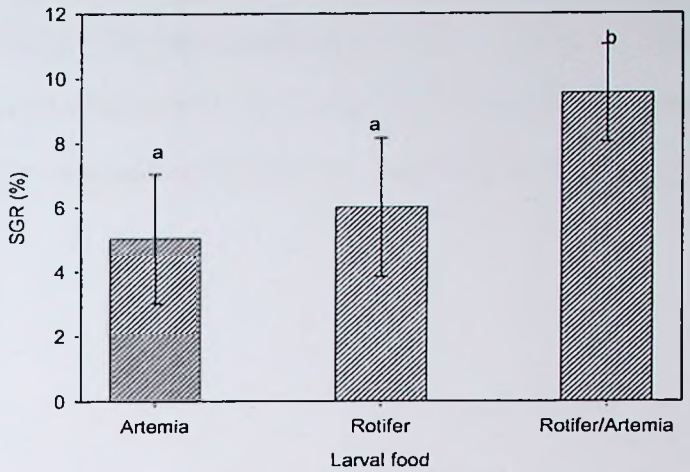

Figure 4.4: Specific Growth Rate (SGR % ± SD) of African catfish larvae fed on three experimental diets (*Artemia*, rotifer, and a mixture of *Artemia* and rotifer) [Different superscripts on bars indicate significant difference ($p < 0.05$) in SGR while similar superscripts on bars indicate no significant difference ($p > 0.05$)].

4.3 Fatty acid profiles of catfish larvae fed on different diets for three days

The analysis of fatty acids in the African catfish larvae samples indicated variation in fatty acid composition across feed treatments (Table 4.2). The composition of saturated fatty acids was higher in rotifer-fed African catfish larvae (38.92±0.65) % as when compared to *Artemia*-fed (35.16±1.41) % and Rotifer/*Artemia*-fed larvae (34.66±0.67) %. Noteworthy was the higher concentrations of 16:0 and 18:0 of the saturated fatty acids presented across the diets and with a higher concentration in the rotifer-fed catfish larvae. Rotifer-fed catfish larvae demonstrated a comparably low composition of total monounsaturated fatty acids (MUFAs) (16.18±0.45) %. *Artemia* + Rotifer-fed larvae had a higher composition of total MUFAs (26.96±1.10) % followed by *Artemia* fed larvae (21.3±0.30) %. Of the MUFAs present, 16:1n5, 18:1n7 and 18:1n9 were generally high across diets but comparably much higher in *Artemia*-fed larvae than in Rotifer-fed larvae. The composition of polyunsaturated fatty acids was higher in Rotifer-fed larvae

(43.10±1.51) % followed by *Artemia* + Rotifer-fed larvae (38.38±1.38) and finally *Artemia* -fed larvae (37.85±2.15) %. The high composition of DHA; 22:6n3 (17.68±0.43) % in Rotifer-fed larvae as when compared to *Artemia*-fed larvae (14.58±0.86) % was notable. Rotifer- fed larvae also recorded higher proportions of DHA/EPA and AA/EPA than was the case for *Artemia*-fed larvae.

Table 4.2: Main fatty acid composition (% of total fatty acids ±SD) of catfish larvae fed on three different experimental diets

Fatty acid	Artemia	Rotifer	Artemia + Rotifers	
14:0	0.59±0.00	0.54±0.01	0.86±0.03	
15:0	0.80±0.16	1.02±0.03	0.78±0.01	
iso15:0	0.37±0.03	0.47±0.04	0.47±0.01	
iso 17:0	0.0	0.38±0.00	0.40±0.00	
16:0	18.39±0.73	20.18±0.33	18.80±0.27	
17:0	0.92±0.04	0.99±0.07	1.03±0.01	
18:0	14.11±0.45	15.35±0.19	12.33±0.33	
∑SFAs	**35.16±1.41**	**38.92±0.65**	**34.66±0.67**	**F=20.979, P=0.017**
14:1n5	0.0	0.0	0.36±0.03	
16:1n4	0.88±0.05	1.17±0.03	0.86±0.01	
16:1n5	3.35±0.08	1.54±0.03	5.04±0.45	
16:1n7	0.48±0.06	0.49±0.04	0.74±0.07	
17:1n9	0.0	0.0	0.85±0.09	
18:1n7	5.10±0.05	3.30±0.06	6.11±0.22	
18:1n9	11.49±0.05	9.67±0.28	13.00±0.23	
∑MUFAs	**21.3±0.30**	**16.18±0.45**	**26.96±1.10**	**F=140.182, P=0.001**
18:2n6(LA)	6.50±0.37	6.49±0.16	8.25±0.23	
18:3n3(LNA)	1.45±0.19	1.89±0.26	2.77±0.06	
20:2n6	1.13±0.05	1.41±0.06	0.98±0.01	
20:3n6	1.98±0.15	2.47±0.02	1.84±0.01	
20:4n6(ARA)	6.20±0.37	7.32±0.25	5.50±0.18	
20:5n3(EPA)	3.71±0.01	3.16±0.15	4.43±0.16	
22:5n6	0.93±0.14	1.37±0.10	0.84±0.07	
22:5n3	1.36±0.02	1.30±0.09	1.18±0.01	
22:6n3(DHA)	14.58±0.86	17.68±0.43	12.59±0.67	
∑PUFAs	**37.85±2.15**	**43.10±1.51**	**38.38±1.38**	**F=13.492, P=0.032**
DHA/EPA	3.93	5.59	2.84	
AA/EPA	1.67	2.32	1.24	
DHA/AA	2.35	2.42	2.29	

Note: ∑SFAs: Sum of Saturated Fatty Acids; ∑MUFAs: Sum of Monounsaturated Fatty Acids; ∑PUFAs: Sum of Polyunsaturated Fatty Acids; DHA: Docosahexaenoic Acid; EPA: Eicosapentaenoic Acid; AA: Arachidonic Acid; LA: Linoleic Acid; LNA: Linolenic acid

Of the essential fatty acids, variation in composition was similarly observed across diets (Figure 4.5). Fish larvae fed on Rotifer indicated significantly high percentage composition of Docosahexaenoic acid (DHA, 22:6n3; F=28.740, P=0.011) and Arachidonic

Acid (AA, 20:4n6; F=22.292 P=0. 016). On the other hand, *Artemia*-fed larvae, had slightly higher levels of Linoleic Acid (LA, 6.5%) and Eicosapentaenoic Acid (EPA, 3.71%), than Rotifer - fed larvae. Consequently, larvae fed on a combination of *Artemia* and Rotifer had significantly higher percentages of three of the essential fatty acids: Linoleic acid (LA, 18:2n6; F= 29.405, P=0.011), Linolenic acid (LNA, 18:3n3; F=25.926, P=0. 013) and Eicosapentaenoic acid (EPA, 20:5n3; F=51.908, P=0.005), and higher composition of all the three essential fatty acids when compared to *Artemia* only-fed larvae.

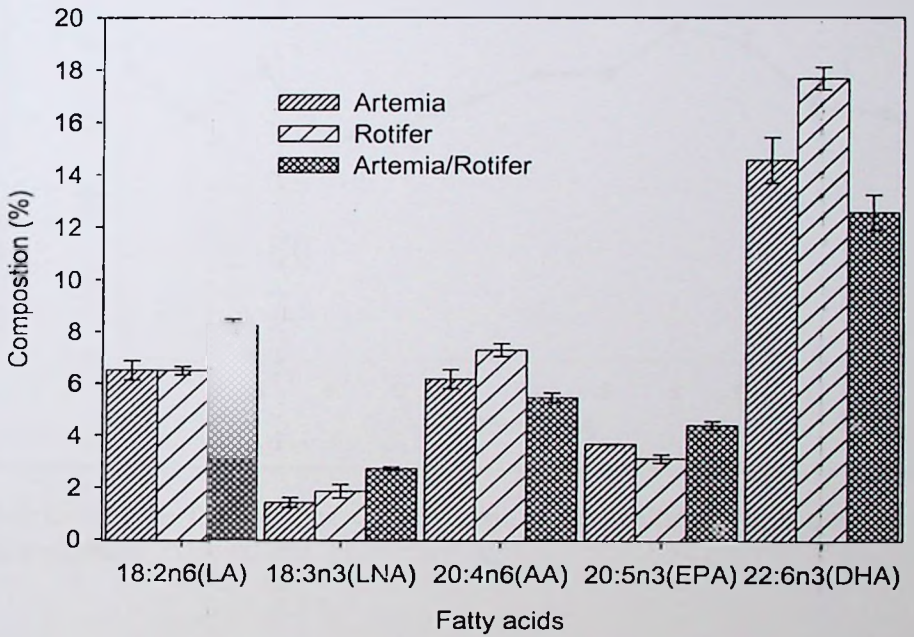

Figure 4.5: Essential fatty acid composition of African catfish larvae fed on different starter feeds.

4.4 Cost effectiveness of utilizing *B. calyciflorus* and *Artemia* -Chinese strain as a live starter feed for African catfish larvae

While culturing algae (*Chlorella* spp.), 95% growth of isolated *Chlorella* spp. was achieved and the maximum ($2.09\times10^5\pm4.26$) cells of *Chlorella* spp. (cell.ml^{-1}) recorded on day nine as indicated by the growth trend (Figure 4.6). However, maximum (334± 48.14) *B. calyciflorus* individuals (ind.ml^{-1} were obtained at day five (Figure 4.7)

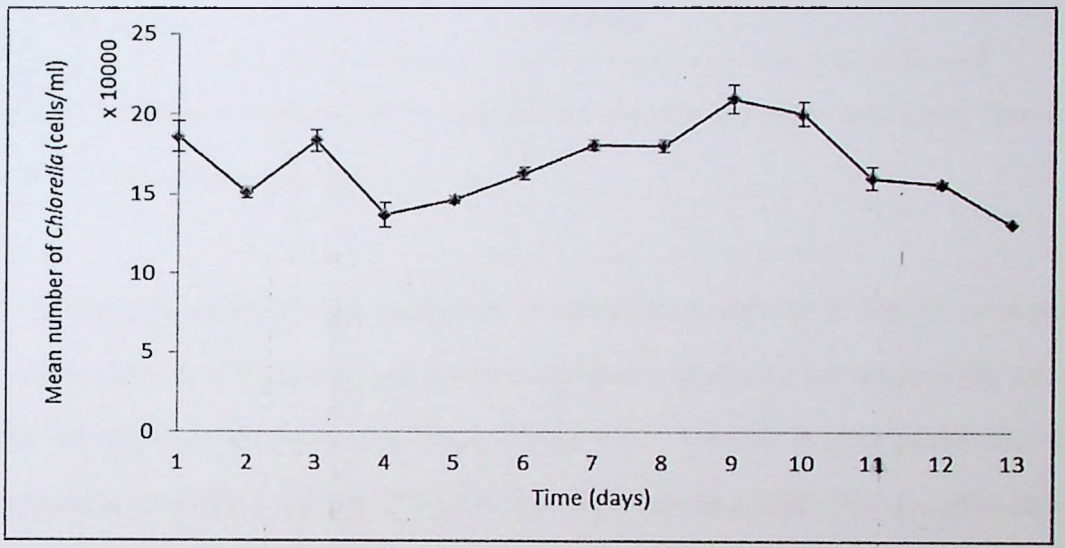

Figure 4.6: Growth performance of *Chlorella* sp. (mean cells/ml) with time as observed in DAP/UREA medium.

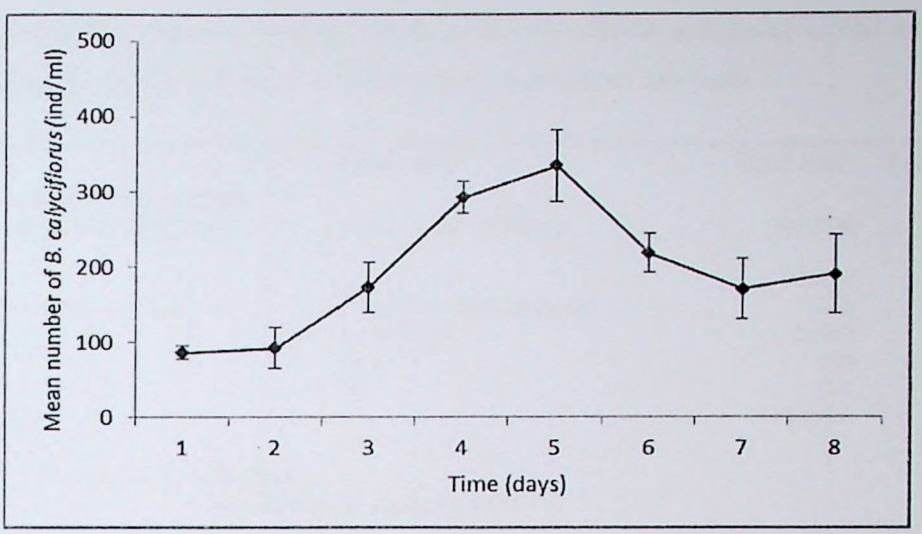

Figure 4.7: Growth performance of *B. calyciflorus* (mean rotifers/ml) with time, observed in *Chlorella sp.* medium.

The total cost of inputs involved in production of sufficient numbers of *B. calyciflorus* that were used to feed African catfish larvae was obtained together with the cost of *Artemia* that was used to grow the same number of the fish larvae (Table 4.3). Overall, *B. calyciflorus*- fed Africa larvae required inputs that cost least (169,641.2=), while rearing 27,000 African catfish larvae as compared to *Artemia*- fed larvae (199,676.29=) for the same number of fish larvae. Similarly, *B. calyciflorus*- fed African catfish larvae had performed better in terms of growth; 10.34±0.45 mm (mean ± SD) on the fourth day compared to *Artemia*-fed larvae; 8.98±0.44 mm (mean ± SD) with similar initial average Total length (mm) of the African catfish larvae was 7.24 ± 0.34 (mean ± SD) .

Table 4.3: Cost effectiveness of utilizing Rotifer (*B. calyciflorus*) and decapsulated *Artemia* (Chinese strain) as live starter feeds on a Commercial fish farm

Item	Quantity	Unit cost	Total Cost
Fixed costs for *B calyciflorus*			
Indoor glass tanks for raising algae	6 tanks (each 25 litres)	180,000	1,080,000
Tank stands	4	10,000	40,000
Jerry cans for rearing rotifers	8 Jericans (each 20 litres)	5,000	40,000
Buckets	1 (20litres)	10,000	10,000
Tubings	20m	500	10,000
Air stones and T-joints	14	5,000	70,000
Electric pump	1	150,000	150,000
Total fixed costs			1,400,000=
Fixed cost amortized for 20 years			
Total Fixed costs (TFC)			**70,000=**
Variable costs *B calyciflorus*			
Fertilizer(DAP/UREA) for growing algae	3kgs for DAP, 3kgs for UREA	1,000	6,000
Labour for raising *B. calyciflorus*	14 days	5,000	70,000
Aluminium foil	1piece	5,000	5000
Water	200litres	3.08	617.2
Disinfectants	0.5 litres	20,000	10,000
Electricity (both for algae, rotifers)	30240 watts=30.24units	100	3,024
Basuidine	1	5,000	5,000
Total Variable costs(TVC)			**99,641.2=**
Total cost of production	**Total Variable cost +Total Fixed cost**		**169,641.2=**
Fixed costs for *Artemia*			
Artemia	129.6g	560	72,576
Variable costs *Artemia*			
Jik for decysting Artemia	6 litres	12,000	72000
Vinegar for cleansing *Artemia*	4litres	5,000	20000
Labour for decysting *Artemia*	3 days	5,000	15000
Water	15 litres	3.08	46.29
Disinfectants	1 litre	20,000	20000
Electricity (decysting *Artemia*)	540 watts=0.54 units		54
Total Variable costs(TVC)			**127,100.29**
Total cost of production	**(Total Variable cost +Fixed cost)**		**199,676.29=**

CHAPTER FIVE

5.0 Discussion

5.1 Comparison of growth of catfish larvae fed on 3 different strains (USA - *Artemia franciscana*, Asia - *Artemia salina* and China -*Artemia* strain)

In this study, the survival and growth performance of African catfish larvae did not significantly differ amongst the *Artemia* diet treatments contrary to previous studies. Seidel *et al.*, (1982) and Patrick *et al.*, (1988) revealed that the nutritional value of *Artemia* is not constant but varies with geographical source and time scale justifying the nutritional ineffectiveness of some commercially available sources of *Artemia* as reported by Olney *et al.*, (1980) and Schauer *et al.*, (1980). This explains the observed growth differences of the African larvae during this study. Naser and Sorgeloos (2005) accounted for such differences based on variations of Essential Fatty Acids (EFAs) levels with geographical strain and even from batch to batch as may be caused by fluctuations in biochemical composition of the primary producers available to the adult population. The observed differences in growth performance of African catfish larvae fed on the different *Artemia* strains could also be attributed to the fact that the quality of *Artemia* strains may decline because of differences in the technology of harvesting or processing Vanhaecke *et al.*, (1990) favouring some strains over others. Similarly, the possibility that some of the branding facilities or companies for *Artemia* may not necessary be the harvesters of *Artemia* from a given locality but possibly processors and packers of strains sourced from different localities may conceal the impact of the reported nutritional differences among *Artemia* strains.

5.2 Comparison of growth of catfish larvae fed on different starter live food (*B. calyciflorus*, *Artemia* (Chinese strain) and *B. calyciflorus-Artemia* mixture)

The superior growth performance of catfish larvae fed on a combination of rotifer *B. calyciflorus* and *Artemia* agrees with previous studies that proved that live feed mixture containing different live feeds provides a wide spectrum of live feeds for choice over the experimental period (Gamal, 2001; Herath and Atapaththu, 2012). In this case, the availability of different prey sizes as the mouth gape of fish larvae undergoes ontogenic development provide all the possibilities of preferred prey size for the growing larvae.

The observed differences in growth performance of *B. calyciflorus*- fed catfish larvae compared to *Artemia* could be attributed to several reasons; The fact that *B. calyciflorus* rotifers offer a much smaller live feed than the size of decysted *Artemia*, could have favoured the initial stages of African catfish larvae. Jeje (1992) reports that the larvae of African catfish are small at hatching less or equal to 4mg and 7mm in weight and length respectively. The small size of this larvae possibly thrives better on small zooplanktons especially rotifers whose ideal size ranges from 50-200 microns (Pronob *et al.*, 2012) compared to decysted Artemia cysts whose size range from 200 to 300 microns), depending upon the strain (Verreth *et al.*, 1987; Granvil, 2000). This size suitability coupled with their relative mobility makes it easier for the rotifers to be found and captured as food with lower energetic cost (Ludwig and Lochmann, 2000).

Additionally, differences in the nutritional composition of rotifers and *Artemia* could explain the growth trends revealed in this study. Rotifer-fed larvae were richer in essential fatty acids together with the combination with rotifers and *Artemia*, which accounts for improved growth

rate. This observation is in accordance with other previous researchers (Watanabe *et al.,* 1983 and Dhert *et al.*, 2001)) who indicated that rotifers confer better nutritional benefits to fish larvae since they are able to transfer fatty acids and other nutrients through the algae-rotifers-larvae food chain.

The better growth performance of rotifer-fed African catfish larvae also corresponds with the high levels of DHA in the rotifer diet. The larval fatty acid profiles are always reflection of the diet profiles (Lund *et al.*, 2007). DHA, an essential fatty acid that accumulates in the brain of fish during early development and functions to increase neural functions (Bell *et al.,* 1995), is easily incorporated in rotifers, unlike *Artemia* which catabolizes this fatty acid (Dhert *et al.*, 1993). Docosahexaenoic acid (DHA) also has important structural and functional roles in all membranes, but especially neural membranes (Feller, 2008; Wassell and Stillwell, 2008; Tocher, 2010). Sargent (1996) also noted that n-3 polyunsaturated (PUFA), principally DHA, has a role in maintaining the structure and functional integrity of fish cells with a specific and important role in neural (brain and eyes) cell membranes. Fish larvae are thought to be visual feeders, adapted to attacking moving prey in nature (Conceica *et al.*, 2010). Therefore higher DHA content in rotifer-fed larvae compared to *Artemia*- fed larvae in this study could explain better growth due to improved visual performance of the larva leading to more larval feeding responses. These results are in accordance with some studies that revealed that DHA is very paramount for various physiological functions, including survival, growth, and pigmentation success (Kanazawa, 1993; and Sorgeloos *et al.*, 1995).

5.3 Fatty acid profiles of catfish larvae fed on different diets for three days

Lipids occur naturally in the *Artemia* embryos (cysts) and zooplankton like rotifers, and because of their significance in fish nutrition, fatty acid composition was used to evaluate the quality of the feed for larval fish in this study. The fatty acids of *B. calyciflorus* rotifers and *Artemia* were *not* determined since fish larvae fatty acid composition reflects the composition in their diets (Millamena *et al.*, 1985; Sorgeloos *et al.*, 1998).

The predominance of PUFAs and SFAs as when compared to MUFAs across the test diets in this study, is in agreement with previous findings (Wiegand, 1996). Preferential catabolism of MUFAs along with preferential retention of DHA, EPA and AA, and specific SFAs, usually 16:0 or 18:0 by embryos of a variety of species has been recorded (Wiegand, 1996). This reflects the essential structural role of DHA in membranes, the importance of AA and EPA in eicosanoid production and specific roles of SFAs in the sn-1 position of structural phospholipids (Rainuzzo, 1993; Tocher, 2003). Amongst the SFAs, Palmitic acid (16:0) was predominant. This is in agreement with other previous researchers (Ackman, 1988; Osibona *et al.*, 2006) who observed that Palmitic acid (C16:0) is a key metabolite in fish.

In this study, total replacement of *Artemia* with *B. calyciflorus* rotifers resulted into quantitatively higher concentration of three (LNA, AA, DHA) of the five recorded essential fatty acids. Interestingly, DHA concentration was remarkably high compared to AA and LNA compositions. This suggests higher DHA concentration in rotifers, attributed to the ability of the rotifer *B. calyciflorus* to convert linoleic acid (18:3n3) to AA and finally DHA as reported by *Sargent et al.*, (2002). Similarly, rotifer *B. calyciflorus* converts linolenic acid (18:3n3) to EPA

(20:5n3) and consequently to DHA (22:6n3) as reported by Cutts *et al.*, (2006). This further explains the reason for higher DHA levels in the *B. calyciflorus*- fed fish larvae compared to *Artemia* fed- larvae thus demonstrating the nutritional superiority of *B. calyciflorus* rotifers.

This higher concentration of DHA in the *B. calyciflorus*-fed larvae in this study corresponded with high specific growth rate of the larvae which agrees with studies by Kanazawa, 1993; Koven *et al.*, 1993; Watanabe and Kiron, 1994; and Sorgeloos *et al.*, 1995, that revealed the significance of DHA in controlling various physiological functions such as growth and survival. The remarkably high level of polyunsaturated fatty acids in rotifer-fed larvae in this study is a reflection of the fatty acid levels obtained from their food (rotifers). This observation is in accordance with other researchers; Hache and Plante (2011) who demonstrated that rotifers catabolize fats easily and can store highly unsaturated fatty acids (HUFA). These fatty acids are later passed onto the fish along the food chain.

The high concentration of AA in *B. calyciflorus* rotifer-fed catfish larvae in this study may further explain improved growth performance of catfish larvae as compared to when *Artemia* was used. Arachidonic acid (20:4n6; AA) is believed to be the chief source of eicosanoids in fish (Tocher, 2010). Eicosanoids produce highly bioactive molecules following regulated dioxygenase enzyme-catalysed oxidation of HUFA 20:4n6 (AA) and 20:5n3 (EPA). In fish, these molecules are involved in a great variety of physiological functions including blood clotting, immune and inflammatory responses, cardiovascular tone, renal and neural functions (Schmitz and Ecker, 2008).

The low levels of essential fatty acids in *Artemia* fed fish larvae in this study also agrees with other previous studies (Bell *et al.*, 2003; Olivotto *et al.*, 2006 and Tizol-Correa *et al.*, 2006) who reported that, *Artemia* are deficient of essential fatty acids necessary in larval development, larval health, proper growth, prevention of anemia and survival. Bell *et al.*, 2003 study also reports that the concentration of DHA in the polar lipid fraction of *Artemia* is very low further explaining the low levels of DHA in *Artemia*-fed larvae in this study.

Larvae fed on a diet combination had the highest composition of 2 essential fatty acids (LA and LNA) and thus explaining better growth performance of larvae fed on diet combination. Henderson and Tocher, (1987) and Sargent *et al.*, (2002) made a similar observation and explained that LA and LNA are true EFAs in freshwater fish species. This is because fish, like other vertebrates, cannot synthesize de novo polyunsaturated fatty acids and consequently require a dietary supply of these essential fatty acids (EFAs) since they do not possess the Δ12 and Δ15 desaturase enzymes. These enzymes are necessary to produce 18:2n−6 (linoleic acid, LA) and 18:3n−3 (linolenic acid, LNA), respectively from 18:1n−9 (oleic acid, OA), and because freshwater teleosts have an innate capacity to desaturate and elongate LA to 20:4n−6 (arachidonic acid, AA) and LNA to 20:5n−3 (eicosapentaenoic acid, EPA) and ultimately 22:6n−3 (docosahexaenoic acid, DHA) (Sargent *et al.*, 2002).

Although freshwater fishes have this ability to modify dietary LA and LNA; the rate at which they do so may be too low to satisfy the high DHA requirement especially during early larval growth (Vengadeshperumal *et al.*, 2010). Besides young fish deposit zooplankton fatty acids in total lipids with little change (Wetzel 1998), therefore DHA, EPA and AA can be designated as

"Essential Fatty Acids" for fish larvae too. In addition, according to Tocher, (2010), all vertebrate species require both n-6 and n-3 PUFA, but the biologically active forms of EFA are generally the C20 and C22 metabolites of 18:2n-6 and18:3n-3, specifically 20:4n-6 (arachidonic acid -ARA)), 20:5n-3 (eicosapentaenoic acid -EPA) and 22:6n-3 (docosahexaenoic acid -DHA), which in aquaculture are often termed highly unsaturated fatty acids (HUFA). Sargent *et al.*, 1999 and Hasan, 2001, also noted that EPA (20:5n-3) and DHA (22:6n-3) acids are essential fatty acids for larviculture of both marine and freshwater fish and crustaceans.

5.4 Cost effectiveness results of utilizing *B. calyciflorus* and *Artemia* (Chinese strain) as a live starter feed for African catfish larvae

This study showed that sufficient numbers of rotifers needed to feed fish larvae in a hatchery could be raised and utilized more cost effectively as when compared to *Artemia* cysts. Considerations of utilizing rotifers as an alternative to *Artemia* use are therefore probable. Dhert *et al.*, (1998, 2001) recommended for use of cheaper alternative diets with comparable nutritional quality to *Artemia* since the high price of *Artemia* cysts increases fish production cost. According to Ajah (1998), the choice of rotifers for early fish larvae has the advantage for providing a low cost, highly predictable and reliable production of food organisms which is acceptable and easily caught by the fish larvae. This study has proved that using *B. calyciflorus* as a choice rotifer by Ugandan fish farmers in African catfish larvae feeding would be a better alternative to *Artemia* since both better growth rates that would result in a shorter hatchery period combined with low costs of production inputs have been recorded.

CHAPTER SIX

6.0 Conclusion and recommendations

6.1 Conclusion

It can be inferred from the results obtained from the study that *B. calyciflorus* rotifers confer a better specific growth rate to the African catfish larvae as compared to the case of utilizing decysted *Artemia* cysts. Notably however, is the best specific growth rate conferred to the African catfish larvae by a diet combination of *B. calyciflorus* and *Artemia*. Secondly, the better growth performance of *B. calyciflorus*-fed catfish larvae can be explained well based on the nutritional superiority of *B. calyciflorus* coupled with its suitable size of prey and mobility qualities as compared to *Artemia*. Besides *B. calyciflorus* yielding good growth performance, results indicate that it is cost effective to utilize *B. calyciflorus* as starter feed as compared to *Artemia* in larval fish nutrition. A combination of these factors favours B. *calyciflorus* as suitable substitutes for *Artemia* in the feeding of African catfish larvae. However, a combined diet of decysted *Artemia* and *B. calyciflorus* as investigated in this study provides even a better substitute to *Artemia* alone.

6.2 Recommendation

Results and conclusions of this study call for further efforts in mass culture of *B. calyciflorus* as a prerequisite for its subsequent use as an alternative to *Artemia* by Ugandan fish farms because they can compete favourably with *Artemia* in fish larvae growth performance and are cost effective. In addition, farmers should consider a combined use of both rotifers and *Artemia* as live starter feeds. While this study covered four experimental feeding days for only larval

feeding using a live starter feed (*B. calyciflorus*), a recommendation is made for a study that will further assess the growth performance of these same larvae during the weaning period.

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APPENDICES

Appendix 1: Culture set up of *Brachionus calyciflorus* rotifers housed at the Department of Biological Sciences, Makerere University.

Appendix 11: Questionnaire used in determine the commonly used *Artemia* strain in Ugandan catfish hatcheries

Commercial fish hatcheries practices in Uganda; Tool for obtaining information on the *Artemia* use in African catfish hatcheries in Uganda.

Name of interviewer:.....Questionnaire serial No.....

A. General information

Date	
Name of respondent	
Title of respondent	(1) Owner (2) Farm Manager (3) Relative (4) Group member (5) Spouse (6) Other
Gender of owner	1) Male juvenile (2) Male adult (3)Female adult (4) Female juvenile
Name of farm	
Owner of farm	
Age of farm owner	
Education level of owner	1)None (2) Primary (3) Secondary (4) Certificate (5) Diploma (6) Degree (7) Others (specify.....)
Contact: Telephone & or e-mail	
Year farm started	

B. Location of farm

GPS coordinates	Altitude:	N:	E:
Name of village &parish			
Name of sub-county			
Name of district			

C. Farm operations and production

Fish farming practice	1. Grow-out 2. Hatchery 3. Both 1& 2
Purpose of production	1. Sale 2. Stocking own-farm 3. Domestic consumption
Products from the farm	1. Fish seed 2. Table fish

D. Fish seed production annually

Species	Number of fingerlings	Average weight of fingerlings at harvest/sale(g)	Number of fingerlings sold	Number of fingerlings sold
1.Catfish				
2. Nile tilapia				
3. Others (specify…)				
How often did you sell the fingerlings? 1. Once a year 2. Twice a year 3. More than three times a year 4. Never				

E. Feeds and feeding management in fish seed production

Fish species	**Growth stage**	**Feed type**

1 = African catfish, 2 = Nile tilapia, 3=Mirror carp, 4=Others (specify)
1 = Fingerling, 2= Juvenile 3= Adult size
1 = *Artemia*, 2 = Rotifers, 3 = Moina, 4= Mixture of live feeds, 5=Others (specify)

F. *Artemia* use in fish seed production

Fish species	*Artemia* strain used	Growth stage

1 = African catfish, 2=others (specify)
1=*Artemia* strain-China, 2=*Artemia salina*-Asia, 3= *Artemia franciscana* –USA, 4=others (Specify)
1 = Fingerling, 2= Juvenile 3= Adult size

G. Constraints in Fingerling Production

Constraint	Suggested solutions
1. Expensive feed 2. High mortalities 3. Poor water quality 4. High operational costs 5. Diseases 6. Theft 7. Predators 8. Lack of market 9. Lack of technical advice 10. Scarcity of manufactured feed 11. Lack of power 12. Lack of skilled human resource 13. None 14. Others……..	1. Provide regular and adequate extension services 2. Fencing 3. Technologies for making feeds on-farm 4. Training in marketing strategies 5. Improve pond security 6. Provide loans/grants 7. Ensure availability of affordable feeds 8. None 9. Avail power 10. Provide training to farm managers 11. Others……

Any other comments?

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……

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……

……

THANK YOU VERY MUCH FOR YOUR TIME

Appendix 111: African catfish larval growth (TL±SD) mm data when fed on decapsulated *Artemia* (China strain), *B. calyciflorus* rotifers and a combination of the 2 diets

Days	*Artemia*	Rotifer	Rotifer/*Artemia*
1	8.06± 0.48	8.54±0.89	8.48±0.59
2	8.83±0.51	9.34±0.53	9.81±0.50
3	8.78±0.54	9.04+0.58[a]	10.04±0.45

Appendix 1V: African catfish larval growth (TL±SD) mm data when fed on 3 *Artemia* strains

Day	*Artemia* strain-China	SD	*Artemia salina-Asia*	*SD*	*Artemia franciscana-USA*	*SD*
1	8.98	0.577267543	8.77	0.670585801	9.03	0.766087162
2	9.35	0.763142023	9.11	0.483141183	9.53	1.075989641
3	10.17	0.553552685	10.31	0.604387597	10.11	0.672476744

Appendix V: Common name and scientific name of fatty acids identified from African catfish larvae fed on three experimental diets, (*Artemia* decapsulated cysts, *Brachionus calyciflorus* rotifers and a mixture of *Artemia* and rotifers).

Fatty acid	common name	Scientific name
14:0	Myristic Acid	Tetradecanoic acid
iso15:0		14-methyl pentadecanoic
15:0		Pentadecanoic
16:0	Palmitic Acid	Hexadecanoic acid
16:1n7		cis 9-hexadecenoic
16:1n5		cis 11-hexadecenoic
16:1n4		
iso 17:0		16-methyl heptadecanoic
17:0	Heptadecanoic acid	Heptadecanoic
17:1n9	Heptadecenoic acid	
18:0	Stearic Acid	Octadecanoic acid
18:1n9	Oleic acid	9-Octadecenoic
18:1n7	Oleic acid	11-Octadecenoic
18:2n6	Linoleic Acid	9,12-Octadecadienoic acid
18:3n3	Alpha-Linolenic Acid (ALA)	9,12,15-Octadecatrienoic acid
20:2n6	Eicosadienoic acid	11,14-Eicosadienoic

20:3n6	Eicosatrienoic acid (omega6)	8,11,14-Eicosatrienoic
20:4n6	Arachidonic Acid (AA)	5,8,11,14-Eicosatetraenoic acid
20:5n3	EPA	5,8,11,14,17-Eicosapentaenoic acid
22:5n6	Clupanodonic acid(omega6)	
22:5n3	Clupanodonic acid(omega3)	
22:6n3	DHA	4,7,10,13,16,19-Docosahexaenoic acid