DETERMINATION OF BEST BIOTIC AND ABIOTIC FACTORS FOR FINGERLING PRODUCTION FOR OREOCHROMIS KARONGAE (TREWAVAS 1941)

HASSIB SAINAN

BSc. (AQUACULTURE AND FISHERIES), MALAWI

A THESIS SUBMITTED TO THE FACULTY OF NATURAL RESOURCES IN PARTIAL FULFILMENT OF REQUIREMENTS FOR AWARD OF THE DEGREE OF MASTER OF SCIENCE IN AQUACULTURE.

LILONGWE UNIVERSITY OF AGRICULTURE AND NATURAL RESOURCES

BUNDA CAMPUS

JULY, 2020

DECLARATION

I hereby certify that this research thesis is my own original effort and work and that is to the best of my knowledge. The findings have never been previously presented to Lilongwe University of Agriculture and Natural Resources or elsewhere for the award of any academic qualification. Proper references and acknowledgements have been provided where assistance was sought.

Hassib Sainan

Signed:

Date

CERTIFICATE OF APPROVAL

We, the undersigned, certify that this thesis is a result of the author's own work, and that to the best of our knowledge, it has not been submitted for any other academic qualification within the Lilongwe University of Agriculture and Natural Resources or elsewhere. The thesis is acceptable in form and content, and that satisfactory knowledge of the field covered by thesis was demonstrated by the candidate through an oral examination held on _____

Major Supervisor: Prof. Daud Kassam

Signature: _____Date: _____

Co-Supervisor: Prof. Jeremiah Kang'ombe

Signature: _____Date: _____

Co-Supervisor: Dr. Bernd Ueberschär

Signature: _____Date: _____

Co-Supervisor: **Dr. Joshua Valeta, Associate Professor**

Signature: _____Date: _____

DEDICATION

This work is dedicated to late Che Naliwa, my Grandfather, who thrived hard to make sure that I access education from the village during the time when going to school was considered as a waste of time and resources. May the Almighty Allah shower His mercy on him and reward him and those who encouraged him abundantly.

ACKNOWLEDGEMENTS

My sincere gratitude to the following people and departments for their contribution towards the successful completion of the study:

My Supervisors: Prof. Daud Kassam, Prof. Jeremiah Kang'ombe, Dr. Bernd Ueberschär and Dr. Joshua Valeta for encouragement and technical guidance throughout the course of study. I can't thank you all enough.

My parents, my family and all my friends that helped in creating a conducive environment for my studies

Dr. M. Gebert, Dr. S. Rakers, Jan Giebichenstein Christopher Haiduk, Frederik Kaiser and Bjarne Scholz who contributed a lot in terms classroom and out of class lessons during my stay in Germany.

Apatsa Chelewani, Kareem Longwe, Peter Chimangeni, Mr Isaac Chagwa, Mofolo Sifo, Mr Benson Lusangasi, Isaac Kaunda and the entire team at the aquaculture farm under the Department of Aquaculture and Fisheries, Lilongwe University of Agriculture and Natural Resources (LUANAR).

The entire members of staff at the Department of Aquaculture and Fisheries, Bunda College, LUANAR.

The department of Fisheries under the Ministry of Agriculture, Irrigation and Water Development, and the National Aquaculture Centre in Domasi, Zomba.

Technical teams at the Fraunhofer Research Institution for Marine Biotechnology and Cell Technology (EMB) in Lübeck and the Association for Marine Aquaculture (GMA) in Büsum, Germany.

This material is based upon work financially supported by the German Federal Ministry of Food and Agriculture (BMEL) based on the decision of the parliament of the Federal Republic of Germany through the Federal office for Agriculture and Food (BLE) under the project , 'Improving Community Health-Nutrition linkages through Solar energy-based Fish and crop integrated value chains' – Ich Liebe Fisch (I love fish), in partnership with Africa Center of Excellence in Aquaculture and Fisheries Science (AquaFish ACE) at LUANAR.

ABSTRACT

Fingerling production remains determinant of quality and quantity of fish harvests. In Malawi, lack of hatchery management expertise and understanding of best factors for fingerling production hinder availability and accessibility of good fingerlings. Studies were conducted to evaluate factors for Oreochromis karongae fingerling production. Four feed types (Malawi-Bunda, Malawi-Bunda plus live feeding, Zambia Novatek, German-Coppens) under three densities (2500, 5000, 7500 fry/m^3) were investigated. Results showed no significant interaction between the two factors on growth performance. Feed type and density significantly affected growth, fish fed with Coppens recorded highest final weight followed by Malawi-Bunda. Novatek feed was third and Malawi feed with live feeding was the least. Based on these findings, Malawi-Bunda feed (considering accessibility) and 2500 hatchlings /m^3 density were used to test effects of water temperatures. Fry were cultured in two different temperature levels (24 and 28^{o}C). Results indicated that 28^{o}C significantly improved weight gain. Based on these results, effect of photoperiod on fry growth was investigated. Fry were cultured in different photoperiod (16:8 and 24:0 LD). Results indicated that 24-hour photoperiod significantly improved weight gain. Another experiment tested hormone doses (20, 30, 60 and 90 mg/ml/Kg) administered to fry to determine efficiency. A 60mg/ml/kg of 17α-MT produced highest male proportion. The study concludes that proper feeds with suitable densities, water temperature and light regimes are crucial for maximised fry growth and proper hormonal dosage can help produce highest male percentage. The study recommends that hatcheries should use quality feeds and good culture conditions to improve growth performance.

TABLE CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

ACE	:	Africa Centre of Excellence
ADWG	:	Average Daily Weight Gain
AFW	:	Average Final Weight
AWG	:	Average Weight Gain
BLE	:	Bundesanstalt Fur Landwirtschaft Und ErnAhrung
BMEL	:	Bundesministerium für Ernährung und Landwirtschaft
CP	:	Crude Protein
CZAM	:	Zambian (Novatek Feed)
DAC	:	Decapsulated Artemia Cysts
DO	:	Dissolved Oxygen
DoF	:	Department of Fisheries
EFA	:	Essential Fatty Acids
EMB	:	Fraunhofer Research Institution for Marine Biotechnology and Cell Technology

FAO	:	Food and Agriculture Organisation
FCR	:	Feed Conversion Ratio
GDP	:	Gross Domestic Products
GEM	:	Germany (Coppens Feed)
GMA	:	Association for Marine Aquaculture
GoM	:	Government of Malawi
LD	:	Light to Darkness
LED	:	Light Emitting Diode
LMW	:	Live Feeding Malawian-Bunda feed
LUANAR	:	Lilongwe University of Agriculture and Natural Resources
MALDECO	:	Malawi Development Corporation
MT	:	Methyl Testosterone hormone
MW	:	Malawian-Bunda feed
NAC	:	National Aquaculture Centre
NFAP	:	National Fisheries and Aquaculture Policy

NRC	:	National Research Council
NSO	:	National Statistics Office
PUFA	:	Polyunsaturated Fatty Acids
SGR	:	Specific Growth Rate
UNDP	:	United Nations Development Programme
WHO	:	World Health Organisation

CHAPTER 1

INTRODUCTION

Background

Fisheries and aquaculture have been and they remain important sources of a health food, nutrition, income and livelihoods for hundreds of millions of people around the world. According to Food and Agriculture Organisation (FAO, 2016), global per capita fish supply reached a record of 20 kg in 2014. By the year 2016, fish provided 17% of animal protein consumed globally representing 6.7% of all protein consumed. Fish is a source of easily digestible and high-quality protein which contains all essential amino acids. It is also a major source of essential fats such as the long chain omega-3-fatty acids. Vitamins (such as Vitamin B, D and A) as well as minerals like calcium and iron can be obtained from fish (FAO, 2016). Global fish production was estimated at 171 million tonnes (Figure 1.1:) in the year 2016 of which aquaculture comprised of 47% (FAO, 2018). According to the same report, 110.2 million tonnes of aquaculture products was produced in the year 2016.

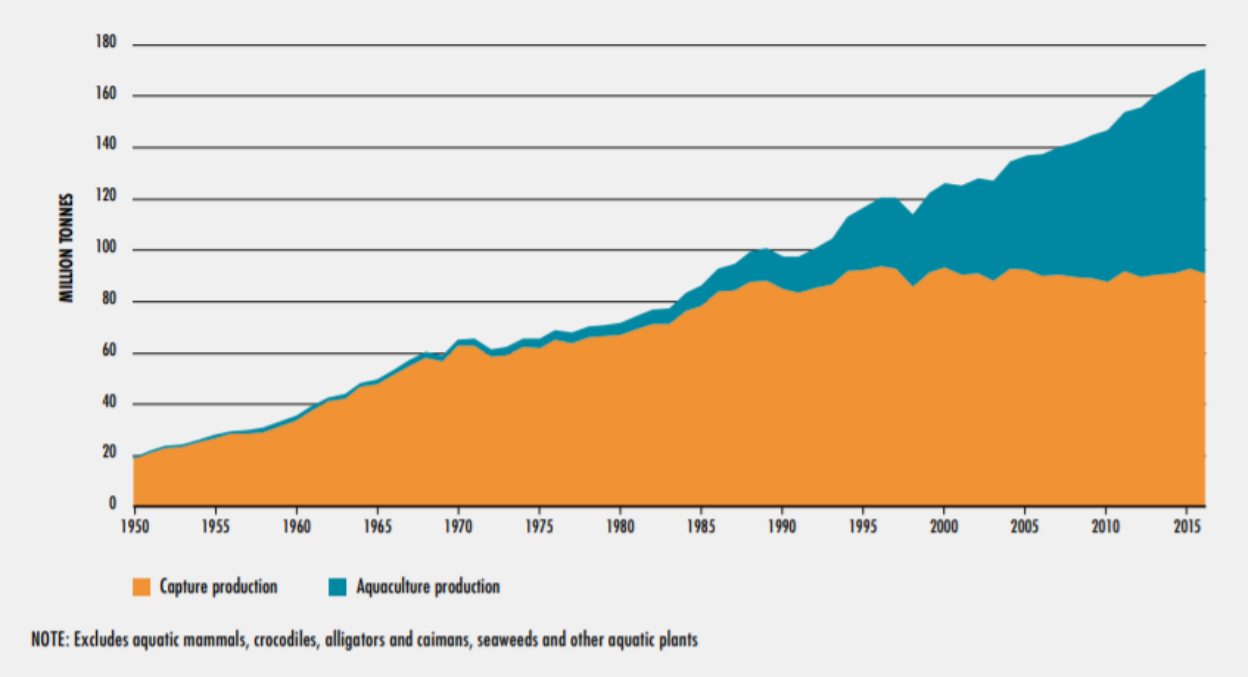


Figure 1.1: World capture fisheries and aquaculture production (Source: FAO Report, 2018)

Aquaculture development in Africa has faced many challenges over the past 50 years. Its development has followed a long and bumpy road (Machena and Moehl, 2001). Despite the application of technologies and promotion of various initiatives for aquaculture by most nations, the per capita fish supply has been stagnant for the last decades in sub –Saharan Africa (FAO, 2015). Paradoxically, recent studies show alarming signs of decline in fish per capita consumption in many African countries. For example, per capita supply of fish sharply declined from 6.1 kg/year in 1999 to 2.8 kg/year in 2005 in Kenya. Similarly, Zambia used to consume 16.5 kg per capita until early 1970s but now the data indicates to have declined to 6.2 in the year 2000. In Malawi per capita supply declined from 14 kg in the 1970s to 8kg in the year 2015 representing over 60% decline. (Department of Fisheries, 2016). However recent economic report for Malawi indicates

that currently the per capita supply has improved from 10.7 kg/year in 2016 to 12.47 kg/year in 2017. This improvement is regarded as a major achievement towards attaining the World Health Organisation (WHO) recommended standards (Malawi Government Annual Economic Report, 2018). The current trends show that majority of African countries are operating at a far lower per capita fish consumption than the recommended per capita consumption of 13 to 15kg by the World Health Organization (WHO) (Bhujel , 2011). This shows a real threat to food and nutrition security in African countries.

1.1.1 Country Profile for Malawi

Malawi is in the southern part of Africa (13.2543° S, 34.3015° E) and is a largely agricultural country, with about 85 percent of its population living in rural areas (FAO, 2015). Despite making significant structure and economic reforms to sustain growth, Malawi remains one of the poorest countries (World Bank, 2018). It is a landlocked country sharing borders with Tanzania, Zambia and Mozambique. Its surface area is approximately 118,000 square kilometres of which 20 percent is covered by Lake Malawi (Kauye and Mafuta, 2007). Over 51.5% of the population lives below the income poverty line, and 20.1 percent are considered in severe poverty (National Statistics Office, 2018). Although poverty is more widespread in rural areas than urban areas, income inequality is significantly more pronounced in urban areas (World Bank, 2018). Malawi's economy is predominantly agrarian, with 85.1%of households engaged in agricultural activities and agriculture accounting for 30 percent of Malawi's GDP and 80 percent of its exports mainly tobacco (FAO, 2015).

1.1.2 Capture fisheries

The main source of fish production is Lake Malawi which runs from Karonga in the northern region to Mangochi in the southern region. The lake has an area of 24,208 km^2 and produces almost 40-50% of the total annual landings (Donda and Njaya, 2007). The other water bodies include Lake Malombe (390 km^2) located in Mangochi and parts of Machinga and Balaka districts, Lake Chilwa (1,800 km^2) shared by Machinga, Zomba and Phalombe districts and Lake Chiuta (200 km^2) in Machinga, upper and Lower Shire River

Fish is one of the most important commodities in the economy of Malawi. Currently, the fisheries sector contributes about 1.6% to the GDP and supplies over 70% of the dietary animal protein intake of Malawians and 40% of the total protein supply (GoM, 2018). According to the department of fisheries, the sector indirectly employs over half a million people through fishing (GoM, 2019). In addition, the development of the fishing industry has promoted the growth of support industries which offer employment to over 500,000 individuals in fish-related activities such as fish processing, trading, marketing and boat building. furthermore 1.6 million people along the lakeshore districts derive their livelihoods through fishing and fishing related activities (DoF, 2016). Recent data have indicated an overwhelming increase in production of Engraucypris sadella, commonly known as Usipa. Overall fish catch has increased from 95,724 tonnes in 2010 to 233,075 tonnes in 2020 (GoM, 2018). However, fisheries production have been fluctuating with the catches of Tilapias significantly dwindling. More crucial is the fact that the population of Malawi was estimated at 17.56 million with an average annual growth rate of 2.9% (NSO report, 2018). Hence, the demand for fish as one of protein sources, is increasing in Malawi and prospect of fish contribution to national economy is high. While increasing fish importation could be a

quick solution to meet increasing demand which has been noticed recently, this only puts pressure on balance of payment through outflow of needed forex.

1.1.3 Status of Aquaculture in Malawi

Fish farming in Malawi begun around 1906 when rainbow trout Onchorhynchus mykiss, got introduced for angling (FAO, 2005). However, the culture of indigenous species in fish farming began in 1956/57 with Oreochromis shiranus, commonly known as Makumba and Coptodon rendalli known as Chilunguni. In 1957 Domasi Experimental Fish Farm located in Zomba district was established to boost culture of these species. The place is now called the National Aquaculture Centre (NAC). The centre was involved in the breeding and distribution of C. rendalli and O. shiranus fingerlings to farmers. Today the number of fish species raised in the sector has increased to five, and there are several fish breeding hatcheries which are run by the government as well as the private sector. Among others, these include the Mzuzu Fisheries Research Station, Aquaculture and Fisheries Department-Bunda College, LUANAR, Chambo Fisheries, MALDECO and Kasinthula Fisheries Station. Fingerling production in Malawi has been progressively improving over the past five years with 2017 recording the highest figure of 9,511,755 (

).

Table 1.1: Trends in fingerling production from public and private hatcheries in Malawi.

Year	Public Hatchery (Mzuzu and NAC)	Private Hatchery, Maldeco	Public +Private
2013	785,906	5,006,011	**5,791,917**
2014	731,756	5,613,964	**6,345,720**
2015	965,811	6,423,307	**7,389,118**
2016	1,670,526	6,625,000	**8,295,526**
2017	1,891,835	7,619,920	**9,511,755**

Source: Malawi government, Ministry of Finance, Economic Planning and Development Annual Economic Report 2018

According to the Department of Fisheries in the Malawi government annual economic report (2018), there are 15,465 fish farmers, of which 61.51 percent are males and 38.49 percent are females. In 2017, there were around 10,007 fish ponds across the country, and aquaculture production from ponds and cages was at 12,217 metric tonnes (**Fehler! Verweisquelle konnte nicht gefunden werden.**).

While the sector is dominated by small-scale fish farmers, there has been an emerging interest for investment in commercial aquaculture with some operators involved in the subsector for the past decade (DoF, 2016).

Table 1.2: Malawi's estimated aquaculture production levels (tonnes) and value (USD) of major cultured fish species (2013 – 2017)

	Estimated			**Years**		
Species	**Units**	**2013**	**2014**	**2015**	**2016**	**2017**
	production(t)	2,578	3,300	3,422	7,080	8,624
Oreochromis shiranus/mossambicus	value (US$)	2,704,783	3,462,123	3,849,012	18,893,010	23,498,202
Coptodon rendalli	production(t)	641	820	851	142	2,593
	value (US$)	813,640	1,041,229	957,027	377,860	6,919,542
Clarias gariepinus	production(t)	333	426	508	212	900
	value (US$)	339,786	434,926	571,915	566,790	2,452,316
Cyprinus carpio	production(t)	71	91	94	118	44
	value (US$)	88,884	113,772	106,005	314,884	119,861
Oncorhynchus mykiss	production(t)	82	105	109	94	56

	value (US$)	103,511	132,494	122,428	251,907	152,589
Total major species(t)		**3,705**	**4,742**	**4,984**	**7,646**	**12,217**
Total value USD		**4,050,425**	**5,184,543**	**560,637**	**20,404,451**	**33,142,539**

Source: Department of fisheries in the 2018 Annual Economic Report

Five main species are cultured in Malawi namely Clarias gariepinus commonly known as catfish, C. rendalli, O. mossambicus commonly known as Makakana, and O. shiranus and Oreochromis karongae. Lake Malawi Chambo, O. karongae (**Fehler! Verweisquelle konnte nicht gefunden werden.**) is one of the most preferable fish by most consumers and it has proven to exhibit superior growth characteristics in earthen ponds over all tilapia species raised in Malawi (Msiska et al., 1996). Although limited in fingerling availability, this fish species has been adopted by both small scale and commercial fish farmers in the country.

Figure 1.2: Lake Malawi Chambo, (O. karongae) commonly known as Chambo (Photo by B. Ueberschär)

This species is a mouth brooder but breeding in captivity is still not as successful as its sister species. Production of grow-out fish species starts with mass production of good quality fingerlings and good quality fingerlings production starts with proper management of broodstock, and nursing of fry to fingerlings. Expertise in the process of egg collection, hatchery management and nursing of the fry to fingerling sizes is a fundamental key towards a successful fish production.

1.1.4 Challenges Facing the Aquaculture Industry in Malawi

Despite the overwhelming increase in the number of farmers venturing into the aquaculture industry in Malawi, several challenges hinder the industry from realizing its potential level of production. The main constraints of aquaculture development include; lack of quality fingerlings and lack of quality fish feed. Additionally, policy limitations , poor technological advancement and weak institutional support are constraining aquaculture development in the country (National Fisheries and Aquaculture Policy, 2012),. Valeta et al (2013) reported that scarcity of fingerlings remains is a key constraint in aquaculture production. Nevertheless, slow growth of the cultured fish species available, which have also been observed to have poor feed conversion ratios (FCR) creates a barrier towards aquaculture development. Malawi's most cultured species O. shiranus for example, shows fast growth when young but matures early and may become stunted (Msiska et al., 1996). Despite being highly demanded by most farmers, C. rendalli's broodstock produces relatively few fingerlings. Furthermore, unavailability of quality feeds and high cost of fish feed is also another challenge facing the industry.

Feed manufacturers in Malawi mostly produce sinking feed due to unavailability of suitable infrastructure like extruding feed mills and technical expertise. The sinking feeds, however, increase the cost of fingerling production. When sinking feeds are used in column feeding species, which are common in Malawi, some portion of the feed sinks to the bottom making it hard for some fish species such as column feeders to access the feed, (NFAP, 2016). The sinking feed on

its own may not be the limiting factor, the nutritive value of the feed is of paramount importance. Thus floating or sinking, feed formulations must contain the essential nutrients for fish growth.

1.1.5 Potential for Aquaculture in Malawi

Aquaculture has a potential to contribute to food security and poverty reduction goal by supplementing capture fisheries (NFAP, 2016). Although the aquaculture industry has been facing a great number of challenges, there is great potential for aquaculture growth which can have a significant contribution to the economy of the country. More than 11, 650 km^2 of land in Malawi (about 15%) has potential for aquaculture (Chimatiro and Chirwa, 2005). There is plenty of space in the natural water bodies and if such areas could be investigated in terms of organic load and discharge, there could be more opportunities for the establishment of medium and large-scale cage culture facilities. By focusing on large scale operations and promoting aquaculture as a business at various operational levels (small, medium or large), fish supply can increase and thereby improve the food and nutrition security.

1.1.6 Biotic and Abiotic Factors in Seed Production

Fingerling production is at various stages affected by several factors. These factors could be physical as well as biological. Factors such as fish species cultured and scarcity of brood fish stocks (Akankali, 2011) affect the success of quality fingerling produced. Other factors such as water temperature, photoperiod and stocking densities are said to affect the rate of cannibalism (Szczepkowski, 2009). High growth rates can be experienced at high levels of photo period. For

instance, Abdel Fattah et al., (2003) observed high growth rates in catfish juveniles at 24-hour light and 0 hours' darkness. Reducing the light phase during fry nursing leads to significantly retarded fry performance (El-sayed, 2004).

1.1.7 Problem statement and justification

Despite growing effort to increase supply of tilapia fingerlings in Malawi, demand for fingerlings from species like O. karongae remains high. Production of tilapias such as O. karongae through fish farming has been limited due to inconsistent supply of fingerlings, both in quality and quantity. This has largely been caused by scarcity of brood fish, low fecundity, and high mortality rates (Msiska, 1998). Poor feed quality and improper feeding practices have contributed to the limited supply of fingerlings. Kapeleta (2001) documented that inadequate seed production in Malawi has been due to low fecundity of some tilapia species, lack of proper fingerling production techniques, lack of proper feed and heavy predation. These challenges have not been adequately addressed since the sector still experiences inadequate supply of fingerlings. Furthermore, there is still not yet much known about the optimal rearing conditions for O. karongae in terms of abiotic and biotic conditions such as temperature, dissolved oxygen, photoperiod and ammonia levels, feed types and feeding regimes among other factors.

There is therefore, a need to take appropriate efforts in addressing the challenges facing the tilapia fingerling production industry. This research was thus conducted to determine some of best culture conditions for production of O. karongae fingerlings. The research attempts to provide the best culture techniques in terms of management of environmental parameters that includes adjustments

in water temperature, dissolved oxygen levels, photoperiod and ammonia levels and determination of feed types and stocking densities for O. karongae fry that can support standardization of survival and growth performance with a higher predictability.

1.1.8 Objectives of the Study

1.1.9 Main Objective

To determine feed types, biotic and abiotic conditions that can enhance production and supply of good quality O. karongae fingerling.

1.1.10 Specific Objectives

- To determine the best amongst four different feed types; Malawi-Bunda, Malawi-Bunda plus live feeding, Zambia Novatek and German-Coppens, as well as best stocking density for growth, survival and feed utilization of Oreochromis karongae fry that will help to improve quality fingerling availability.

- To determine the best temperature between 24°C and 28°C, suitable to maximize growth, survival and feed utilization of Oreochromis karongae fry.
- To determine the best photoperiod between 16:8 LD and 24:0 LD, for growth, survival and feed utilization of Oreochromis karongae fry, which subsequently will improve fish feeding behaviors.
- To assess the efficiency of using hormonal feed in producing all male O. karongae fingerlings which can help to increase access to all-male Tilapia seed.

1.1.11 Research hypotheses

The hypotheses tested by this research were as follows:

I. Feeding O. karongae fry with different formulated diets under certain feeding regimes in varying stocking densities can significantly affect their growth and survival rates.
II. Rearing O. karongae larvae as well as fry under certain water temperatures can significantly impact their growth performance and survival.
III. Rearing O. karongae larvae as well as fry under short or prolonged photoperiod in various fish culture facilities significantly impact their growth performance and survival.
IV. There is the best level of 17α-Methyl Testosterone hormone that can be incorporated to feed to be administered to O. karongae fry to attain aall male populations.

CHAPTER 2

LITERATURE REVIEW

Tilapia fish farming

Tilapia is regarded as the second most cultured aquaculture species globally because of its easy to adapt in tropical and sub-tropical areas of the world (Shelton, 2002). Tilapia is the generic name

of a large range of fish species of cichlids. The original distribution of this group was south-central Africa northward into Syria, where more than 70 species have been identified (Popma and Phelps, 1998). Tilapia have numerous advantages as an aquaculture species (Teichert-Coddington et al., 1997). Reports suggest tilapia were successfully produced in ponds for the first time in (Democratic Republic of Congo DRC) in 1946 (Machena and Moehl, 2001). By the end of the 1950s, there were almost 300 000 ponds in production in Africa (Satia, 1989). According to Beveridge and McAndrews, (2012) tilapia are highly opportunistic feeders, they have shown to ingest a wide range of items, algae, zooplankton, detritus, insect larvae, fish eggs, and embryos. Reproduction cycle for tilapia is well known, thus fingerlings readily available the entire spawning season. In captivity, tilapia will attain sexual maturity at small size from 50 to 100 grams, at an early age within 6-8 months of hatching), and can spawn frequently year-round in warm water 25 to 30ºC) (Phelps and Popma, 2000). In Malawi, O. shiranus one of the mostly cultured species can spawn at an early size of 30 grams.

2.1.1 Tilapia Seed Production

Fish farming still depends on various stages of fish seeds which could be larvae, juveniles, fry and fingerlings collected from the wild (Bhujel, 2014). Several indigenous species of fish found in different localities are still dependent on wild seed supply because there is still lack of understanding of their breeding behaviours. Natural breeding of tilapia in open ponds is the simplest method of seed production which is still common in many parts of the world including Africa. Early maturation and frequent spawning are management challenges when working with tilapia (Phelps and Popma., 2000). The problems common for many mixed sex tilapia culture systems are the reduction of growth rates at the onset of sexual maturity and precocious and

excessive reproduction, leading to various sizes of small fish production (Lèveque, 2002). Monosex culture by either manually or mechanically selecting males results in half of the potential fish seed being rejected (Popma et al., 1984)

Recently, there have been various production technologies applied in tilapia seed production. These technologies have aimed at producing all male populations. Tilapia males have high weight gain and better FCR compared to their females (Drummond et al., 2009). According to Macintosh and Little (1995) males can grow 18-25% faster than females. All male fingerlings are also produced in order to avoid unwanted spawning in a production unit, (Adel et al., 2006).

2.1.2 Hormonal Sex Reversal Techniques

This technique involves the administration of hormonal feed during the early stages of fry. Exogenous steroids given during the gonadal development period can control the phenotype overriding the expression of the genotypic ally determined sex (Phelps and Popma, 2000). All male fish populations are commonly produced using methyl-testosterone (MT) mixed with high quality feed as early as possible. Efforts are needed to achieve overall survival from eggs to fry (>60%) and male percentage (>99%). These include determining the optimum dose of methyl testosterone in feed, frequency and length of feeding period (Bhujel and Program, 2011). According to Popma and Green (1990), levels of 17 α-methyl testosterone (MT) administered to fish was determined to be at 60mg/Kg of feed. However, the main challenge with this technique is that it requires skillful labour to produce the hormonal feed and also the limitation on the accessibility and the use of the hormones is another outstanding challenge. Although it has been

documented that hormones do not have adverse effects on the flesh of the fish after cessation of the treatment, one is not sure on its effects on vital organs (liver, kidney, pancreas and gills. Furthermore escapes from such hatcheries into the natural water bodies would alter the dynamics of the environment due to unforeseen consequences (Megbowon and Mojekwu, 2013).

2.1.3 Factors Affecting Larvae Rearing

2.1.3.1 Effects of temperature on larvae rearing

Temperature is very essential for egg production and growth of fry in a hatchery system. The effects of water temperature on growth and development of fish have been well documented for many species. Optimal environmental conditions are needed to reach the best growth performance. The optimal temperature for the growth of most tilapia species is 25–28°C; reproduction stops at 22°C and feeding below 20°C (Kamel et al., 2008). Excessive temperature may hamper egg production and growth of fry and the hatchery operator cannot reach its optimal production. (Faruk, 2012). According to Brummett (1995) Nile tilapia did not lay eggs when water temperatures went down below 19ºC. The most productive period was observed with the rise in water temperature to 22-27°C where spawning rate averaged 40 and 73% of total females under dark and natural photoperiod conditions, respectively.

Temperature also has an effect on the growth rate, survival in the process of sex reversal of Nile tilapia (Drummond et al., 2009). In a study conducted by Ali Faruk et al (2012) with Nile tilapia, it was found that egg production was highest at 26°C and lowest at 29°C. Furthermore growth of

mono sex fry was highest at 33°C and lowest at 25°C. It is therefore important to understand the role of temperature on egg production, hatchability, growth and survival of fish fry.

2.1.3.2 Effects photoperiod on larvae rearing

Fish growth is influenced by photoperiod which stimulates the endocrine System and influence circulating growth hormone (Björnsson, 1997). Also, Purchase et al., (2000) reported that according to Porter et al., (1999) several fish species react to longer photoperiod. Beyond the natural conditions, light applications directly improve their feed efficiency rate or reduce the incidence of sexual maturation so enabling redirection of energy from gonad development to muscle tissue and fat in the abdominal cavity. In another study, El-Sayeed et al., (2004) documented that photoperiod significantly affected Nile tilapia fry growth performance, but not fingerlings reared in indoor, re-circulatory systems. Photoperiod had no significant difference in fertilization rate, mean final weight, mean weight gain, and specific growth rate of fry, but had a significant effect on hatching rate and survival rate when its effect was investigated in C. gariepinus larvae (Ataguba et al., 2015). In another study by Valeta et al., (2013), on the effect of temperature on egg development, hatchability and survival rates for O. karongae, high temperature reduced the hatching period from 14.7 days at 25°C to 7.3 days at 29°C

The determination of light conditions is further complicated by the fact that there may be different light requirements for different populations of the same species as reported by Puvanendran and Brown (1998).

2.1.3.3 Effects of dissolved oxygen (DO) on larvae rearing

The availability of ambient oxygen affects the metabolism and growth of fishes. Thus all factors affecting changes in dissolved oxygen, including the lowering of oxygen and resultant hypoxia and the diel flux of oxygen can affect production of juvenile fish. In fish culture facilities, oxygen availability is affected by organic matter decomposition, temperature, diffusion caused by wind and consumption by fish. Dissolved oxygen is a limiting factor that sets an upper limit for total metabolism under hypoxia (Niklitschek and Secor, 2009). There is considerable information on how ambient oxygen limits growth in fishes. It may be incidentally mentioned that very high concentration of DO leading to a state of super saturation sometimes becomes lethal to fish fry during the rearing of spawn in nursery ponds (Bhatnagar and Devi, 2013). Availability of dissolved oxygen affects the feeding behavior of fish. As reported by Mallya (2007), maintaining oxygen level near saturation or even at slightly super saturation at all times increases growth rates, reduce the food conversion ratio and increase overall fish production.

2.1.3.4 Effects of ammonia levels on larvae rearing

Ammonia is one of the major water quality parameters that need to be well managed when rearing fish. Ammonia toxicity to fish depends on the concentration of unionized ammonia (NH_3) (Handy and Poxton 1993). According to Netten et al., (2013) both temperature and pH increase the NH_3 concentration. Generally, at high pH, levels of NH_3 and its toxicity increases. It is toxic to

fish if allowed to accumulate in fish production systems. When ammonia accumulates to toxic levels, fish cannot extract energy from feed efficiently. If the ammonia concentration gets high enough, the fish will become lethargic and eventually fall into a coma and die (Hargreaves and Tucker, 2004). Ammonia affects metabolism or inhibition of physical activity which lead to retarded growth. Fish larvae exposed to a high concentration of unionized ammonia NH3-N 0.5–0.6mg/l) show clinical signs such as malformation in yolk sac, body shortening, darkening in eye and cardiac edema, spine curvature development. Fish exposed to ammonia levels above 3 mg NH3-N/l 60 days after hatching show pathological lesions represented as degeneration of some secondary lamellae, hyperplasia of epithelial cells on the top of filaments and aggregated amount of blood cells inside vacuolations that are located on the margin of filaments (El-Greisy et al., 2016). On the other hand, when ammonia is dissolved in water, it is partially ionized depending upon the pH and temperature. The ionized ammonia is called Ammonium and is not toxic to the fish (Ogbonna and Chinomso, 2010).

2.1.3.5 Effects of pH on larvae rearing

The water pH has a great impact on the quality of water and thus affect the survival of fish. An increase in pH value causes a rise in the fraction of unionized ammonia and the water becomes toxic to fish (El-Greisy et al., 2016). Water pH has an effect on the fish feeding, in that feed consumption is reduced at lower pH levels there by causing growth reduction (El-Sherif and El-Feky , 2009). According to Saha et al. (2002) and Scott et al., (2005) the excretion of ammonia increase with an increase in pH (alkalinity), and that inversely affects fish growth. El-Sherif and

El-Feky (2009) also reports that the optimum levels of pH for Nile tilapia fingerling growth was found to be 7-8.

2.1.3.6 Effects of stocking densities on larvae survival and growth

Stocking density refers to the number of organisms cultured in a given unit space. It is one of the important aspect to be considered when rearing fish. It can be expressed as the number of organism per square meter (fish/m^2) or per cubic meter (fish/m^3). It is well-known fact that growth rate progressively increases as the stocking density decreases and vice versa. This is because a relatively less number of fish of similar size in a pond could get more space, food, less competition and more dissolved oxygen (Daudpota, 2014). Mensah et al., (2013) observed that social interactions through competition for food and/or space negatively affect fish growth, hence higher stocking densities lead to increased stress, resulting into increase in energy requirements thereby causing a reduction in growth rate and food utilization. In a study conducted by Zidana et al., (2015) growth performance for C. rendalli fry was observed to significantly increase when the stocking densities were low (30fry/m^3) unlike at high densities of (90fry/m^3).

2.1.4 Importance of proper feeding in larvae rearing

Newly hatched fry are given a complete diet of powdered feed. The feed is usually high in protein about (50%) and energy to meet the demands of the fast-growing fry. Diet has been known to improve the ability of fish to tolerate low temperatures. In striped bass, white bass and their hybrids

for example, Kelly and Kohler (1999) reported that fish fed their natural diet suffered no mortality and had higher levels of unsaturated lipids than those artificially fed. Similarly, dietary supplementation of L-carnitine at different levels led to higher cold tolerance in an ornamental cichlid, Pelvicachromis pulcher (Charo-Karisa et al., 2003).

The lipid content in larval feed is mostly higher, compared to feed for juveniles and adults, since fat is used as the main energizer for the metabolism, specifically in young stages. The feeding rates as well as the feeding frequency are also of paramount importance in proper rearing of fry to fingerings. The feeding rates vary in that when fish weight increases, the percent body weight fed decreases. The interval between feedings may be more important than the total number of feedings. This helps to avoid fish starvation (where the intervals are longer than required) and also minimizes feed wastage when there is too much administering of feed into the culture facilities than the fish would require. Feeding strategies for tilapia have traditionally been to feed a little bit of feed at frequent intervals (Riche and Garling, 2003).

2.1.4.1 Effects of feeding larvae with planktons

The success in the hatchery production of fish fingerlings for stocking in the grow-out production system is largely dependent on the availability of suitable live food organisms for feeding fish larvae, fry and fingerlings (Webster et al., 2006). Many fish and crustacean larvae require live food at the onset of exogenous feeding. The use of zooplankton as live food for tilapia and catfish decreases the ash percentage about 40-50% and increase the protein and fat content of fish (El Fattah h et al., 2008). Feeding fry with zooplankton may result into suitable growth than artificial feed (Hepher, 1988). The protein content of natural food ranges between 550 and 700 g/kg on a

dry matter basis (Hepher, 1988). The availability of large quantities of live food organisms such as marine rotifer (Brachionus plicatilis and Brachionus rotundiformis) and Artemia nauplii to feed fry at different stages production has contributed to the successful fry production.

Zooplankton abundance is often linked to lake primary productivity in lentic systems, and high productivity likely facilitated growth and survival of larval fish in these lakes. For instance, Wang et al., (2005) documented that survival rate was significantly higher in larvae fed live food than in larvae fed formulated diets. Adult condition may also positively be affected by high primary productivity (Knuth, 2007). However, it is important to note that a variety of environmental factors are known to affect zooplankton production. Furthermore, Tilapia is believed to change feeding habits as they grow, it is therefore crucial to adjust the feeding strategies when producing Tilapia fingerlings

2.1.4.2 Protein requirement for fish larvae

Protein is one of the most important elements in aquaculture diets, because it is the basic component of animal tissue and body fluids, and is, therefore, essential to survival and growth (Tacon, 1989). On the other hand, protein levels above optimum requirement reduce growth performance of fish due to high metabolic nitrogen excretion demand , thus for catabolising than protein disposition (Khan and Maqbool, 2017). Dietary protein levels affect the proximate composition of fish, with the later increasing as the dietary levels increase. The moisture content however gradually decreases with the increase in the dietary protein levels (Singh et al., 2006). The method of feeding in terms of the form (pellets or powder), timing, frequency and duration of

feeding administered to fry are the key factors that should be considered when rearing fry. Poor management of these factors will lead not only to poor quality of fingerlings, but also it will result into wastage of feed which will in turn increase the cost of producing fingerlings and deterioration of water quality thereby increasing the occurrences of fungal and disease attack. Therefore, the selection of proper quantity and quality of dietary protein is extremely important for successful Tilapia larval rearing (El-Sayed, 2004). Among other factors protein requirements for optimum growth of the fish seem to be affected by temperature, salinity, fish age and size (Cowey, 1976). El-Sayed et al., (2003) documented that fry growth was improved with increasing protein level at different water salinities.

2.1.4.3 Energy requirements for fish larvae

Fish have lower dietary energy requirements because they do not have to maintain a constant body temperature, they use less energy in protein waste excretion. However, the expensive protein fraction should be optimally utilized for growth rather than for maintenance of fish (Shiau and Lin, 1993). National Research Council, (NRC, 1983) indicated that diets formulations for fish are done considering the minimum protein requirement for optimal growth. Where all other nutrients are not provided in their appropriate levels, protein in the diet becomes an alternative source of energy and this impairs fish growth. The combination of other nutrients such as carbohydrates and lipid in the diet therefore spares protein for growth. To meet the requirement for essential fatty acids in fish , Long Chain (LC) Polyunsaturated Fatty Acids (PUFA) should be supplied in the diet for fish (Hixson, 2014). The qualitative and quantitative essential fatty acids (EFA) requirements of fish, however, vary among species.

It should also be mentioned that excess energy may produce fatty fish, reduce feed consumption (reducing total protein intake) and inhibit proper utilization of other feedstuffs (Winfree, 1981).

2.1.4.4 Mineral and vitamin requirements for fish larvae

Micronutrients, such as vitamins are organic substances that are essential for growth, health, reproduction and maintenance of animals, but required in small amounts. Since fish cannot synthesize vitamins at all or can only synthesise in insufficient quantities for normal development, growth and maintenance , they must be supplied in the diets (Shiau and Lin, 2006). Vitamins are essential for the growth and survival of juvenile fish. Juvenile fish growth and survival significantly increase with an increase in the levels of Ascorbic acid included in the diets (Nsonga et al., 2009).

Iron is essential in the fish's body for it is responsible for binding and carrying capacity of oxygen in the haemoglobin (Bury et al., 2003). In a research on Nile tilapia fingerlings, El-Serafy et al, (2007) documented that increased dietary levels of iron in fish diets was associated with an increase in red blood cells, haemoglobin and haematocrit. However iron can both be toxic and beneficial hence its levels should be well regulated. Calcium is essential for skeletal development , normal growth and other physiological processes (Lall, 2002).Calcium is also essential for blood clotting , proper nerve impulse transmission, osmoregulation and as a co-factor for enzymic processes (NRC, 1993).

The following deficiency signs have been reported for Nile tilapia: calcium- reduced growth, poor feed conversion and bone mineralization; magnesium- whole-body hyper calcinosis, and manganese- reduced growth and skeletal abnormalities. In a study by Dabrowska et al., (1989) with Nile tilapia, excess magnesium (0.32%) in a low-protein (24%) diet produced severe growth retardation and showed a significant decrease in blood parameters, haematocrit and hemoglobin content, and magnesium deficiency in a high-protein (44%) diet caused whole-body hyper calcinosis. A dietary magnesium content of 0.059-0.077% was adequate for optimum performance of this species

CHAPTER 3

MATERIALS AND METHODS

Area of study

The study was conducted at the Aquaculture and Fisheries Department's fish farm, Bunda College (Figure 3.1: Arial view of Bunda Aquaculture Farm, in Lilongwe). The series of experiments begun during the month of February, 2018 (for the all-male production trials) and these were carried out in the indoor fish hatchery except for grow-out period where the juveniles were then raised in hapas-in-ponds. The subsequent experiments begun in December 2018, to May 2019.

Figure 3.1: Arial view of Bunda Aquaculture Farm, in Lilongwe **(**Photo by J. Straiger**)**

Broodstock collection and establishment

Broodstock for O. karongae with body weight range of 100 to 200g were collected from various sites across the country (Benga in Nkhotakota, Mpondasi, Maldeco and Malembo in Mangochi, Shire River-Liwonde site and Chingale in Zomba). These were then brought to Bunda for acclimatization prior to the breeding program. Males and females were separated by hand-sexing and then conditioned by rearing them in separate ponds and these were stocked at a density of 3fish/m^2. The fish were fed with broodstock feed containing 32% CP produced by Coppens from Germany under the Ich-Liebe Fisch project. Feeding was done at 3% body weight and the fish were fed two times a day by manually introducing the feed into the ponds through broadcasting.

After two months, the fish were checked for readiness by observing the onset of breeding colours and also by observing the male fish papilla which is usually reddish when the fish is ready for breeding. One hundred and fifty fish (both males and females) with an average weight of 150g were stocked at a sex ratio of 1: 2 (male to female).

Developing rearing protocol with recommendations for best settings of abiotic and biotic factors

To develop rearing protocol for assessment of biotic and abiotic factors, series of experiments were conducted upon successfully breeding the fish.

3.1.1 Determining the best larval feed and stocking densities

To determine the optimal larval rearing and stocking densities in O. karongae, an experiment was conducted consisting of two factors that were tested, the feed type and stocking densities. This was done to determine the best feed and optimal stocking density. Four types of feed were tested; Malawi-Bunda feed formulated from local ingredients (with zooplankton starter in the first week of feeding and then switched to Malawi-Bunda feed), Malawi-Bunda feed formulated from local ingredients but without live feeding, Zambia (Novatek) feed and Germany (Coppens) feed. Three levels of stocking densities were tested. These were; the low stocking density level (2500 hatchlings/m^3), medium densities (5000 hatchlings/m^3) and the high stocking densities (7500 hatchlings/m^3). The two factors were tested together in a completely randomised design with 36 experimental units.

3.1.1.1 Preparation of experimental buckets

Since the design of the solar powered hatchery installed at Bunda aquaculture farm has two major tanks with three small tanks in each one of them, there is a limitation on the number of treatments one can test, two treatments to be specific. Hence small 36-1L plastic buckets (**Fehler! Verweisquelle konnte nicht gefunden werden.**A) were used. Holes were drilled and a mesh was used to cover the holes to make sure that there was enough water exchange between the bucket and the surrounding. A buoyant material (a small PVC pipe, (**Fehler! Verweisquelle konnte nicht gefunden werden.**B) was used to make sure that these buckets can freely float in the tank.

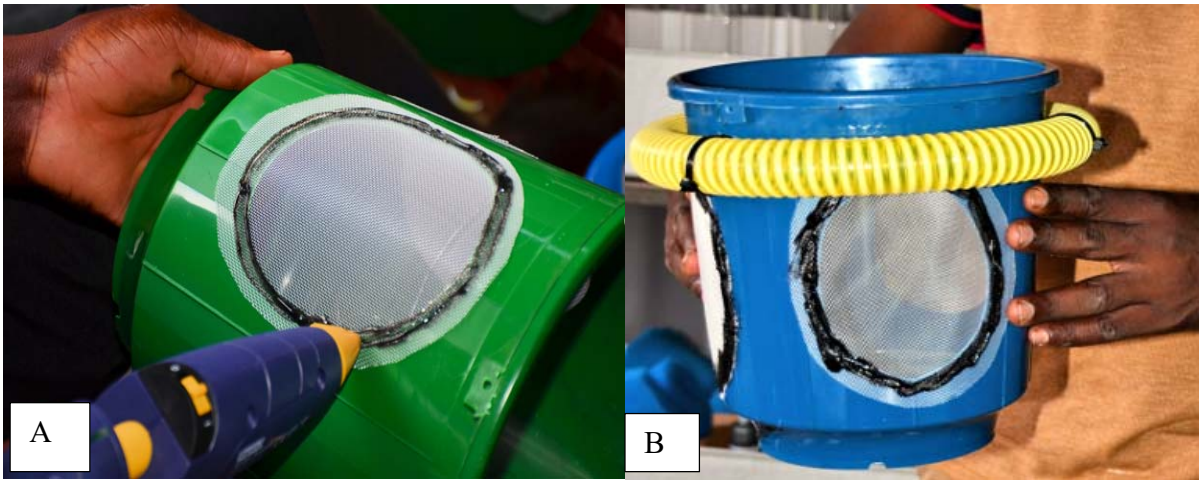


Figure 3.2**:** Portable fry nursing buckets covered with a 300µm mesh **(**Photo by B. Ueberschär**)**

3.1.1.2 Egg collection

Eggs were collected from the brooders' mouth and these were well rinsed to remove all the debris (: Egg collection from female O. karongae brooder (A), stage 2 O. karongae eggs (B)) which could cause bruises to eggs. The eggs were then put in the incubation jars of the Mc Donald unit (**Fehler!**

Verweisquelle konnte nicht gefunden werden.)Fehler! Verweisquelle konnte nicht gefunden werden. installed in the hatchery.

Figure 3.3: Egg collection from female O. karongae brooder (A), stage 2 O. karongae eggs (B) (Photo by B. Ueberschär)

Water circulation, temperature and general water quality were monitored in the incubation unit. Dissolved oxygen levels, temperature and pH were taken twice a day, at 08:00 hours and at 14:00 hours, while ammonia were measured every four days (this was done considering that ammonia levels do not drastically change on daily basis). Francis et al., (2009) recommends that ammonia measurements should be done every 10 to 14 days for pond culture and every week for tank culture. Water was only changed at the end of the egg incubation period, thus after transferring the swim up fry into the nursing buckets.

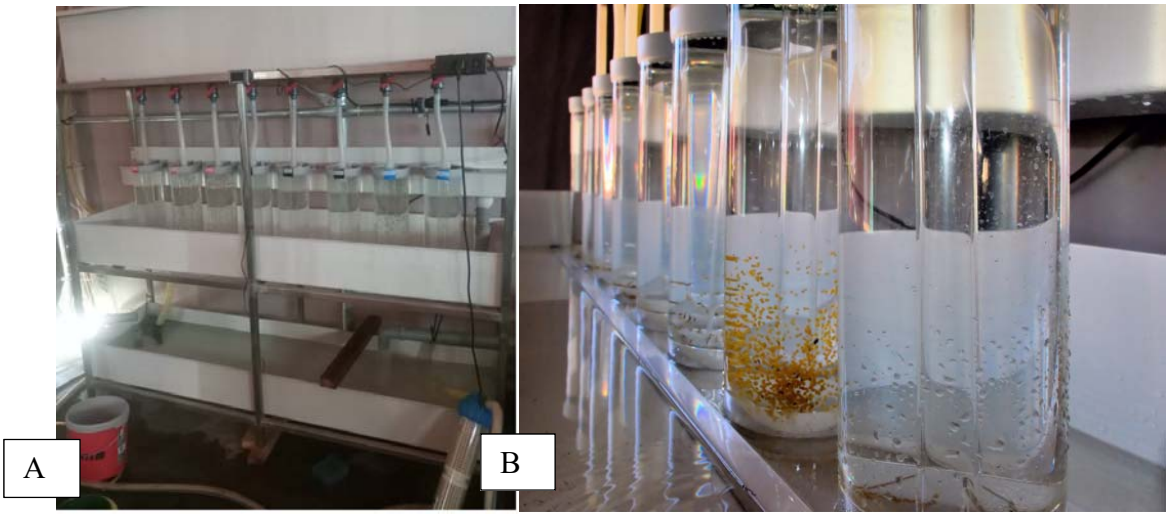


Figure 3.4: Egg incubation in the Mac Donald Incubation unit (Photo by H. Sainan)

During egg collection exercise unfertilised and fertilised were not separated to reduce handling stress that may lead to injuries. Only stage one eggs were collected during and these eggs are those newly fertilised which are brownish in colour and without spot or single structure. Unfertilised eggs are whitish and usually less dense.

3.1.1.3 Stocking of larvae

Upon reaching a swim up stage (: Egg incubation in the Mac Donald Incubation unit (Photo by H. Sainan)

), the larvae were transferred into the nursing buckets which were randomly put in the six small tanks inside the two big tanks. The larvae were then left to complete their yolk sacs before introducing them to the different types of feed. Swim up fry of the same age were targeted in this trial. This was done by selecting only those fry that hatched on the same day.

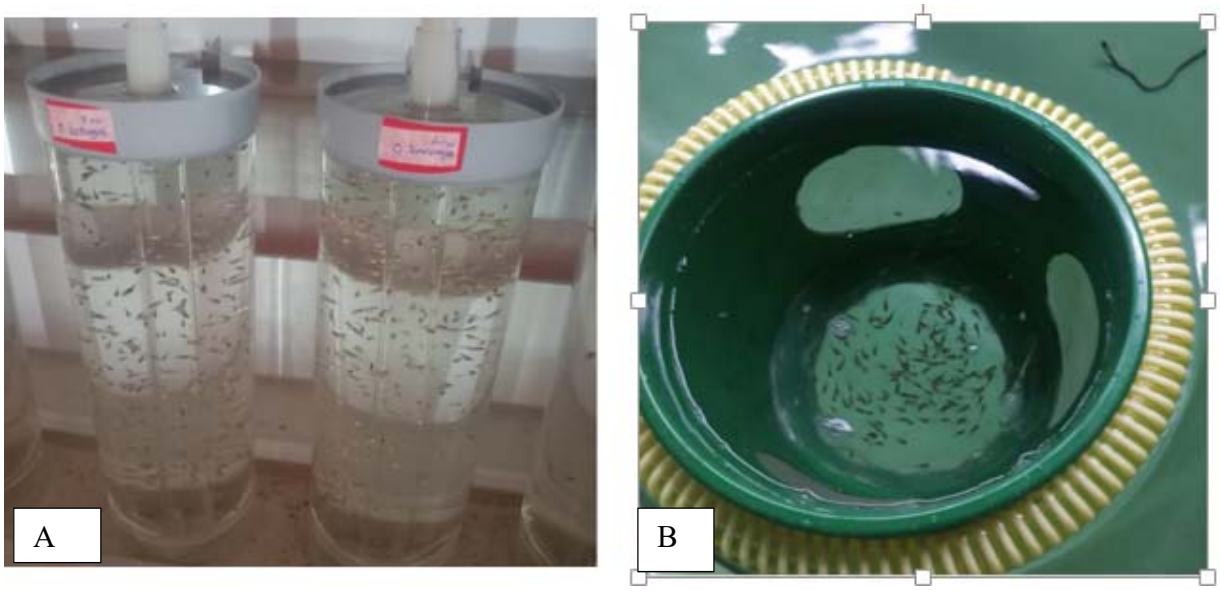


Figure 3.5**:** Swim-up O. karongae larvae in jars (A), stocked nursing bucket (B) (Photo by H. Sainan)

3.1.1.4 Feed types and feeding regimes

Fry feeding commenced as soon as the yolk sacs were observed to have been completed. Small cups were assigned to the different levels of stocking density to properly monitor feed consumption.

3.1.1.5 Culture and harvesting of zooplankton

As one way of determining the best starter food for O. karongae fry, live food was introduced in one of the experimental diets to test if starting with live food would improve fry growth. Zooplankton (**Fehler! Verweisquelle konnte nicht gefunden werden.**Figure 3.6) were thus cultured in a 500 m^2pond which was fertilised with chicken manure to boost primary production a rate of 200g/m^2/week. These were collected using a harvesting net. To determine the amount of

zooplanktons in the water 10ml water was sampled to estimate the amount of Zooplankton per ml. On average 200 zooplanktons were counted in every 10 ml sample of pond water. During feeding, 100ml of the pond water was administered into the buckets twice a day, with an estimated population of 2000 zooplanktons. After 14 days from the day of hatching, formulated diet was then introduced.

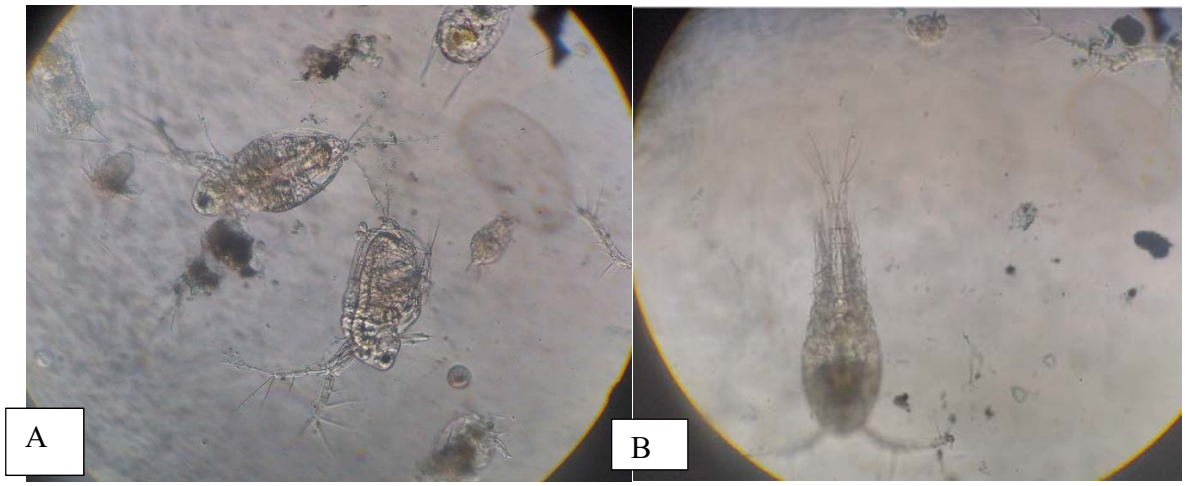


Figure 3.6: Zooplanktons taken from the pond (Cladocera-A and copepods-B (Photo by H. Sainan)

Every morning the feed was weighed into the small cups and quantity in each cup depended on the stocking density of the specific buckets assigned. During the first days of feeding, small feeding bottles were filled with 10 grams , 15 grams and 20 grams of feed respectively and these were assigned to densities of 2500 fry/m^3 , 5000 fry/m^3 and 7500 fry/m^3 respectively. These were then adjusted as the fry advanced in weight. Every evening when no more feed could be administered during that particular day, the feed remaining in the bottle could be weighed to determine how much feed has been used up. These were then recorded until the end of the experiment where

AFCRs were calculated based on this data. Although feeding was set be done a 8 hours, 10 hours , 14 hours and 16 hours , feeding frequency was dependant on the rate at which the feed administered has been depleted , hence more feed could be given when a particular bucket was observed to run out of feed. This was done purposively to determine the optimal amount of feed to be given to the fish per day, thus feeding was done ad-libitum (feeding until satiation) while minimising feed wastage.

The Malawi larval Bunda feed was prepared (Figure 3.7A) using the formula being used at the farm (: Feed preparation exercise (A) and Malawi's Bunda Feed (B)). Among the ingredients include fish meal, low and high fat soy, maize meal, rice bran, wheat bran, cassava flour, sunflower and mineral and vitamin premixes (Figure 3.7 B).

Figure 3.7**:** Feed preparation exercise (A) and Malawi's Bunda Feed (B) (Photo by A. Chelewani)

The Zambia Novatek feed (Figure 3.8: A) as well as the Germany-Coppens feed (Figure 3.8: B) were imported from Zambia and Germany, respectively.

Figure 3.8: Zambia Novatek (A) Germany-Coppens (B) larval micro-diet (Photo by H. Sainan).

Both diets (Zambia Novatek and Germany-Coppens feed) were specific for larval to fingerling stages and were both floating. Nutritional composition for three different types of diets used in the experiment is provided in Table 3.1. The Malawi feed samples were sent to Association for Marine Aquaculture (GMA) in Büsum, Germany for the analysis, while for the Novatek and Coppens feed, information provided by the company labelled on packs product was used.

Table 3.1: Proximate Composition (%) for the different feed types.

Feed Type	**Crude Protein (%)**	**Crude Fat (%)**	**Crude Ash (%)**	**Crude Fibre (%)**	**Moisture (%)**
Malawi-Bunda	37.42	12.3	11.49	2.9	11.5
Zambia-Novatek	38	8	10.1	3	10
Germany-Coppens	45	10	8.3	1.9	9.3

3.1.1.6 Fish Sampling

Fish sampling was conducted every 7 days (Bagum et al., 2015) to monitor progress in terms of growth. Growth in juvenile fish is faster than in adult fish hence sampling intervals should be reduced but at the same time consideration should be made to minimise stresses due to handling hence 7 day sampling interval was set for this study. Fish in each bucket were sampled and 6 fish, 8 fish and 12 fish (representing 30% of the total stocked in the buckets) were sampled from the lowest, middle and highest levels of densities respectively. Parameters measured were the wet weight, standard length and the total length, but in this study much focus was put on the body weight. Weight was taken using a balance (Figure 3.9) while the lengths were measured using a measuring ruler. The experiment was terminated after 35 days of rearing period.

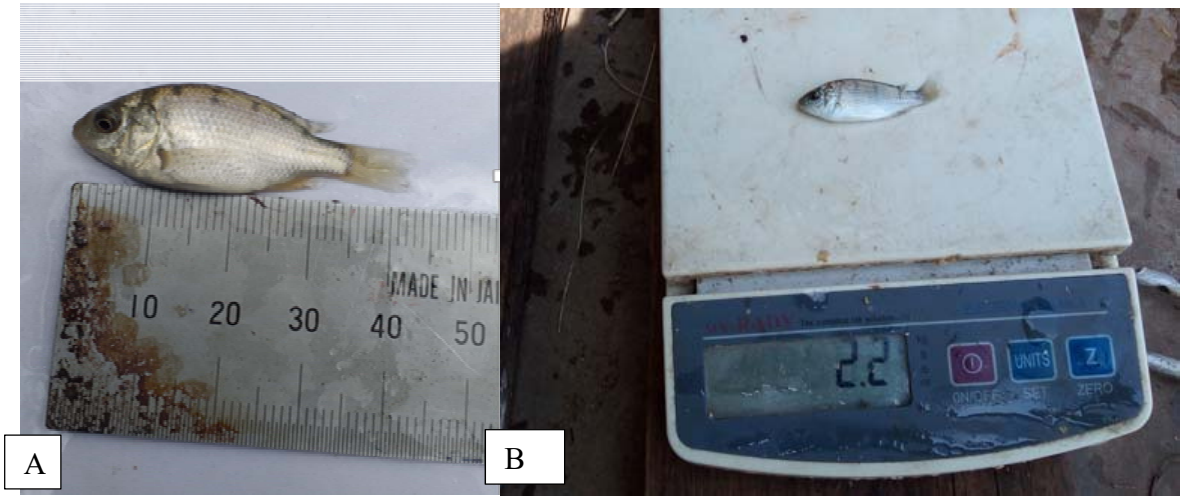


Figure 3.9: Live specimen during sampling, 28 days after hatching (Photo by H. Sainan)

3.1.2 Effect of water temperature on fry growth performance and survival

In this trial**,** two levels of temperature were tested in this experiment using heaters installed in different tanks with varying temperatures set. Two temperature regimes; 24ºC and 28ºC were used and each treatment was replicated three times. A much lower water temperature 24ºC was used to increase the range considering the limitation of culture units in the solar powered indoor hatchery. Both stocking density and the feed type used in this experiment were based on the results that showed best growth from the first (feed type and stocking density) experiment. Although the Germany-Coppens feed performed best among the four different types of feed, the Malawi-Bunda feed was selected considering accessibility. Hence a stocking density of (2500 fry/m^3) and the Malawi-Bunda feed (considering availability) were used in this experiment. In each replicate 13 fry corresponding to a stocking density of 2500 fry/m^3 was used. Heaters were used to set the water temperature at 24ºC and 28ºC for the lower and high water temperature respectively. All the tanks were aerated using an air pump and again all water parameters checked. Six nursing buckets were rrandomly assigned to the tanks.

Feeding was done using the Malawi-Bunda feed which was developed during the first feed type and stocking density) experiment. In both treatments, feeding was done every four-hour time interval from 8 am to 4 pm. Fish Sampling was conducted every 7 days for the entire 5 weeks culture period. Body weight was measured using a weighing balance.

3.1.3 Effect of photoperiodicity on fry growth performance and survival

3.1.3.1 Experimental set up

Based on the results from the two previous experiments (feed type-density and temperature), another experiment investigating the effect of photoperiod on growth performance was set. Malawi-Bunda feed, a stocking density of 2500 fry/m^3 and water temperature of 28°C were used in this trial. Two levels of photoperiod (24:0 LD and 16: 8 LD) were tested by using LED bulbs (**Fehler! Verweisquelle konnte nicht gefunden werden.**) hanged 50 cm above the water tanks (Ridha and Cruz, 2000). In the 16-hour regime treatment, a timer was set to switch the light on at 5 am and again switch the power off at 21 hours for the whole experimental period. All tanks were aerated using an air pump and temperature was set at 28°C. Small plastic buckets (5 litres) were used to raise the larvae, and in each one of these 5-litre- bucket 13 (corresponding to a stocking density of 2500 fry/m^3) O. karongae fry with an average weight of 0.013 grams were stocked. The nursing buckets were randomly assigned to the different facilities with different photoperiod.

Figure 3.10: Suspended fry nursing buckets (Photo by H. Sainan)

In both treatments, feeding was done at four hour -interval for the entire experimental period. In the 16-hour light regime, feeding was stopped at 21 hours but the amount of feed given to each experimental treatment was the same. The Malawi-Bunda feed was used in this experiment.

Fish Sampling was conducted every 7 days for period of 5 weeks. Body weight and body lengths were taken using weighing balances and rulers respectively.

3.1.4 Production of all male O. karongae Fingerlings using hormones

This experiment consisted of four treatments which were of varying levels of 17α-Methyl Testosterone (MT) hormone. Four levels of the hormone were administered to feed, 20mg/mL/kg,

30mg/mL/kg, 60mg/mL/kg and 90mg/mL/kg. These were set in completely rrandomised experimental design.

3.1.4.1 Hormonal feed preparation

Hormonal feed was prepared by incorporating the feed with 17α- Methyl testosterone hormone solution and then dried in the shade. The hormone solution was prepared by mixing the required level of hormone (20, 30, 60 and 90mg/mL/kg) with 1 ml of 95% ethanol (El-Greisy and El-Gamal, 2012).

3.1.4.2 Egg collection and incubation

Fish eggs for O. karongae were collected from brooding females and incubated in plastic tanks. Aeration was done using air stones but no heating was done considering the frequent blackout experienced during the time, since this experiment was conducted before the installation of the solar powered hatchery. Any dead eggs and larvae were removed during the incubation period. Upon hatching, swim up fry were there transferred to nursing tanks where first exogenous feed was administered.

3.1.4.3 Feeding

Feeding in the nursing tanks was done four times a day at 5% body weight for a period of 28 days. Effluent water from these nursing tanks was properly disposed into the ground by digging pit and bury the effluent. After 28 days of hormonal feed administration, the fish were then transferred

into hapas where they were raised up to a size big enough (above 50grams which took 6 months) to hand sex them.

3.1.4.4 Fish sampling

Fish Growth and survival rates were also monitored every 7 days during the time of hormone feed administration and monthly during the grow-out phase. During the final sampling which took place after 6 months, the proportion of males to females was recorded.

3.1.5 Water quality parameters

3.1.5.1 Water management and daily hatchery operations

Every morning the facility was checked to make sure that all the equipment such as water pumps, air pumps and heaters are properly functioning. Water flow and the bio-filtration system was regularly checked. Every morning upon taking water measurement, the tanks were siphoned to remove the uneaten feed accumulating at the bottom of the tanks. Water exchange was done by reducing the volume by : Cleaning the bio filtration substrates and add fresh water every 5 days. All the materials such buckets, stand pipes, feeding bottles, hand nets and siphoning pipes being used in the hatchery were cleaned (: Cleaning the bio filtration substrates) every time after use.

Figure 3.11: Cleaning the bio filtration substrates (Photo by Isaac).

3.1.5.2 Water Measurements

Water temperature, DO, Oxygen saturation levels, and pH were taken twice every day, thus in the morning at hours and in the afternoon at 14 hours and ammonia measurement was done every 4 days. Water temperature, saturation and dissolved oxygen were measured using an ox guard, while a pH meter was used to measure the pH. Ammonia measurements were done using a JBL TESTLAB water parameter measuring kit (**Fehler! Verweisquelle konnte nicht gefunden werden.**) which could also measure phosphate, zinc and many other compounds, but in this trial the focus was the ammonia. The measuring kit's principle is the reaction among the parameter to measure, for example the ammonia in the water and one or two reagents which produce a certain

colour in the sample for a given time. With this method, water sample is filled in two different vials and in one vial reagents are added according to the manufacturer's procedures. A colour develops and then a comparison with the colour code card (which is provided) is then made. Water exchange was done as in the previous experiment.

Figure 3.12: JBL Water parameter testing kit.

3.1.6 Data collection and analysis

3.1.6.1.1 Growth Parameters

Body weights were measured using a weighing balance on wet weight basis and the study assessed Final Weights (FW), amount of Weight Gained (WG) during the rearing period, Daily Weight Gain (DWG), Specific Growth Rates (SGR), Survival Rates (SR) and apparent food Conversion Ratios (AFCR).

a) The Specific Growth Rate SGR) was calculated using the following formula:

$$\text{SGR (\%/day)} = \left(\frac{\text{Ln}_{\text{Wf}} - \text{Ln}_{\text{Wi}}}{\text{t}}\right) 100$$

Where, lnWf = the natural logarithm of the mean final weight g), lnWi = the natural logarithm of the mean initial weight g), t = time days) between lnWf and lnWi

b) The average weight gained was derived from the calculations based on this formula.

$$\text{DWG (g/day)} = \text{Wt2} - \text{Wt1} / \text{t}$$

Where Wt_2 is final mean weight of fish at time t_2, Wt1is Initial mean weight of fish at time t_1 and t is time in days.

c) the amount of feed used to produce a unit mass of biomass, the food conversion ratio (FCR) was calculated using the following formula:

$$\text{FCR} = \frac{\text{dry weight of feed consumed (g)}}{\text{wet weight gain (g)}}$$

d) the total weight gain for an individual fish was calculated using the formula:

$$\text{WG (g)} = Wt_2 - Wt_1$$

Where Wt_2 is final mean weight of fish at time t2, and Wt_1 is initial mean weight of fish at time t_1.

e) The survival rates were expressed as the total number of fish at the end of the rearing period over the initial number of fry stocked, in percentage.

$$\%SR=\frac{\text{number of fingerlings at the end of the experiment}}{\text{number of fry at the start of the experiment}} \text{ x } 100$$

3.1.6.1.2 Statistical analysis

The data collected was subjected to a two -way analysis of variance using R statistical package version 3.5.1. The data collected was first tested for normality and homogeneity using the Wilk-Shapiro test and Fligner-Killeen test, respectively. A post-hoc Tukey test was carried out to determine the differences between the treatments and the parameters tested. Polynomial contrasts was carried out to determine the relationship between stocking density and fry growth as well as to find the optimal stocking density. In the all-male experiment, a Pearson Chi-square test was used to determine the performance of the hormone dosage. Hence only the growth data was subjected to ANOVA test.

CHAPTER 4

RESULTS AND DISCUSSION

Results

The results for normality and homogeneity test indicated that there was no significance. Furthermore means of all water quality parameters not controlled during the experiments were not significantly different and were with acceptable ranges for tilapia culture.

4.1.1 Effect of feed type and stocking density on growth performance and survival of O. karongae fry

The results show that there was no significant interactive effect between the feed type and the stocking density in all the parameters (Table 4.1). These include final weight attained (P=0.35), weight gain (P=0.36), specific growth rates (P=0.92), apparent feed conversion ratio (P=0.69) and Survival rates (P=0.24)

Table 4.1: Fry growth performance for the different feed types and stocking densities

Stocking Density	FT	IW(g)	FW(g)	WG(g)	SGR (%/day)	AFCR	SR (%)
A	CZAM	0.01	1.13	1.12	14.05	6.67	97.44
	GEM	0.01	1.89	1.87	15.91	3.76	97.44
	LMW	0.02	0.92	0.91	13.60	5.80	94.87
	MW	0.01	1.23	1.22	14.62	5.71	92.31
B	CZAM	0.01	0.86	0.849	12.88	5.79	97.30
	GEM	0.01	1.57	1.55	14.40	3.03	97.30
	LMW	0.02	0.74	0.72	12.57	5.23	96.00
	MW	0.01	0.96	0.95	13.74	4.67	98.70
C	CZAM	0.01	0.83	0.82	13.00	6.15	97.14
	GEM	0.01	1.37	1.36	14.50	3.18	98.10
	LMW	0.02	0.63	0.62	12.20	6.27	95.24
	MW	0.01	0.97	0.95	13.70	5.04	61.90
	P-Value		**0.35**	**0.36**	**0.92**	**0.69**	**0.24**

Notes: Notes: Where FT is feed type, A, B and C are stocking densities of 2500, 5000, 7500 hatchlings/m^3 respectively, CZAM is Novatek, GEM is Coppens, LMW is live feeding Malawi-Bunda feed, and MW is Malawi-Bunda feed without live feeding, IW is initial weight, FW is final weight, WG is weight DWG is daily weight gain, SGR is specific growth rates, AFCR is specific growth rate and SR is survival rate.

4.1.1.1 Effect of feed types on fry growth performance

However there was a significant effect of feed type on all the parameters (except survival rates). As indicated in Table 4.2, final weight attained (P<001), weight gain (P<001), daily weight gain (P<001), specific growth rates (P<001), apparent feed conversion ratio (P<001) and Survival rates (P=0.206).

Table 4.2: Overall performance of the different feed types.

Feed	IW(g)	FWT(g)	WG(g)	DWG(g)	SGR (%/day)	AFCR	SR (%)
CZAM	0.01	0.94 ± 0.06^{bc}	0.93 ± 0.06^{bc}	0.03 ± 0.01^{bc}	13.31 ± 0.06^{bc}	6.21 ± 0.09^{a}	95.30 ± 0.12^{a}
GEM	0.01	1.61 ± 0.06^{a}	1.60 ± 0.06^{a}	0.05 ± 0.01^{a}	14.93 ± 0.08^{a}	3.32 ± 0.067^{c}	97.62 ± 0.13^{a}
LMW	0.02	0.77 ± 0.05^{c}	0.75 ± 0.05^{c}	0.02 ± 0.01^{c}	12.80 ± 0.08^{c}	6.77 ± 0.12^{ab}	97.37 ± 0.17^{a}
MW	0.02	1.06 ± 0.05^{b}	1.04 ± 0.05^{b}	0.03 ± 0.01^{b}	14.02 ± 0.05^{ab}	5.14 ± 0.10^{b}	84.29 ± 0.05^{a}
P-value		<0.001	<0.001	<0.001	<0.001	<0.001	**0.206**

Notes: CZAM is Zambia Novatek, GEM is Germany Coppens, LMW is live feed+Malawi, MW is Malawi-Bunda feed without live feeding, IW is initial weight, FWT is final weight, WG is weight gain, DWG is daily weight gain, SGR is specific growth rates, AFCR is feed conversion ratio and SR is survival rate.

Means with different superscripts within the same column are significantly different ($p<0.05$)

The graph (

Figure *4.1*) shows the growth trend for the fish administered with the different types of feed. It shows Coppens feed from Germany had an outstanding growth curve throughout the entire culture period, reaching a final weight of 1.61 g. Fish growth in the Malawi-Bunda feed was not as fast as in the Coppens and Novatek feed during the first week, but later growth was shown to have gained momentum in the last subsequent weeks.

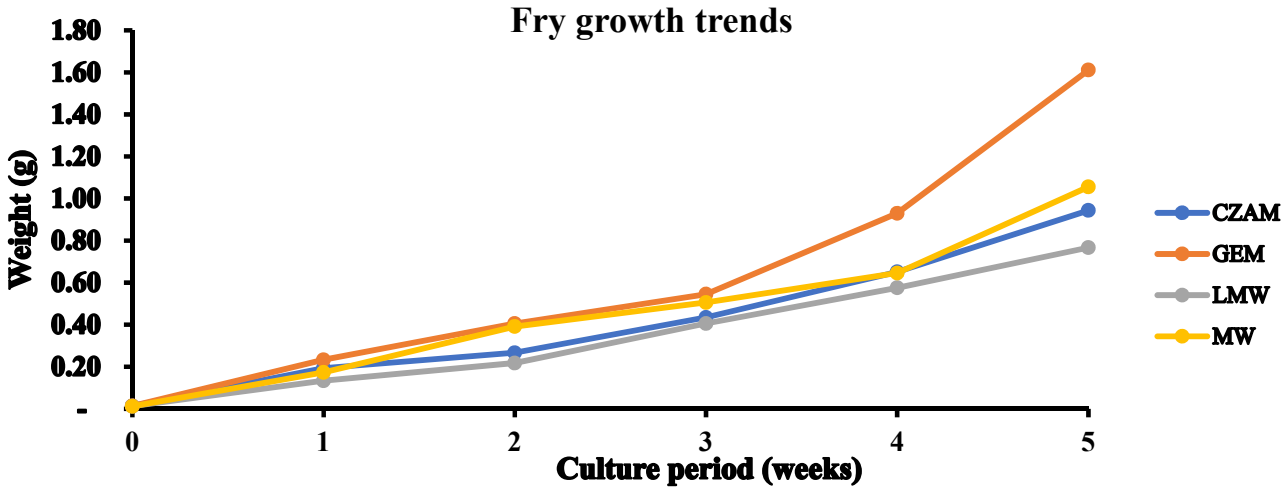


Figure 4.1: Fry growth performance for the four different diets.

Notes: CZAM is Zambia Novatek, GEM is Germany Coppens), LMW is live feed+Malawi and MW is Malawi-Bunda feed without live feeding

4.1.1.1.1 Final weight

The final weight attained by the fry during the course of this trial was found to be significantly (P<0.001) affected by the feed type. There was a significant difference between the 2 commercial diets (Germany-Coppens and the Zambia Novatek feed) (Table 4.2: Overall performance of the different feed types.

Feed	IW(g)	FWT(g)	WG(g)	DWG(g)	SGR (%/day)	AFCR
CZAM	0.01	0.94±0.06bc	0.93±0.06bc	0.03± 0.01bc	13.31± 0.06bc	6.21±0.09a
GEM	0.01	1.61±0.06a	1.60±0.06a	0.05± 0.01a	14.93± 0.08a	3.32±0.067c
LMW	0.02	0.77 ±0.05c	0.75±0.05c	0.02± 0.01c	12.80±0.08c	6.77±0.12ab
MW	0.02	1.06 ±0.05b	1.04±0.05b	0.03± 0.01b	14.02 ±0.05ab	5.14±0.10b
P-value		<0.001	<0.001	<0.001	<0.001	<0.001

Notes: CZAM is Zambia Novatek, GEM is Germany Coppens, LMW is live feed+Malawi, MW is Malawi-Bunda feed without live feeding, IW is initial weight, FWT is final weight, WG is weight gain, DWG is daily weight gain, SGR is specific growth rates, AFCR is feed conversion ratio and SR is survival rate.

Means with different superscripts within the same column are significantly different (p<0.05)

). A comparison between these two feed types showed that the Coppens feed from Germany recorded a higher a final weight (1.61g) than the Zambia Novatek Feed (0.94g). When the two local diets were compared, these indicated to have a significant (P<0.001) impact on the final weight attained by the fish. However the Malawi-Bunda feed that started with live feeding recorded a much significantly lower final weight (0.77g) than the Malawi-Bunda feed that introduced formulated diets right at the on-set of exogenous feeding which recorded a final weight

of 1.06g. The average final weight for the Malawi-Bunda feed was significantly lower than the Germany feed but it was not significantly different from the Zambia feed (Table 4.2: Overall performance of the different feed types.

Feed	IW(g)	FWT(g)	WG(g)	DWG(g)	SGR (%/day)	AFCR
CZAM	0.01	0.94±0.06bc	0.93±0.06bc	0.03± 0.01bc	13.31± 0.06bc	6.21±0.09a
GEM	0.01	1.61±0.06a	1.60±0.06a	0.05± 0.01a	14.93± 0.08a	3.32±0.067c
LMW	0.02	0.77 ±0.05c	0.75±0.05c	0.02± 0.01c	12.80±0.08c	6.77±0.12ab
MW	0.02	1.06 ±0.05b	1.04±0.05b	0.03± 0.01b	14.02 ±0.05ab	5.14±0.10b
P-value		<0.001	<0.001	<0.001	<0.001	<0.001

Notes: CZAM is Zambia Novatek, GEM is Germany Coppens, LMW is live feed+Malawi, MW is Malawi-Bunda feed without live feeding, IW is initial weight, FWT is final weight, WG is weight gain, DWG is daily weight gain, SGR is specific growth rates, AFCR is feed conversion ratio and SR is survival rate.

Means with different superscripts within the same column are significantly different (p<0.05)

).

4.1.1.1.2 Weight gain

Results from this study the average body weight gain and the average daily body weight gain for the fry subjected to the four types of feed were significantly (P<0.001) different (Table 4.2: Overall performance of the different feed types.

Feed	IW(g)	FWT(g)	WG(g)	DWG(g)	SGR (%/day)	AFCR
CZAM	0.01	0.94±0.06bc	0.93±0.06bc	0.03± 0.01bc	13.31± 0.06bc	6.21±0.09a
GEM	0.01	1.61±0.06a	1.60±0.06a	0.05± 0.01a	14.93± 0.08a	3.32±0.067c
LMW	0.02	0.77 ±0.05c	0.75±0.05c	0.02± 0.01c	12.80±0.08c	6.77±0.12ab
MW	0.02	1.06 ±0.05b	1.04±0.05b	0.03± 0.01b	14.02 ±0.05ab	5.14±0.10b
P-value		<0.001	<0.001	<0.001	<0.001	<0.001

Notes: CZAM is Zambia Novatek, GEM is Germany Coppens, LMW is live feed+Malawi, MW is Malawi-Bunda feed without live feeding, IW is initial weight, FWT is final weight, WG is weight gain, DWG is daily weight gain, SGR is specific growth rates, AFCR is feed conversion ratio and SR is survival rate.

Means with different superscripts within the same column are significantly different (p<0.05)

)**Fehler! Verweisquelle konnte nicht gefunden werden.**. The average weight gain for the fish fed with the Germany-Coppens feed was (1.6 g), 0.67 g higher than the Zambia Novatek feed **(**Figure **4.2: Average weight gain** if fish for the different feed types . Amongst the local diets, the Malawi live feed recorded a significantly lower weight gain of (0.75 g), 0.29 g lower than the Malawi-Bunda feed without live feeding. Like the average final weight, the average weight gain for the Malawi-Bunda feed was higher than the Zambia-Novatek feed but this difference was not significant (P=0.202). However fish weight gain for the Malawi-Bunda feed was significantly lower (P<0.001) than that of the Germany-Coppens feed, and fish weight gain for the Malawi-live feed was significantly lower than the rest of the feed types

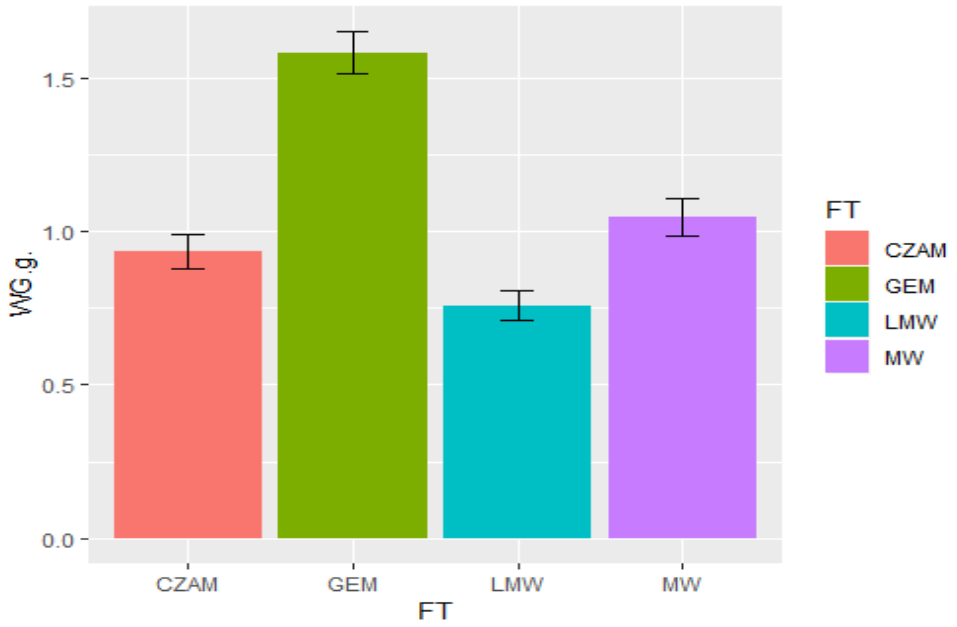


Figure 4.2: Average weight gain if fish for the different feed types

Notes: CZAM is Zambia Novatek, GEM is Germany Coppens, LMW is live feed+Malawi and MW is Malawi-Bunda feed without live feeding, FT is feed type and WG.g is weight gain in grams

4.1.1.1.3 Specific growth rates

Specific growth rate (SGR) is the percentage increase in body weight per day. In this study the SGR was significantly affected (P<0.001) by the feed type (**Fehler! Verweisquelle konnte nicht gefunden werden.**). The Coppens feed recorded an SGR of 14.93%/day, while the Zambia Novatek feed recorded a value of 13.31%/day. On one hand the Malawi diet had an SGR of 14.02% which seconded the Coppens diet in the ranking and this was significantly higher than both the Zambia (P<0.001) and the other Malawi diet. On the other hand the Malawi-live feeding diet

recorded a lower a SGR of 12.8% which was the least in the ranking of the four different feed types.

4.1.1.1.4 Apparent feed conversion ratio (AFCR)

The results of this study show that the feed type significantly (P<0.001) affected the apparent feed conversion ratios (AFCR). Amongst the commercial feed and amongst the local feed, there were also significant differences in their AFCR. The Germany-Coppens feed gave the lowest value (3.32), which was also significantly different to both the live feeding Malawi diet and the ordinary Malawi-Bunda feed (Table 4.2: Overall performance of the different feed types.

Feed	IW(g)	FWT(g)	WG(g)	DWG(g)	SGR (%/day)	AFCR
CZAM	0.01	0.94±0.06bc	0.93±0.06bc	0.03± 0.01bc	13.31± 0.06bc	6.21±0.09a
GEM	0.01	1.61±0.06a	1.60±0.06a	0.05± 0.01a	14.93± 0.08a	3.32±0.067c
LMW	0.02	0.77 ±0.05c	0.75±0.05c	0.02± 0.01c	12.80±0.08c	6.77±0.12ab
MW	0.02	1.06 ±0.05b	1.04±0.05b	0.03± 0.01b	14.02 ±0.05ab	5.14±0.10b
P-value		<0.001	<0.001	<0.001	<0.001	<0.001

Notes: CZAM is Zambia Novatek, GEM is Germany Coppens, LMW is live feed+Malawi, MW is Malawi-Bunda feed without live feeding, IW is initial weight, FWT is final weight, WG is weight gain, DWG is daily weight gain, SGR is specific growth rates, AFCR is feed conversion ratio and SR is survival rate.

Means with different superscripts within the same column are significantly different (p<0.05)

). The highest AFCR value (6.77) was recorded in the live feeding Malawi diet which was also significantly higher than the Zambia diet. When compared to the Zambia feed, the ordinary

Malawi-Bunda feed was shown to have recorded a better FRC (5.14) and this difference was significant (P<0.0007) as well as indicated in the same table (Table 4.2: Overall performance of the different feed types.

Feed	IW(g)	FWT(g)	WG(g)	DWG(g)	SGR (%/day)	AFCR
CZAM	0.01	0.94±0.06bc	0.93±0.06bc	0.03± 0.01bc	13.31± 0.06bc	6.21±0.09a
GEM	0.01	1.61±0.06a	1.60±0.06a	0.05± 0.01a	14.93± 0.08a	3.32±0.067c
LMW	0.02	0.77 ±0.05c	0.75±0.05c	0.02± 0.01c	12.80±0.08c	6.77±0.12ab
MW	0.02	1.06 ±0.05b	1.04±0.05b	0.03± 0.01b	14.02 ±0.05ab	5.14±0.10b
P-value		<0.001	<0.001	<0.001	<0.001	<0.001

Notes: CZAM is Zambia Novatek, GEM is Germany Coppens, LMW is live feed+Malawi, MW is Malawi-Bunda feed without live feeding, IW is initial weight, FWT is final weight, WG is weight gain, DWG is daily weight gain, SGR is specific growth rates, AFCR is feed conversion ratio and SR is survival rate.

Means with different superscripts within the same column are significantly different (p<0.05)

).

4.1.1.1.5 Survival rates (SR)

Feed type in this trial did not show any significant effect (P=0.206) on the number of individuals that survived at the end of the trial as compared to the number of individuals that were stocked (as shown in Table 4.2: Overall performance of the different feed types.

Feed	IW(g)	FWT(g)	WG(g)	DWG(g)	SGR (%/day)	AFCR

CZAM	0.01	0.94±0.06bc	0.93±0.06bc	0.03± 0.01bc	13.31± 0.06bc	6.21±0.09a
GEM	0.01	1.61±0.06a	1.60±0.06a	0.05± 0.01a	14.93± 0.08a	3.32±0.067c
LMW	0.02	0.77 ±0.05c	0.75±0.05c	0.02± 0.01c	12.80±0.08c	6.77±0.12ab
MW	0.02	1.06 ±0.05b	1.04±0.05b	0.03± 0.01b	14.02 ±0.05ab	5.14±0.10b
P-value		<0.001	<0.001	<0.001	<0.001	<0.001

Notes: CZAM is Zambia Novatek, GEM is Germany Coppens, LMW is live feed+Malawi, MW is Malawi-Bunda feed without live feeding, IW is initial weight, FWT is final weight, WG is weight gain, DWG is daily weight gain, SGR is specific growth rates, AFCR is feed conversion ratio and SR is survival rate.

Means with different superscripts within the same column are significantly different (p<0.05)

). As this experiment was carried out in a very well controlled environment, mortalities were not a problem during this trial. The results were the same when comparisons amongst the four different feed types were made. However fish survival was shown to be better in both the Germany-Coppens and the Malawi Live feeding diets (97.62% and 97.37% respectively). The Zambia Novatek recorded an average survival rate of 95.3% while the Malawi diet recorded the least average survival rate 84.29%.

4.1.1.2 Effect of stocking density on growth performance and survival of O. karongae fry

The study results indicate that the growth performance was significantly affected by the stocking density (Table 4.3: Growth performance in the three different stocking densities).

Table 4.3: Growth performance in the three different stocking densities

Density (F/m3)	**IW(g)**	**FW(g)**	**WG(g)**	**DWG(g)**	**SGR(%/day)**	**AFCR**	**SR (%)**
A(2500)	0.02	1.30 ± 0.10^{a}	1.28 ± 0.10^{a}	0.04 ± 0.02^{a}	14.55 ± 0.07^{a}	5.48 ± 0.15^{a}	97.33 ± 0.18^{a}
B(5000)	0.02	1.04 ± 0.09^{ab}	1.02 ± 0.09^{ab}	0.03 ± 0.02^{ab}	13.40 ± 0.07^{b}	4.68 ± 0.14^{a}	95.51 ± 0.10^{a}
C(7500F)	0.01	0.95 ± 0.08^{b}	0.94 ± 0.08^{b}	0.03 ± 0.01^{b}	13.36 ± 0.08^{b}	5.16 ± 0.18^{a}	88.10 ± 0.8^{a}
P value		**0.0508**	**0.0495**	**0.0495**	**0.00595**	**0.314**	**0.302**

Means with different superscripts within the same column are significantly different ($p<0.05$)

IW is initial weight, FW is final weight, WG is weight gain, DWG is daily weight gain, SGR is specific growth rate, AFCR is apparent feed conversion ratio and SR is survival rate

However, the apparent feed conversion ratios (AFCR) and survival rates were not significantly affected as shown in the Table 4.3: Growth performance in the three different stocking densities3. In all the three different densities, growth trend was observed to increase throughout the culture period, with the lowest densities recording the best trend (Figure *4.3*).

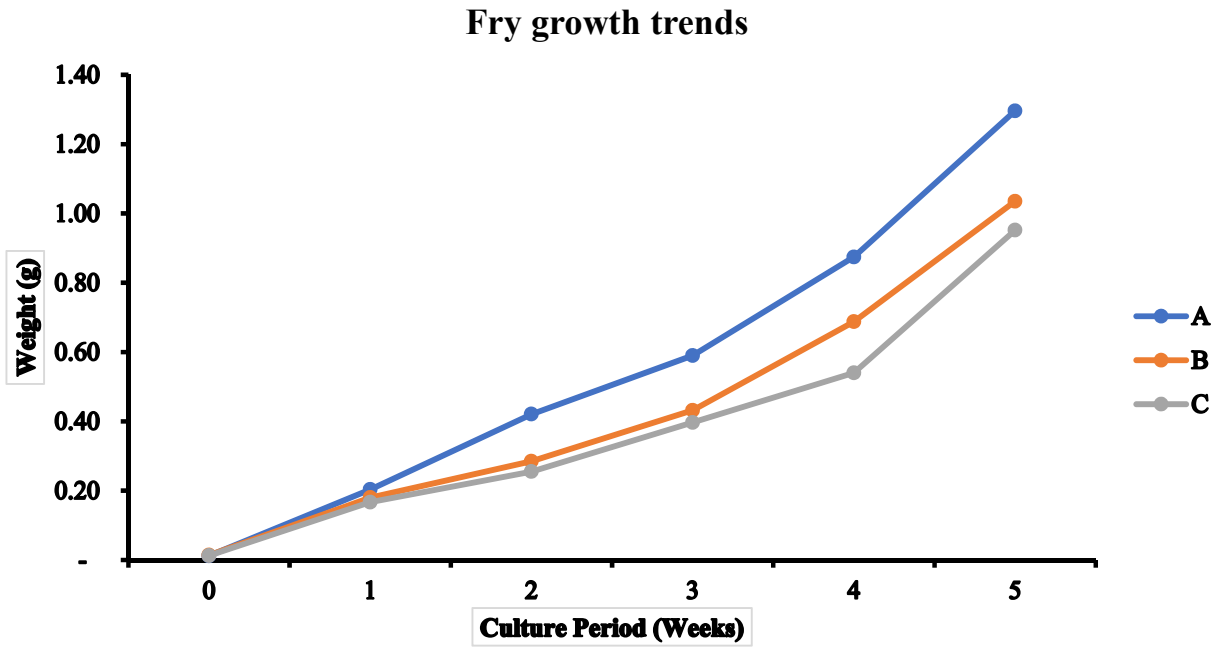


Figure 4.3: Fry growth trends raised under the three different stocking densities

Notes: **A** is 2500fry/m3, **B** is 5000 fry/m3 and **C** is 7500 fry/m3

4.1.1.2.1 Final weight

A polynomial contrast indicates that from a density of 2500frm/m^3 fish growth significantly increased as the density was increase until after a density of 4700fry/m^3 (Figure 4.4). Fry growth begins to decrease as the stocking densities are increased just after the 4700fry/m^3. Fry growth is maximised at this stocking density, thus the optimum stocking density is in this study was 4700 fry/m^3.

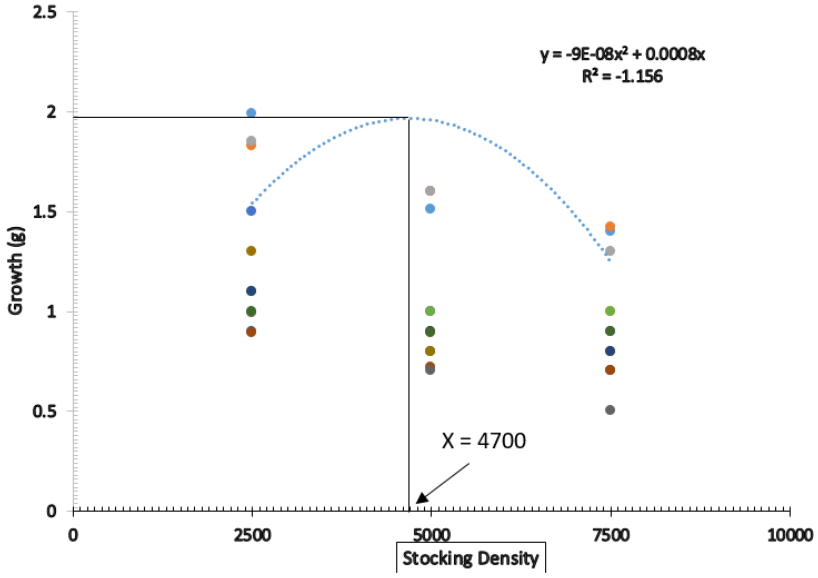


Figure 4.4: fish growth curve in response to stocking densities.

4.1.1.2.2 Weight gain

The average amount of weight gained from the time of stocking to the time of termination was significantly affected (P=0.0495) by the stocking density. On one hand the difference in weight

gain for the fish raised in lower densities was significantly higher than for those raised in medium and high densities (**Fehler! Verweisquelle konnte nicht gefunden werden.**). There was no significant difference (P=0.463) in the weight gain for the fish raised in the medium and the high stocking densities. The fish raised in the medium stocking densities recorded a weight gain of 1.02g, 0.08g better than the value for those raised in the higher densities (**Fehler! Verweisquelle konnte nicht gefunden werden.**). The difference in weight gain between the lower density levels and the higher density levels was 0.34g and this was much significant (P=0.019).

4.1.1.2.3 Specific growth rates

Stocking densities also significantly affected the percentage increase in weight per unit time. The SGR values recorded in fish from the medium and high stocking densities were significantly lower (P=0.005) than those recorded in lower densities (**Fehler! Verweisquelle konnte nicht gefunden werden.** The difference in terms of SGR was not much pronounced in the medium and high stocking densities (0.04%) and this was not significant (P=0.11).

4.1.1.2.4 Apparent feed conversion ratio (AFCR)

A statistical analysis of the results indicated that there was no significant difference (P=0.314) in terms of the apparent feed conversion ratios of the fish stocked in the varying levels of stocking densities (**Fehler! Verweisquelle konnte nicht gefunden werden.**). However the medium

densities had the lowest average value of 4.68 compared to the lower and highest density levels. (Which recorded values of 5.48 and 5.16 respectively) but this difference was not significant.

4.1.1.2.5 Survival rates

The results for this study show that the varying stocking densities did not have a significant effect (P=0.302) on the survival rates. When lower stocking densities was compared to the medium and the high stocking densities, no effect on survival was significant (**Fehler! Verweisquelle konnte nicht gefunden werden.**). Neither was there any significant effect when the medium and high stocking densities were compared. The higher stocking densities recorded an average survival rate of 88.10% while the medium and lower levels recorded average survival rates of 95.51% and 97.33% respectively

4.1.1.3 Water parameters

The results (Table 4.4: Water parameter recordings for the culture facilities during the trial.) obtained for water parameters showed that all the water parameters in the different culture units were within the acceptable range for the culture of tilapia species.

Table 4.4: Water parameter recordings for the culture facilities during the trial.

	DO(mg/l)		Saturation (%)		Temperature (°C)		pH		Ammonia
Facility	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	mg/L
BigTank1	7.34±0.03	7.06±0.03	92.64±0.71	88.95±0.73	25.33±0.07	26.41±1.07	8.49±0.40	8.57±0.36	<0.05
BigTank2	7.27±0.03	7.02±0.03	90.50±0.71	87.28±0.73	24.95±0.07	25.87±1.07	8.50±0.40	8.60±0.36	<0.05
Incubator	7.41±0.03	7.25±0.03	93.48±0.71	88.56±0.73	25.51±0.07	32.51±1.07	10.89±0.40	10.76±0.36	<0.05
T1A	7.24±0.03	7.06±0.03	89.41±0.71	85.99±0.73	25.19±0.07	26.22±1.07	8.49±0.40	8.57±0.36	<0.05
T1B	7.21±0.03	7.04±0.03	89.29±0.71	88.03±0.73	25.21±0.07	26.07±1.07	8.49±0.40	8.57±0.36	<0.05
T1C	7.25±0.03	7.10±0.03	90.08±0.71	84.20±0.73	25.25±0.07	25.98±1.07	8.49±0.40	8.57±0.36	<0.05
T2A	7.25±0.03	7.06±0.03	88.95±0.71	86.32±0.73	25.10±0.07	25.86±1.07	8.50±0.40	8.60±0.36	<0.05
T2B	7.27±0.03	7.13±0.03	89.31±0.71	88.11±0.73	25.02±0.07	25.81±1.07	8.50±0.40	8.60±0.36	<0.05
T2C	7.28±0.03	7.12±0.03	89.17±0.71	85.50±0.73	25.08±0.07	25.88±1.07	8.50±0.40	8.60±0.36	<0.05

Notes: DO is dissolved oxygen, T1A is tank 1A

The graphs (Figure 4.5) show the trends in some of the water parameters during the culture period. There was a variation in water temperature from week to week unlike in the ammonia levels which shows that throughout the culture period it was below 0.05ml/L. Dissolved oxygen levels reduced as the culture period increased. No drastic changes were observed in the pH levels throughout the experimental period.

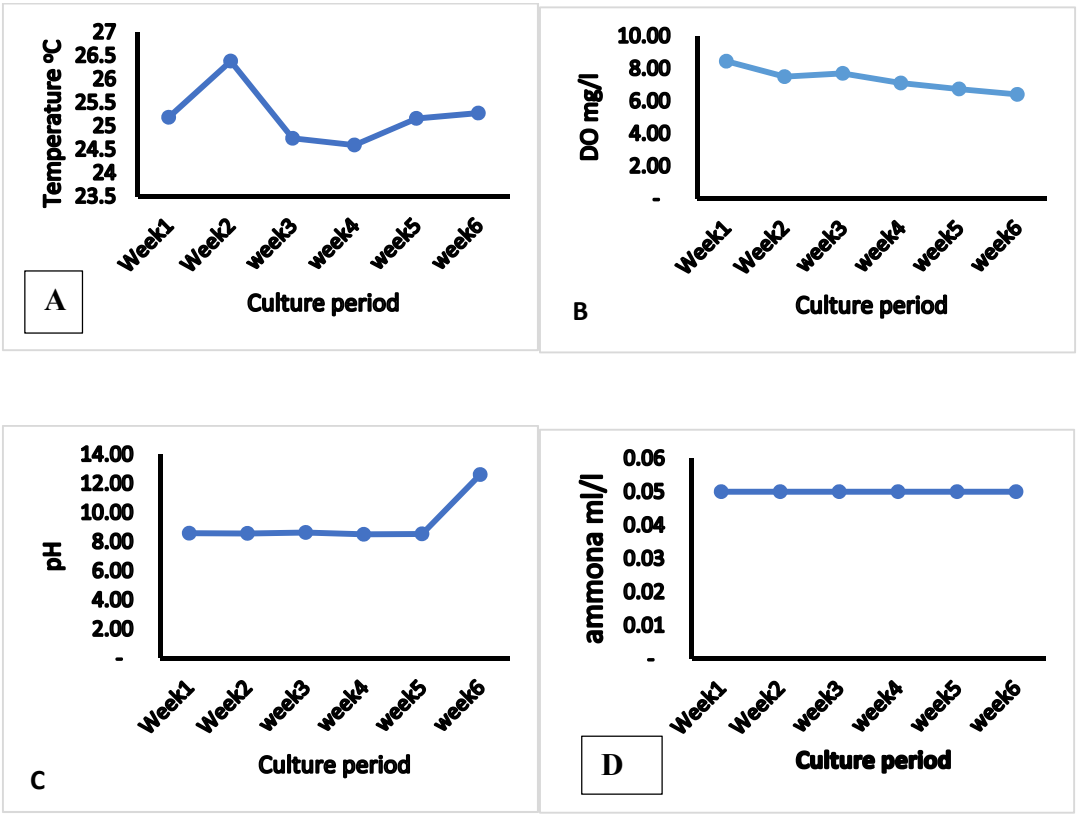


Figure 4.5: Water parameter dynamics in the culture facilities for the entire experimental period **A** water temperature, **B** dissolved oxygen, **C** pH and **D** ammonia**.**

4.1.2 Effect of water temperature on fry growth and survival of O. karongae fry

Table 4.5**:** Fry growth performance in relation to water temperature

Temp (^{o}C)	IW (g)	FW(g)	WG(g)	DWG (g)	SGR (%/day)	AFCR	SR (%)
28	0.09	2.98± 0.09	2.89± 0.10	0.09± 0.02	11.10± 0.10	5.57 ± 0.13	98.6± 0.06
24	0.09	1.69 ± 0.10	1.60± 0.10	0.05± 0.03	9.16 ± 0.09	10.15± 0.28	96.01± 0.06
P-value		**0.004**	**0.004**	**0.008**	**0.011**	**0.008**	**0.287**

6 provides a summary of the results for fish growth parameters recorded and calculated for the two levels of water temperature. The fish cultured in high water temperature (28 ^{o}C) showed better growth performance than the fish raised in the 24^{o}C water temperature. As shown in Table 4.5**:** Fry growth performance in relation to water temperature 6 most of the growth parameters had been significantly affected by the water temperatures.

Table 4.5**:** Fry growth performance in relation to water temperature

Temp (^{o}C)	IW (g)	FW(g)	WG(g)	DWG (g)	SGR (%/day)	AFCR	SR (%)
28	0.09	2.98± 0.09	2.89± 0.10	0.09± 0.02	11.10± 0.10	5.57 ± 0.13	98.6± 0.06
24	0.09	1.69 ± 0.10	1.60± 0.10	0.05± 0.03	9.16 ± 0.09	10.15± 0.28	96.01± 0.06
P-value		**0.004**	**0.004**	**0.008**	**0.011**	**0.008**	**0.287**

Notes: IW is initial weight, FW is final weight, WG is weight gain, DWG is daily weight gain, SGR is specific growth rate, AFCR is apparent feed conversion ratio and SR is survival rate.

The graph (Figure 4.6) outlines the trends in fish growth raised in the two different levels of water temperatures. The growth trend was better in fish raised in high (28°C) water temperatures than in those raise in lower (24°C) water temperatures.

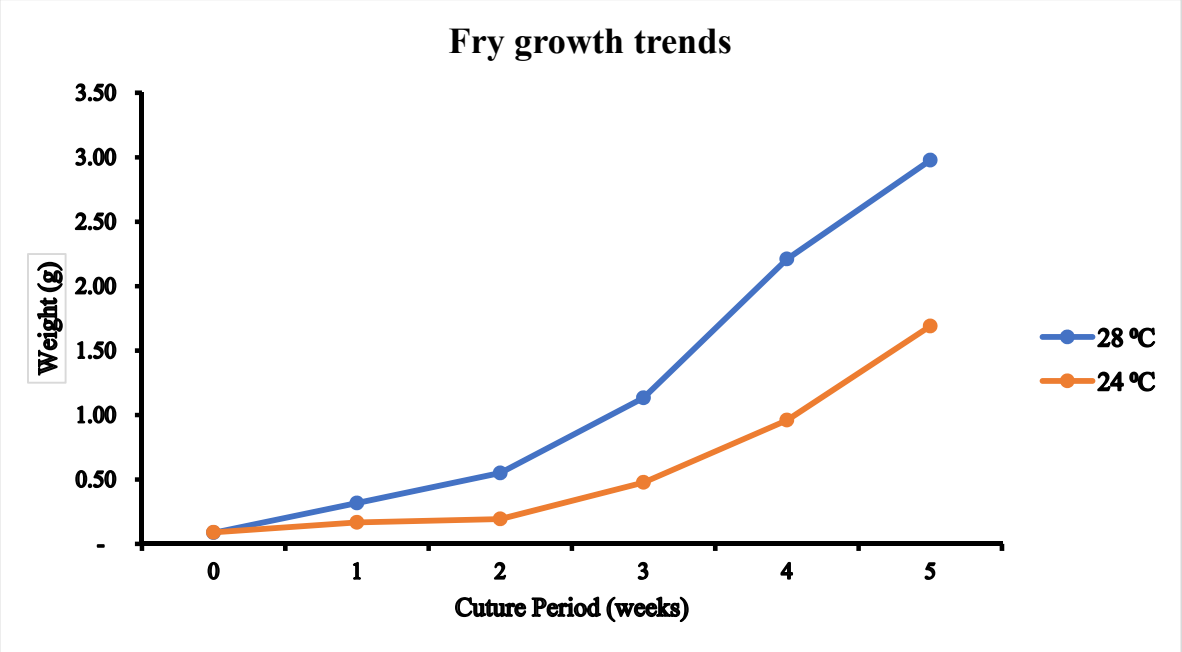


Figure 4.6 : Fry growth trends in relation to water temperature.

4.1.2.1 Final weight

The average final weight attained by the individual fish in this experiment was significantly affected (P=0.003) by temperature (Table 4.5**:** Fry growth performance in relation to water temperature 6**)**. The fish raised in the tank where its water temperature was high recorded a higher average final weight (2.98± 0.29g) , 1.29g higher than the average final weight in the lower water temperature tank (1.69 ± 0.23g).

4.1.2.2 Weight gain (WG)

The average amount of weight an individual fish gained from the time of stocking until the end of the experiment was significantly affected (P=0.003) by the temperature, as indicated in (Table 4.5**:** Fry growth performance in relation to water temperature 6**)**. Weight gain was recorded to be high in the high temperature treatment (2.89± 0.29g), the lower temperature treatment on the other hand recorded a lower average weight gain of 1.60± 0.22g.

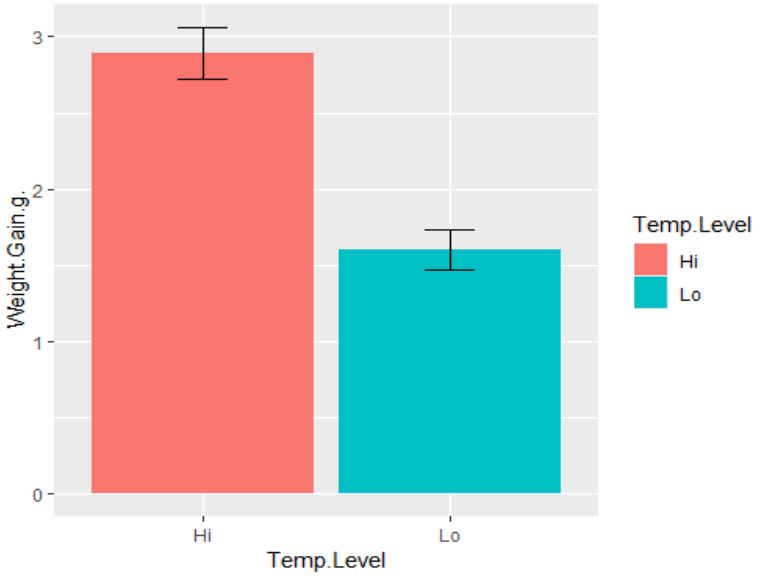


Figure 4.7: Weight gain (g) in relation to water temperature.

Notes: Low tem is 24 ºC and High tem is28 ºC

The trend in weight gain during the culture period was also observed to vary amongst the two different levels of water temperature. The graph in Figure 4.8 : Weight gain trends in relation to water temperature.

indicates an increase in weight gain from week 2 to week 4, week 1 was observed to record minimal weight gain values.

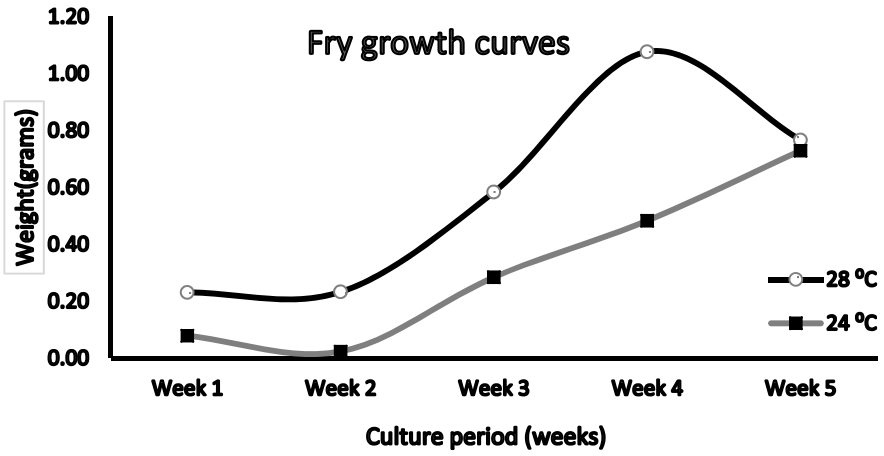


Figure 4.8 : Weight gain trends in relation to water temperature.

In terms of Daily weight gain, there was a significant effect of temperature on the average daily weight gain. The fish raised in the 28°C water temperature which also recorded the highest weight gain, had a higher daily weight gain of 0.09± 0.01g, 0.04g higher than the lower temperature treatment which recorded a daily weight gain of 0.05g.

4.1.2.3 Specific growth rates (SGR)

The percentage increase in weight per unit time, specific growth rate was significantly (P=0.01) affected by the temperature level (Table 4.5**:** Fry growth performance in relation to water temperature 6). The lower temperature level treatment recorded SGR of 11.10 ± 0.10%/day, 1.94% less than in the higher temperature treatments which recorded an SGR of 9.16 ± 0.47%.

4.1.2.4 Apparent feed conversion ratio (AFCR)

In terms of the ability to convert unit mass of feed administered to unit biomass, the fish raised in high water temperature recorded lower values with an average of 5.57 ± 0.54. On the other hand fish that were raised in lower water temperature tank recorded a high AFCR value (10.15± 1.52). Statistical analysis of these results, indicated that the temperature level had a significant (P=0.008) effect on the apparent feed conversion ratio (Table 4.5**:** Fry growth performance in relation to water temperature 6). This indicates that high water temperatures are associated with lower AFCR values.

4.1.2.5 Survival rates

The number of fish that survived during the time of experiment termination was not significantly (P=0.2879) affected by the temperature level, as shown by the statistical p-value of 0.2879 (Table 4.5**:** Fry growth performance in relation to water temperature 6). Comparatively, the high water temperature treatment had a better survival rates (98.01± 1%) than the lower water temperature treatment which had a survival rate of 96.6± 1%. Like in the other feed experiment, most of the fish losses were due to escapes from the small bucket into the big tank.

4.1.2.6 Water parameters, and these were within the tolerable range for tilapia culture.

Table 4.6: Water parameters in in the two different rearing tanks. 7 provides reading for various water parameters obtained during the course of the experiment, and these were within the tolerable range for tilapia culture.

Table 4.6: Water parameters in in the two different rearing tanks.

Temperature (ºC)	**DO(mg/l)**		**Saturation (%)**		**Temperature (ºC)**		**pH**		**Ammonia (mg/l)**
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	
24	7.14 ±0.04	7.16±0.03	93.64±0.73	87.94±1.06	24.33±0.05	24.410.06	8.29±0.04	8.07±0.03	<0.05
28	7.27 ±0.04	7.22±0.03	93.50±0.73	86.18±1.06	28.050.05	28.070.06	8.30±0.04	8.10±0.03	<0.05
P-value	**0.11**	**0.999**	**0.314**	**0.364**	**<0.001**	**<0.001**	**0.33**	**0.363**	**0.34**

Notes: DO is dissolved oxygen.

4.1.3 Effect of photoperiodicity on growth performance and survival of O. karongae fry

The results recorded for the two different light regimes indicate that light regime significantly affected most of the growth parameters in this study (Table 4. 8).

Table 4.7: Fry growth performance in relation to photoperiod

Photoperiod	IW(g)	FWT(g)	WG(g)	ADWG(g)	SGR (%/day)	AFCR	SR (%)
24	0.02	3.18± 0.12	3.16± 0.11	0.08 ± 0.02	17.10± 0.08	3.22± 0.12	97.67 ± 0.07
16	0.01	1.93± 0.06	1.92± 0.12	0.05 ± 0.03	15.65 ± 0.07	5.13± 0.23	99.00± 0.06
P-value		**0.005**	**0.01747**	**0.02131**	**0.02375**	**0.0272**	**0.7247**

Notes: IW is initial weight, FWT is final weight, WG is weight gain, DWG is daily weight gain, SGR is specific growth rate, AFCR is feed conversion ratio, and SR is survival rate.

The growth trends for the fish raised in the two different light regimes are outlined in Figure 4.9: Fry growth trend in relation to photoperiod Fish raised in the higher (24:0 LD) light regime show a better growth trend than those raised in the lower (16:8 LD) light regime.

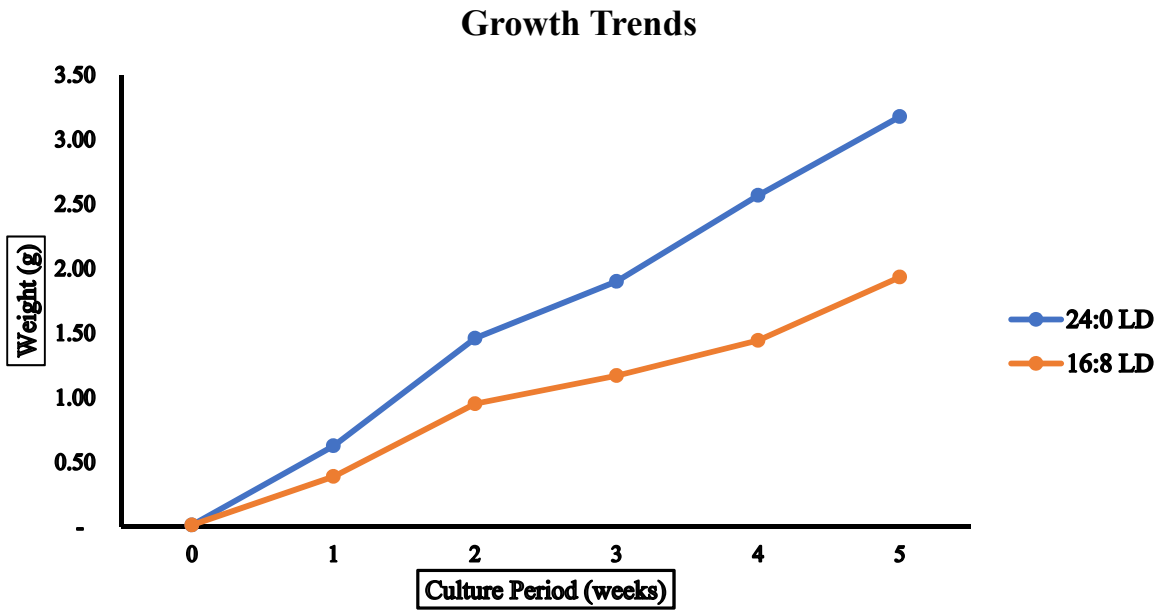


Figure 4.9: Fry growth trend in relation to photoperiod.

4.1.3.1 Final Weight

The average final weight was significantly affected (P=0.005) by the light regime (Table 4. 89). The 24 hour regime recorded the highest final weight of 3.18± 0.3g while 16 hour regime recorded a lower average final weight of 1.93± 0.6g.

4.1.3.2 Weight gain

The weight gain refers to the mount of biomass an individual fish has accumulated during the experimental period. As shown in table 4.8, there was a significant effect (P=0.017) of the photoperiodicity on the amount of weight gained. The 16 hour light regime a recorded a weight gain of 2.09g, 1.07g lower than the 24 hour light regime which recorded an average weight gain of 3.1g (Figure 4.10: Weight gain (in g) for fish in relation to photoperiod0).

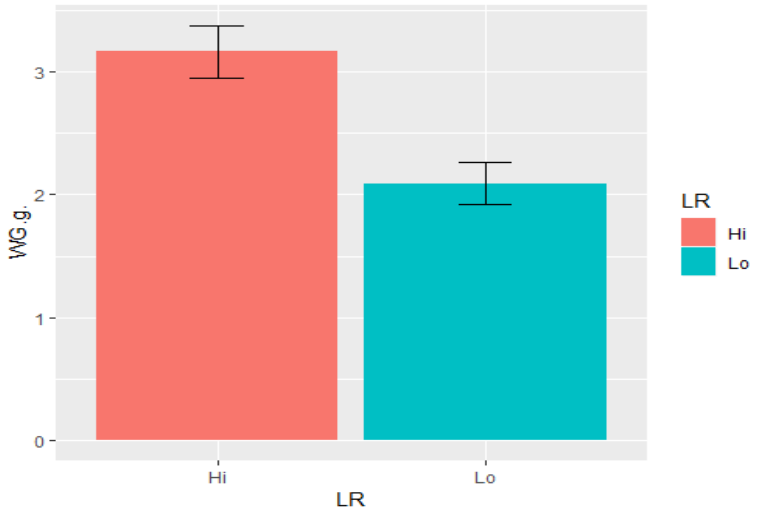


Figure 4.10: Weight gain (in g) for fish in relation to photoperiod.

Notes: Hi light regimeis24 hours light, zero hours darkness and Lo light regimeis16 hours light, 8 hours darkness, WG.g is weight gain LR is light regime/photoperiod)

The trend in weight gain (Figure 4.11 : Weight gain trend in relation to photoperiod.1) during the culture period was also observed to vary amongst the two different levels of photoperiod. The gain in weight was recorded highest (0.83g) for the 24-hour regime and 0.56g for the 16-hour regime) in week 2, after which both treatments' weight gain drastically decreased.

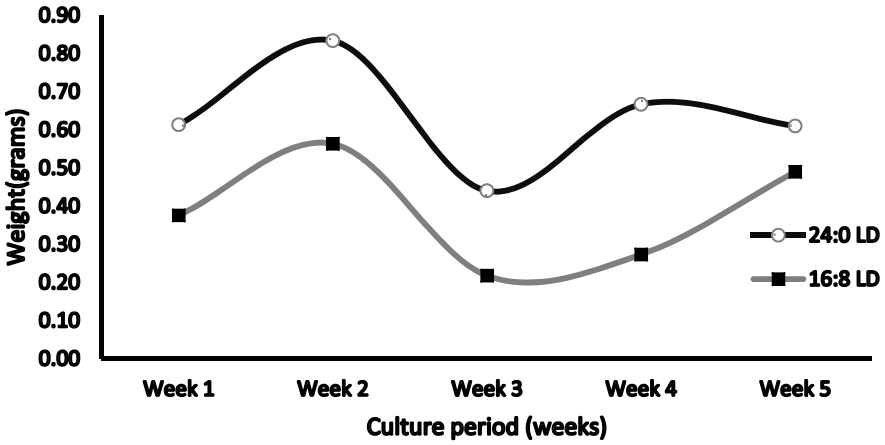


Figure 4.11 : Weight gain trend in relation to photoperiod.

In terms of the average daily weight gain determined for both treatments, it was shown to be significantly affected (P=0.021) by the light regime (Table 4.7). The 24-hour regime had a high ADWG of 0.08g as compared to 0.05g recorded in the 16-hour regime.

4.1.3.3 Specific growth rate (SGR)

The SGR values recorded in this study for the 24-hour and 16-hour light regimes were 17.10± 0.5% of the body weight and 15.65 ± 0.4% /day respectively. This means that the fish weight was

increasing at the rate of 17.1% /day of its individual body weight in the higher light regime and 15.65%/day in the lower light regime. The analysis indicated that the SGR was significantly affected (P=0.0237) by the light regime (Table 4. 8). Table *4.7*: Fry growth performance in relation to photoperiod

4.1.3.4 Apparent feed conversion ratio feed conversion ratio (AFCR)

In this study, light regime had a significant effect (P=0.0272) on AFCR as shown in Table 4.7: Fry growth performance in relation to photoperiod. As indicated in **Fehler! Verweisquelle konnte nicht gefunden werden.**2, the value is higher for the low light regime (5.13± 0.8) than in the higher light regime (3.22± 0.3).

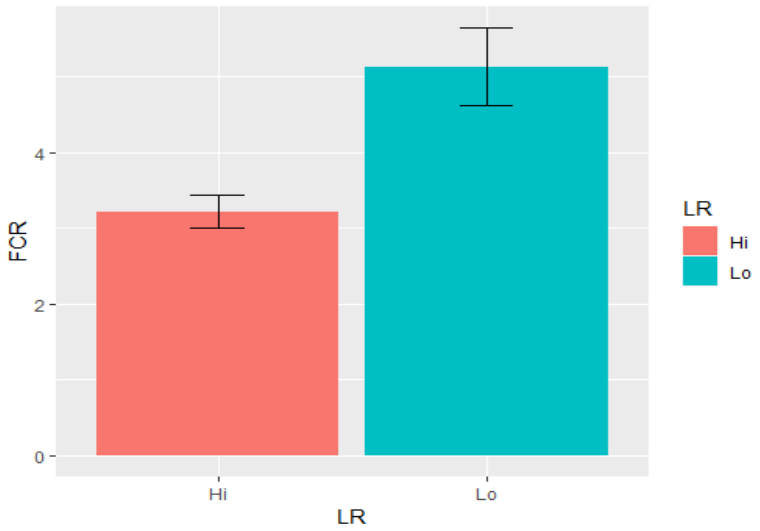


Figure 4.12 : Apparent feed conversion ratios in relation to photoperiod.

Notes: (Hi light regime is 24 hours light, zero hours darkness and Lo light regime is 16 hours light, 8 hours darkness)

4.1.3.5 Survival rates

The survival rates were observed to be high in the lower light regime (99.00± 1.0%) than in the higher light regime (97.67 ± 1.1%), but the difference was not significant (Table 4. 8).

4.1.3.6 Water parameters

Table 4.79 is a summary of recording for water parameters obtained during the experimental period. All water parameters measured during the course of the experiment namely; dissolved oxygen, pH and ammonia were within the standard range for growth and survival of tilapia fry.

Table 4.8: Water parameter recordings for the photoperiod experiment

Photoperiod	**DO(mg/l)**		**Saturation (%)**		**Temperature (ºC)**		**pH**		**Ammonia(mg/l)**
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	
16 hours	7.54±0.03	7.26±0.04	94.64±0.7	89.95±1.04	27.33±0.06	8.31±0.07	8.19±0.03	.47±0.04	<0.05
24 hours	7.67±0.03	7.12±0.04	93.50±0.7	88.28±1.04	27.95±0.07	27.97±0.07	8.30±0.03	8.00±0.04	<0.05
P-Value	**0.11**	**0.368**	**0.238**	**0.915**	**0.634**	**0.577**	**0.37**	**0.382**	**0.422**

Notes: DO is morning dissolved oxygen.

4.1.4 Hormone dosage and its effect on production of all-Male O. karongae fingerlings

As indicated in table 4.10, a Pearson's Chi-square test indicates that sex proportions depend on the dosage of hormone incorporated in the feed. The fish that were subjected to a dose of 60mg/kg of feed resulted into high proportion of males (75.5%) to females (24.5%). The 20 mg/mL/kg group recorded the lowest numbers of males (43%) compared to the number of females (51). The fish that were subjected to hormonal dose of 30 mg/mL/kg of feed produce the least gap (10) between the numbers of males to females (50 males vs. 40 females).

Table 4.9 Output of person's Chi-square test

Hormone dosage	**Females**	**Males**	**Row total**
20mg/ml/kg	51	39	90
30mg/ml/kg	40	50	90
60mg/ml/kg	22	68	90
90mg/ml/kg	39	51	90

Chi^2 = 19.68 d.f = 3 p = 0.0002

4.1.4.1 Weight gain

The weight gained at the end of the experimental period varied amongst the different doses of hormones (Table 4.10). The highest amount of weight gain was realized in the 30mg/mL/kg treatment (81.97g). Fish growth was observed to be limited in the high dose (90mg/mL/kg) which

attained a weight gain of 49.34g with the 8 months of culture period. The 60mg/kg and the 20mg/mL/kg doses recorded an average weight gain of 74.16g and 64.79 g, respectively (Figure 4.15).

Table 4.10 **:** Weight gain (in g) for fish in relation to different doses of hormonal feed

Hormone Dose	**Average of WG(g)**	**Average of FSL(mm)**	**Average of FTL(mm)**
20mg/kg	64.79±0.17^{c}	121.02±0.11^{a}	143.87± 0.12^{a}
30mg /kg	81.97± 0.16^{a}	119.41±0.12^{a}	138.48± 0.13^{a}
60mg /kg	74.16±0.15^{b}	100.16±0.21^{c}	126.20±0.14^{b}
90mg /kg	49.34±0.10^{d}	93.48± 0.18^{b}	100.5±0.18^{c}
P-value	<0.001	<0.001	<0.001

Notes: Means with different superscripts within the same column are significantly different ($p<0.05$) WG is weight gain g, FSL is final standard length in millimetres, FTL is final total length in millimetres.

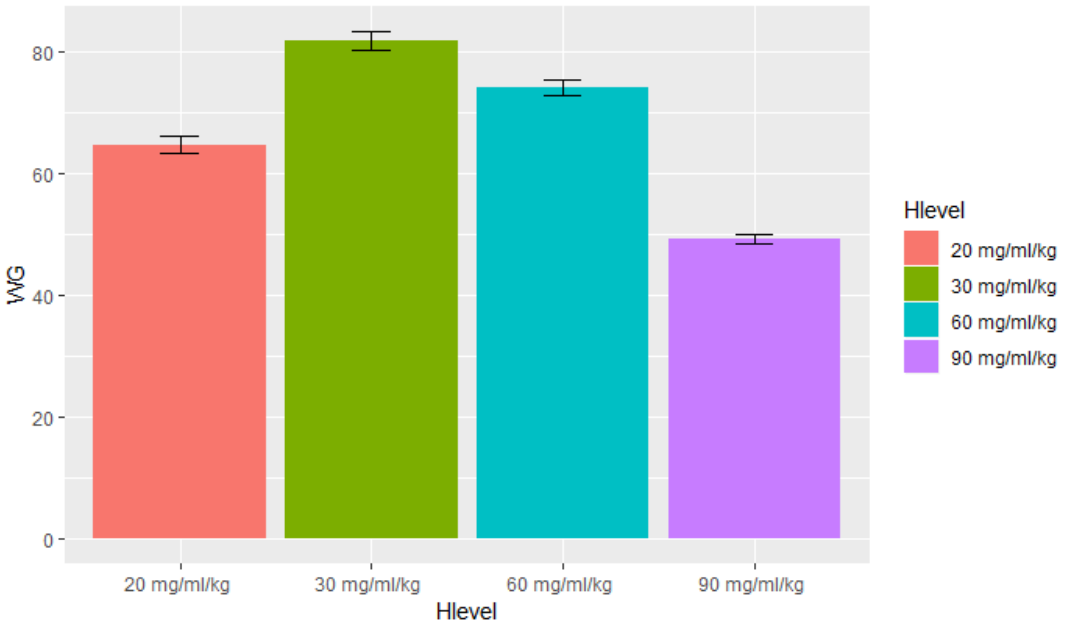


Figure 4.13: Average weight gain (in g) against hormonal dose

As indicated in Table 4.711 the weight gain, standard length and the total length among the treatments, were significantly different.

4.1.5 Effect of feed type on growth performance and survival of O. karongae fry

The results of this experiment where the effect of feed types and stocking densities on fish growth performance was investigated indicates that there was no significant interactive effect of these two factors (feed type and stocking densities) on various growth parameters. This shows that the number of fish raised in a culture facility cannot influence the type of feed but rather the amount. Similar findings were obtained with other authors. Khattab et al., (2001) for example, reported a significant effect of stocking density and feed type, but there was no significant interaction of feed type and stocking density on growth performance, survival, feed utilisation and body composition of Nile tilapia fry.

In this study the two categories of feed (locally prepared and commercial feed) are produced from different countries where there is a huge variation in the feed ingredients used in fish feed formulation, thus the performance of these types of feed when they were tested under uniform environmental condition varied. The results are in agreement with the finding by Fall et al., (2011) who evaluated the effect of different sources of protein on the growth of hybrid Tilapia (O. niloticus x O. aureus). In their study, where the protein sources used were fish meal, soy bean meal squid meal and shrimp meal, a significant effect was recorded on the growth performance and mortality rates.

The results of this study indicate that feeding Tilapia with live food before introducing formulated feed does not improve growth performance. Many studies have indicated that live food improves growth in tilapia where the live food is used as a supplemental diet. For example García-Ulloa et

al. (2013) reported that use of 90 percent starter diets and 10 % decapsulated Artemia cysts (DAC) in O. niloticus fry improved growth than in those diets where 100% starter diets were used. Similar results were obtained by Akbary et al., (2010) where comparison between live food and artificial diet on survival rate, growth and body chemical composition of Oncorhynchus mykiss larvae was investigated. In this study live food was not used as a supplement, it was rather used as a starter. Furthermore the zooplanktons used was generalised, no specific identification of the type of zooplanktons suitable for fry was conducted. These could be the aattributing factors for the poor performance of the live feeding.

Fish will utilise energy available for various body processes, some of which include maintenance and body building. The unit ratio of biomass an individual fish accumulates for every unit amount of feed given is referred to as the feed conversion ratios. It defines the proficiency of a certain individual in utilising the feed given and translate it into biomass. It also rates the quality of feed being administered to the fish as to how good in terms of nutrient composition that particular feed is. The lower the value of AFCR, the better the quality of feed. In this study the Coppens Feed from Germany recorded the lowest AFCR value **(**3.32**)** and highest weight gain values. This signifies that the Coppens Feed performed better than the rest of the diets that were investigated. These results would be attributed to various factors within the diets some of which could be palatability and digestibility. However the overall AFCR values obtained in this study were high than those obtained by others in similar studies. Like in a study conducted by Kaya and Bilgüven, **(**2015**)**, the highest recorded was 1.36, Ali and El-Feky **(**2013**)** recorded an average FCR of 1.2 and Ahmed et al., **(**2013**)** found an average FCR of 1.51. This variation could be attributed to the fact that feed staffs that passed through the mesh used in covering the small nursing buckets could

no longer be accessible to the fish inside the buckets. This entails that if the fish were raised in the tanks the FCR could even be much smaller. Effect of stocking density on growth performance and survival of O. karongae fry

The digestibility of feed ingredients is considered as one of the important factors in fish growth. During the course of feeding, it was observed that the two Malawi-Bunda and the Zambia-Novatek feed had accumulation of undigested feed that remained at the bottom of the buckets (Figure 4.14: Un-eaten feed remains at the bottom of the nursing bucket, Novatek (A) and Malawi (B) (photo by H. -left). This problem was more frequent in the buckets fed with the Zambia Novatek feed. De Silva et al., (1990) documented that dry matter digestibility may vary in diets depending on the nature and proximal composition of ingredients. Thus the type and nature of the ingredients used to formulate the diets can affect the digestibility consequently affecting the feeding efficiencies and growth performances.

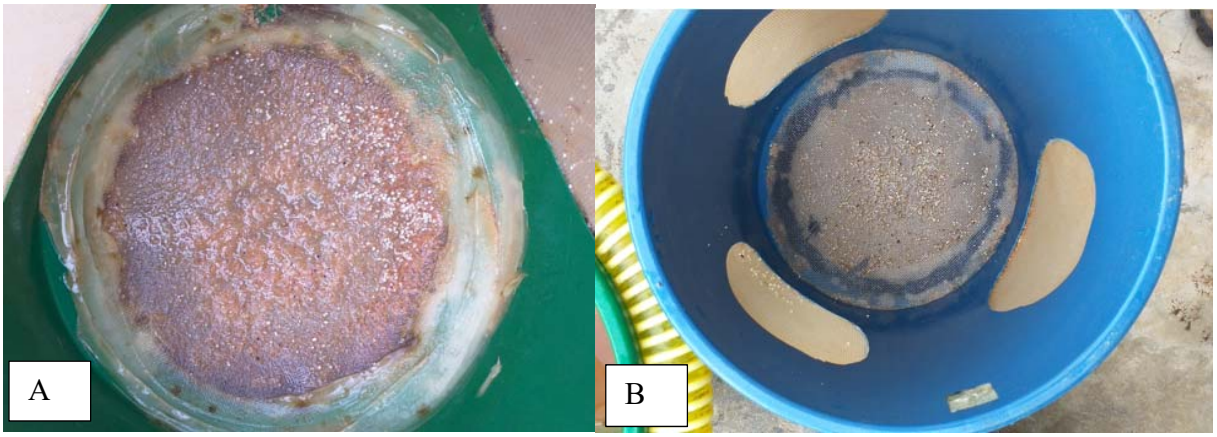


Figure 4.14: Un-eaten feed remains at the bottom of the nursing bucket, Novatek (A) and Malawi (B) (photo by H. Sainan)

The results for growth performance of fish in the various stocking densities for this study indicate that stocking densities had impacted significantly (P=0.04) on the amount of weight gained. Studies conducted by other researchers found similar result when similar investigations were carried out. Diana et al., (2004) documented that high densities are associated with poor growth performance due to voluntary appetite dominance, competition for food and living space, and more energy expenditure because of aggressive developmental contact. Rahmatullah et al. (2010) reported an inverse relationship between weight gain and stocking density in an experiment where the effect of stocking density on fry growth in aquaponic system was been evaluated

In this study stocking densities were found to have a significant (P=0.005) impact on the specific growth rate of fish. These results agree with findings from a number of authors who have document similar trends in other related research work. For example Bagum et al., (2015) found that SGR was significantly affected by the stocking densities when an investigation was carried out to determine the effect of stocking density on growth and survival of hormone fed O. niloticus fry .

SGR of 12.24%, 11.25%/ and 9.90% per day, were observed in lower, medium and high stocking densities, respectively.

The survival rates obtained in the current study were not significantly (P=0.302) affected by the feed type as well as the stocking densities not even the interaction between the two factors. This could be attributed to the fact that the experiment was conducted in well controlled environment. Similar findings have been documented by other authors. Rodmongkoldee and Leelapat (2017) reported that feed type did not significantly affect survival of sex reversed Nile tilapia

Similar results were also obtained in other experiments where effect of stocking densities on fish survival was tested. El -Sayed (2002), in an experiment where the effect of stocking density and feeding levels on growth and feeding efficiency of Nile Tilapia was investigated, documented that fish survival was reasonably good in all levels of stocking densities ranging from 90 to 98%, which indicated that the stocking density had a negligible effect on fish survival. Rahmatullah et al (2010) also report that survival rates in three different stocking densities were 99%, 98%, 96% for the lower (106 fish/m^3) , medium (142 fish/m^3) and high (177 fish/m^3)stocking densities , and these values were not significantly different. Another research conducted by Duffy and Epifanio (1994) reported that the instantaneous mortality rates were not significant during an experiment where the effect of stocking density on growth and survival of weakfish was investigated. A polynomial contrast results for fry growth in the various stocking densities indicates that the optimum stocking density for fry nursing is 4700 fry/m^3.

4.1.6 Effect of temperature on growth performance and survival of O. karongae fry

In this study water temperature has shown to have a significant influence on fish growth. Fish raised in high water temperatures (28°C) recorded significantly better growth parameters than those raised in water temperature of around 24°C. High temperature is associated with high growth rates because of the increased metabolic and feeding activities. A research conducted by Subasinghe and Sommerville (1992), where the effect of temperature on hatchability, development and growth of eggs and yolk sac fry of O. mossambicus was investigated, indicated that even yolk absorption was faster at high temperatures.

This result is in line with what other authors have found, for example in another study conducted by Yang et al., (2016) where the effect of temperature on growth, survival and occurrence of skeletal deformities in the golden pompano was investigated, results indicated that at 29 and 33°C growth was significantly faster than those at 23 and 26°C, but temperatures more than 29°C were associated with high incidences of jaw and skeletal deformities. Drummond et al., (2009) conducted a research to investigate the growth and survival of tilapia O. niloticus, and the findings indicated that weight gain, size and fry survival were significantly affected by temperature. Water temperatures of 28°C and 30°C produced the highest weight means of 0.349mg and 0.286mg respectively as compared to temperatures of 26°C and 32°C which recorded low weight values of 0.244mg and 0.211mg, respectively.

Another study conducted by Pandit and Nakamura (2010) where they were investigating the effect of high temperature on survival, growth and feed conversion ratio of Nile tilapia , found that Nile tilapia fry and juveniles grew better in water temperature range of 27-32°C . The study also

concludes that temperatures above 32°C resulted in reduced feeding efficiency, slow growth and increased mortality. A low AFCR is an indication of an organism's appropriate digestion efficiency. In the current study, the high water temperature can be associated with a heating effect which consequently can improve the palatability of fish diets. This can in turn improve fish feeding efficiency and hence improve growth performance.

However the findings of this study contradicts with Subasinghe and Sommerville (1992), who documented that fry survival of O. mossambicus was close to 100% within a temperature range of 24.3 and 34.0°C, however survival was lower than 60% at 20°C. In this study water temperature did not have a significant impact on the survival because this experiment was conducted in a much-controlled environment. Rearing fish such environments minimises stress thereby improving fish survival.

In this study 28°C water temperature has been associated with better growth of O. karongae fry. In some studies conducted by other researchers, similar temperature optima ranges have been noted in other members of the Cichlidae family. For example, O. mossambicus has an optimum temperature for growth at 30°C and Coptodon zilli has an optimum feeding temperature between 28.8 and 31.4°C (Boeuf and Payanb, 2001).

4.1.7 Effect of photoperiodicity on growth performance and survival of O. karongae fry

The results of this study indicate that photoperiod significantly affects growth performance of Tilapia fry. It was observed that the fish raised in the 24-hour light regime had long periods of feeding time compared to those in the 16L: 8D regime suggesting that most feed was consumed in the 24-hour light tanks than in the other treatment, resulting in better growth. The results agree

with what other authors have documented. In a study by Barlow et al.,(1995) who did work on barramundi, Lates calcarifer (Bloch), longer photoperiod recorded were associated with improved growth performance and survival. In the same study, they also observed that the fish in almost all the treatments could cease feeding at 1500 hrs. Their guts were observed to be mostly empty at around 21 hours and those raised in prolonged light regimes could resume feeding upon emptying their guts. Similar results were reported by other authors, for instance long period light intensity was reported to produce significantly greater number of fingerlings in O. niloticus (Ridha and Cruz, 2000). In other studies by Campos-Mendoza et al., (2004), El-Sayed & Kawanna, (2004) and Rad et al. (2006) have documented that when cichlids such as O. niloticus fry are exposed to continuous or long periods of light, the growth of the fish is stimulated. Boeuf and Le Bail (1999) have reported that, the effects of light intensity or specific photoperiod for some fish species depend on the stage of the life cycle. This is so because distribution in the habitat and food habits are modified according to fish growth.

A study by Aragón-Flores (2017) observed that feed intake of the C. beani cichlid continued as long as there was light in the culture facilities and that few un-eaten feed were observed in such facilities.

This trend in weight gain is in agreement with a study by Elsbaay (2013), where the effects of photoperiod and different artificial light colours on Nile Tilapia growth rate was tested. In this study a 24-hour regime produced the highest percentage weight gain (1037.8%) compared to a 16L: 8D regime which recorded a lower weight gain of 890.4. Another study by El-Sayed and Kawanna (2004) also observed a similar trend in O. niloticus when cultured in photoperiods of 24:00, 18:06, 12:12 and 06:18 (L: D).

The SGR obtained from this study agrees with another study by Elsbaay (2013), it was documented that in terms of SGR, a 24-hour regime produced a higher value of 4.05% , which was 0.23% higher than the 16L:8D regime.

The results for the light regime trials indicate that fish in the 24-hour light regime were better in terms of converting every unit mass of feed into biomass than those in the lower light regime. The effect of light on the feed conversion ratio is related to the effect of light on feeding. In a study conducted by Biswas and Takeuchi (2002), where they studied the effects of photoperiod on the metabolic rate of fed and unfed young and adult Nile tilapia, it was concluded that Nile tilapia conserve energy when raised under photoperiods with longer light phases. They found that metabolic rate and energy loss were negatively correlated with light periods, thus prolonged light regimes are associated with reduction in standard metabolic rates.

The results of this experiment indicate that light regime did not significantly (P=0.7247) affect fish survival rates between the two tanks under different light regime. However, it was difficult to identify the source of this difference because some fish were observed to have escaped from the small buckets into the big tank, hence one could not attribute this minimal fish losses to either being as a result of mortalities or escapes, though mortalities were hardly experienced. In agreement to this finding is a study conducted by Aragón-Flores et al., (2017) no significant effect was found on survival rates of this cichlid juveniles. The study conducted by Barlow et al., (1995) also found that survival rates were not significantly affected by the light regimes. However, Boeuf and Le Bail (1999) reported that stressful conditions that can sometimes cause mortalities as a result of too little or an excessive amount of light.

In a similar study, influence of photoperiod on growth, uniformity, and survival of larvae of the Amazonian ornamental Heros severus conducted by Veras et al., (2016), showed that no significant impact of photoperiod on the water quality was recorded.

4.1.8 Hormone dosage and its effect on production of all male O. karongae fingerlings

Results for the all-male seed production experiment demonstrate that a dose of 60 mg/kg of feed recorded a high proportion of males to females. Similar results were obtained by El-Greisy and El-Gamal (2012) who investigated the effect of using different doses of MT hormone with respect to sex stability within a certain period of time. Their findings indicated that proportion of males in Nile tilapia and survival rates were significantly high in fish administered with 60 mg of 17α-MT hormone per kg of feed. However, in the current study the proportions of males to females was lower as compared to what other studies have reported. This study used locally formulated feed whose quality was far much lower than the formulated diet and this may have affected the feed utilisation thereby affecting the effectiveness of the hormones incorporated in the feed.

At higher dosage (90g/kg), the proportion of males has been observed to decrease (56%). This finding is in line with what Khalil et al., (2011) who documented that high doses of the hormone were associated with reduction in the proportions of males because treated fry were associated with methyl-testosterone being able to induce molecular genetic viability and also increased number of spermatozoa. There are other approaches that have been used in producing all –male tilapia fish. Hybridisation for example has been used to produce inter-specific hybrids to increase growth, reduce unwanted reproduction by producing sterile pupations, improve productivity through hybrid vigour and transfer traits (Rahman et al., 2015). The technique which involves

crossing interspecific fish species. For example, two species of tilapias, female Nile tilapia Oreochromis niloticus) and male blue tilapia Oreochromis aureus) have been crossed and produce Nile-Blue hybrid tilapia in ponds (Qiuming and Yi, 2004). This method produces highly skewed ratio of males (up to 90%) in the offspring. Runemark et al., (2018) Outlines that hybridisation can affect viability , sexual conflicts and sexual dimorphism. This my in turn affect the sex ratios of the hybrids. Furthermore the other effects of hybridisation have been reported growth ad fecundity. In Malawi two interspecific species, O. karongae and O. shiranus have been successfully cross-bred to produce hybrids. According to Kassam and Sangazi (2016) crosses between O. shiranus male and O. karongae females produced significant high growth rates in terms of weight gain and specific growth rate.

In other nations, another technology called YY-male t has been applied to produce all-male populations**.** This is a chromosome manipulation technique employs the use of males with YY chromosomes, often called 'super males' which are produced by crossing between hormonally sex-reversed females (genotypic males) and normal males. This approach is preferred because the use of hormones to produce all males is widely being criticized rearing healthy and environmental concerns (Alcántar-vázquez et al., 2014). However, successful YY technology depends on the production of XY females and their identification is complicated because they are indistinguishable from normal females. The YY-males are distributed to the hatcheries so that they can cross with any females to produce all-male fry, in principle (Bhujel and & Program 2011). However, the results have not been consistent in different environment (Pham et al., 1998). There are some other factors including environmental factors in determining the sex. This technology

has not been widely adopted because of its complicity and requires high levels of technicality and knowledge of fish genetics.

The amount of weight gained at the end of the experiment was significantly ($P<0.001$) affected by the dosage of hormone administered. In a research conducted by Ridha and Lone (1990) who tested the effect of oral administration of different levels of hormone on Tilapia Oreochromis spilurus (Giinther) in brackish water, found that hormone dose had a no significant effect on growth, SGR and FCE, but there were significant effect observed in grow-out for the treated fish, specifically the specific growth rates and feed conversion efficiencies. Administration of methyl testosterone treatment can both improve growth and bring in a negative feedback depending on the dosage. In study conducted by Khalil et al., (2011), methyl testosterone treatment can result into DNA damage especially during the first few weeks of hormone treatment. This however disappears after considerable time and the DNA gets stabilised which may occur to an increase in the immune system as the fish grows.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

Conclusion

This study focused on identifying some key factors that could be improved to uplift fingerling production of cultured O. karongae. Based on the findings of this study, it is concluded that Feed type has a big influence on fry growth performance. Feeding fry with good quality formulated diets improves growth performance significantly. However the results of this study has demonstrated that not all commercial feed can perform better than the home-made, local diets. It is also concluded that fry growth is better when stocking densities are lower until a certain level beyond which growth decreases with an increase in stocking densities, thus fish stocking density is also an important factor to consider when nursing fry of the species. In terms of water temperature, the study concludes that high water temperature (up to a certain limit), is suitable for

maximised fry growth and in this study a 28°C water temperature has been shown to improve fry growth of O. karongae. Furthermore raising O. karongae fry in a facility with 24-hour photoperiodicity improves fry growth performance. Thus photoperiod, in fish culture facilities has an influence on growth performance. Low light regime, according to this study, significantly decreases fry growth rate. The study also concludes that highest percentage of males can be attained if every kg of feed is mixed with 60mg of 17 α-methyl testosterone hormone. This component of the study was however faced with big challenges for it took a long period of time to raise the fish to a size big enough to identify its sex. This led to high loses in terms of mortalities and escapes.

In general controlling most water parameters during fry nursing and proper management of water quality have proven to improve growth performance which eventually decreases the fry-fingerling culture period. In this study fish has been shown to attain up to 3 grams within a month. A fish weight that has been taking up to three months to be attained in ponds and tanks.

Recommendations

This study has helped to elucidated some of the key factors suitable for seed production for O. karongae. Therefore, based on the results of this study, the following recommendations are proposed;

- Quality feed should be produced using locally available ingredients which will reduce the cost of producing fingerlings. The Malawi- Bunda larval feed used in this experiment can be promoted and made available to other fingerling producers.

- Fry stocking densities should always be considered as one of the important factors in raising fry to fingerlings. According to this study a stocking density of 47000 fry/m^3 should be recommended for all hatchery operators producing O. karongae fingerlings.
- The use of indoor hatchery with controlled environmental conditions should be advocated for, considering the challenges associated with power generation in the country. Commercial farmers should be encouraged to invest the solar powered hatchery systems for this has proven to minimise fingerling loses through mortalities and predation.
- The study also recommends that water temperature as high as 28ºC , photoperiod of 24 hours dissolved oxygen levels of 7.0-7.5mg/L, ammonia of <0.05 and pH of 7-8.5 should be set to improve growth and survival of fry in seed production.
- There is need to do more research on feed formulation using the locally available ingredients to minimise cost of importation of fish feed. A lot of efforts should also be invested in coming up with specified diets in terms of nutritive value, size of pellets forms and colour that can best be used to maximise fish growth at various stages of development.
- There is need to incorporate the findings of this study with the existing hatchery guidelines in the country to help hatchery operators mass produce good quality fingerlings

REFERENCES

Adel, M. E. S., Ashraf, A., Ramadan, A. &Khattab, A. E. (2006). Sex reversal of Nile tilapia fry using different doses of 17 a-methyl testosterone at different dietary protein

levels. Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharkia Governorate, Egypt.

Ahmed, G. U., Sultana, N., Shamsuddin, M., &Hossain, M. B. (2013). Growth &production performance of monosex tilapia (Oreochromis niloticus) fed with homemade feed in earthen mini ponds. Pakistan journal Biological science 16(23), 1781

Akankali, J.A., Seiyaboh, E.I. &Abowei, J.F.N. (2011). Fish hatchery management in Nigeria. Advance journal of food science &technology, 32, 144-154.

Akbary, P., Imanpoor, M., Sudagar, M. &Makhdomi, N.M. (2010). Comparison between live food &artificial diet on survival rate, growth &body chemical composition of Oncorhynchus mykiss larvae. Iranian Journal of Fisheries Sciences, 91, 19-32.

Alcántar-Vázquez, J.P., Moreno-de la Torre, R., Calzada-Ruíz, D. &Antonio-Estrada, C. (2014). Production of YY-male of Nile tilapia Oreochromis niloticus (Linnaeus, 1758) from atypical fish. Latin American Journal of Aquatic Research, 423, 644-648.

Ali, M.A.M. &El-Feky, A.M.I. (2013). Effect of different photo periods on growth performance, survival rate &skin Colour of Nile tilapia fingerlings. Egyptian Journal of Animal Production, 503, 186-192.

Aragón-Flores, E.A., Martínez-Cárdenas, L., Hernández-González, C., Barba-Quintero, G., Zavala-Leal, O.I., Ruiz-Velazco, J.M., Hernández-Almeida, O.U. &Juárez-López, P. (2017). Effect of light intensity &photoperiod on growth &survival of the Mexican

cichlid, Cichlasoma beani in culture conditions. Latin American journal of aquatic research, 452, 293-301

Ataguba, G. A., Okomoda, V. T., &Azave, V. T. (2015). Effect of photoperiod on hatching success of eggs &early fry performance of Clarias gariepinus (Siluriformes, Burchell 1822). Trakia Journal of Sciences, 13(2), 171-174

Bagum, N., Hossain, M. S., Doulah, M. A., Bhadra, A., &Shaheen, M. B. A. (2015). Effect of stocking density on growth &survival of hormone treated Tilapia (Oreochromis niloticus) fry reared in hapas-in-pond system. Trends in Fisheries Research, 4, 1-6.

Barlow, C. G., Pearce, M. G., Rodgers, L. J., &Clayton, P. (1995). Effects of photoperiod on growth, survival &feeding periodicity of larval &juvenile barramundi Lates calcarifer (Bloch). Aquaculture, 138(1-4), 159-168.

Beveridge, M. C., &McAndrew, B., (2012). Tilapias: biology &exploitation Springer Science &Business Media (Vol. 25).

Bhatnagar, A., &Devi, P. (2013). Water quality guidelines for the management of pond fish culture, 36, 1980–2009. https://doi.org/10.6088/ijes.2013030600019

Bhujel, R. C. (2014). A manual for tilapia business management, Aquaculture &Aquatic Resource Management, School of Environment, Resource &Development, Asian Institute of Technology, Pathumthani, Thailand.

Bhujel, R. C., &Program, A. (2011). Strategic review on aquaculture seed supply, Partnership for African Fisheries (PAF) Aquaculture Working Group, NEPAD report, University of Sterling, UK.

Biswas, A. K., &Takeuchi, T. (2002). Effect of different photoperiod cycles on metabolic rate &energy loss of fed &unfed adult tilapia Oreochromis niloticus: Part II. Fisheries science, 683, 543-553.

Björnsson, B. T. (1997). The biology of salmon growth hormone: from daylight to dominance. Fish Physiology &Biochemistry, 17(1-6), 9-24.

Boeuf, G., &Le Bail, P. Y. (1999). Does light have an influence on fish growth? Aquaculture, 177(1-4), 129-152.

Boeuf, G., &Payan, P. (2001). How should salinity influence fish growth? Comparative Biochemistry &Physiology Part C: Toxicology &Pharmacology, 130(4), 411-423.

Brummett, R. E. (1995). Environmental regulation of sexual maturation &reproduction in tilapia. Reviews in Fisheries Science, 3(3), 231-248.

Bury, N. R., Walker, P. A., &Glover, C. N. (2003). Nutritive metal uptake in teleost fish. Journal of experimental biology, 206(1), 11-23.

Campos-Mendoza, A., McAndrew, B. J., Coward, K., &Bromage, N. (2004). Reproductive response of Nile tilapia (Oreochromis niloticus) to photoperiodic manipulation; effects on spawning periodicity, fecundity &egg size. Aquacultue, 231(1-4), 299-314.

Charo-Karisa, H., Rezk, M. A., Bovenhuis, H., &Komen, H. (2003). Effects of rearing conditions on low-temperature tolerance of Nile tilapia, Oreochromis niloticus, juveniles, Fish Culture &Fisheries Group, Wageningen Institute of Animal Sciences (WIAS), Wageningen University, Wageningen, The Netherlands

Chimatiro, S.K. &Chirwa, B.B. (2005). National Aquaculture Sector Overview-Malawi. National Aquaculture Sector Overview Fact Sheets, 22, FAO Fisheries &Aquaculture Department [online]. Rome.

Cowey, C. B. (1976). Use of synthetic diets &biochemical criteria in the assessment of nutrient requirements of fish. Journal of the Fisheries Board of Canada, 33(4), 1040-1045.

Dabrowska, H., Meyer-Burgdorff, K., &Günther, K. D. (1989). Interaction between dietary protein &magnesium level in tilapia (Oreochromis niloticus). Aquaculture, 76(3-4), 277-291.

Daudpota, A.M., Kalhoro, I.B., Shah, S.A., Kalhoro, H. &Abbas, G. (2014). Effect of stocking densities on growth, production &survival rate of red tilapia in hapa at fish hatchery Chilya Thatta, Sindh, Pakistan. Journal of Fisheries, 23,180-186.

De Silva, S.S., Shim, K.F. &Ong, A.K. (1990). An evaluation of the method used in digestibility estimations of a dietary ingredient &comparisons on external &internal markers, &time of faeces collection in digestibility studies in the fish Oreochromis aureus Steindachner. Reproduction Nutrition Development, 302, pp.215-226

Diana, J. S., Yi, Y., &Lin, C. K. (2004, September). Stocking densities &fertilization regimes for Nile tilapia (Oreochromis niloticus) production in ponds with supplemental feeding.

In Proceedings of the sixth international symposium on tilapia in aquaculture, R. Bolivar, G. Mair &K. Fitzsimmons, Eds. Manila, Philippines, BFAR, Philippines (pp. 487-499).

DoF. (2015). Annual Economic Report, Fisheries Sector Contribution Prepared by Department of Fisheries Ministry of Agriculture, Irrigation &Water Development, Lilongwe, Malawi

DoF. (2016). Tilapia Seed Rearing Manual, Agriculture Technology Transfer, department of Fisheries, Ministry of Agriculture, Irrigation &Water Development, Lilongwe, Malawi

Donda, S., &Njaya, F. (2007). Food security &poverty alleviation through improved valuation &governance of river fisheries in Africa, Fisheries Department, Mangochi, MALAWI

Drummond, C. D., Murgas, L. D. S., &Vicentini, B. (2009). Growth &survival of tilapia Oreochromis niloticus (Linnaeus, 1758) submitted to different temperatures during the process of sex reversal. Ciência e agrotecnologia, 33(3), 895-902.

Duffy, J. T., &Epifanio, C. E. (1994). Effects of larval density on the growth &survival of weakfish, Cynoscion regalis in large-volume enclosures. Marine Ecology-Progress Series, 104, 227-227.

El Fattah, I. M. S. A., Ahmed, M. H., &A, M. A. (2008). Zooplankton as Live Food for Fry &Fingerlings of Nile Tilapia (Oreochromis Niloticus) &Catfish (Clarias Gariepinus) In Concrete Ponds. In From the pharaohs to the future. Eighth International

Symposium on Tilapia in Aquaculture, Elghobashy, H., Fitzsimmons, K., &Diab, A. S. Eds. AQUAFISH Collaborative Research Support, Program Proceedings. Cairo, Egypt, 12-14 October, 2008 (pp. 757-771).

El-Greisy, Z. A. E. B., Elgamal, A. E. E., &Ahmed, N. A. M. (2016). Effect of prolonged ammonia toxicity on fertilized eggs, hatchability &size of newly hatched larvae of Nile tilapia, Oreochromis niloticus. The Egyptian Journal of Aquatic Research, 42(2), 215-222.

El-Greisy, Z. A., &El-Gamal, A. E. (2012). Monosex production of tilapia, Oreochromis niloticus using different doses of 17α-methyl testosterone with respect to the degree of sex stability after one year of treatment. The Egyptian Journal of Aquatic Research, 381, 59-66.

El-Sayed, A. F. M. (2004). Protein nutrition of farmed tilapia: searching for unconventional sources. In New dimensions in farmed tilapia: proceedings of the Sixth International Symposium on Tilapia Aquaculture, Bolivar, R. B., Jimenez, E. B. T., Sugue, J. R., &Brown, C. L, Eds. January, 2004 (pp. 364-378).

El-Sayed, A. F. M., & Kawanna, M. (2004). Effects of photoperiod on the performance of farmed Nile tilapia Oreochromis niloticus: I. Growth, feed utilization efficiency &survival of fry &fingerlings. Aquaculture, 231(1-4), 393-402.

El-Sayed, A. F. M., Mansour, C. R., &Ezzat, A. A. (2003). Effects of dietary protein level on spawning performance of Nile tilapia (Oreochromis niloticus) broodstock reared at different water salinities. Aquaculture, 220(1-4), 619-632.

El-Sayed, A.F.M. (2002). Effects of stocking density &feeding levels on growth &feed efficiency of Nile tilapia Oreochromis niloticus L. fry. Aquaculture research, 338, 621-626.

Elsbaay, A.M., (2013). Effects of photoperiod &different artificial light colours on Nile tilapia growth rate. Journal of Agriculture &Veterinary Science, 33, 5-12.

El-Serafy, S., El-Ezabi, M., Shehata, T., &Esmael, N. (2007). Dietary iron requirement of the Nile tilapia, Oreochromis niloticus (L.) fingerlings in intensive fish culture. Egyptian Journal of Aquatic Biology &Fisheries, 11(2), 165-184.

El-Sherif, M. S., &El-Feky, A. M. I. (2009). Performance of Nile tilapia (Oreochromis niloticus) fingerlings. I. Effect of pH. International Journal of Agriculture &Biology, 11(3), 297-300.

Fall, J., Tseng, Y., Ndong, D., &Sheen, S. S. (2011). The effects of different protein sources on the growth of hybrid tilapia (Oreochromis niloticus× O. aureus) reared under fresh water &brackish water. African Journal of Agricultural Research, 6(21), 5024-5029.

FAO (2005). Characteristics, structure &resources of the sector, National Aquaculture Sector Overview, Food &Agriculture Organization , Fisheries &Aquaculture Department , Rome, Italy.

FAO (2016), the State of World Fisheries &Aquaculture, Contributing to Food Security &Nutrition for All. Rome, Italy.

FAO. (2015). Country fact sheet on food &agriculture policy trends, Food &Agriculture Policy Decision Analysis (FAPDA), fapda@fao.org, [Accessed on February 25, 2020]

FAO. (2018). The State of World Fisheries &Aquaculture 2018-Meeting the sustainable development goals. Food &Agriculture Organization of the United Nations, Rome, 2018

Faruk, M. A. R., Mausumi, M. I., Anka, I. Z., Hasan, M. M., &Mausumi, M. I. (2012). Effects of temperature on the egg production &growth of monosex Nile tilapia Oreochromis niloticus fry. Bangladesh Research Publication Journal, 7(4), 367-377.

Francis-Floyd, R., Watson, C., Petty, D., &Pouder, D. B. (2009). Ammonia in aquatic systems. University of Florida IFAS Extension Publication, USA, https://thefishsite.com/articles/ammonia [Accessed on February 25, 2020]

García-Ulloa, M. A. N. U. E. L., Gallo-García, M. C., Ponce-Palafox, J. T., Rodríguez-González, H. E. R. V. E. Y., &Góngora-Gómez, A. M. (2013). Growth &survival of Nile tilapia (Oreochromis niloticus) fry fed with a dietary inclusion of decapsulated Artemia cysts. Journal of Aquaculture in the Tropics, 28(1-4), 67-73.

GoM (2016), Post-disaster needs assessment, Ministry of Disaster &Management Affairs Brummett, R.E., 1995. Environmental regulation of sexual maturation &reproduction in tilapia. Reviews in Fisheries Science, 33, 231-248.

GoM (2018). Annual Economic Report, Fisheries Sector Contribution Prepared by Ministry of Finance, Economic Planning &Development, Lilongwe, Malawi

GoM (2019). Annual Economic Report, Fisheries Sector Contribution Prepared by Ministry of Finance, Economic Planning &Development, Lilongwe, Malawi

Handy, R. D., &Poxton, M. G. (1993). Nitrogen pollution in mariculture: toxicity &excretion of nitrogenous compounds by marine fish. Reviews in Fish Biology &Fisheries, 3(3), 205-241.

Hargreaves, J. A., &Tucker, C. S. (2004). Managing ammonia in fish ponds Stoneville, MS: Southern Regional Aquaculture Centre, United States Department of Agriculture, Cooperative State Research, Education, &Extension Service, USA.

Hepher, B. (1988). Principles of fish nutrition. In: Fish Culture in Warm water systems, Problems &Trends Shilo, M. &Sarig, S, CRC Press, New York, USA, pp. 121–142

Hixson, S. M. (2014). Fish Nutrition &Current Issues in Aquaculture: The Balance in Providing Safe &Nutritious Seafood, in an Environmentally Sustainable Manner, 53. https://doi.org/10.4172/2155-9546.1000234 [Accessed on September 2, 2019]

Kamel, E. A., Elghobashy, H. A., &Farag, M. A. (2008). Performance of Growth &Survival Rates of Oreochromis Aureus Juveniles during Hard Winter Condition in Egypt. In From the pharaohs to the future. Eighth International Symposium on Tilapia in Aquaculture, Elghobashy, H., Fitzsimmons, K., &Diab, A. S. Eds. AQUAFISH Collaborative Research Support, Program Proceedings. Cairo, Egypt, 12-14 October. (pp. 319-327).

Kapeleta, M. V. (2001). Aquaculture development in Malawi: Tilapia culture. Promotion of sustainable commercial aquaculture in Sub-Saharan Africa. Experiences of selected

developing countries, FAO Fisheries circular No Cyprinus carpio, 971. http://www.fao.org/tempref/docrep/fao/007/y2277b/y2277b04.pdf [Accessed 0n 2 September 2019]

Kassam, D., &Sangazi, M. (2016). Comparative Growth Performance of Oreochromis Hybrids &Selectively-Bred Strain (F8) in Malawi. Sustainable Agriculture Research, 5, no. 526-2017-2643 (2016).

Kauye, F. &Mafuta, C. (2007). Malawi. International Psychiatry, 41, 9-11., https://www.ncbi.nlm.nih.gov/pubmed/31507874 [Accessed 0n 9 November 2019]

Kaya, G. K., &Bilgüven, M. (2015). The effects of feeding frequency on growth performance &proximate composition of young Nile tilapia (Oreochromis niloticus L.). Journal of Agricultural Faculty, 29(1), 11-18.

Kelly, A. M., &Kohler, C. C. (1999). Cold tolerance &fatty acid composition of striped bass, white bass, &their hybrids. North American Journal of Aquaculture, 614, 278-285.

Khalil, W. K., Hasheesh, W. S., Marie, M. A. S., Abbas, H. H., &Zahran, E. A. (2011). Assessment the impact of 17α-methyl testosterone hormone on growth, hormone concentration, molecular &histopathological changes in muscles &testis of Nile tilapia; Oreochromis niloticus. Life Science Journal, 8(3), 329-343.

Khan, I. A., & Maqbool, A. (2017). Effects of Dietary Protein Levels on the Growth, Feed Utilization &Haemato-Biochemical Parameters of Freshwater Fish, and Cyprinus carpio Var. Specularis. Fisheries &Aquaculture Journal, 8, 2-12.

Khattab, Y., Abdel-Tawwab, M., &H Ahmad, M. (2001). Effect of protein level &stocking density on growth performance, survival rate, feed utilization &body composition of Nile tilapia fry (Oreochromis niloticus L.). Egyptian Journal of Aquatic Biology &Fisheries, 5(3), 195-212.

Knuth, D. S. (2007). Effects of abiotic &biotic factors on bluegill reproduction &growth in seven unexploited surface coal mine lakes. Department of Zoology, Southern Illinois University at Carbondale, Illinois.

Lall, S.P. (2002). The minerals. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition, 3rd ed. Academic Press, San Diego, CA, pp. 259-308.

Lèveque, C. (2002). Out of Africa: the success story of tilapias. Environmental Biology of Fishes, 64: 461- 464

Machena, C., &Moehl, J. (2001). African aquaculture: a regional summary with emphasis on Sub-Saharan Africa, Network of Aquaculture Centres in Asia-Pacific, Bangkok (Thailand) FAO, Rome (Italy).

Macintosh, D. J., &Little, D. C. (1995). Nile tilapia (Oreochromis niloticus), Broodstock management &egg &larval quality, Willy Blackwell, United States of America.

Malawi National Statistics Office, (2018) Malawi population &housing census, Zomba, Malawi.

Mallya, Y. J. (2007). The effects of dissolved oxygen on fish growth in aquaculture. The United Nations University Fisheries Training Programme, Final Project, Kingolwira National

Fish Farming Centre, Fisheries Division Ministry of Natural Resources &Tourism, Tanzania.

Megbowon, I., &Mojekwu, T. O. (2013). Tilapia sex reversal using methyl testosterone (MT) &its effect on fish, man &environment, In: 28th Annual Conference of the Fisheries Society of Nigeria (FISON) , 25-29 Nov 2013 ,Abuja, Nigeria, pp. 217-219

Mensah, E. T. D., Attipoe, F. K., &Ashun-Johnson, M. (2013). Effect of different stocking densities on growth performance &profitability of Oreochromis niloticus fry reared in hapa-in-pond system. International Journal of Fisheries &aquaculture, 58, 204-209.

Moffitt, C. M., &Cajas-Cano, L. (2014). Blue growth: the 2014 FAO state of world fisheries &aquaculture. Fisheries, 3911, 552-553.

Msiska, O. V. (1998). Reproduction biology &growth of Oreochromis (Nyasalapia) karongae in ponds &open water in Malawi, Doctoral dissertation, Biology Department, University of Malawi.

Msiska, O. V., &Costa-Pierce, B. A. (1996). Growth performance of Oreochromis lidole, O. squamipinnis, O. shiranus &O. karongae, new candidate species for aquaculture in open water &fishponds in Malawi. In The Third International Symposium on Tilapia in Aquaculture, Pullin, R. S., Legendre M., Pauly D. (Ed.), 1996, Abidjan, Cote d, Ivoire. ICLARM Conference Proceedings (Vol. 41, pp. 129-134).

NAC. (2003). National Aquaculture Center Annual Report, 2002/2003, Department of Fisheries, Ministry of Agriculture, Irrigation &Water Development, Lilongwe, Malawi

National Fisheries &Aquaculture Policy, (2016), Department of Fisheries, Ministry of Agriculture, Irrigation &Water Development, Lilongwe, Malawi

National Research Council NRC, (1983). Nutrient requirements of warm water fishes &shell fishes. National Academy Press, Washington D.C. 102p

National Research Council. (1993). Nutrient requirements of fish. National Academies Press.

Netten, J. J., van der Heide, T., &Smolders, A. J. (2013). Interactive effects of pH, temperature &light during ammonia toxicity events in Elodea canadensis. Chemistry &Ecology, 29(5), 448-458.

NFAP. (2012). National Fisheries Policy 2012 – 2017, Department of Fisheries, Ministry of Agriculture , Irrigation &Water Development, Lilongwe , Malawi.

NFAP. (2016). National fisheries &aquaculture policy, Department of Fisheries, Ministry of Agriculture, Irrigation &Water Development, Lilongwe , Malawi

Niklitschek, E. J., &Secor, D. H. (2009). Dissolved oxygen, temperature &salinity effects on the ecophysiology &survival of juvenile Atlantic sturgeon in estuarine waters: II. Model development &testing. Journal of Experimental Marine Biology &Ecology, 381, S161-S172.

Nsonga, A. R., Kang'ombe, J., Mfitilodze, W., Soko, C. K., &Mtethiwa, A. H. (2009). Effect of varying levels of dietary vitamin C (ascorbic acid) on growth, survival &hematology

of juvenile tilapia, Oreochromis karongae (Trewavas 1941) reared in aquaria. Brazilian Journal of Aquatic Science &Technology, 13(2), 17-23.

Nzohabonayo, E., Kang'ombe, J., &Kassam, D. (2017). Effect of hybridisation on fecundity of Oreochromis karongae (Trewavas 1941). The Egyptian Journal of Aquatic Research, 43(3), 241-244.

Ogbonna, J. &Chinomso, A. (2010). Determination of the concentration of ammonia that could have lethal effect on fish pond. Journal of Engineering &Applied Sciences Asian Research Publishing Network, 5, 1-5.

Pandit, N. P., &Nakamura, M. (2010). Effect of high temperature on survival, growth &feed conversion ratio of Nile tilapia, Oreochromis niloticus. Our Nature, 8(1), 219-224.

Pham, T. A., Little, D. C., &Mair, G. C. (1998). Genetic effects on comparative growth performance of all-male Oreochromis niloticus. Aquaculture, 159, 293-302.

Phelps, R. P., &Popma, T. J. (2000). Sex reversal of tilapia. Tilapia aquaculture in the Americas, World Aquaculture Society, Baton Rouge, Louisiana, United States 2, 34-59.

Popma, T. J., &Green, B. W. (1990). Sex reversal of tilapia in earthen ponds. Aquaculture production manual. Alabama: Auburn University, Alabama Research &Development.

Popma, T. J., &Phelps, R. P. (1998). Status report to commercial tilapia producers in monosex fingerling production techniques. Latin American Chapter. World Aquaculture Society, Aquaculture Brazil, 98, 2-6.

Popma, T.J., F.E. Ross, B.L. Nerrie &J.R. Bowman. (1984). The Development of Commercial Farming of tilapia in Jamaica 1979–1983. Research &Development Series No. 31. International Center for Aquaculture, AlabamaAgricultural Experiment Station, Auburn University, Auburn, AL, USA

Purchase, C. F., Boyce, D. L., &Brown, J. A. (2000). Growth &survival of juvenile yellowtail flounder Pleuronectes ferrugineus (Storer) under different photoperiods. Aquaculture Research, 31(6), 547-552.

Puvanendran, V., &Brown, J. A. (1998). Effect of light intensity on the foraging &growth of Atlantic cod larvae: interpopulation difference? Marine Ecology Progress Series, 167, 207-214.

Qiuming, L., &Yi, Y. (2004). Tilapia culture in mainland China. In 6th International Symposium on Tilapia in Aquaculture, ISTA, 12-16 September 2004 Philippine International Convention Centre Roxas Boulevard, Manila, Philippines.

Rahman, M. A., Arshad, A., Marimuthu, K., Ara, R., &Amin, S. M. N. (2013). Inter-specific hybridization &its potential for aquaculture of fin fishes. Asian Journal Animal Veterinary. Advances, 8(2), 139-153.https://doi.org/10.3923/ajava.2013.139.153, [Accessed on May 15 2019

Rahmatullah, R., Das, M. &Rahmatullah, S.M. (2010). Suitable stocking density of tilapia in an aquaponic system. Bangladesh Journal of Fisheries Research, 141(2), 29-35.

Riche, M., &Garling, D. (2003). Feeding Tilapia in Intensive Recirculating Systems. North Central Regional Aquaculture Center, Extension Fact Sheets. 6. Available at http://lib.dr.iastate.edu/ncrac_factsheets/6 pp.1-6. [Accessed on December 27, 2019]

Ridha, M.T. &Cruz, E.M. (2000). Effect of light intensity &photoperiod on Nile tilapia Oreochromis niloticus L. seed production. Aquaculture Research, 317, 609-617.

Ridha, M.T. &Lone, K.P. (1990). Effect of oral administration of different levels of 17α-methyl testosterone on the sex reversal, growth &food conversion efficiency of the tilapia Oreochromis spilurus Günther in brackish water. Aquaculture Research, 214, 391-397.

Rodmongkoldee, M. &Leelapat, W. (2017). Effect of Feed Type on Growth Performance &Sex Reversal of Nile Tilapia Oreochromis niloticus. Burapha Science Journal 221, 14-23.

Rombough, P.J. (1994). Energy partitioning during fish development: additive or compensatory allocation of energy to support growth? Functional Ecology, 8(2), 178-186

Runemark, A., Eroukhmanoff, F., Nava-Bolaños, A., Hermansen, J. S., &Meier, J. I. (2018). Hybridization, sex-specific genomic architecture &local adaptation. Philosophical Transactions of the Royal Society B: Biological Sciences, 373(1757), 20170419.

Saha, N., Kharbuli, Z. Y., Bhattacharjee, A., Goswami, C., &Häussinger, D. (2002). Effect of alkalinity (pH 10) on ureogenesis in the air-breathing walking catfish, Clarias batrachus. Comparative Biochemistry &Physiology Part A: Molecular &Integrative Physiology, 132(2), 353-364.

Satia, B. (1989). A regional survey of the aquaculture sector in Africa south of the Sahara, AGRIS, Food &Agriculture Organisation, available at http://agris.fao.org/agris-search/search.do?recordID=XF9095229, [accessed on 19th November 19 2019]

Scott, D. M., Lucas, M. C., &Wilson, R. W. (2005). The effect of high pH on ion balance, nitrogen excretion &behaviour in freshwater fish from a eutrophic lake: a laboratory &field study. Aquatic Toxicology, 73(1), 31-43.

Shelton, W. L. (2002). Tilapia culture in the 21st century. In Tilapia Farming in the 21st Century, Proceedings, 25-27 February 2002 International Forum on Tilapia Farming in the 21st Century, Raphael, D. Guerrero, M., Rizalina C, Eds. Philippine Fisheries Association, Philippine.

Shiau, S. Y., &Lin, S. F. (1993). Effect of supplemental dietary chromium &vanadium on the utilization of different carbohydrates in tilapia, Oreochromis niloticus× O. aureus. Aquaculture, 110(3-4), 321-330.

Shiau, Y. S., &Lin, Y. H. (2006). Vitamin requirements of tilapia. A Review. En: Editors Cruz Suares LE. et al., Avances on Nutrition Acuicola VIII. VIII. Symposium

Internacionalde Nutricion Acuicola. 15- 17 Noviembre. Universidad Autonoma de Nuevo Leon, Monterrey, Nuovo Leon, Mexico. ISBN 970-694-333-5

Singh, P. K., Gaur, S. R., &Tiwari, S. P. (2006). Growth response, survival, feed conversion &protein utilization in fingerlings of rohu, Labeo rohita (Hamilton) to diets of different protein levels. Journal of Fish &Aquatic Science, 1, 97-101.

Subasinghe, R.P. &Sommerville, C. (1992). Effects of temperature on hatchability, development &growth of eggs &yolk sac fry of Oreochromis mossambicus Peters under artificial incubation. Aquaculture Research, 231, 31-39.

Szczepkowski, M. (2009). Impact of selected abiotic &biotic factors on the results of rearing juvenile stages of northern pike Esox lucius L. in recirculating systems. Archives of Polish Fisheries, 17(3), 107-147.

Tacon, A. G. J. (1989). Nutrición y alimentación de peces y camarones cultivados. Manual de capacitación. Programa cooperativo governmental, FAO-Italia. FAO, Documento de campo, 4, 572.

Teichert-Coddington DR, Popma TP &Lovshin LL (1997). Attributes of tropical pond cultured fish. In: Egna HS &Boyd CE, eds. Dynamics of Pond Aquaculture. CRC Press, Boca Raton, FL, USA. pp. 183–198

UNDP (2015). Human development report 2015: Work for human development. United Nations Development Programme, Rome, Italy.

Valeta, J.S., Likongwe, J.S., Kassam, D. &Maluwa, A.O. (2013). Temperature-dependent egg development rates, hatchability &fry survival rate of Lake Malawi tilapia Chambo, Oreochromis karongae Pisces: Cichlidae. International journal of fisheries &aquaculture, 54, 55-59.

Van Dam, A. A., Beveridge, M. C., Azim, M. E., &Verdegem, M. C. (2002). The potential of fish production based on periphyton. Reviews in fish biology &fisheries, 12(1), 1-31.

Veras, G.C., Paixão, D.J.D.M.R., Brabo, M.F., Soares, L.M.O. &Sales, A.D. (2016). Influence of photoperiod on growth, uniformity, &survival of larvae of the Amazonian ornamental Heros Severus Heckel, 1840. Revista Brasileira de Zootecnia, 457, 422-426.

Wang, C., Xie, S., Zheng, K., Zhu, X., Lei, W., Yang, Y., &Liu, J. (2005). Effects of live food &formulated diets on survival, growth &protein content of first-feeding larvae of Plelteobagrus fulvidraco. Journal of Applied Ichthyology, 21(3), 210-214.

Webster, C. D., &Lim, C. (2006). Tilapia: biology, culture, &nutrition. CRC Press, New York.

Winfree, R. A., &Stickney, R. (1981). Effects of dietary protein &energy on growth, feed conversion efficiency &body composition of Tilapia aureus. The Journal of nutrition, 111(6), 1001-1012.

World Bank (2018). Systematic Country Diagnostic: Breaking the Cycle of Low Growth &Slow Poverty Reduction, Malawi country tem, a region, World Bank group, http://documents.worldbank.org, [Accessed on February 25, 2020]

Yang, Q., Ma, Z., Zheng, P., Jiang, S., Qin, J.G. &Zhang, Q. (2016). Effect of temperature on growth, survival &occurrence of skeletal deformity in the golden pompano Trachinotus ovatus larvae. Indian Journal of Fisheries, 631, 74-82.

Zidana, H., Phiri, F., Kapute, F., Ajiboye, O., &Singini, W. (2015). Effect of stocking density &rearing facility on growth &profitability of Coptodon rendalli fry. International Journal of Aquaculture, International Journal of Aquaculture, 5(24), 1-5

APPENDEX

Glossary of terms

Abiotic factors	Non-living chemical and physical parts of the environment that affect living organisms and the functioning of ecosystems.
Ad libitum feeding	Proving feed to organism at one's pleasure, desire or discretion.
Agrarian	Community whose economy is based on producing and maintaining crops and farmland.
Aquaculture	The farming of fish, molluscs, algae, crustaceans, aquatic plants and other organisms.
Aquaponic	Refers to any system that combines conventional aquaculture with plants in water in a symbiotic environment.
Biotic factors	Living component that affects another organisms or shapes the ecosystem.
Capture fisheries	Refers to all kinds of harvesting of naturally occurring living resources in both marine and freshwater environments.
Detritus	Dead particulate organic material, as distinguished from dissolved organic material.
Fecundity	The natural capability to produce offspring, measured by the number of gametes (eggs), seed set, or asexual propagules.

Feed conversion ratio	Feed conversion rate is a ratio or rate measuring of the efficiency with which the bodies of livestock convert animal feed into the desired output.
Feed utilisation	Refers to the feed intake of an organism and the associated response by that particular organism.
Fingerling	Refers to a development stage of young fish.
Fish sampling	The act of collecting information about fish populations and communities through capturing or observations.
Fry nursing	Rearing of newly hatched fish to juvenile or fingerling stage.
Gonads	A mixed gland that produces the gametes (sex cells) and sex hormones of an organism.
Grow-out	The final phase of aquaculture production where juveniles are grown to adulthood.
Haematocrit	The volume percentage of red blood cells in blood.
Hatchling	It is a newly hatched fish, reptile, bird or amphibian.
Hybridisation	The process of combining different varieties of organisms to create a hybrid.
Lentic	Stationary or relatively still water.
Mouth brooder	Care given by some groups of animals to their offspring by holding them in the mouth of the parent for extended periods of time.
Organic load	The amount of volatile organic dry matter entering the anaerobic digester over time - measured in pounds (lbs.) per ft^3 digester volume and day.

Photoperiod	The period of time each day during which an organism receives illumination; day length.
Sinking feed	As opposed to floating feed, sinking feed is a type of feed that sinks to the bottom of water column when introduced into the water.
Specific growth rate	Refers to growth rate relative to size usually within a specified time.
Stocking density	Refers to the number of organisms that are kept on a given unit of space.
Survival rate	Percentage of organisms that survive to the end of given trial period.
Weight gain	An increase in body weight in a given period of time
Zooplankton	Are a type of heterotrophic plankton that range from microscopic organisms to large species.

Water parameter ranges used during the study

Water Parameter	Units	Recommended Range
Temperature	^{o}C	26-28
Photoperiod	L: D	24:0
Dissolved Oxygen	mg/L	7.0-7.5
Saturation	%	90-95
Ammonia	mg/L	<0.05
pH		7-8.5

Output for the data analysis

Testing for interaction: Feed types*stocking density

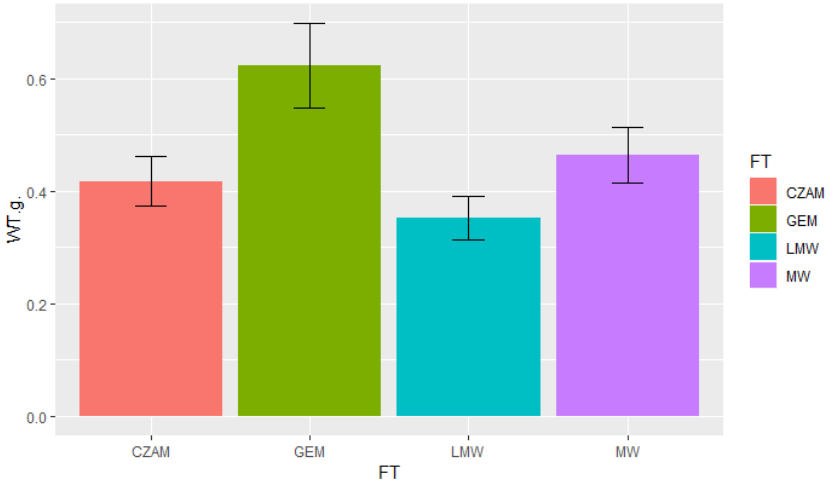
```
Response: WT.g.
           Df  Sum Sq Mean Sq F value   Pr(>F)
FT          3  2.1652 0.72173  4.7068 0.003365 **
SD          2  1.2198 0.60989  3.9775 0.020204 *
FT:SD       6  0.0907 0.01511  0.0985 0.996464
Residuals 204 31.2806 0.15334
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Effect of Feed type on weight gain

```
lm(formula = WT.g. ~ FT, data = fri)


Residuals:
     Min       1Q   Median       3Q      Max
-0.61215 -0.26462 -0.08542  0.21675  1.36685


Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.41702    0.05336   7.816 2.51e-13 ***
FTGEM        0.20613    0.07546   2.732  0.00683 **
FTLMW       -0.06509    0.07546  -0.863  0.38931
FTMW         0.04680    0.07546   0.620  0.53581
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```



Effect of stocking density on weight gain

```
contrasts(sat$SD)=comps

> analysis<-aov(WT.g.~SD,data=sat)

> summary(analysis, split=list(SD=list("A vs BC"=1, "B vs CC"=2)))

                Df Sum Sq Mean Sq F value  Pr(>F)

SD               2   1.22  0.6099   3.874 0.02226 *

  SD: A vs BC    1   1.12  1.1235   7.136 0.00814 **

  SD: B vs CC    1   0.10  0.0963   0.611 0.43515

Residuals      213  33.54  0.1574
```

```
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.56597    0.04676  12.103   <2e-16 ***
SDB         -0.12714    0.06613  -1.922   0.0559 .
SDC         -0.17885    0.06613  -2.704   0.0074 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Effect of temperature on weight gain

```
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   1.2122     0.2113   5.738 1.89e-06 ***
TempLo       -0.6161     0.2988  -2.062   0.0469 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Effect of Photoperiodicity on weight gain

```
Coefficients:

            Estimate Std. Error t value Pr(>|t|)

(Intercept)   3.1667     0.1944  16.292 8.31e-05 ***

LRLo         -1.0733     0.2749  -3.905   0.0175 *

---

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Pearson's Chi-squared test

```
CrossTable(sat$Tretment, sat$final.sex, digits=3, expected =FALSE, prop.r=FAL
SE, prop.c=FALSE,prop.t =FALSE, prop.chisq = TRUE, chisq = TRUE, fisher =FALS
E, mcnemar = FALSE,missing.include = TRUE )
```

```
   Cell Contents

|-------------------------|

|                       N |

| Chi-square contribution |

|-------------------------|
```

Total Observations in Table: 360

	sat$final.sex		
sat$Tretment	females	males	Row Total
20 mg/ml/kg	51	39	90
	4.250	3.141	
30 mg/ml/kg	40	50	90
	0.080	0.059	
60 mg/ml/kg	22	68	90
	6.904	5.103	
90 mg/ml/kg	40	50	90
	0.080	0.059	
Column Total	153	207	360

```
Statistics for All Table Factors

Pearson's Chi-squared test

------------------------------------------------------------

Chi^2 =  19.67604     d.f. =  3     p =  0.0001981085
```