EFFECT OF PERIPHYTON TECHNOLOGY ON THE GROWTH PERFORMANCE AND FECUNDITY OF NILE TILAPIA, *Oreochromis niloticus* (Linnaeus, 1758) CULTURED IN EARTHEN PONDS

BY

MAVINDU MUTHOKA

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN AQUATIC SCIENCE

SCHOOL OF PHYSICAL AND BIOLOGICAL SCIENCES

MASENO UNIVERSITY

DECLARATION

I confirm that the work reported in this thesis has never been submitted for the award of a degree or related program in any other institution of learning. References and citations have been used to properly acknowledge all sources of information.

Signature ………………………………. Date ……………………………

MAVINDU MUTHOKA

MSC/SC/00117/019

We affirm that the work presented in this thesis was undertaken under our supervision by the aforementioned student and that it was forwarded for examination with our consent.

Signature ……………………………….   Date ……………………………

Dr. Erick Ogello, Ph.D.

Department of Animal and Fisheries Sciences,

School of Agriculture, Food Security, and Environmental Sciences,

Maseno University.

Signature ……………………………….   Date ……………………………

Dr. Henry Ouma, Ph.D.

Department of Zoology,

School of Physical and, Biological Sciences,

Maseno University.

ACKNOWLEDGEMENT

First and foremost, I'd want to thank God for the good health He granted me while working on this thesis. Without Him, this work would not have been possible. I'd want to express my gratitude to the administration of the Kenya Marine and Fisheries Research Institute, Sang'oro station, for the kind welcome and assistance they provided me throughout my research.

I’m grateful to my supervisors Dr. Erick Ogello (Department of Animal and Fisheries Sciences) and Dr. Henry Ouma (Department of Zoology), for their invaluable support and constant supervision during the duration of this work. Their wisdom and experience, patience, insightful criticisms, and encouragement, were invaluable in the development of this work.

My gratitude extends to Mr. Daniel Mungai Ndegwa and Mr. Nicholas Outa for their invaluable guidance during writing and data analysis. Further, I want to thank my colleague, FabriziaOdoje, for all the assistance she accorded me. Last but not least, I would like to thank my parents, Augustine and Pauline, for their encouragement, financial and spiritual support throughout this work.

DEDICATION

This thesis is dedicated to the Almighty God and to my dear parents, Augustine, and Pauline who raised me to be a dependable, hardworking, and focused person via their efforts, advice, and care.

ABSTRACT

Aquaculture plays a big role in eliminating poverty and malnutrition. However, the current pond-based systems rely on the supply of large amounts of low-quality commercial feeds that limit aquaculture productivity and have the potential to cause environmental problems such as eutrophication in the receiving waters. Poor brood stock productivity remains a major constraint in aquaculture growth. Early sexual maturation of *O.niloticus* before it attains market size results to stunted growth affecting its productivity. The present study was therefore guided by the following specific objectives: i) To determine the effect of periphyton technology (PPT) on the growth performance indicators of *O. niloticus* reared in earthen ponds ii) To determine the effect of PPT on the fecundity of *O. niloticus*iii) To determine the effect of PPT on plankton diversity and abundance iv) To determine the effect of PPT on pond water quality parameters. The research was done at Kenya Marine and Fisheries Research Institute (KMFRI), Sang'oro.Six earthen ponds used for the study were limedat a rate of 4 $g.m^{-2}$ and filled with water one week after liming. The ponds were fertilized using chicken manure. Two treatments, i.e., PPT-and control-ponds, were evaluated in the study and triplicated. The PPT ponds were fitted with 2-m long eucalyptus poles of 5 cm diameter at 50 cm interval with the inclusion of molasses as carbon source. The control ponds were not treated. Tilapia fish (mean weight, 12.35±0.15g) were stocked in all ponds at a density of 3fish/m^2, and fed on a commercial diet with 20 % crude protein (CP) twice daily at 3 % body weight. 30 fish were sampled weekly from each pond for growth, and 10 fish were sampled bi-weekly for the determination of fecundity. Water samples were collected from the ponds to determine plankton abundance and diversity. Selected physicochemical water parameters were monitored *in situ* weekly using multi-parameter meter. Total ammonia nitrogen (TAN) was measured in a laboratory using standard procedures. R software version 3.2.1 was used to conduct statistical analysis. The effects of PPT on growth performance, fecundity of *O. niloticus*, water quality parameters, plankton diversity and abundance were analyzed using independent t-test. The PPT-ponds registered significantly higher mean weight (150.69 ± 0.99 g), SGR (2.75 ± 0.01), and lower FCR (1.29 ± 0.01),than the control ponds: Mean weight (99.23 ± 0.96 g), SGR (2.29 ± 0.00), and FCR (1.58 ± 0.01).There was significantly higher fecundity in the PPT-ponds (2.28 ± 0.09 g) than control (1.74 ± 0.06 $g.fish^{-1}$) with prolific spawning behavior starting earlier in the fourth week in control pond (fish mean weight, 29.19 ± 0.26) but delayed to the eighth week in the PPT-ponds with fish having a mean weight of 81.37 ± 0.51. PPT-ponds had a higher phytoplankton and zooplankton diversity index of 3.19 and 3.42, respectively, compared to 2.57 and 2.34, respectively, in control-ponds. There was a significantly higher zooplankton mean abundance in the PPT-ponds (2771.83 ± 313.11)than in control (262.67 ± 16.78). The control-ponds recorded significantly higher concentrations of nitrite (0.07 ± 0.01 $mg.L^{-1}$), ammonia (0.21 ± 0.03 $mg.L^{-1}$) and ammonium (0.20 ± 0.04 $mg.L^{-1}$)compared to PPT-ponds: nitrite (0.02 ± 0.01 $mg.L^{-1}$), ammonia (0.06 ± 0.01 $mg.L^{-1}$), and ammonium(0.02 ± 0.01 $mg.L^{-1}$). This study demonstrated that PPT is a quality natural fish feed that can be used with supplemental feed to replace the costly commercial fish feeds. PPT also has a high potential of improving the water quality of the culture system, thereby providing the ideal conditions for maximum growth of fish. The present study recommends the adoption of PPT in replacing the costly commercial fish feeds used in tilapia culture. Further studies are recommended to explore other substrates for installing PPT in ponds.

TABLE OF CONTENTS

ABBREVIATIONS AND ACRONYMS

ADG	Average Daily Growth Rate
Cm	Centimeter
C/N	Carbon Nitrogen ratio
CP	Crude Protein
DO	Dissolved Oxygen
F	Food
FCR	Feed Conversion Ratio
GDP	Gross Domestic Product
g/m^2	grams per square metre
Ha	Hectare
H_o	Null hypothesis
IBM	International Business Machines
Kg	Kilogram
KMFRI	Kenya Marine and Fisheries Research Institute
ln	Logarithm
MWG	Mean Weight Gain
N	Nitrogen
P	Phosphorus
PM	Past Midday
PEL	Protein Efficiency Level
PPT	Periphyton technology
PTAN	Production of Total Ammonia
PWI	Percentage Weight Increase
SAI	Survival Activity Index
SGR	Specific Growth Rate
TAN	Total Ammonia Nitrogen

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

1.1 Background information

Aquatic food consumption is now a subject of discussion in the global food system analysis due to many health benefits attributed to fish proteins (Naylor et al. 2021). To maintain the current human aquatic food consumption demand, there is a need for expansion and sustainable intensification of the aquaculture sector in the next five decades (Henriksson et al. 2018). The intensification of aquaculture production requires the implementation of sustainable and regenerative fish production technologies that ensure faster and higher production volumes with minimal cost of input. In most developing countries, the aquaculture sector is facing several challenges, including limited land, water, seed,and feeds,which have led to production stagnation (Munguti et al. 2014; Ogello and Munguti, 2016).

Fish feed is an important factor in the aquaculture industry which now accounts for 50 - 70 % of total production cost (Munguti et al. 2021). Most of the fish feeds used by small-scale fishermen in the current pond-based aquaculture systems are inefficient and unproductive as only about thirty percent of fish feed is transformed into flesh resulting to reduced growth performance (Gross et al. 2000; Krishnani et al. 2019). About 70 % of the dietary protein therefore dissolves in water and can accumulate to toxic levels leading to fish mortality (Krishnani et al. 2019). The nitrogenous compounds also result to severe nuisance algal blooms which yield unpleasant odor and reduce the aesthetic appeal of the water. As the algae die and decompose, a lot of oxygen is consumed making the cultured fish suffer and die due to low oxygen levels(Mbuthia, 2020). To prevent losses, frequent water exchange is often done, where the water is released to natural ecosystems.

The intensification of aquaculture has raised concerns about its environmental and social impacts due to the subsequent discharge of immense amount of pollutants generated from fecal, uneaten feed, and decomposed organic and inorganic materials (Troell et al., 2017). The effluents contain toxic nitrogenous compounds which cause eutrophication in the receiving waters and exotic species which may alter the dynamics of natural ecosystems (Carballeira-Brana et al. 2021). The increased water exchange rate has led to increased chances of the introduction of pathogenic bacteria. Escapees from ponds during water exchange also continue to compromise the aquaculture industry’s long-term viability (Naylor et al. 2021).

Kenya is also considered a chronically water scarce country(Mogaka et al. 2006). Despite this, the high-water exchange rates due to water pollution increases water budget limiting the intensification of the aquaculture (Emerenciano et al. 2017). Extreme abstraction of water from limited underground aquifers can result to subsidence and salinization. Plankton counts, which are primary sources of feed in ponds, have been reported to decrease gradually over time in aquaculture systems without additional areas of attachment (Garcia et al. 2012).Most of these plankton communities control water quality and have all the nutritional requirements required for fish growth (Emerenciano et al. 2017).Their absence therefore reduces the amount of nutrients available leading to reduced growth performance.

Poor brood stock productivity remains a major significant constraint on tilapia production. Tilapia seed production problems are associated with poor nutrition and early sexual maturity of *O. niloticus* (Tahoun et al. 2008).Early sexual maturity in *O. niloticus* results in overpopulation and stunted growth rates (Omasaki, 2017).The large number of fingerlings produced during these early stages consume the feeds and dissolved oxygen intended for the stocked *O. niloticus* (Shoko et al. 2015). During the breeding season, *O. niloticus* females have to mouth brood the fries, and therefore during the period, they do not feed. This results to reduced feed conversion ratio (Opiyo et al. 2020). There is a need for regenerative or bio-circular aquaculture technologies that can generate natural food materials within the system to sustain the aquaculture units, with supplementary feeding, which can be done with low-quality and cheap diets.

Culture systems such as periphyton technology (PPT) have been reported to be highly productive, efficient and ensure proper utilization of natural resources such as land, water, and feeds (Guttman, 2019).PPT was derived from traditional fishing methods in West Africa and was lately improved in India and Bangladesh through the introduction of substrates in the polyculture of Indian carps (Beveridge et al. 1998; Yadav et al. 2017). The introduced substrates positively affected the quality of the water, yields of cultured species, and periphyton development(Beveridge et al. 1998).

The present study will therefore evaluate the effects of PPT on growth performance and fecundity of *O. niloticus*by modifying the conventional fish ponds through balancing C/N ratio and introducing underwater substrates on which a community of bacteria, fungi, crustaceans, protozoa, chironomids, oligochaetes, snails, and may flies colonize. Sufficient surface area is provided by the substrates for the growth of periphyton communities which enhance natural primary productivity, facilitate good water quality and create food for the farm edaquatic animals (Li et al. 2019a; Miao et al. 2021). The single-cell microbial feed ingredients that make up the periphyton have also been reported to be a better replacement for the costly and scarce fishmeal, an ingredient that is the chief protein source in most fish diets (Promthale et al. 2019).The growth of the heterotrophic bacterial community is enhanced by ensuring a higher carbon-nitrogen ratio of about 10-15 (Ogello et al. 2018; Guo et al. 2020).A higher C/N ratio is achieved through the introduction of a meal with low protein content or the addition of carbohydrate source in addition to the supplemental feed (Avnimelech, 1999; Tinh et al. 2021). The use of a low protein diet consequently translates to higher profitability for fish farmers because of the cheap cost of feeds. The heterotrophic microorganisms convert the total ammonia nitrogen (TAN) generated in the system to microbial biomass (Aisyahet al. 2021) or the nitrifying microorganisms convert TAN into nitrite in aerobic conditions and then into nitrate allowing other microorganisms to form less harmful molecules (Abakari et al. 2020).

Periphyton is nutritionally attractive as it comprises of sufficient crude protein, carbohydrates, and lipid, which are superior compared to commercial feeds utilized for the growth of *O.niloticus*, and thus all nutrient constituents are present on the periphytic microhabitat (Ogello et al. 2014). Since tilapia relies on natural source of nutrients in the form of plankton, periphyton (Abdel-Fattah, 2020), and microbial floc (Mugwanya et al. 2021), this study hypothesizes that PPT will enhance the growth performance of the cultured fish due to the high-quality nutrition. Quality nutrition is a factor of high fecundity and quality of tilapia eggs. Mostly, tilapia males invest in mating while the female tilapia invest in good and “hot” males, which are assessed based on the size and qualities of the spawning territory (Nelson, 1995). The presence of good spawning sites over stimulates females for reproduction resulting to overpopulation in tilapia culture ponds.

The introduction of substrates is expected to cause restrictions in nest-making by depriving space for tilapia to make good nests that act as a sexual selection character (Mendonca & Goncalves-de-Freitas, 2008), thus affecting tilapia breeding behavior. The balancing of the C/N ratio in the PPT ponds is expected to change the plankton and microbial dynamics in the ponds, thus affecting the quality of water and the tilapia growth performance. However, the adoption of PPT in local aquaculture initiatives has not been adequately explored, and many technical and biological functions are not yet clear for fish farmers. The present study aims at evaluating the effects of PPT on growth performance and breeding behavior of *O.niloticus* cultured in earthen ponds.

1.2 Statement of the problem

An ideal fish pond system should be productive and ecologically sound to fasten fish production. This can be achieved through the implementation of efficient management practices. The current pond-based aquaculture systems rely on the supply of large amounts of costly and low-quality commercial feeds that lead to low fish yields. Tilapia seed production in conventional fish ponds are faced by productivity problems that are associated poor nutrition and early sexual maturity. The necessity of dependable supply of quality *O. niloticus* fry in many parts of Kenya is imperative, but the problem of mass production remains a challenge. The early sexual maturation in *O. niloticus* before it attains marketable size leads to a stunted growth rate, reducing the economic benefits despite the use of costly commercial feeds. Kenya is generally considered a water scarce country. Despite this, the high-water exchange rates in the current pond-based aquaculture systems result to consumption of huge quantities of water which further hinders intensification of the aquaculture sector. The PPT system has not been adequately explored in the Kenyan aquaculture sector, and scientific information on its possible impact on growth rate, pond water quality parameters, and fecundity of *O. niloticus* remains unclear. The present study will therefore modify the conventional fish ponds through introduction of substrates and balancing of C/N ratio and evaluate its effects on growth performance and fecundity of *O. niloticus*.

1.3 Objectives of the study

1.3.1 General objective

To determine the effect of Periphyton Technology (PPT) on the growth performance and fecundity of Nile Tilapia, *Oreochromis niloticus* L. cultured in earthen ponds

1.3.2 Specific objectives

i. To determine the effect of periphyton technology on the growth performance indicators of *O. niloticus* reared in earthen ponds

ii. To determine the effect of periphyton technology on the fecundity of *O. niloticus*

iii. To evaluate the effect of periphyton technology on plankton diversity and abundance.

iv. To determine the effect of periphyton technology on pond water quality parameters

1.4 Null Hypothesis

H_{o1}: Periphyton technology does not have an effect on growth performance indicators of *O.niloticus* reared in earthen ponds.

H_{o2}: Periphyton technology does not affect the fecundity of *O.niloticus*.

H_{o3}: Periphyton technology does not have an effect on plankton diversity and abundance.

H_{o4}: Periphyton technology does not have an effect on pond water quality parameters.

1.5 Justification of the study

Sustainable intensification of aquaculture will not be achieved unless accompanied by sustainable improvements in biological and technological aspects that address the social and economic environment within which aquaculture industry is placed.Animal protein demand is predicted to be two-thirdsmore than the current demand by the year 2050 (Henchion et al. 2017). With the current low production yields of *O. niloticus* as a result of early sexual maturation, which has caused stunted growth rates, it will be close to impossible to meet the high animal protein demand in 2050. The high animal demand is also expected to exert high pressure on the natural resources including water, land, and feeds, which are required for producing animal protein (Makkar et al. 2014).The increased water demand will cause negative environmental impacts, especially in aquaculture systems, generating and accumulating large amounts of waste which are deposited to natural water ecosystems (Henchion et al. 2017). Addressing this “perfect storm” necessitates sustainable production technologies. It is envisioned that the present study will provide fish farmers with productive fish farming technology which will improve the growth performance, and lead to mass production of quality fingerlings.

1.6 Significance of the study

The present study will provide a cheap and climate-smart fish farming technique for farmers especially in developing countries, which minimizes the utilization of resources such as water and fish feed hence reducing the cost of production. The findings on the effect of the periphyton system on the fecundity will help fish farmers in maximizing fish seed productivity. The study will also provide a methodical framework for establishing periphyton technology, which is a new culture system for improving aquaculture performance in Kenya.

1.7 Limitations of the study

The sex of the tilapia post-fingerlings used in this study could not be visually determined due to the small size of genital papilla. High sex ratio of males, which have high feed conversion ratio compared to females could lead to higher growth performance in one of the treatments.

CHAPTER TWO

LITERATURE REVIEW

2.1 Status of aquaculture production in Kenya

Kenya has a tremendous potential for the aquaculture sector to flourish because it’s endowed with natural resources, including wetlands, rivers, lakes, streams, and dams, that favor the culture of aquaculture species (FAO, 2006a; Munguti et al. 2021). Based on Ngugi et al. (2007), the fishery industry began in Kenya in the 1900s with trout introduction into river systems for sport fishing. The aquaculture sector, however, became popular in the 1960’s (FAO, 2016)but had a slow growth rate until the year 2014, when the aquaculture sector production rose to 24,096 MT from 4,218 MT in 2006. This ranked Kenya as the 4th major producer of aquaculture in Africa after Nigeria, Uganda, and Ghana (KMFRI, 2017). The freshwater aquaculture sub-sector recently registered a depressed performance, with whole aquaculture productivity declining by 33.77% from 18,656MT in 2015 to 12,356 MT in 2017(Munguti et al. 2021). There was, however, a production increase in 2018 and 2019 with 15,320 MT and 18,542 MT, respectively (fig. 1) (KNBS, 2020), which has been attributed to the progressive use of quality fish feeds (Munguti et al. 2021).

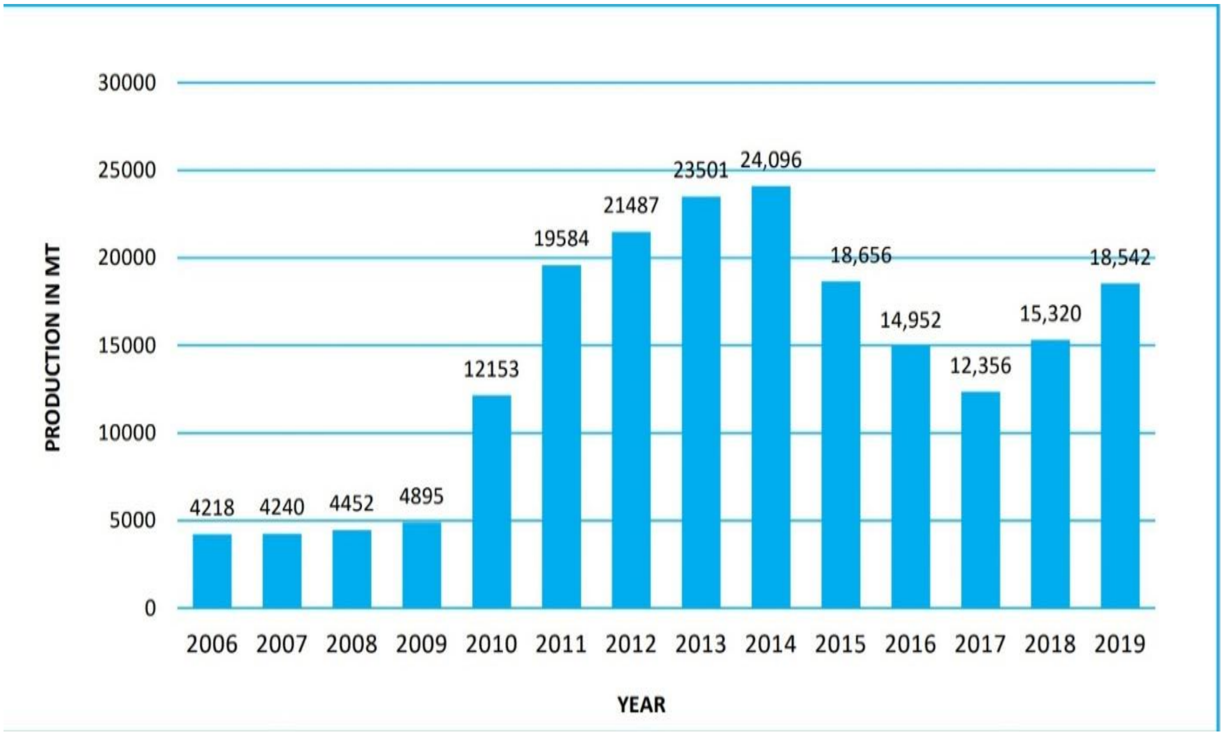


Fig.1: Kenya Aquaculture production trend from 2006 to 2019 (KNBS, 2020).

Aquaculture production in Kenya comprises of four main fishes,i.e., Nile tilapia(*Oreochromisniloticus*), African catfish (*Clariasgariepinus*), common carp (*Cyprinus carpio*), and rainbow trout (*Oncorhynchus mykiss*). Other fishes such as *Labeovictorianus* have not been fully accepted as culturable fishes due to their biological and ecological technicalities (Munguti et al. 2014). *O.niloticus* is found in all aquatic ecosystems and is the main cultured species in Kenya. Out of the 18,545 MT of fish harvested in 2019,*O.niloticus* contributed to the bulk of the fish, accounting for 81.42%,followed by *C.gariepinus,* contributing 12.94%. According to Simoes et al. (2007), *O.niloticus* is amongst the most high-yielding and globally commercialized food fishes because of the lack of intramuscular spines and its white flesh with a firm texture .*O.niloticus* species is also more preferred because it's easy to farm, it's a good protein source, and it has wide acceptance with no reported socio-cultural restrictions (Uddin et al. 2007). It has been termed the 'aquatic chicken' because it's cultured in almost all fish farms

(Ogello et al. 2014). The prosperity of *O.niloticus* has also been attributed to the high tolerance in adverse environmental conditions and is the most physiologically adaptable species to new technologies where cultivation is at high densities (Perez-Fuentez et al. 2016). *O.niloticus* species also can be cultivated at high suspended particulate densities (Emerenciano et al. 2013).

The culturing of fish in Kenya is mostly based on semi-intensive culture systems. More than 90% of farmed fish species are reared in earthen ponds ranging from 150 to500m^2 (Ngugi et al. 2007). Other culture systems used include tanks, cages, re-circulating and integrated systems (KMFRI, 2017). The farmer's decision on the culture unit to be used is determined by managerial, economic, and environmental factors that affect the farmers (De Graaf et al.2005).Most farmers, however are unaware of the economic strengths of alternative aquaculture farming techniques (Kaliba et al. 2007). Innovations in fish farming techniques such as the adoption of efficient systems are some of the challenges that need to be addressed (Munguti et al. 2014). This is because the aquaculture output rate needs significant acceleration to meet the fish demand since Kenya still imports over30,000 MT yearly from Pakistan, Uganda, India, Japan, China, and Korea(FAO, 2012; SDF, 2014).

2.2Productivity of current conventional ponds systems in Kenya

Aquaculture production in Kenya is generally made of extensive (output ranging0.5-1.5tonnesper ha every year) to semi-intensive systems (output approximately three tonnes per ha every year) (Obwanga and Lewo, 2017). However, studies show that newsubsistence fish farmers are switching to intensive farming systems such as cage culture, ponds, integrated systems, raceways, and recirculating culture systems though still at the infancy stage(KMFRI, 2017).

In Kenya, earthen ponds are the most popular systems because they are inexpensive to setup, favorable climate, and presence of favorable soils in many regions of the country (Musa et al. 2012). Under the Economic Stimulus Programme (ESP) in 2009 introduced by the government of Kenya, the number of ponds increased from 4,000 in 2009 to 69,194 in 2013. Despite the expectation that government efforts would increase production, the aquaculture sector registered a depressed performance, with the total number of active ponds reducing to 60,277 in 2015 from a peak of 69,194 in 2013 (SDF, 2016).

The reduction was attributed to inadequate fish farming infrastructure and low technical support (SDF, 2016; Obwanga and Lewo, 2017), which creates room for a more innovative approach. Most of the ponds are owned by individual smallholder farmers with a maximum of 60 ponds and a minimum of 1 pond ranging 200-500 m^2 (Obwanga and Lewo, 2017). In well-managed systems, three fish per square metre is the standard stocking rate in the ponds to produce 1 kg/m^2, with fish gaining daily weight of about 1.5 to 2.0 g (FAO, 2016).

2.3How pond-based periphyton technology works

Periphyton is an amalgamation of attached living micro-organisms such as algae, bacteria, protozoan, and free-swimming organisms such as cladocerans and rotifers that grow on free surfaces of submerged objects in water (Azim et al. 2002). Periphyton productivity ranges between 1-3 $g.m^{-2}$substrate.Day^{-1} (Azim et al. 2005).The growth of these microorganisms requires the presence of oxygen, control of the C/N ratio, and introduction of substrates for attachment in the euphotic layer where there is maximum light penetration (Azim, 2001).

The introduction of substrates promotes the growth of bio films, accelerating the biological elimination of nitrogenous toxic pollutants (Crab et al. 2007) and increasing fish production by enhancing food availability (Azim et al. 2004) through periphyton development.The formation of the periphyton layer on substrates is initiated by the buildup of electrostatic charges of a layer of dissolved organic compounds, to which microbial populations are pulled by hydrophobic reaction and attach themselves by use of mucilaginous strands. The process is stimulated by the presence of free-floating particulates in the water column(van Dam et al. 2002).Bamboo substrates have been highly recommended because of their durability, ease of use, high-quality periphyton, and availability in the tropics. The periphyton community in these substrates is also highly diverse, comprising of inorganic matter, organic nutrients, heterotrophic and autotrophic organisms (Azim et al. 2001a). The growth of these periphytic organisms helps in controlling water quality while providing the fish feed.

A large percentage of the feed used in culture systems is not consumed by fish and ends up accumulating in water as ammonia (Franco-Nava et al. 2004). The eaten feed is also not fully transformed into harvestable products and part of it is released as feces (Jimenez-Montealegre et al. 2002). The unconsumed feed and the feces (approximately 75 % of the total feed) result to high concentrations of nitrite and TAN, which may lead to fish mortalities (Jimenez-Montealegre et al. 2002). In a periphyton system, the TAN and nitrite can be eliminated in three main nitrogen transformation path ways, i.e., The transformation of toxic ammonia by heterotrophic bacteria to bacterial biomass, the transformation of ammonia nitrogen to nitrate by chemoautotrophic bacteria, and photoautotrophic process(Ebeling et al. 2006).

The heterotrophic and chemoautotrophic conversion of ammonia is promoted by the addition of a source of carbon or increasing carbon content of the fish feed because organic carbon gets limited for bacterial growth in pond environments, while nitrogen that is dissolved in water is at high levels reducing the water quality (Emerenciano et al. 2013; Xu et al. 2016). The added carbon stimulates the growth of microbial communities on which both autotrophic and heterotrophic microorganisms thrive (De-Schryver &Verstraete, 2009). These microorganisms are responsible for the degradation of toxic compounds that lead to water pollution (Ebeling et al. 2006).

Heterotrophic bacteria immobilize ammonia more quickly than autotrophic bacteria because their growth rate and bacterial biomass production per unit substrate are a factor of ten higher (Hargreaves, 2006),hence the system is dominated mainly by heterotrophic bacteria and will play the biggest role in maintaining water quality and providing feed ingredients to fish. Heterotrophic bacterial cells require a fixed ratio of both organic carbon and inorganic nitrogen that reflects their composition (Hargreaves, 2013). The addition of a carbon source such as molasses makes the heterotrophic bacteria create a demand for inorganic nitrogen (Hargreaves, 2013). The heterotrophic bacteria thus colonize the unconsumed feed and feces, taking up nitrogen from the water generating large amounts of bacterial biomass used as fish feed (Avnimelech, 2015; Emerenciano et al. 2017). The process occurs rapidly and elevated levels of TAN can be reduced within 1-3 days if a sufficient quantity of organic carbon is added. The consumption of these microbial feed ingredients contributes to increased growth rate, doubled feed utilization, and decreased expenses in the purchase of feed (Avnimelech, 2015). Other organisms fed on by fish such as snails, chironomids, mayflies, and crustaceans (Fig. 2) also feed on the periphyton. This allows for quicker growth than in a traditional production system while avoiding resource competition with the microorganisms that form bio films. (Ebeling et al. 2006).

The autotrophic bacteria,i.e., nitrifying bacteria found in the microbial community, also play a major function in controlling the quality of water in the periphyton system. The presence of nitrite and ammonia normally facilitates the growth of nitrifying bacteria (Emereniano et al. 2017). The autotrophic process involves two phases achieved by two different kinds of bacteria. Ammonia-oxidizing bacteria of genera *Nitrosospira, Nitrosococcus, Nitrosovibrio, Nitrosomonas, and Nitrosolobus*are the first group of bacteria. In the presence of oxygen, they convert ammonia which develops from feces, unconsumed feed, prawn shell molts, dead algae, and zooplankton into nitrite. The second group of bacteria isnitrite-oxidizing bacteria of genera *Nitrospira, Nitrobacter, Nitrospina, and Nitrococcus,* and metabolizes nitrite to nitrate(Timmons et al. 2002; Abakari et al. 2020). The nitrate can be utilized by algae and phytoplankton to produce chlorophyll, and in turn, consumed directly as fish feed and indirectly via secondary trophic levels such as benthos, prawns, zooplankton, and invertebrates (Azim et al. 2001a), and the cycle repeats. The system facilitates other nitrogen conversion mechanisms, including denitrification (Hu et al. 2014), which results in the formation of dinitrogen, a harmless gas that bubbles out of the system, and photoautotrophic N uptake (Emerenciano et al. 2013), which depend on prevailing environmental conditions.

Photoautotrophic removal by algae involves the assimilation of ammonia and nitrite directly to produce algal biomass through the biosynthesis process(Emerenciano et al.2017).The decomposing organic matter ,i.e., unconsumed feed, dead organisms, and fecal wastes, release nutrients that are quickly absorbed and deposited in algal cells. The conversion of ammonia and nitrite to algal biomass requires energy input which is derived from solar energy. Algae and phytoplankton are the main contributors to primary production (Uddin et al. 2007) and source of dissolved oxygen which is very vital for fish growth and production. Provision of dissolved oxygen by algae can lead to an increase in pH by 1 unit, leading to calcium phosphate precipitation and deposition of carbonate-phosphate complexes (Yadav et al. 2017).

According to Rodriguez et al. (2012) periphytic algae are more productive than phytoplankton per unit water surface. According to Rodriguez et al. (2012) periphytic algae are more productive than phytoplankton per unit water surface. Filter feeding on algae provides energy requirements and micro-nutrients to herbivorous fishes unlikely to be provided by phytoplankton (Dempster et al. 1995). The local biotic and a biotic variables including grazing, level of nutrients, light, and substrate quality are also frequently connected to periphyton species richness, which is mostly made up of algae (Hillebr and, 2002; Albay & Akcaalan, 2003).

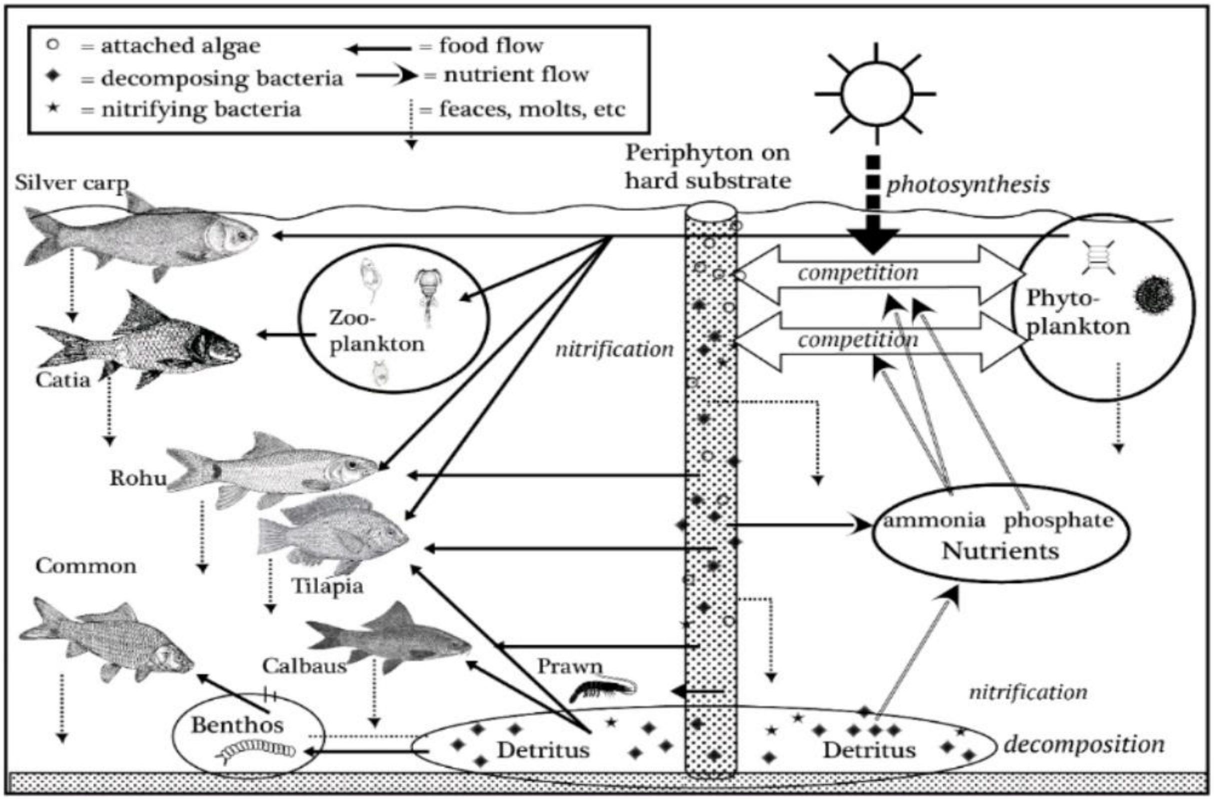


Fig.2: A cross-section of a single bamboo stick under water showing the food web and flow of energy and ecological interactions of microscopic flora, decomposing matter, zooplankton, and fish communities under the water mass in a periphyton aquaculture (Yadav et al. 2017).

2.4Growth performance indicators of *O. niloticus*

2.4.1 Specific growth rate

The development of aquaculture is constrained by a lack of cheap, and high-quality feed, which can result increased tilapia growth. The major factor that should be considered to improve the growth rate of *O. niloticus* is feed which is the single highest item of expenditure in semi-intensive as well as intensive systems. The type and value of fish feed that farmers choose depend on financial resources available, market, culture system, and the value of the fish (Hasan, 2007). Conventional systems retain 20 to 30 % of the nitrogen in fish biomass (Azim et al. 2003), and other percentage that is not harvested as fish biomass islost to the aquatic environment as organic nitrogen and ammonia in the form of feed residue and feces, resulting to the accumulation of ammonia in the water column which is harmful to the aquaculture species (Avnimelech, 2007). Overfeeding results to reduced growth rates and increased production cost (Biswas et al. 2006), and the deteriorated water quality may result to an outbreak of diseases (Samocha et al. 2004).

A number of diets have been evaluated in the culture of *O. niloticus*. Fish meal from the diminishing capture fisheries has been used as the key ingredient in fish feeds resulting to unsustainable aquaculture (Naylor et al. 2000). The high rate of inclusion of fishmeal in aquaculture is attributed to its components like protein, vitamins, amino acids, essential fatty acids and digestible energy that meets the nutritional requirements of *O. niloticus* (Tacon, 1993). Fish meal such as *Rastrineobolaargentia* is also used by human beings as source of food. This has created competition which has resulted overexploitation putting it at risk of depletion (Promthale et al. 2019). To reduce dependency on fishmeal, scholars have tried other viable alternatives such as vegetable plant materials (Alfred, et al. 2020). Plants products are believed to be in abundance, with high content of protein and are cheaper compared to fishmeal (Hardy, 2010). Plants materials however comes with several drawbacks such as environmental impacts resulting from high phosphorus and solid waste, lower levels of Omega-3 acids, endogenous anti-nutritional factors that reduce the growth rate of fish thus extending the time the fish takes to reach maturity age (Hardy, 2010; Naylor et al., 2009).Farmers have also been forced to use locally available materials such as maize bran and wheat bran as a source of fish feed (Liti et al. 2006). This is because high percentage of the commercial feeds available in the market are too expensive for them to afford. Most of these fish feeds are made of single ingredients with low protein levels and high crude fiber content that lowers their palatability and digestibility, resulting to poor fish performance (Liti et al. 2006). Commercial feeds are mostly applied in fish species reared for commercial purposes in intensive systems to avoid cost implications (Munguti et al. 2014).

Farmers have also been using farm-made feeds formulated by use of locally available ingredients that are more affordable than commercial feeds. The ingredients are purchased from suppliers whom they may not be able to access the information on how they need to design feeding regimes. The feed ingredients are also poorly milled during processing, failing to meet the feed processing standards. Therefore, a high percentage of the feed is lost in the water column as waste leading to poor rates of ingestion (Craig &Helfric, 2002).This creates a need for an alternative cheaper and quality protein source to be used as a source of fish feed.

The promotion of bacterial growth in periphyton culture systems will reduce the need for costly feeds and fertilizers while increasing the growth rate of *O. niloticus* (Azim, 2001). The culture unit is only fertilized during establishment to stimulate the growth of microbial organisms (Perez-Fuentez et al. 2016). The fertilization of water and control of the C/N ratio results to the growth of heterotrophic bacteria which converts nitrogenous wastes inform of uneaten feed into micro algae and bacterial biomass to be used as the source of lipids and proteins hence improving the specific growth rate of the farmed species (Wasielesky et al. 2006; Avnimelech, 2007; Emerenciano et al. 2011).The substrates will provide surface area for attachment of nutrients, and a high submersed area leads to greater benefits from the periphyton, such as more natural food inform of periphyton (Abwao et al. 2014).

2.4.2Feed conversion ratio (FCR) of *O. niloticus*

The FCR, which is the ratio between feed intake and body weight gain, is of major interest for improving aquaculture sustainability through reduced costs in feed and environmental impacts (Rodde et al. 2020). Meeting the future animal protein demand requires improving the ability of the cultured fish to convert the feed consumed into flesh (de Verdal et al. 2018). FCR is determined by the individual feed efficiency and survival, as fish that die during the culture period consume feed until death, but they are not accounted in total fish biomass harvested. *O. niloticus* mortality rates vary considerably, with 20-71 % mortality being reported for *O. niloticus* cultured in fertilized ponds with or without supplemental feeding (Abdelghany& Ahmad, 2002). Fish mortalities that happen during the later stages of fish development have the largest economic effect due to the accumulated cost of production (Abdelghany& Ahmad, 2002). In areas where the cost of fish feed is high, a slight increase in FCR could considerably result to increased variable cost. Therefore, underperformance in terms of FCR is a major concern as it strongly and negatively affects the profitability of fish farms. FCR can be improved through changes in feed composition, husbandry and selective breeding (NRC, 2011). Improving FCR in selective breeding program is a major problem as it is difficulty to accurately measure FCR at individual level on a large number of fish.

Determining the individual feed consumed of a large number of fish is difficulty as the fish are reared in groups, and the share of a meal eaten by each fish is not easily recorded. Several methods have been proposed to determine feed consumed by each individual fish species, including X-radiography with radio-opaque pellets (Grima et al. 2008) or use of video recording of small groups of fish distinguished by coloured T-bar tags (de Verdal et al. 2017). The other option that is normally used is the rearing of individual fish in aquaria with collection of all uneaten pellets (Besson et al. 2019). This method is however tiresome though it has the potential to be used for selective breeding through the identification of Quantitative Trait Loci (QTLs) or utilization of genomic selection (Besson et al. 2019). Determining the FCR of individual fish in post-juvenile stages is essential as the quantity of feed consumed during these stages is higher compared to the amount consumed during the younger stages (Alanärä et al. 2001). De Verdal (2017) reported that its important to determine the correlations between FCR measured at various stages of development, in order to evaluate the ability to use data from one given stage to select efficiently for FCR over the entire rearing period.

The FCR of pelleted feeds for *O. niloticus* cultured in semi-intensive aquaculture systems varies considerably, and is dependent on the fish stocking size, densities, and fertilization regimes (El-Sayed, 2013). The FCR achieved using pelleted feed for fingerlings ranges from 1.7:1 to 2.1:1, while that for floating feeds range from 1.2:1 to 1.6:1. For the adult stages of the production cycle, the FCR of pelleted and extruded feeds range from 1.3:1 to 1.8:1 and 1:1 to 1.4:1, respectively. FCR is considered acceptable when it’s not higher than 2 (Craig, 2009). However, these values may differ when the fish are fed on natural feeds as they are inherent component of total food consumption (El-Sayed, 2013).

2.5 Fecundity of *O. niloticus*

Fecundity is considered as the reproductive capacity of a fish species in each spawning season (Izquierdo et al. 2001; Orlando et al. 2017). *O.niloticus* is the most commonly species cultured in ponds because of its rapid growth and palatability. They, however have a problem of early sexual maturation under captivity which affects its fecundity and leads to reduced growth due to slow somatic growth in favor of sexual maturation (Fashina-Bombata&Megbowon, 2012). According to Bucur et al. (2012), the wild *O. niloticus* attains reproductive age at a length of 20-30 cm, while according to Suresh &Bhujel (2012),it attains reproductive age at a length of 8-13 cm while under captivity. The fingerlings produced by the fish under captivity lead to reduced dissolved oxygen intended for the cultured *O. niloticus* stocks decreasing their growth rate. There is also lack of uniformity of the product at harvest due to the fast growth of the males, which have better food conversion efficiency than females (Megbowon & Mojekwu, 2014). According to Munguti et al. (2014), fish reared for aquaculture should not breed before reaching a body size acceptable for harvest.

This precocious maturation has driven scientists to find a remedy to this problem, and the administration of 17α-methyl testosterone, a hormone used for the production of mono sex males, has been widely promoted and adopted (El-Greisy& El-Gamal, 2012). The production of mono sex males' results to enhanced growth and prevention of unwanted reproduction (Jensi et al. 2016). There are other methods of achieving uniformity in sizes, such as chromosomal manipulation, hybridization, and manual separation of males and females. However, the utilization of methyl testosterone for sex reversal is most preferred because it's the least expensive and efficient (Megbowon &Mojekwu, 2014). This study, however raises a concern on the effect of the hormone on the farmers, consumers, and the surrounding water bodies on which the effluents are discharged.

It’s advisable that farmers use techniques that will achieve the desired objective with no adverse effects on farmers, consumers, and the surrounding environment. The hormone-laced feed is prepared by the culturists, who are exposed to risks such as coming into contact with the hormone and also inhalation, which may result to anabolic and presumed libido-enhancing characteristics of the hormone (FAO, 2006a; Megbowon & Mojekwu, 2014).The use of 17-αMethyl testosterone may also have negative impacts to the surrounding water bodies. The fry’s that have already been fed the hormone-laced feed may escape from the culture units and end up to the nearest water bodies. The escapees will have lost the capacity to reproduce and interbreed with wild stock altering the dynamics of the water ecosystem, as reproductive potential is dictated by the total number of active females (Shore & Shemesh, 2003).

Megbowon (2011) also revealed that the hormone accumulates in pond sediments and persists for about three months after the treatment is stopped. When the effluents are released in natural water bodies, it may also alter the sex dynamics of other populations resulting to recruitment failure. The effluents containing the hormone mix with water in rivers used for drinking purposes, especially in rural areas. The water, if consumed by humans, may cause reversible infertility, breast atrophy and menstrual disturbances, and voice deepening in females. In males, it can cause breast enlargement and spontaneous erections, increased aggressiveness, and liver damage may occur in both sexes (Kicman, 2008; Llewellyn, 2009).

Ekasari et al, (2013) reported that the production system chosen might improve the reproductive performance of *O. niloticus*. The stocking density also strongly influences sexual differentiation pathways. According to Davey &Jellyman (2005), high fish stocking density can produce a large number of males. Through this, the farmer can be able to achieve the desired objective reducing the costs involved in purchasing sex reversal hormone, 17-αMethyl testosterone, and avoiding negative impacts that may be caused by the hormone to the consumers and the surrounding environments where effluents are deposited. Experiments done by Ekasari et al. (2016) on the fecundity of African catfish *Clariusgariepinus* revealed that the brood stock fed on microbial feed ingredients had 26 % higher production than the control system. The experiment also done by Ekasari et al. (2015) showed an increased size of eggs and the number of fries per spawn in fish fed on microbial feed ingredients than in the control setup with clear water.

Male tilapias invest mostly in mating, whereas female tilapias seek for good and "hot" males based on the size and quality of the spawning zone (Nelson, 1995). Females are over stimulated for reproduction in the presence of suitable spawning places, resulting in overcrowding in tilapia culture ponds. In the present study, introduction of substrates in the PPT is expected to deprive tilapia of space to build nice nests, which operate as a sexual selection trait (Mendonca & Goncalves-de-Freitas, 2008). This will prevent the early breeding behavior which results to overpopulation of fish ponds.

2.6 Plankton community in aquatic ecosystems

The exposure of the periphyton system to solar energy results to a positive effect on the growth of plankton communities (Garcia et al. 2012). The plankton communities continue to grow despite high grazing pressure from the culture organisms (Azim et al. 2003). Phytoplankton is a primary producer and is at the food chain’s bottom, supporting higher trophic levels (Azim et al. 2005). They are, therefore, the main ecological component in defining and characterizing the environmental dynamics of the culture system (Margalef, 1983). The type of nutrients available determines the phytoplankton composition and ecology. *O. niloticus*is an herbivorous filter feeder and is able to filter these planktonic organisms from the water surface using their gill rakers (Azim et al. 2005).

Garcia et al. (2012), however, reported that plankton counts in ponds without substrates decrease gradually over time. Phytoplankton is, therefore, more efficient and dynamic in culture systems with additional areas of attachment. The presence of substrates favors the growth of herbivorous *O. niloticus* because their mouths are well adapted to browse on attached planktonic biota (Garcia et al. 2012). According to Dempster et al. (1993), the ingestion rates of attached algae by tilapia are 25 times higher than when suspended on the water column. The additional substrates, therefore, increase the conversion efficiencies in the aquaculture system (Azim et al. 2003). The presence of substrates also improves the physiochemical water parameters, thus providing the necessary conditions required for maximum growth of the planktons.

The mostly observed phytoplankton community in culture systems with additional carbon source belong to families Euglenophyceae, Cyanophyceae, Bacillariophyceae, and Chlorophyceae (Emerenciano et al. 2017). These phytoplankton groups assimilate nitrogenous wastes to produce biomass, controlling water quality and acting as a source of fish feed. Chlorophytes have been reported to be the major contributors to increased oxygen levels in aquaculture systems (Schrader et al. 2011). The inclusion of a source of carbon has also been reported to result to a high population of zooplankton due to the presence of organic matter (Galvez et al. 2015). The zooplankton species that grow in these systems are dependent on the type of organic matter added to the culture system. Copepods, rotifers, protozoa, and ciliates, which are essential food items for most aquatic organisms during juvenile stages, dominate C/N controlled culture systems (Emerenciano et al. 2017).

2.7Water quality

2.7.1 Conservation of water

The aquaculture industry's long-term viability is largely dependent on effective resource management, particularly water (FAO, 2018).Based on the availability of freshwater resources per capita in relation to international standards, Kenya is considered a chronically water-scarce country (Mogaka et al. 2006).Organic matter and nitrogenous wastes are a big challenge in aquaculture (Emerenciano et al. 2017). The promotion of bacterial growth that controls water quality in periphyton culture units makes it a sustainable culture system through minimal or zero water exchange. The microbial community's vigorous interactions are crucial for maintaining stability in the zero-water exchange system (Emerenciano et al. 2017).

Specifically, the growth of heterotrophic and autotrophic bacteria that colonize the surface of substrates degrade wastes controlling anoxic conditions in the water (Emerenciano et al. 2017). The heterotrophic and autotrophic microbes control ammonia concentration in the water column via incorporation into microbial biomass. The removal of toxic wastes in the periphyton system minimizes the quantity of water used throughout the culture cycle. In the case of insufficient nitrification, the toxic ammonia can develop to levels that are lethal to the fish species hence constraining the intensification of aquaculture (Avnimelech, 2007).

The microorganisms also keep a healthy ecosystem on the farm and nearby aquatic environment by reducing the amount of waste released into natural water sources (Tucker et al. 2015). Minimum water exchange also prevents temperature fluctuation in the culture units

(Crab et al.2009). The maintenance of water quality and limitation of the TAN accumulation in the waters makes periphyton system highly efficient.

2.7.2 Bio security agent

Diseases have been a limiting factor in aquaculture and result to substantial economic losses (FAO, 2012).The high-water exchange rates, which is a major source of entry of the pathogenic organisms, has resulted to careless handling of antibiotics making most drugs less effective in combating pathogenic bacteria (Defoirdt et al. 2011). However, several studies have observed that aquaculture species have a high growth performance and are also healthiest in farming systems that have a high abundance of natural biota of the periphytic community (Kuhn et al. 2009).

Microbial community acts as an immune stimulant providing immunological potential against disease-causing bacteria (Vazquez et al. 2009). According to Xu & Pan (2013), the haemocyte phagocytic activity and the total number of haemocyte of aquaculture species fed on bacterial biomass is statistically higher compared to those cultured in non-microbial culture units. The control of the C/N ratio leads to the production of diverse bacterial strains which contain about 0.9-16 % poly-β-hydroxybutyrate, a bacterial storage compound which is able to prevent Vibrio infection (De-Schryver & Verstraete, 2009). The added carbohydrates also act as a source of prebiotics and can positively influence the microbial community in the gut, which is essential in improving the aquaculture species’ health status (Ahmad et al. 2017).

2.8Case studies of PPT

Several trials have been done to investigate the production of the periphyton system. Trials done at Bangladesh Agricultural University (BAU), Bangladesh, gave statistically higher fish yields in treatments with introduced substrates compared to control treatment without substrates (Azim, 2001). Azim et al. (2001a) reported that fish production can be increased by introducing substrates without the use of supplementary feeds. Production output of up to 5tonnes per ha have been documented by Azim et al. (2001b) without supplementary feeds and providing substrata corresponding to the surface area of the pond with fertilization of Triple Super Phosphate at 150 kg per ha, cow dung of 4.5tonnes per ha, and urea at 0.15tonnes per ha. The fish yields were eight folds higher compared to the control treatment that lacked substrates. Uddin et al. (2007) also observed improved feed conversion ratio of *O. niloticus* grown on bamboo substrates compared to control ponds without substrates.

The use of abandoned sprinkler pipes as underwater hard substrates was used in an experiment to enhance natural production of food for *O. niloticus* while lowering extra feed expenses, and within four months, *O. niloticus* grazing on natural periphyton and commercial feed indicated the similar harvesting weight, produce, and rate of growth(Milstein and Omri, 2003). The addition of substrates thus enhances production and improves FCR for *O. niloticus* (Abwao et al. 2014) by creating natural food for the cultured organisms.

It’s estimated that aquaculture systems use more than five million liters of water to produce a kilogram of fish (Tiago &Gianesella, 2003). Perez-Fuentez et al. (2013) observed that in the application of heterotrophic microbial population to control the quality of water, the consumption of fresh water throughout the Prawn *Macrobrachiumrosenbergii* culture period is 6.8 m^3 while that of *O. niloticus* is0.071 m^3/kg of fish. Traditional fresh water fish farming consumes approximately 16.9 m^3 / kg of fish production (Verdegem & Bosma, 2009), and this clearly shows that in the use of bio films to control water quality, very little water is used because of the minimal water exchange rate throughout the culture cycle (Avnimelech, 2009).

The source of carbon used is also very important in the stimulation of periphyton growth due to aerobic microbial metabolism, which affects the levels of dissolved oxygen. According to Khanjani et al. (2017), molasses has proved to be a better source of carbon compared to other sources such as wheat flour, starch, and a combination of them. The C/N ratio should be above 10:1 (Burford et al. 2003),with Schneider et al. (2005),recommending a C/N ratio of 15:1.According to the findings of Asaduzzaman et al. (2010), the control of the C/N ratio and introduction of substrates for development of periphyton led to higher freshwater prawn yields by 75 % due to increased survival. The added substrates decreased feed-conversion ratios by 13 % by supplying microbial biomass as an extra source of food. Garcia et al. (2017) investigated the periphyton system profitability in *O. niloticus* cultured in cages. The introduction of bamboo substrates in cages improved fish growth rates, reduced culture cycle length, and feed conversion ratio of *O. niloticus* species. The author also reported a periphyton profitability index of up to 87 % higher and up to 57 % higher annual operating income than traditional farming systems. Periphyton technology is, therefore an effective farming system to lower production expenses and makes organic *O. niloticus* farming economically viable and can be adopted in various parts of Kenya, especially in areas with water shortages and by the poor fish farmers who are not able to afford the commercial feeds in the market.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The research work was carried out at Kenya Marine and Fisheries Research Institute (KMFRI) Sang’oro located at latitude: 0°20ꞌ 28.730ꞌꞌ N and Longitude: 34°48ꞌ 37.598ꞌꞌ E in Kisumu County. The farm is stationed on a land characterized by clay soil, which is good for earthen ponds due to its high-water retention capacity, and near River Sondu-Miriu, which supplies it with water all-year-round.

3.2 Experimental design

An experimental study design was adopted. Six earthen ponds, each measuring81m^{2} with an average depth of 1m,wereused for the study. The ponds were limed using quick lime, i.e., calcium carbonate ($CaCO_3$)based on soil pH at 4 g.m^{-2} (Boyd et al. 2002),and left for 1 week. Eucalyptus poles (a local tree mainly used for fencing as well as a building material) with a mean diameter of 5cm and about 2m long were used as substrates in the periphyton culture units. Eucalyptus poles were used due to their ease of processing and use, high availability in tropics, and durability. The poles were vertically inserted into the pond bottom at an interval spacing of 50cm in between the poles, according to Hoque et al. (2018).Water was filled into the ponds and fertilized using chicken manure at a rate of 50 g.m^{-2}. The manure was enclosed in gunny bags and placed at the inlet area of each pond.

The ratio of carbon-nitrogen(C: N) of the pond water in the periphyton pond was adjusted to 15:1using molasses as the main carbon source according to Schneider et al. (2005), and the ponds left for 10 days to allow sufficient production and colonization of periphyton on the substrates before stocking. This study had two treatments, i.e., PPT- and control-ponds (Plate 1). Each treatment was triplicate and completely randomized to avoid biasness that may occur due to extraneous factors. The control ponds had similar treatment and management practices as PPT except the substrates and addition of molasses.

Plate 1: Constructed PPT-ponds in KMFRI Sangoro in which *O. niloticus* fish were cultured. The inserted eucalyptus poles were used as substrates upon which periphyton community attached for fish to graze.

3.3 Experimental fish

Mixed-sex of *O. niloticus* post-fingerlings were obtained from KMFRI. At the fry stage, the fish were conditioned using live sources of food (i.e.,cladocerans, copepods, and rotifers) produced in the biofloc system protocol of Ogello et al,(2019) (Fig.3) for 2 weeks. The fish were then introduced to a co-feeding regime involving live food and a commercial diet (20 % Crude Protein) until they attained post-fingerling stage of about 12 g. The fish were conditioned, initial measurements taken and then stoked in the ponds. All the ponds were stocked at a rate of 3 fish.m^{-2}.

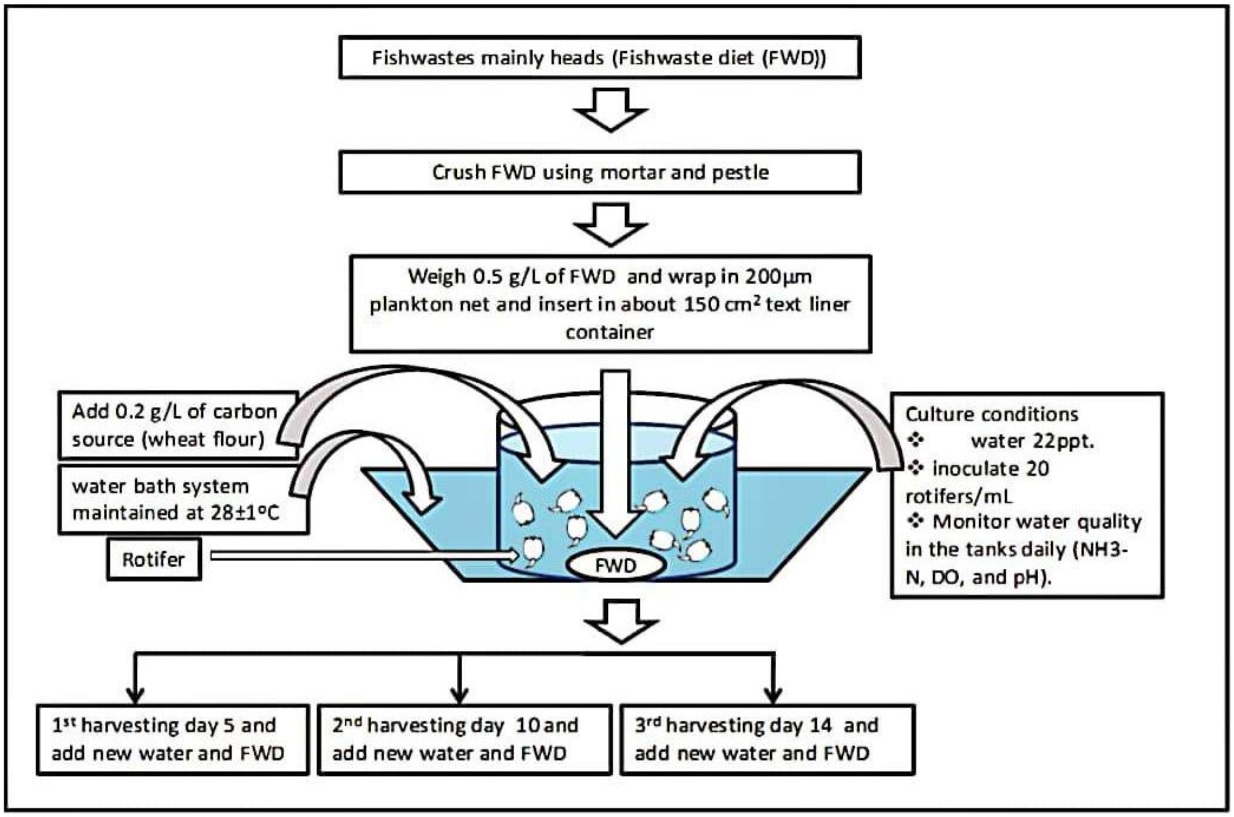


Fig. 3: Live-food dispenser used for the production of live food resources using environmental wastes as substrates (adopted from Ogello et al. 2019).

3.4 Pond management, feed formulation, and feeding

There was no water exchange in the ponds to preserve the periphyton community. This further ensured reduced discharge of nitrogenous wastes into natural water ecosystems which could raise environmental concerns. However, water depth was monitored daily, and water only added to compensate for losses from evaporation. For three months, the fish were fed a commercial feed of 20% CP (Table 1) twice daily at a 3 percent body weight daily ration (i.e., at 9.00 am and 3.00 pm).The 20% CP diet is relatively cheap for most farmers and was important in determining the effect of PPT on fish growth. The fish diet was formulated according to the KMFRI feed production manual. The protein content of 20 % was achieved using an Excel fish Diet-Formulator. The Excel Diet-Formulator has been modified in a such a way that it is only fed with the ingredients to be used and the total quantity of the feed required. The Excel diet formulator is then able to give ratios required for each ingredient based on their crude protein levels.

Table 1: Ingredients of formulated feed with 20 % crude protein level

Ingredient	Crude protein content (%)	Amount used (%)
Rastrineobolaargentia meal (Omena)	55	14
Soy bean meal	48	25
Maize bran	10.64	15.6
Wheat pollard	17.15	20
Sunflower seedcake	30	3
Cassava meal	12.45	22
Mycoban binder	0	0.2
Vitamin premix	13.5	0.1
Trace mineral	5	0.1

3.5 Data collection

3.5.1Sampling

Fish was sampled weekly for growth measurements and bi-weekly for determination of fecundity. For the growth experiment, 30 fish were sampled randomly from each pond (using seine nets) at each sampling time for length and weight determination before returning them into the pond. For weight determination, an empty container containing little amount of water was placed on the weighing balance and the reading was adjusted to 0 g. Each individual fish was placed on container containing water and the weight was recorded. Zero adjustment of the weighing balance was made after each individual fish weighed. For length determination, individual fish was placed on a measuring board that had a measuring scale on it and on which the fish was laid to determine its total length. For fecundity, 10 fish were sampled, and the mouth checked for eggs. The eggs were weighed and recorded (Plate 2).

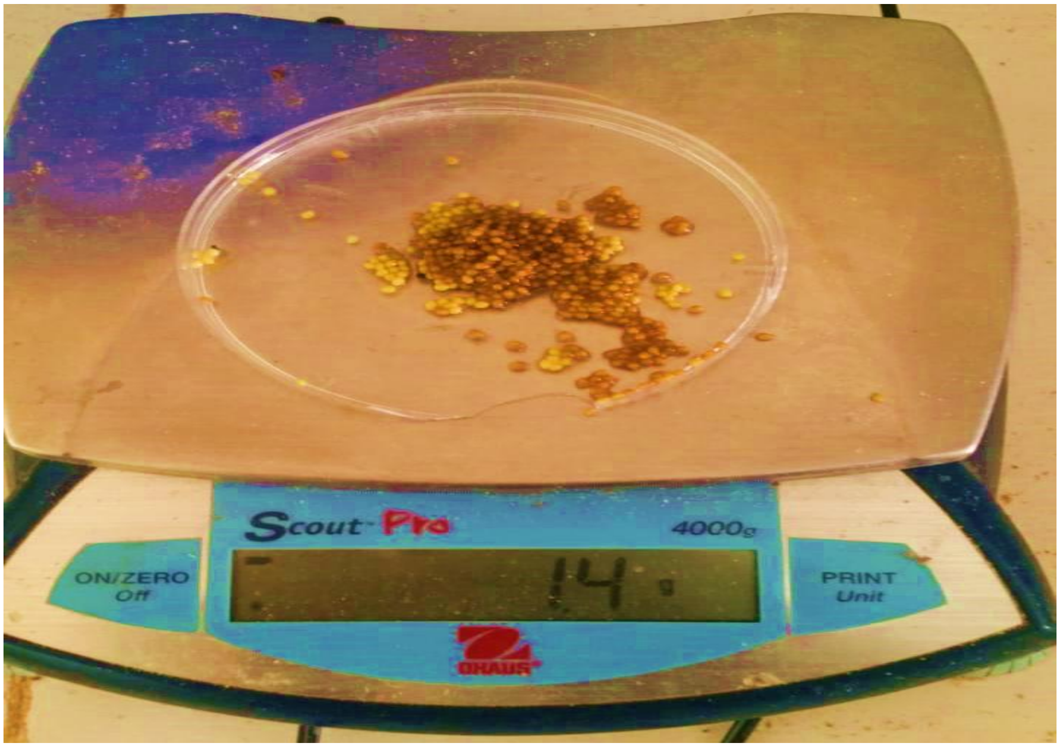


Plate 2: *O. niloticus* eggs weight determination in the laboratory**.**

3.5.2 Survival activity index (SAI)

The experiments on hatching rate and survival activity index (SAI) were done to assess the quality of the tilapia eggs and the survivability of fish larvae during starvation, respectively. 20 eggs were put in a 500 ml beaker with 300 ml of water and kept at 25 ℃ without feeding or aeration and in total darkness. Dead larvae were enumerated and removed every 24 h until complete larval mortality was attained. The treatments were triplicated, and the hatching rate and survival activity index were calculated using the observations. After every 24 h, the proportion of eggs that hatched normally was calculated by the formula: $\frac{\text{Normal hatched larvae}}{\text{Total number of eggs}} \times 100\ \%$,(Pertiwi et al. 2017).

SAI was calculated using the formular of Matsuo et al. (2006);

$$\text{SAI} = \frac{1}{N}\sum_{i=1}^{K}(N - hi) \times i$$

Where N is the quantity of larvae observed, hi is the cumulative mortality by the i-th day, and K is the duration of time taken till all larvae died of deprivation.

3.5.3 Determination of plankton communities

Water samples from the ponds were collected during the last week of the study period. In each pond, the water was collected in three spots at 25 cm under the water surface and filtered using a 50 µ mesh plankton net. The water samples were then put on a measuring cylinder and made up to 100ml. One Milliliter of water sample was then pooled from the measuring cylinder and placed on Sedge wick-Rafter (S-R) chamber. The plankton communities were observed under light microscope under ×400 magnification. Five fields of the chamber were randomly selected, counted, and used to estimate the total number of the plankton communities. The abundance was expressed as the number of individuals/5 ml. Zooplankton species were identified using the keys constructed by Jeje (1988), and Fernando (1994), while phytoplankton were identified using the keys compiled by Withford & Schumacher (1973) and Edmonson (1959).

Estimation of the total number of plankton was calculated using the formular: $N= (n\times v)/V$; Where, N is the quantity of plankton cells per litre of initial water; n is the average count of plankton cells in 5 fields; v is the volume of the final concentrate of the sample (ml); V is the volume of total filtered pond water (L) (Patkar et al. 2021).

Diversity of the plankton was determined by the Shannon-Wiener diversity index using the formular:

$H = \sum[(pi)\times\ln(pi)]$ (Kumar et al. 2013).

Where H is the Shannon diversity index;
I is the division of the number of individuals of species I by total number of samples
ln is natural logarithm
Pi = Proportion of the total sample represented by species i

Shannon-wiener diversity index was used because its universally accepted as it accounts for entropy in representative of samples, i.e., it accounts for both species richness and its evenness (Daly et al. 2018). Other method for determination of fecundity i.e., Simpson index, only estimates dominance of species and therefore does not account for species richness (Daly et al. 2018).

3.5.4 Physico-chemical parameters

Throughout the study period, selected physico-chemical water parameters were monitored weekly. These parameters include temperature (℃), pH, conductivity ($\mu S.cm^{-1}$), dissolved oxygen – DO (mg.l^{-1}), and total dissolved solids. Water temperature, pH, conductivity DO, and total dissolved solids were determined *insitu* using a multi parameter water quality meter model number WQC-24, DKK-TOA Corporation, Japan. Total ammonia nitrogen (TAN) was determined weekly by the indophenol spectrophotometric method using spectrophotometer (model Varian-Cary® 50 UV Spectrophotometer, Varian, Inc., USA)(Li et al. 2019b).

3.5.5 Growth analysis

The following growth parameters were measured using standard protocols (Khanjani et al. 2017).

i. Mean Weight Gain (MWG)= Final mean weight (W_1) – initial mean weight

ii. Specific Growth Rate (SGR) = [ln (final mean weight) – ln (initial mean weight)]/(time in days)×100.

iii. Feed Conversion Ratio (FCR) =Feed given/body weight gain)

3.5.6 Calculation of C/N ratio

The carbon sources applied the balancing of C/N in heterotrophic systems are often cheap and locally available by-products from human or animal food industry (Emerenciano et al. 2017). Figure 4 below shows the methodology used in calculating the quantity of molasses required to maintain low toxic nitrogenous compounds.

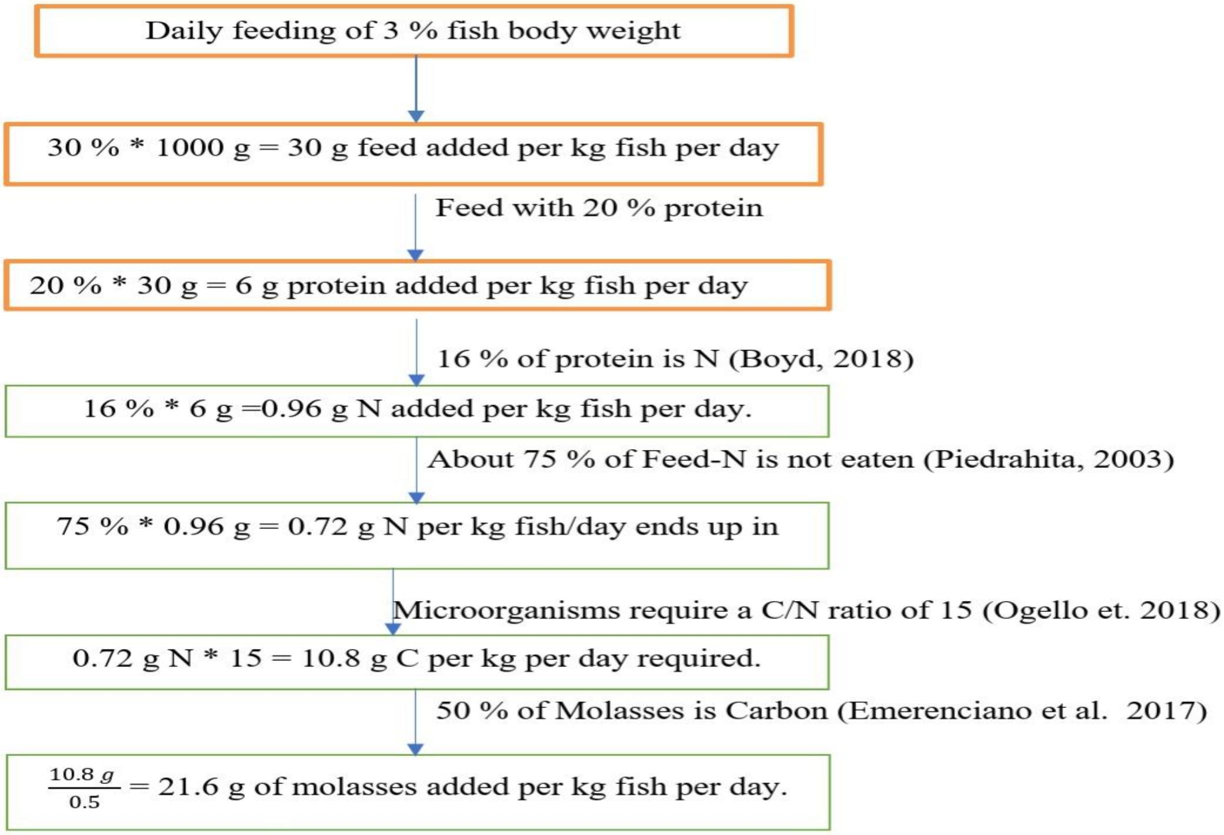


Fig.4: Schematic calculation of the amount of molasses required for removal of total ammonia nitrogen (TAN) in the PPT-ponds

3.6 Statistical data analysis

R software (version 3.2.1 of the R Foundation for Statistical Computing Platform © 2015 R Foundation) was used for statistical data analysis. Histograms were plotted to test data normality, while the Bartlett test for homogeneity of variances was used to test for the equality of variance. The effects of PPT on fish mean weight, weight gained, SGR, FCR, and fecundity between the two treatment was determined using independent t-test. The abundance of plankton between the two groups was compared using independent t-test, while within the plankton groups, one-way ANOVA was used. Data was presented using graphs in SPSS version 20.Significant differences were accepted at $P < 0.05$ while values were reported as mean ± standard error of the mean (SEM).

CHAPTER FOUR

RESULTS

4.1 Growth performance indicators of *O. niloticus*

Significant differences ($P < 0.05$) were recorded in the final weight, final length, weight gain, SGR, and FCR, with fish cultured under PPT condition recording superior performance than those cultured in control. The growth performance indicators of *O. niloticus* cultured under PPT and control treatments are summarized in Table 2.

Table 2: *O. niloticus* fish growth performance indicators comparing the control- and periphyton- ponds. The values represent mean ± S.E. Common superscript in the same row shows that the measurements were not statistically different as determined by unpaired t-test, non-identical superscripts show statistically significant differences ($P < 0.05$., a < b; n = 30).

Variable	**trol**	**phyton**
Initial weight (g)	$12.35 \pm 0.15^{\mathbf{a}}$	12.35 ± 0.15^{a}
Initial length (cm)	$8.44 \pm 0.09^{\mathbf{a}}$	8.44 ± 0.09^{a}
Final weight (g)	$3 \pm 0.96^{\mathbf{a}}$	150.69 ± 0.99^{b}
Final length (cm)	$17.58 \pm 0.06^{\mathbf{a}}$	21.08 ± 0.21^{b}
Weight gain (g)	$86.88 \pm 0.81^{\mathbf{a}}$	138.34 ± 0.84^{b}
Specific growth rate (SGR)	$2.29 \pm 0.00^{\mathbf{a}}$	2.75 ± 0.01^{b}
Food conversion ratio (FCR)	1.58 ± 0.01^{a}	1.29 ± 0.01^{b}

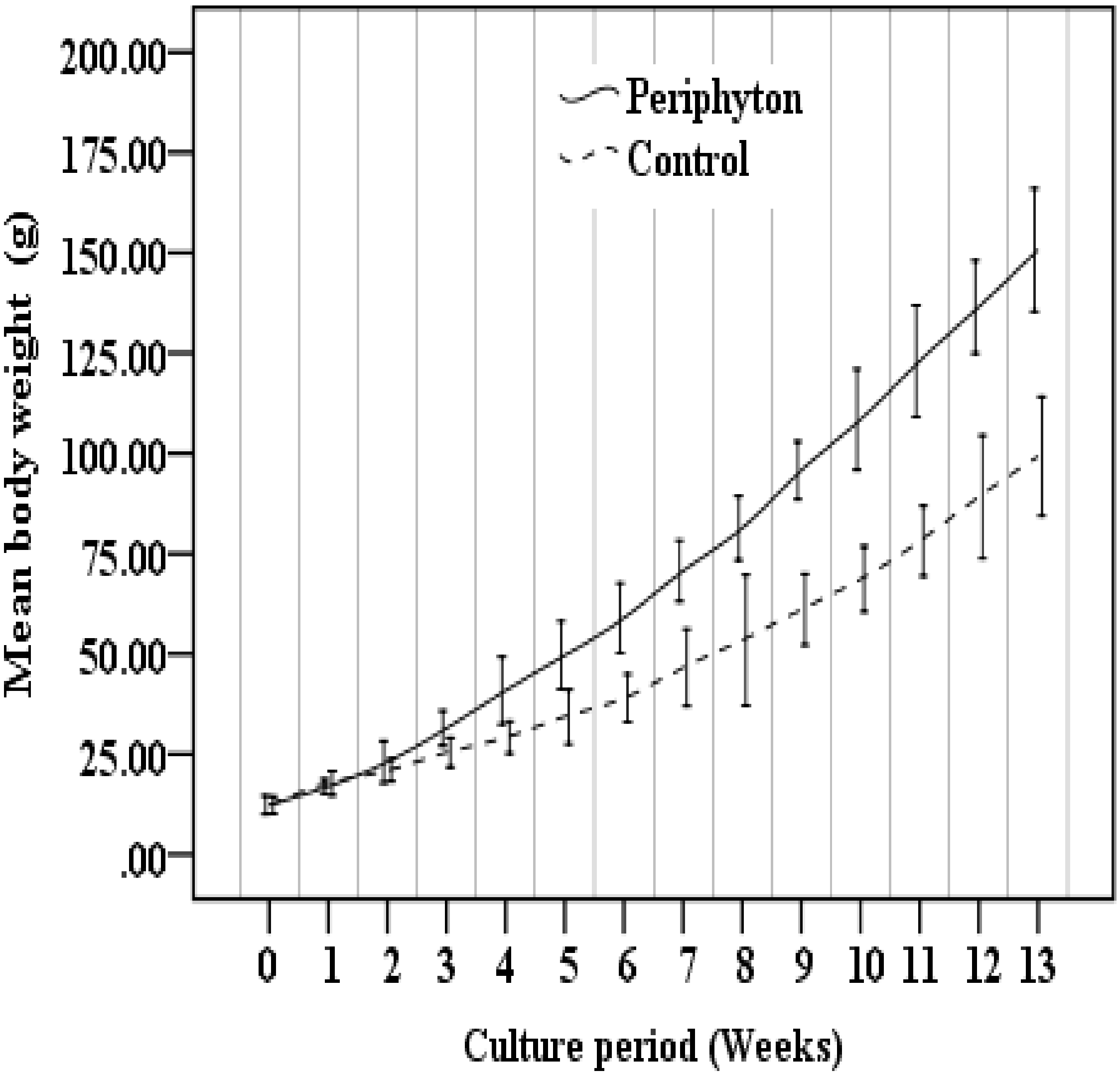


Fig.5: Mean body weight trend in PPT and control-ponds over the experimental period

There was no significant difference (*P*> 0.05) in mean body weight within the first 4 weeks of the experiment between PPT- and Control fish. However, the difference was eminent from week 5 to 13, with fish cultured in the PPT condition having a higher mean body weight than those cultured in control conditions (Fig. 5).

4.2 Fecundity of *O. niloticus*

PPT significantly affected fish fecundity estimates (*P* = 0.001), with the PPT- ponds showing a higher mean weight of eggs (2.28 ± 0.09) than the control-ponds (1.74 ± 0.06). There was a significant difference in fish spawning schedule, with fish in control ponds which had a mean weight of 29.19 ± 0.26 experiencing early spawning (from the fourth week) while fish in the PPT with a mean weight of 81.37 ± 0.51 started spawning at the eighth week (Fig.6).

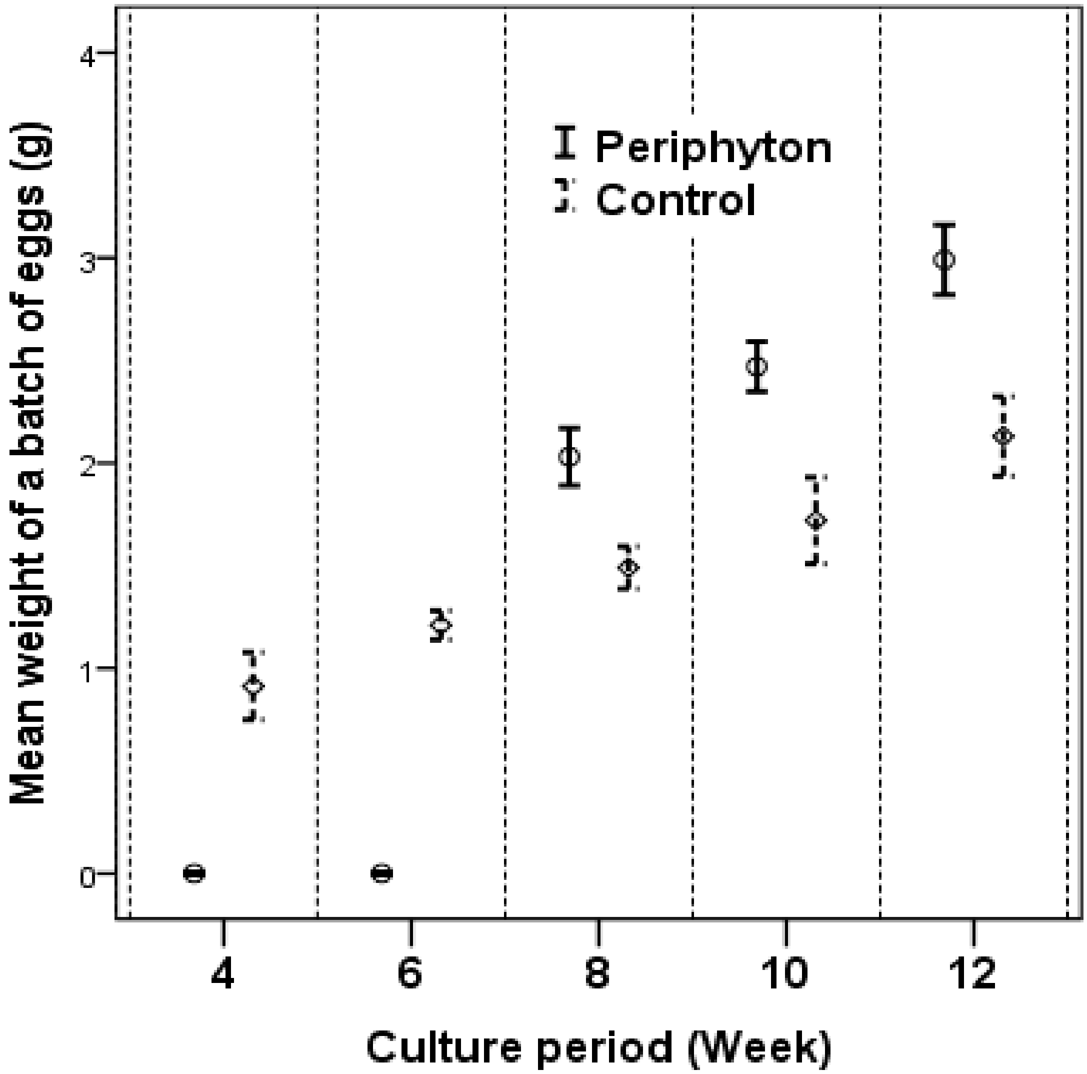


Fig.6: Mean weight of a batch of eggs of *O. niloticus* fish at every week of sample collection

The hatching rate of eggs in the PPT (96.40 ± 1.98) was significantly higher than the hatching rate of eggs in the control (88.75 ± 0.45). Fish larvae from PPT-ponds survived up to the eighth day of starvation with a survival activity index (SAI) of 8.94 ± 1.7 while all the control fish died by the fifth day, indicating SAI of 5.29 ± 2.1.

4.3 Plankton Diversity and Abundance

4.3.1 Phytoplankton

The PPT-pond's had a higher phytoplankton diversity index of 3.19 compared to control ponds with 2.57. There was no statistical difference (*P*= 0.071) in phytoplankton mean abundance between control (1061.6 ± 145.46) and the periphyton treatment (689.67 ± 127.71).Cyan bacteria dominated the control ponds while diatoms dominated the PPT- ponds. Table 3 summarizes the mean abundances of each phytoplankton group.

Table 3: Mean abundance of phytoplankton in both control and PPT-ponds

The values represent mean ± S.E. Different superscript in the same row shows that the measurements were statistically different as determined by independent t-test ($P<0.05$; a > b., n = 9).

Phytoplankton	trol	phyton
Cyanobacteria	4.59 ± 2.31^{a}	0.67 ± 1.76^{b}
Diatoms	2.48 ± 4.97^{a}	3.24 ± 3.84^{b}
Heterokontophytes	1.65 ± 4.43^{a}	32 ± 3.71^{b}
Chlorophytes		0.26 ± 5.03
Dinophytes	35 ± 8.46	-
Euglenophytes	5.10 ± 4.78^{a}	346.67 ± 3.53^{b}

4.3.2 Zooplankton

The frequency of each zooplankton species was first recorded. The proportion of each species was then calculated by dividing the frequency of individual species by the total number of all the species. The natural logarithm of each proportion was then determined and multiplied with the proportions. The sum of the values obtained after multiplying the natural logarithm of each proportion and the proportion was used the Shannon wiener diversity index. The PPT-treatment recorded a higher diversity of 3.42 compared to the control ponds, which registered a diversity index of 2.34. There was significantly higher (P=0.00) mean zooplankton abundance in the PPT-ponds than in the control-ponds. The PPT- and control-ponds registered zooplankton mean abundance of 2771.83 ± 313.11 and 262.67 ± 16.78, respectively. Rotifera was the most abundant, while Cladocera were the least abundant in both Control and PPT treatment. The mean abundances of each zooplankton group have been summarized in Table 4.

Table 4: Mean abundance of zooplankton in the control and PPT treatment

The values represent mean ± S.E. Different superscript in the same row shows that the measurements were statistically different as determined by the independent t-test (P< 0.05; a > b., n = 9).

Zooplankton	Control	Periphyton
Rotifera	315.37 ± 0.34^{a}	4466.00 ± 5.29^{b}
Copepoda	241.39 ± 3.18^{a}	3354.67 ± 4.67^{b}
Protozoa	312.67 ± 0.39^{a}	2327.35 ± 6.06^{b}
Cladocera	181.37 ± 3.52^{a}	1739.31 ± 5.70^{b}

4.4Physicochemical parameters

Physicochemical parameters are key determinants of the survival and growth performance of *O. niloticus*. The PPT- ponds recorded significantly lower ($P < 0.05$) nitrite, ammonia, and ammonium levels than the control ponds. The mean temperature range, pH, and dissolved oxygen did not differ significantly ($P > 0.05$).The mean values for various water physicochemical parameters have been recorded in table 5.

Table 5: Water quality parameters in control and PPT-ponds

Unidentical superscripts per row show a significant difference between the ponds as determined by unpaired t-test ($P < 0.05$; a > b., n = 39).

Variable	Control	Periphyton	Ideal ranges
Temperature (^{o}C)	27.43 ± 0.45^{a}	27.76 ± 0.41^{a}	20 – 35 (Ngugi et al. 2007)
	8.30 ± 0.19^{a}	8.10 ± 0.13^{a}	6.5 – 9.0 (Deswati et al. 2020)
Total Dissolved Solids ($mg.L^{-1}$)	204.54 ± 0.96^{a}	97.15 ± 6.21^{b}	< 500 (FME, 2001)
Conductivity (μScm^{-1})	431.92 ± 16.05^{a}	233.54 ± 15.51^{b}	100 – 500 (Russel et al. 2011)
Dissolved Oxygen ($mg.L^{-1}$)	6.71 ± 0.18^{a}	6.49 ± 0.13^{a}	> 4.0 (Emerenciano et al. 2017)
Nitrite ($mg.L^{-1}$)	0.07 ± 0.01^{a}	0.02 ± 0.01^{b}	<1 (Emerenciano et al. 2017)
Ammonia ($mg.L^{-1}$)	0.21 ± 0.03^{a}	0.06 ± 0.01^{b}	< 1 (Emerenciano et al. 2017)
Ammonium ($mg.L^{-1}$)	0.20 ± 0.04^{a}	0.02 ± 0.01^{b}	< 1(Emerenciano et al. 2017)

CHAPTER FIVE

DISCUSSION

5.1 Fish growth performance indicators

This study evaluated the effects of periphyton-based aquaculture on the growth performance and fecundity of *O. niloticus*. Ponds have proved periphyton-based aquaculture as a robust source of high-quality natural feed (Saikia and Das, 2009) which can be used to improve aquaculture production (Keshavanath *et al*. 2004). Other earlier researchers have also reported improved growth performance of cultured fish in natural heterotrophic biota (Adineh et al. 2019; Sarkar et al. 2021). In the present study, periphyton treatment registered a significantly higher ($P<0.05$) mean weight, SGR, and a lower FCR than control-ponds. Fish were observed regularly browsing on the large periphyton biomass attached on the eucalyptus substrates in the periphyton ponds. This study, therefore, hypothesizes that the attached periphyton was a highly preferred fish feed which might have contributed to the high tilapia growth rate compared to control ponds. After grazing, the periphyton mass would quickly regenerate, and this could be due to the symbiotic relationship between periphyton and phytoplankton. The periphyton community thus acted as a major source of nutrients for aquatic organisms. Recent studies have confirmed the microbial organisms are rich sources of dietary stimulants (Wang et al. 2015), bioactive compounds (Xu & Pan, 2013), growth and immune boosters (Supamattaya et al. 2005; Kuhn et al. 2010) that when combined with supplemental feed, supply the cultured aquatic species with a nutritious meal (Khanjani & Sharifinia, 2020).

The high growth performance is also attributed to the presence of periphytic algae on the PPT ponds. According to Dempster et al (1995), filter-feeding on only plank tonic algae may not fulfill the energy needs of *O. niloticus*. *O. niloticus* being an herbivorous fish species, requires larger-sized food items such as algal-based detritus and benthic algae to augment phytoplankton consumption (Dempster et al. 1993). Becker (2007) reported that microalgae are composed of high nutritional value, which is even superior to conventional plant protein sources, while van Dam et al. (2002) noted that the protein/metabolizable energy (P/ME) ratio of periphyton ranges from 10 to 40 kj.g^{-1}. The control-ponds lacked the presence of substrates that are required for attachment of the larger benthic algae hence, reduced energy and nutrient transfer efficiencies compared to the PPT-ponds. According to Huchette et al. (2000), algae attached on periphyton substrates and the coexisting zooplankton and bacterial biomass are directly utilized by tilapia species leading to higher growth than in ponds without substrates. The 24-h availability of nutritious fish feed, maintenance of optimum water quality, and reduced early sexual maturation could also have contributed to an increased growth rate. Most of the energy that could have been spent in reproduction due to early sexual maturation was directed to somatic growth.

The present findings support those of Van Dam et al. (2002), who found that the addition of substrates in culture units led to increased fish production compared to the culture units without substrates. Mirzakhani et al. (2019) &Khanjani et al. (2020) observed better FCR in tilapia cultured in C/N-controlled systems. The current findings also corroborate the results of Sakr et al. (2015), who found improved growth performance in tilapia fed with low protein content in treatments with periphyton substrates compared to the treatments fed on high protein levels in the absence of periphyton substrates. Huchette and Beveridge (2003) concluded that the periphyton can only be used to partially replace the pelleted feeds to reduce the production costs. The findings were similarly consistent with those of Garcia et al. (2016), who observed that the inclusion of substrates in cage cultures improved the fish weight gain. According to

Garcia et al. (2016) the introduction of substrates led to the production of 52 kg/m^3 of *O. niloticus* with 32 % less feed in a 20 % shorter time compared to control groups without PPT substrates. The current results further confirmed the findings of Harraz et al. (2018), who reported a significantly higher final mean weight, and SGR of *O. niloticus* in C/N controlled culture systems. Avnimelech (2007) reported that the bacterial biomass stimulated by the added carbon source provides 50 % of the protein requirement of tilapia. Therefore, the high growth rate recorded in the periphyton treatment shows efficient utilization of the natural microbial feed.

5.2 Fecundity of *O. niloticus*

Low egg quality is one of the major problems that have restricted aquaculture expansion. PPT-ponds in the present study registered statistically higher ($P<0.05$) mean weight of eggs than the control-ponds. According to Gómez-Márquez et al. (2003) and Lupatsch et al. (2010), fecundity of *O. niloticus* can vary based on the fish size or the farming technology involved. The periphyton treatment also had a higher hatching rate, and SAI than the control-ponds. According to Rocha (2008), egg quality is determined by ecological factors (e.g., water quality), physiological factors (e.g., mobilization of energy reserves), and nutrition of the female. The lipids and proteins in fish feeds are assimilated into the eggs during yolk formation inform of enzymes, lipoproteins, and enzymes (Orlando et al. 2017). The larvae depend solely on the nutritive reserves as they hatch. The time that they take to exhaust the endogenous reserves determine the quality of eggs. The higher reproductive performance in PPT-ponds could therefore be attributed to high-quality periphyton because the nutrients present in the diet are deposited into the oocytes during yolk formation. The microbial feed also contains vitamin C (Crab et al. 2012) and polyunsaturated fatty acids (Ekasari et al. 2010), which have been observed to contribute to the high quality and quantity of eggs (Dabrowski & Ciereszko, 2001). Ecological factors such as poor water quality caused fish stress, eventually reducing reproductive performance in the control-ponds. The produced low-quality eggs resulted to poor egg survival and low hatching rates, and gracile larvae with reduced stress resistance. The presence of high abundance of cyano bacteria in the control-ponds is an important ecological factor that contributed to low reproductive performance. Semyalo et al. (2011) reported that cyano bacteria contain microcystin that lowers the reproductive success of tilapia.

The sustainability of *O. niloticus* culture has been limited by its precocious maturation. In the current study, the use of periphyton substrates seems to be a possible solution to reduce the overpopulation in farmed tilapia by preventing early sexual maturation. The prolific spawning behavior started earlier (in the fourth week) in control-ponds where fish had a mean weight of 29.19 ± 0.29, but delayed to the eighth week in PPT-ponds with fish recording a mean weight of 81.37 ± 0.51. This resulted to reduced growth rates in control-ponds because tilapia species spend a lot of energy during their generative process, including for the pugnacious behavior of the males, territorial defense, mating, and mouth brooding of the eggs. If the energy reserved for the reproductive process is not enough, tissue proteins are mobilized and catalyzed to perform these functions (Orlando et al. 2017). Further, the early spawning periods make the ponds to be overpopulated with fries and fingerlings that bring competition for feeds and deprive oxygen meant for the cultured tilapia.

According to Mendonca & Goncalves-de-Freitas (2008), tilapia males that have a high investment in nest building increase the chances of mating and thus increased prolific breeding in tilapia culture ponds. In the PPT-ponds, the presence of periphyton substrates restricted tilapia spawning by reducing the space available for male tilapia to make good and large nests which are the basis for sexual selection by females, thus reducing lekking activities. This, therefore, reduced the prolific breeding which contributes to overpopulation in ponds and energy that could be directed to maintaining their reproductive capacity during the early stages of growth was translated to somatic growth. These results concur with McKaye et al. (1990), who reported that reproduction in cichlid species is based on the kind and size of the nests formed by the males, and thus, nest is an important trait to females for male selection. Mendonca & Goncalves-de-Freitas (2008) also found a positive correlation between investment in nests and female nest visitation frequency in *O. niloticus*.

5.3 Plankton diversity and abundance

According to Diana et al. (1997), the amount and quality of nutrients are among the key aspects that influence the growth, diversity, and abundance of plankton. Plankton are, therefore, indicators of production conditions of a culture system and act as direct and indirect fish feed source (Azim, 2001). The PPT-ponds registered higher phytoplankton and zooplankton diversity index of 3.19 and 3.42, respectively, compared to 2.57 and 2.34, respectively, in control-ponds. The high diversity index of plankton in PPT-ponds indicates that the PPT-ponds had high plankton diversity than the control-ponds. The high diversity index values could be as a result of better ecological conditions such as increased water quality in the PPT- ponds. The presence of substrates and the introduction of external carbon source increase species diversity by providing the required growth conditions for their maximum growth. Saikia et al. (2011) also indicated that grazers' presence in the periphyton system increases the heterogeneity and primary productivity of periphytic algal communities.

Phytoplankton is reported to be the chief energy source in traditional aquaculture systems but its more productive and efficient in culture systems with extra attachment sites (Garcia et al.

2012). In the present study, both control- and PPT-ponds were fertilized at the same rate to stimulate the growth of natural micro biota, which are natural food items in pond environments. Five species of phytoplankton were identified in each treatment. The identified phytoplankton communities include cyano bacteria, diatoms, heterokontophyte, chlorophytes, dinophyte, and Euglenophytes. Chlorophytes were identified only in the periphyton ponds, while dinophytes were identified only in the control ponds.

Similar phytoplankton groups were observed by Gangadhara &Keshavanath (2008) harbored in both biodegradable and non-biodegradable substrates. These findings were likewise comparable to those of Anand et al. (2013), where from the qualitative analysis of periphyton, algae belonging to the groups Bacillariophyceae, Cyanophyceae, Chlorophyceae, and Euglenophyceae were identified. In the current study, diatoms and chlorophytes were the dominant phytoplankton in the periphyton treatment. Diatoms have a lot of health benefits and contribute substantially to improved water quality (Llario et al. 2018). The results are similar to those of Sakr et al. (2015),where diatoms and chlorophytes were the most dominant communities of phytoplankton attached on periphyton substrates.

In the PPT-ponds, it’s expected that *O. niloticus* affected the abundance of the phytoplankton indirectly by re suspension of nutrients and directly by grazing. According to Ibrahim et al. (2015), tilapia fries and juveniles have high grazing pressure on zooplankton and shift to filter-feeding during their adult stages. Azim et al. (2003) further reported that tilapia is omnivorous and performs better while feeding on algal biomass attached on substrates than filtering algae floating on the water surface. The observed low mean values of phytoplankton, therefore, indicate a high preference of tilapia on the phytoplankton species present in the periphyton ponds. The results are consistent with the study of Haque et al. (2016), who indicated that phytoplankton abundance reduces over time, and this is reflected from the results where the periphyton treatment records a significantly higher specific growth rate and weight gain.

The slightly higher abundance of phytoplankton in the control-ponds than the periphyton treatment was due to the high abundance of cyano bacteria. Cyano bacteria have low nutritional value (Llario et al. 2018), and fish are not likely to feed on cyano bacteria, possibly due to their toxicity effects (Semyalo, 2009). Malbrouck and Kestemont (2006), from their laboratory and field experiments, reported that aqueous and cell-bound cyanotoxins in fish diets are not good for their behavior, morphology, and physiology. Cyanobacteria have also been reported to produce allopathic chemicals that affect photosynthesis and growth of algae (Gantar et al. 2008).

In both control and PPT treatments, four species of zooplankton were identified. The identified zooplanktons were represented by Rotifera, Copepoda, Protozoa, and Cladocera. The same animal assemblages were reported by Das et al. (2017), in PPT ponds treated with cow manure and biogas slurry. The PPT treatment mostly favored the growth of rotifers and copepods. According to Cuvin-Aralar (2003), these are the main food items in tilapia diet. The present results also concur with the work of Anand et al. (2013) and Haque et al. (2016), who found rotifers to be the most dominant communities in a periphyton-based culture system.

5.4 Water quality physicochemical parameters

Physicochemical parameters of water are critical in ensuring healthy growth of aquatic organisms and are a major constraint (Sharifinia et al. 2020). From earlier findings, periphyton extracts nutrients from water (Abwao et al. 2014) hence influencing the physicochemical water parameters. The water quality parameters recorded in the present study were within the acceptable range (Emerenciano et al. 2017) for the culture of aquatic species though some differed statistically between the treatments.

Temperature is a major factor that influences the abiotic and biotic components of the culture system. Temperature also affects the metabolic behavior, degree of ammonia toxicity, and feeding rates of the cultured species (Kumar et al. 2021). The temperature recorded in the control (27.43 ± 0.45 ℃) and PPT treatment (27.76 ± 0.41 ℃) were both within the acceptable limits for fish culture according to optimum ranges reported by Emerenciano et al. (2017) and El-Shafiey et al. (2018). Kausar and Salim (2006) reported that the ideal temperature for optimum growth of tilapia in ponds should range from 25 to 27 ℃. Ngugi et al. (2007) recommended temperatures ranging from 20 to 35 ℃ while FAO (2006b) recommends values ranging from 27.6 to 30 ℃ as optimum for maximum production in aquaculture. The temperature ranges of both treatments in the present work are consistent with previous findings.

Total ammonia nitrogen (TAN) is the amount of nitrogen in the form of ionized ammonia (NH_4^+) and un-ionized ammonia (NH_3). Ammonia is directly harmful to cultured species even at low levels, unlike other forms of nitrogen which are only toxic at high concentrations (EPA, 2013). The adjustment of the C/N ratio in PPT- ponds using molasses as source of carbon kept the total ammonia concentration at very low levels. Control-ponds recorded higher ammonia (0.21 ± 0.03 $mg.L^{-1}$) and ammonium ($0.20 \pm 0.04 mg.L^{-1}$) concentration values than the PPT-ponds ($NH_3=0.06 \pm 0.01 mg.L^{-1}$; $NH_4^+=0.02 \pm 0.01 mg.L^{-1}$).

The level of TAN concentration recorded in the periphyton treatment was significantly lower than the values reported by Khanjani et al. (2020) and Mirzakhani et al. (2019). This was due to the presence of chemoautotrophic nitrifying bacteria that attach and develop on the substrates near the water column where there is sufficient oxygen required for the nitrification process that reduces the nitrogen compounds in water. There is also development of heterotrophic organisms that develop faster than autotrophic organisms. The presence of heterotrophic organisms is stimulated by the balancing of the C/N ratio. Heterotrophic bacteria convert ammonia resulting from non-consumed feeds and feces into non-toxic compounds more rapidly compared to chemoautotrophic bacteria (Hargreaves, 2006) and, therefore, are the major contributors to reduced ammonia levels in the PPT treatment. The reduction of supplemental dietary protein also reduced the nitrogen load in the ponds.

This confirms the earlier study of Anand et al. (2012; 2013), who postulated that the presence of submerged substrates where periphyton attach on helps in reducing the TAN levels. The values of the PPT treatment were also consistent with the reports of TNAU (2008), where the optimum range of ammonia (NH_3) should range between 0.02 and 0.05 mg.L^{-1}. According to Emerson et al. (1975), chronic exposure of fish to un-ionized ammonia concentration of 0.06 mg.L^{-1} can result to reduced growth rate or gill and kidney damage, while un-ionized ammonia concentrations of 0.6 mg.L^{-1} can lead to fish mortality even when exposed briefly. The low mean values of un-ionized ammonia observed in the PPT-ponds indicate proper development of heterotrophic organisms, which grow and reduce TAN levels at a high rate.

The use of simple carbohydrate such as molasses as a carbon source that is absorbed and degraded faster results to high populations of heterotrophic microbes (Khanjani et al. 2020) which could also have contributed to lower TAN levels. The stimulation of heterotrophic bacterial growth in culture systems with minimal water exchange rates reduces nitrogen load in the water (García-Ríos et al. 2019). The results were consistent with the finding of Panigrahi et al. (2018), who recorded low levels of nitrogenous metabolites in the C/N controlled system due to high heterotrophic bacterial growth.

Nitrite is an intermediate formed by chemoautotrophic bacterial activity in the process of conversion of ammonia to nitrate. The nitrite concentration in the PPT-ponds (0.02 ± 0.01mg.L^{-1}) was significantly lower ($P<0.05$) than in the control (0.07 ± 0.01 mg.L^{-1}). Emerenciano et al. (2017) suggested nitrite level below 1 mg.L^{-1} once the C/N controlled system is stable. From the observed mean values of nitrite, it is therefore evident that the PPT treatment has a high potential of removing nitrite from water, which is toxic to tilapia.

pH is a critical factor in fish culture as it affects the toxicity of other compounds such as ammonia to fish (Alam and Al-Hafedh, 2006). Aquatic species are adapted to specific pH ranges, and any wide variation can affect the metabolism and physiological processes of the cultured species. pH can enhance the susceptibility of tilapia to diseases, reduce production, create stress, and cause slow growth rates. pH is also correlated with the alkalinity and conductivity of the water (Kumar et al. 2021). The average pH values observed in the present study were 8.30 ± 0.19for the control-ponds and8.10 ± 0.13 for the PPT-ponds. The values were consistent with the results of Keremah et al. (2014) and Deswati et al. (2020), who indicated that pH values ranging 6.5-9.0 as an ideal pH for culturing freshwater fish. The values are also in agreement with the pH range of 6.6-8.5 recommended by Khan et al. (2017) as the standard pH range for fish farming.

Total dissolved solids (TDS) is a crucial water quality parameter which accounts for all organic and inorganic solids dissolved in water (Agbaire et al. 2015). TDS have been reported to be an indicator of the degree of pollution in culture water (Tripathy and Adhikary, 1990). TDS in water is comprised of nitrates, chlorides, carbonates, bicarbonates, phosphates, salts, organic matter, and other particles. Control-ponds registered a significantly higher ($P<0.05$) TDS than the PPT treatment. The TDS mean values were 97.15 ± 6.21 $mg.L^{-1}$ and 204.54 ± 0.96 $mg.L^{-1}$ for PPT-and control-ponds, respectively. The mean values for TDS on control-and PPT treatment were less than the recommended limit of 500 $mg.L^{-1}$ (FME, 2001).

Conductivity is the measure of the potential of water to conduct electric current, which relies on water temperature on measurement, total concentration of dissolved salts, their mobility, and valence (Sarkar et al. 2020). Conductivity is therefore a good indicator of water quality since it indicates the ionic strength of the water. The control- ponds (431.92 ± 16.05$\mu S.cm^{-1}$) had statistically higher conductivity compared to that in PPT-ponds (233.54 ± 15.51 $\mu S.cm^{-1}$). Both mean values however, corroborated the findings of Russel et al. (2011), who noted that water conductivity ranging from 100 to 500 $\mu S.cm^{-1}$ is optimal for fish culture.

Dissolved oxygen (DO) is one of the most critical water quality parameters in the growth of aquatic organisms since it has a great impact on feeding rates, metabolism, and disease resistance. Biochemical and chemical processes in the culture system are highly dependent on the availability of oxygen. High levels of DO, therefore, maintain high forms of biological life, keeping due balance of different wastes, thus making the water healthy (Kumar et al. 2021).DO average values in the present study were 6.71 ± 0.18 $mg.L^{-1}$ for control-ponds and 6.49 ± 0.13 $mg.L^{-1}$ for PPT-ponds and were not statistically ($P>0.05$) different from each other. The observed mean values were within the suitable range for the growth of *O. niloticus. O. niloticus* is known to be one of the most tolerant fish species that can survive with even oxygen levels as low as 3 $mg.L^{-1}$ (Ross, 2002). However, according to Riche and Garling (2003), for the optimum growth of *O. niloticus*, the DO level should exceed 5 $mg.L^{-1}$. The observed DO ranges were also above 4.0 mg.L^{-1} as recommended by Emerenciano et al. (2017), while in the PPT- ponds, the DO levels were similar to those of Khanjani et al. (2020) in the rearing *O. niloticus* in a C/N controlled culture system.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

Four key findings arose from the study. First, the periphyton treatment reported superior performance in terms of mean weight, SGR, and FCR. Second, there was significantly higher fecundity in the PPT-ponds than control ponds with prolific spawning behavior starting earlier in the fourth week in control ponds but delayed to the eighth week in the PPT-ponds. Third, PPT-ponds had a higher phytoplankton and zooplankton diversity index and abundance compared to control-ponds. Lastly, the control-ponds recorded significantly higher concentrations of nitrite, ammonia, and ammonium compared to PPT-ponds.

6.2 Conclusion

Based on the results obtained in this study the following conclusions were drawn:

1. The periphyton treatment recorded significantly higher final mean weight, specific growth rate, and feed conversion ratio compared to the control ponds. The high growth performance is attributed to better feed utilization efficiency of the natural feed in the form of plankton and periphyton.
2. The batch of eggs from *O. niloticus* cultured in the PPT-ponds exhibited higher hatching rate, survival rate, and mean weight compared to the control ponds implying that the periphyton results to mass production of quality eggs.
3. The plankton abundance and diversity were significantly higher in the periphyton ponds than the control ponds due to ideal water conditions that favour their maximum growth.

4. Low nitrite, ammonia, and ammonium levels were recorded in the PPT treatment compared to the control. This suggests that PPT ponds had a higher potential of reducing the amount of toxic nitrogenous compounds in the pond compared to the control.

6.3 Recommendations

6.3.1 Recommendations for the present study

1. The high growth performance recorded in PPT treatment suggests that PPTis a quality natural fish feed that should be adopted to replace the low-quality commercial fish feed used in *O. niloticus* culture.
2. There is very high demand for quality fingerlings in most hatcheries. The study recommends the adoption of PPT to increase supply of quality*O. niloticus* fingerlings in the hatcheries.
3. Phytoplankton was more efficient and dynamic in the PPT-ponds with additional areas of attachment. The present study therefore recommends the modification of the conventional fish ponds by introducing substrates and balancing C/N to increase the diversity and abundance of planktons which are beneficial in controlling water quality and are major contributors to primary production.
4. The present study recommends the adoption of PPT to reduce water consumption in aquaculture and provide ideal water conditions required for healthy growth of *O. niloticus*.

6.3.2 Suggestions for further research

1. Future studies should consider working with other species such as *Clariusgariepinus* and evaluate their growth performance in periphyton technology.
2. Future studies should perform an economic analysis to determine the full economic potential of culturing *O. niloticus* in periphyton technology.
3. Future studies should consider varying the periphyton substrate density and spacing in the ponds and evaluate its effect on *O. niloticus* growth performance.
4. Further studies are recommended to explore other substrates for installing PPT in ponds.

REFERENCES

Abakari, G., Luo, G., & Kombat, E. O. (2020). Dynamics of nitrogenous compounds and their control in biofloc technology (BFT) systems: A review. *Aquaculture and Fisheries*, https://doi.org/10.1016/j.aaf.2020.05.005.

Abdel-Fattah, M. E. (2020). Tilapia culture. Taxonomy and basic biology, 2nd eds, *Academic Press*, pp. 21-31.

Abdelghany, A. E., & Ahmad, M. H. (2002). Effects of feeding rates on growth and production of Nile tilapia, common carp and silver carp polycultured in fertilized ponds. *Aquaculture Research*, 33(6): 415–423.

Abwao, J., Boera, P. N., Munguti, J., Orina, S., & Ogello E. (2014). The potential of periphyton based aquaculture for Nile tilapia (*Oreochromis niloticus L.*) production. A review. *International Journal of Fisheries and Aquatic Studies,* 2(1):147-152.

Adineh, H., Naderi, M., Hamidi, M. K., &Harsij, M. (2019). Biofloc technology improves growth, Innate immune responses, oxidative status, and resistance to acute stress in common carp (*Cyprinus carpio*) under high stocking density. *Fish and Shellfish Immunology*, 95: 440-448.

Agbaire, P. O., Akporido, S. O. &Emoyan, O. O. (2015). Determination of some physiochemical parameters of water from artificial concrete fish ponds in Abraka and its environs, Delta State, Nigeria. *International Journal of Plant, Animal and Environmental Sciences*, 5(3): 70-76.

Ahmad, I., Babitha, R. A. M., Verma, A. K., & Maqsood, M. (2017). Biofloc technology: an emerging avenue in aquatic animal healthcare and nutrition. *Aquaculture International,* 25: 1215-1226.

Aisyah,.Yustiati, A., &Andriani, Y. (2021). Evaluation of various sources of bacteria on biofloc productivity. *Asian Journal of Fisheries and Aquatic Research*, 12(2): 30-39.

Alam, A., & Al-Hafedh, Y. S. (2006). Diurnal dynamics of water quality parameters in an aquaculture system based on recirculating green water technology. *Journal of Applied Science and Environmental Management*; 10:19-21.

Alanärä, A., Kadri, S. &Paspatis, M., (2001). Feeding management. In: Houlihan, D., Boujard, T., Jobling, M. (Eds.), Food Intake in Fish. Blackwell Science Ltd, Oxford, UK, pp. 332–353.

Albay, M., &Akcaalan, R. (2003). Comparative study of periphyton colonization on common reed (Phragmites australis) and artificial substrate in a shallow lake, Manyas, Turkey. *Hydrobiologia*, 506: 531-540.

Alfred, O., Shaahu, A., Jibung, G. G., Amon, T. E., Msaakpa, T. S., Orban, D. A., Egwenomhe, M. (2020). Fish nutrition: Plant Source as an alternative. International Journal of Research and Scientific Innovation 7(12):109-115.

Anand, P., Kumar, S., Panigrahi, A., Ghoshal, T., Dayal, J., Biswas, G., Sundaray, J., De, D., Ananda, R., Deo, A., Pillai, S., & Ravichandran, P. (2012). Effects of C:N ratio and substrate integration on periphyton biomass, microbial dynamics and growth of *Penaeus monodon* juveniles. *Aquaculture International,* 21:511-524.

Anand, P., Kohli, M., Roy, S., Sundaray, J., Kumar, S., Sinha, A., Pailan, G., & Sukham, K. (2013). Effect of dietary supplementation of periphyton on growth performance and digestive enzyme activities in Penaeus monodon. *Aquaculture,* 392-395: 59-68.

Asaduzzaman, M., Rahman, M. M., Azim, M. E., Islam, M. A., Wahab, M. A., Verdegem, M. C. J., Verreth, J. A. J. (2010). Effects of C/N ratio and substrate addition on natural food communities in freshwater prawn monoculture ponds. *Aquaculture,* 306(1-4):127-136.

Avnimelech, Y. (1999). Carbon and nitrogen ratio as a control element in Aquaculture systems. *Aquaculture,* 176(3-4): 227-235.

Avnimelech, Y. (2007). Feeding with microbial flocs by tilapia in minimal discharge bioflocs technology ponds. *Aquaculture,* 264:140-147

Avnimelech, Y. (2009). Biofloc Technology: A practical guide book. *The world Aquaculture Society, Baton Rounge, Louisiana, United States,* 182.

Avnimelech, Y. (2015). Biofloc technology – a practical guide book. 3rd edition. *The world Aquaculture Society, Baton Rouge, Louisiana, USA,* pp. 21-73.

Azim, M. E. (2001). The potential of periphyton-based aquaculture production systems. *PhD thesis. Wangeningen, the Netherlands*.

Azim, M. E., Wahab, M. A., van Dam, A. A., Beveridge, M. M. &Verdegem, M. J. (2001a). The potential of periphyton-based culture of two Indian major carps, rohu *Labeorohita* (Hamilton) and *goniaLabeogonius* (Linnaeus). *Aquaculture Research*, 32(3):209-216.

Azim, M. E., Wahab, M. A., van Dam, A. A., Beveridge, M. C. M., Milstein, A., &Verdegem, M. C. J. (2001b). Optimization of fertilization rate for maximizing periphyton production on artificial substrates and the implications for periphyton-base aquaculture. *Aquaculture Research*, 32:749-760.

Azim, M. E., Verdegem, M. C. J., Khatoon, H., Wahab, M. A., van Dam, A. A., & Beveridge, M. C. M. (2002). A comparison of fertilization, feeding and three periphyton substrates for increasing fish production in fresh water pond aquaculture in Bangladesh. *Aquaculture*, 212:227-243.

Azim, M. E., Verdegem, M. C. J., Singh, M., van Dam, A. A., & Beveridge, M. C. M. (2003). The effects of periphyton substrate and fish stocking density on water quality, phytoplankton, periphyton and fish growth. *Aquaculture Research*, 34 (9): 685-695**.**

Azim, M. E., Wahab, M. A., Biswas, P. K., Asaeda, T., Fujino, T., & M. C. J. Verdegem, M.C.J. (2004). The effect of periphyton substrate density on production in freshwater polyculture ponds. *Aquaculture,* 232: 441-453.

Azim, M. E., Beveridge, M. C. M., van Dam, A. A., &Verdegem, M. C. J. (2005). Periphyton and aquatic production: an introduction. Azim, M.E., Verdegem, M.C.J., van Dam, A.A., & Beveridge, M.C.M. (Eds), Periphyton: ecology, exploitation and management. *CABI Publishing,* Wallingford, pp. 1-13.

Becker, E. W. (2007). Micro-algae as a source of protein. Biotechnology Advances 25: 207-210.

Besson, M., Allal, F., Chatain, B., Vergnet, A., Clota, F., &Vandeputte, M., (2019). Combining individual phenotypes of feed intake with genomic data to improve feed efficiency in sea bass. Front. Genet. 10, 219.

Beveridge, M., Verdegem, M., Wahab, M., Keshavanath, P., Baird, D. (1998). Periphyton-based aquaculture and the EC-funded PAISA Project. *The International Centre for Living Aquatic Resources Management (ICLARM) Quarterly*, 21:49-50

Biswas, G., Jena, J. K., Singh, S. K. &Muduli, H. K. (2006). Effect of feeding efficiency on growth, survival and feed utilization in fingerlings of *Catlacatla* (Hamilton), *Labeorohita* (Hamilton) and *Cirrhinusmrigala* (Hamilton) in outdoor rearing systems. *Aquaculture Research*, 37(5): 510-514.

Boyd, C. E. (2018). Protein conversion efficiency in Aquaculture. *Blobal Aquculture Advocate*, 1-4.

Boyd, C., Wood, C. W., & Thunjai, T. (2002). Aquaculture Pond Bottom Soil Quality Management. Pond Dynamics/Aquaculture Collaborative Research Support Program Oregon State University, Corvalis, Oregon 97331-1641.

Bucur, C., Mioara C., Oprea, D., & Marica, N. (2012). Studies and observations on the Spawning of *Oreochromis niloticus* Species Reared at SCDP Nucet-Dambovita. *Animal Science and Biotechnologies,* 45(2).

Burford, M. A., Thompson, P. J., McIntosh, R. P., Bauman, R. H., & Pearson, D. C. (2003). Nutrient and microbial dynamics in high intensity, zero-exchange shrimp ponds in Belize. *Aquaculture*, 219(1-4):393-411.

Carballeira-Brana C. B., Cerbule, K., Senff, P., & Stolz, I. K. (2021). Towards Environmental Sustainability in Marine Finfish Aquaculture. *Frontiers in Marine Science, 8:666662. doi: 10.3389/fmars.2021.666662*

Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P., & Verstraete, W., (2007). Nitrogen removal techniques in aquaculture for a sustainable production. *Aquaculture*, 270: 1-14.

Crab, R., Defoirdt, T., Bossier, P. &Verstraete, W. (2012). Biofloc technology in aquaculture: beneficial effects and future challenges. *Aquaculture* 357: 351–356.

Crab, R., Kochva, M., Verstraete, W., &Avnimelech, Y. (2009). Bioflocs technology application in over-wintering of tilapia. *Aquacultural Engineering*, 40(3): 105-112.

Craig, S. (2009). Understanding Fish Nutrition, Feeds, and Feeding. In. Virginia Polytechnic Institute and State University, publication No. 420-256. Available from URL: https://vtechworks.lib.vt.edu/handle/10919/80712/ Accessed 11-7-2021

Craig, S., & Helfrich, L. A. (2002). Understanding fish nutrition, feeds and feeding. *Cooperative extension service, publication Virginia State University*, USA. 420-256.

Cuvin-Aralar, M. L. C. (2003). Influence of Three Nitrogen: Phosphorus Ratios on Growth and Chemical Composition of Microalgae and Its Utilization by Nile Tilapia, *Oreochromis niloticus* (L.). Verlag Grauer, Beuren, pp. 159.

Dabrowski, K. &Ciereszko, A. (2001). Ascorbic acid and reproduction in fish: endocrine regulation and gamete quality. *Aquaculture Research* 32: 623–638.

Daly, A. J., Baetens, J. M., & De Baets, B. (2018). Ecological Diversity: Measuring the Unmeasurable. Mathematics 6, 119.

Das, M., Behera, P. R., & Dash, B. (2017). Periphyton communities in carp culture ponds treated with cow manure and biogas slurry. *Aquatic Living Resources*, 30(25).

Davey, A. J. & Jellyman, D. J. (2005). Sex determination in freshwater eels and management options for manipulation of sex. *Reviews in Fish Biology and Fisheries*, 15: 37-52.

De Graaf, G. J., Dekker, P. J., Huisman, B. &Verreth, J. A. J. (2005). Simulation of Nile tilapia (*Oreochromisniloticus*L.) culture in ponds, through individual-based modeling, using a population dynamic approach. *Aquaculture Research,* 36: 455-471.

de Verdal, H., Komen, H., Quillet, E., Chatain, B., Allal, F., Benzie, J. A. H., & Vandeputte, M., (2018). Improving feed efficiency in fish using selective breeding: a review. Rev. Aquac. 10, 833–851.

de Verdal, H., Mekkawy, W., Lind, C. E., Vandeputte, M., Chatain, B., Benzie, J. A. H. (2017). Measuring individual feed efficiency and its correlations with performance traits in Nile tilapia, *Oreochromis niloticus*. *Aquaculture*, 468, 489–495.

Defoirdt, T., Sorgeloos, P., & Bossier, P. (2011). Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Current Opinion in Microbiology*, 14:251-258.

Dempster, P. W., Beveridge, M. C. M. & Baird, D. J. (1993). Herbivory in the tilapia Oreochromis niloticus: a comparison of feeding rates on phytoplankton and periphyton. *Journal of Fish Biology,* 43: 385-392.

Dempster, P., Baird, D. J., & Beveridge, M. C. M. (1995). Can fish survive by filter-feeding on microparticles? Energy balance in tilapia grazing on algal suspensions. *Journal of Fish Biology*, *47*(1): 7–17. https://doi.org/10.1111/j.1095-8649.1995.tb01868.x.

De-Schryver, P., & Verstraete, W. (2009). Nitrogen removal from aquaculture pond water by heterotrophic nitrogen assimilation in lab-scale sequencing batch reactors. *Bioresource Technology*, 100:1162-1167.

Deswati, D., Safni, S., Khairiyah, K., Yani, E., Yusuf, Y., & Pardi, H. (2020). Biofloc technology: water quality (pH, temperature, DO, COD, BOD) in a flood and drain aquaponic system. *International Journal of Environmental and Analytical Chemistry*, DOI:10.1080/03067319.2020.1817428.

Diana, J. S., Szyper, J. P., Batterson, T. R., Boyd, C. E., & Piedrahita, R.H. (1997). Water quality in ponds. In: Egna, H.S., Boyd, C.E. (Eds.), Dynamics of Pond Aquaculture. CRC Press, Boca Raton, New York, pp. 53–72.

Ebeling, J. M., Timmons, M. B., &Bisogni, J. J. (2006). Engineering analysis of the stoichiometry of photoautotrophic, autotrophic and heterotrophic removal of ammonia-nitrogen in aquaculture systems. *Aquaculture,* 257: 346-358.

Edmonson, W. T. (1959). Freshwater Biology. Wiley, New York, USA.

Ekasari, J., Crab, R. & Verstraete, W. (2010). Primary nutritional content of bio-flocs cultured with different organic carbon sources and salinity. *Hayati Journal of Biosciences* 17: 125–130.

Ekasari, J., Rivandi, D. R., Firdausi, A. P., Surawidjaja, E. H., Zairin, M., Bossier, P. (2015). Biofloc technology positively affects Nile tilapia (*Oreochromis niloticus*) larvae performance. *Aquaculture,* 441: 72-77.

Ekasari, J., Suprayudi, M. A., Wiyoto, W., Hazanah, R. F., Lenggara, G. S., Sulistiani, R., Alkahfi, M., & Zairin, M. (2016). Biofloc technology application in African catfish fingerling production: The effects on the reproductive performance of brood stock and the quality of eggs and larvae. *Aquaculture,* 464: 349-356.

Ekasari, J., Zairin, M., Putri, D. U., Sari, N. P., Surawidjaja, E. H., & Bossier, P. (2013). Biofloc-based reproductive performance of Nile tilapia *Oreochromis niloticus*L. broodstock. *Aquaculture Research,* 46: 509-512.

El-Greisy, Z. A., & El-Gamal, A. E. (2012). Monosex production of tilapia, *Oreochromis niloticus* using different doses of 17α-methyltestosterone with respect to the degree of sex stability after one year of treatment. *The Egyptian Journal of Aquatic Research,* 38(1):59-66.

El-Sayed, A. F. M. (2013). On-farm feed management practices for Nile tilapia *(Oreochromis niloticus)* in Egypt. *In* M.R. Hasan and M.B. New, eds. *On-farm feeding and feed management in aquaculture.* FAO Fisheries and Aquaculture Technical Paper No. 583. Rome, FAO. pp. 101–129.

El-Shafiey, M. M., Mabroke, R. S., Mola, H. R. A., Hassan, M. S. and Suloma, A., (2018). Assessing the suitability of different carbon sources for Nile tilapia, *Oreochromis niloticus*culture in BFT system. *Aquaculture, Aquarium, Conservation, &LegislationBioflux*, 11(3): 782-795.

Emerenciano, M., Ballester, E. L. C., Cavalli, R. O., &Wasielesky, W. (2011). Biofloc Technology application as a food source in a limited water exchange nursery system for pink shrimp *Farfantepenaeusbrasiliensis* (Latreille, 1817). *Aquaculture Research*, 43:447-457.

Emerenciano, M., Cuzon, G., Paredes, A., &Gaxiola, G. (2013). Evaluation of biofloc technology in pink shrimp *Farfantepenaeusduorarum*culture: growth performance, water quality, microorganisms' profile and proximate analysis of biofloc. *Aquaculture International,* 21: 1381-1394.

Emerenciano, M., Martinez-Cordova, L., Martinez-Porchas, M., & Miranda-Baeza, A. (2017). Biofloc technology (BFT): a tool for water quality management in aquaculture In: Water quality. Tutu H (ed), *IntechOpen*, DOI: 10:5772/66416.

Emerson, K., Russo, R. C., Lund, R. E., & Thurston, R. V. (1975). Aqueous ammonia equilibrium calculations: effect of pH and temperature. *J Fish Res Board Can*. 32:2379–83.

EPA. (2013). Aquatic Life Ambient Water Quality Criteria for Ammonia – Freshwater. (EPA 822-R-13-001) US EPA: Washington, DC.

FAO (Food and Agriculture Organization of the United Nations). (2006b). State of World Aquaculture. 2006. FAO Fisheries Technical paper 500. Rome: FAO Fisheries Department.

FAO. (2006a). Aquaculture Production Statistics 1997-2006. Food and Agriculture Organization, Rome, Italy, Pg: 239.

FAO. (2012). Food and Agriculture Organization. The state of World Fisheries and Aquaculture, Rome, FAO. 209.

FAO. (2012). *The State of World Fisheries and Aquaculture* (209th ed.).

FAO. (2016). Food and Agriculture Organization of the United Nations, 2016. Fishery and Aquaculture Country profiles. Kenya, 2016. Country Profile Fact Sheets. Rome: p.42.

FAO. (2018). The state of World Fisheries and Aquaculture. Meeting the sustainable development goals. Food and Agriculture Organization, Rome.

Fashina-Bombata, H., &Megbowon, I. (2012). Proximate composition and breeding description of an unidentified cichlid of Epe Lagoon, Lagos, Southwest, Nigeria commonly called Wesafu. *International Journal of Nutrition and Metabolism*, 4:57-63.

Fernando C. H. (1994). Zooplankton, fish and fisheries in tropical freshwaters. *Hydrobiologia*272(1-3):105-123.

FME (Federal Ministry of Environment) (2001). Guidelines and standards for water quality. *Nigerian Publication Federal Ministry of Environment*, Pp. 114.

Franco-Nava, M. A., Blancheton, J. P., Deviller, G., Charrier, A., & Le-Gall, J. Y. (2004). Particulate matter dynamics and transformations in a recirculating aquaculture system: application of stable isotope tracers in seabass rearing. *Aquacultural Engineering*, 31: 135-155.

Galvez, A. O., Campos, C. V., dos Santos, G. S., Marinho, Y. F., Vinatea, L., & Brito, L. O. (2015). Plankton communities in shrimp monoculture, integrated biofloc system. *Global Aquaculture Advocate*, 1-9.

Gangadhara, B., &Keshavanath, P. (2008). Planktonic and Biochemical Composition of Periphyton Grown on Some Biodegradable and Non-Degradable Substrates. *Journal of Applied Aquaculture*, 20(3): 213-232

Gantar, M., Berry, J. P., Thomas, S., Wang, M. L., Perez, R., and Rein, K. S. (2008). Allelopathic activity among Cyanobacteria and microalgae isolated from Florida freshwater habitats. *Federation of European Microbiological Society (FEMS) Microbiology Ecology*, 64:55-64.

Garcia, F., Romera, D. M., Sousa, N. S., Paiva-Ramos, I., &Onaka, E. M. (2016). The potential of periphyton -based cage culture of Nile tilapia in a Brazilian reservoir. *Aquaculture*, doi: 10.1016/j.aquaculture.2016.06.031.

Garcia, F., Sabbag, O. J., Kimpara, J. M., Romera, D. M., Sousa, N. S., Onaka, E. M., & Ramos, I. P. (2017). Periphyton-based cage culture of Nile tilapia: An interesting model for small-scale farming. *Aquaculture*, 479:838-844.

Garcia, J. J., Correa, G. A., & Pardo-Carrasco, S. C. (2012). Phytoplankton and periphyton in ponds with Nile tilapia (*Oreochromis niloticus*) and bocachico (*Prochilodusmagdalenae*). *RevistaColombiana deCienciasPecuaris*, 25(4):603-614.

García-Ríos, L., Miranda-Baeza, A., Emerenciano, M. G. C. and Huerta-Rábago, J. A., Osuna-Amarillas, P., (2019)**.** Biofloc technology (BFT) applied to tilapia fingerlings production using different carbon sources: Emphasis on commercial applications. *Aquaculture*, 502: 26–31.

Gómez-Márquez, J. L., Peña-Mendoza, B., Salgado-Ugarte, I. H., & Guzmán-Arroyo, M. (2003). Reproductive aspects of *Oreochromis niloticus*(Perciformes: Cichlidae) at Coatetelco lake, Morelos, Mexico. *Revista de Biología Tropical*, 51:221-228.

Grima, L., Quillet, E., Boujard, T., Robert-Granié, C., Chatain, B., &Mambrini, M. (2008). Genetic variability in residual feed intake in rainbow trout clones and testing of indirect selection criteria. Genet. Sel. Evol. 40, 607–624.

Gross, A., Boyd, C. E., & Wood, C. W. (2000). Nitrogen transformation and balance in channel catfish ponds. *Aquacultural Engineering,* 24:1-14.

Guo, H., Huang, L., Hu, S., Chen, C., Huang, X., Liu, W., Wang, S., Zhu, Y., Zhao, Y. & Zhang, D. (2020). Effects of Carbon/Nitrogen ratio on growth, intestinal microbiota and metabolome of shrimp (*Litopenaeusvannamei*). *Frontiers of Microbiology,* 11:652

Guttman, L. (2019). Periphyton For Biofiltration and Fish feeding in An Integrated Multi-Trophic Aquaculture System: A Case Study in The Gulf of Aqaba. *Open Access Journal of Environmental and Soil Science,* 3(5).

Haque, M., Islam, M., Wahab, M., Hoq, Md., Rahman, M., & Azim, M. (2016). Evaluation of production performance and profitability of hybrid red tilapia and genetically improved farmed tilapia (GIFT) strains in the carbon/nitrogen controlled periphyton-based (C/N-CP) on-farm prawn culture system in Bangladesh. *Aquaculture Reports,* 4:101-111.

Hardy, R. W. (2010). Utilization of plant proteins in fish diets: Effects of global demand and supplies of fishmeal. *Aquaculture Research*, *41*(5), 770–776.

Hargreaves, J. A. (2006). Photosynthetic suspended-growth systems in aquaculture. *Aquacultural Engineering,* 34:344-363.

Hargreaves, J. A. (2013). Biofloc production systems for aquaculture. *Southern Regional Aquaculture Centre SRAC,* Publication No. 4503, 12 pp.

Harraz, Y., El-Hawarry, W., &Shourbela, R. (2018). Culture performance of Nile tilapia (*Oreochromis niloticus*) raised in a biofloc-based intensive system, *Alexandria Journal of Veterinary Sciences,* 58 (1): 166-172.

Hasan, M. R. (2007). Economics of aquaculture feeding practices in selected Asian countries. *FAO Fisheries Technical Paper*; No. 505. Rome, FAO. 205.

Henchion, M., Hayes, M., Mullen, A. M., Fenelon, M., Tiwari, B. (2017). Future Protein Supply and Demand: Strategies and Factors Influencing a Sustainable Equilibrium. Foods, 6, 53; doi: 10.3390/foods6070053.

Henriksson, P. J. G., Belton, B., Jahan, K. M., & Rico, A. (2018). Measuring the potential for sustainable intensification of aquaculture in Banglandesh using life cycle assessment. *Proceedings of the National Academy of Sciences*, 1-6.

Hillebrand, H. (2002). Top-down versus bottom-up control of autotrophic biomass: a meta-analysis on experiments with periphyton. *Journal of the North AmericanBenthological Society*. 21: 349-369.

Hoque, M.I., Rahman, A.K.M.F., Mansur, M.A., Rahman, S. (2018). Effects of periphyton on monoculture of *Puntius gonionotus*. *International Journal of Agriculture Research Innovation & Technology,* 8(2): 13-23.

Hu, Z., Lee, J. W., Chandran, K., Kim, S., Sharma, K., &Khanal, S. K. (2014). Influence of carbohydrate addition on nitrogen transformations and greenhouse emissions of intensive aquaculture system. *Science of The Total Environment,* 470: 193-200.

Huchette, S. M. H., Beveridge, M. C. M., Baird, D. J. & Ireland, M. (2000) The impacts of grazing by tilapias (*Oreochromis niloticus* L.) on periphyton communities growing on artificial substrate in cages. *Aquaculture*, 186: 45-60.

Huchette, S., & Beveridge, M. (2003). Technical and economical evaluation of periphyton-based cage culture of tilapia *(Oreochromis niloticus)* in tropical freshwater cages. *Aquaculture*, 218(1-4): 219-234.

Ibrahim, A. N. A. F., Noll, M. S. M. C., &Valenti, W. C. (2015). Zooplankton capturing by Nile Tilapia, *Oreochromis niloticus* (Teleostei: Cichlidae) throughout post-larval development. *ZOOLOGIA,* 32 (6): 469-475.

Izquierdo, M. S.; Fernandez-Palacios, H. and Tacon, A. G. J. (2001). Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture,* 197:25-42.

Jeje, C. Y. (1988). A revision of the Nigerian species of the genera *Mesocyclops*Sars, 1914 and *Thermocyclops*Kiefer, 1927 (Copepoda: Cyclopoida). *Hydrobiologia,* 164(2):171-184.

Jensi, A., Marx, K. K., Rajkumar, M., Shakila, R. J., Chidambaram, P. (2016). Effect of 17 α-Methyl testosterone on sex reversal and growth of Nile tilapia (*O. niloticus* L., 1758). *Ecology, Environment and Conservation,* 22 (3): (1493-1498).

Jimenez-Montealegre, R., Verdegem, M. C. J., van Dam, A., &Verreth, J. A. J., (2002). Conceptualization and validation of a dynamic model for the simulation of nitrogen transformation and fluxes in fish ponds. *Ecological Modelling*, 147(2): 123-152.

Kaliba, A. R., Ngugi, C. C., MAckambo, J., &Quagrainie, K. K. (2007). Economic profitability of Nile tilapia (*Oreochromisniloticus*L.) production in Kenya. *Aquaculture Research,* 38(11): 1129-1136.

Kausar, R., & Salim, M. (2006). Effect of Water Temperature on Growth Performance and Feed Conversion Ratio of *Labeorohita*. http://www.oalib.com/paper/2147234#. WfMKSRLg97k.

Keremah, R. I., Davies, O.A. and Abezi, I. D (2014). Physico-Chemical Analysis of Fish Pond Water in Freshwater Areas of Bayelsa State, Nigeria. *Greener Journal of Biological Sciences.* 4 (2):033-038.

Keshavanath P., Gangadhar, B., Ramesh, T. J., Van Dam, A. A., Beveridge, M. C. M., Verdegem, M. C. J., (2004). Effect of bamboo substrate and supplemental feeding on growth and production of hybrid red tilapia fingerlings (*Oreochromis mossambicus-Oreochromis niloticus*). *Aquaculture,* 235:303-314.

Khan, W., Vahab, A., Masood, A., & Hasan, N. (2017). Water quality requirements and management strategies for fish farming (A case study of ponds around Gurgaon canal Nuh Palwal). *Internal Journal of Trend in Scientific Research and Development*, 2(1):388-393.

Khanjani, M. H. and Sharifinia, M. (2020). Biofloc technology as a promising tool to improve aquaculture production. *Reviews in Aquaculture*, 12(**3**): 1836-1850.

Khanjani, M. H., Alizadeh, M., Mohammadi, M., &Sarsangi Aliabad, H. (2020). Biofloc system applied to Nile tilapia (*Oreochromis niloticus*) farming using different carbon sources: Growth performance, carcass analysis, digestive and hepatic enzyme activity. *Iranian Journal of Fisheries Sciences*, 20(2):490-513.

Khanjani, M. H., Sajjadi, M. M., Alizadeh, M., &Sourinejad, I. (2017). Nursery performance of Pacific white shrimp (*Litopenaeusvannamei*Boone, 1931) cultivated in a biofloc system: the effect of adding different carbon sources. *Aquaculture Research*, 48(4): 1491-1501.

Kicman, A. T. (2008). “Pharmacology of anabolic steroids” *British Journal of Phamacology.* 154 (3):502-521.

KMFRI. (2017). Kenya’s aquaculture brief 2017: Status, Trends, Challenges and Future Outlook. Kenya Marine and Fisheries Research Institute, Mombasa, Kenya pp.12.

KNBS (2020). Economic Survey 2020. Kenya National Bureau of Statistics.

Krishnani, K. K., Kathiravan, V., Meena, K. K., Sarkar, B., Kumar, S., Brahmane, M. P., Kumar, N., &Kailasam, M. (2019). Bioremediation of aquatic toxicants: Application of multi-omic approaches. *Advances in Fish Research,* 7: 371-398.

Kuhn, D. D., Boardman, G. D., Lawrence, A. L., Marsh, L., & Flick, G. J. (2009). Microbial floc meals as a replacement ingredient for fish meal and soybean protein in shrimp feed. *Aquaculture,* 296:51-57.

Kuhn, D. D., Lawrence, A. L., Boardman, G. D., Patnaik, S., Marsh, L., & Flick, G.J. (2010). Evaluation of two types of bioflocs derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, *Litopenaeusvannamei*. *Aquaculture*, 303: 28–33.

Kumar, P., Mishra, R., & Singh, D. K. (2013). Species Composition of Periphyton Community in Ponds of Chapra District, Bihar, India. *International Journal of Zoology Research*, 3(1): 49-53.

Kumar, S., Jain, V., & Raghuvanshi, S. K. (2021). Physico-chemical characteristics of AKsharVihar pond in Bareilly, U.P. *International Journal of Advanced Research in Biological Sciences*, 8(3):30-36.

Li, D., Li, Z., Wang, C., Wang, T., & Xu, X. (2019b). Spectroscopic method for detection and determination of ammonia nitrogen in aquaculture water. *Institute Of Physics (IOP) Science*, 4(1): 4-13.

Li, Z., Wang, G., Yu, E., Zhang, K., Yu, D., Gong, W., &Xie, J. (2019a). Artificial substrata increase pond farming density of grass carp (*Ctenopharyngodon Idella*)by increasing the bacteria that participate in nitrogen and phosphorus cycles in pond water. *PeerJ Life and Environment*, DOI.10.7717/peerj.7906.

Liti, D. M., Mugo, R. M., Munguti, J. M., &Waidbacher, H. (2006). Growth and economic performance of Nile tilapia (*Oreochromis niloticus* L.) fed on three brans (maize, wheat and rice) in fertilized ponds. *Aquaculture Nutrition*, 12:239-245.

Llario, F., Rodilla, M., Escriva, J., Falco, S., &Sebastia-Frasquet, T. (2018). Phytoplankton evolution during the creation of a biofloc system for shrimp culture. *International Journal of Environmental Science and Technology*, https://doi.org/10.1007/s13762-018-1655-5.

Llewellyn. W. (2009). Anabolics. Molecular Nutrition Llc. Pp.16, 19,22,39, 291-293.

Lupatsch, I., Deshev, R., & Magen, I. (2010). Energy and protein demand for optimal egg production including maintenance requirements of female tilapia (*Oreochromis niloticus*). *Aquaculture Research*, 41:763-769.

Makkar, H. P. S., Tran, G., Heuze, V., &Ankers, P., (2014). State-of-the-art on use of insects as animal feed. *Animal Feed Science &Technology*, 197:133

Malbrouck, C. &Kestemont, P. (2006). Effects of microcystins on fish. *Environmental Toxicology and Chemistry* 25:72-86.

Margalef, R. (1983). *Limnologia*. Barcelona: Ediciones Omega. Pg. 1010.

Matsuo, Y., Kasahara, Y., Hagiwara, A., Sakakura, Y., & Arakawa, T. (2006). Evaluation of larval quality of viviparous scorpion *Sebasticusmarmoratus.Fisheries Science*; 72: 948-954.

Mbuthia, M. G. (2020). Assessment of water quality in aquaculture ponds in Tigoni, Kiambu county, Kenya. MSc Thesis, Kenyatta University.

McKaye, K. R., Louda, S. M., Stauffer, J. R. (1990). Bower size and male reproductive success in a cichlid fish lek. *The American Naturalist*, 135 (5): 597-613.

Megbowon, I. &Mojekwu, T. O. (2014). Tilapia sex Reversal Using Methyl Testosterone and its Effect on Fish, Man and Environment. *Biotechnology*, 13: 213-216.

Megbowon, I. (2011). Tilapia production in Nigeria. Vol.4, Fisheries Society of Nigeria Quarterly Publication, Nigeria, pp.18-22.

Mendonca, F. Z., & Goncalves-de-Freitas (2008). Nest deprivation and mating success in Nile tilapia (Teleostei: Cichlidae). *RevistaBrasileira de Zoologia*, 25 (3): 413-418.

Miao, L., Wang, C., Adyel, T. M., Zhao, J., Yan, N., Wu, J., & Hou J. (2021). Periphytic Biofilm Formation on Natural and Artificial Substrates: Comparison of Microbial Compositions, Interactions, and Functions. *Frontiers in Microbiology*, 12:684903.

Milstein, A., &Omri, L. (2003). Organic tilapia culture in Israel.

Mirzakhani, N., Ebrahimi, E., Jalali, S. A. H.,&Ekasari, J. (2019). Growth performance, intestinal morphology and nonspecific immunity response of Nile tilapia (*Oreochromis niloticus*) fry cultured in biofloc systems with different carbon sources and input C:N ratios. *Aquaculture*, 512: 734235.

Mogaka, H., Gichere, S., Richard, D. & Rafik, H. (2006). Climate variability and water resources degradation in Kenya: improving water resources development and management. *World Bank Working Paper No*. 69.

Mugwanya, M., Dawood, M. A. O., Kimera, F., &Sewilam, H. (2021). Biofloc Systems for Sustainable Production of Economically Important Aquatic Species: A review. *Sustainability*, 13: 7255.

Munguti, J. M., Mugiranea, J. K., &Ogello, E. O. (2014). An overview of the Kenyan aquaculture sector; current status, challenges and opportunities for future development. *Fisheries and Aquatic Sciences*, 17: 1-11.

Munguti, J., Obiero, K., Orina, P., Mirera, D., Kyule, D., Mwaluma, J., Opiyo, M., Musa, S., Ochiewo, J., Njiru, J., Ogello, E. & Hagiwara, A. (Eds) (2021). State of Aquaculture Report 2021: Towards Nutrition Sensitive Fish Food Production Systems. *Techplus Media House*, Kenya. 190 pp.

Musa, S., Aura, M. C., Owiti, G., Nyonje, B., Orina, P., &Charo-Karisa, H. (2012). Fish farming enterprise productivity program (FFEPP) as an impetus to *Oreochromis niloticus*(L.) farming in Western Kenya: lessons to learn. *African Journal of Agricultural Research*, 7(8): 1324-1330.

Naylor, R. L., Goldburg, R. J., Primavera, J. H., Kautsky, N., Beveridge, M. C. M., Clay, J., Folkes, C., Lubchenco, J., Mooney, H., &Troell, M. (2000). Effect of aquaculture on world fish supplies. *Nature*, 405:1017-1024.

Naylor, R. L., Hardy, R. W., &Buschmann, A. H. (2021). A 20-year retrospective review of global aquaculture. *Nature* 591: 551-563.

Naylor, R. L., Hardy, R. W., Bureau, D. P., Chiu, A., Elliott, M., Farrell, A. P., Forster, I., Gatlin, D. M., Goldburg, R. J., Hua, K., & Nichols, P. D. (2009). Feeding aquaculture in an era of finite resources (Proceedings of the National Academy of Sciences of the United States of America (2009) 106, (15103-15110) DOI: 10.1073/pnas.0905235106). *Proceedings of the National Academy of Sciences of the United States of America*, *106*(42), 18040.

Nelson, M. C. (1995). Male size, spawning pit size and female mate choice in a lekking cichlid fish. *Animal Behavior*, 50 (6): 1587 – 1599.

Ngugi, C. C., Bowman, J. R., &Omolo, B. O. (2007). A New guide to fish farming in Kenya. Aquaculture Collaborative Research Support Program. Oregon State University (U.S.A), Moi University and Ministry of Livestock and Fisheries Development, Government of Kenya. ISBN 978-0-9798658-0-0.

NRC, (2011). Nutrient Requirements of Fish and Shrimp. Animal Nutrition Series. National Research Council of the National Academies. The National Academies Press, Washington, DC, USA.

Obwanga, B., &Lewo, M.R. (2017). From aid to responsible trade: driving competitive aquaculture sector development in Kenya; Quick scan of robustness, reliability and resilience of the aquaculture sector. Wageningen, Wageningen University & Research, Report 2017-092 3R Kenya. 68 pp

Ogello, E. O., &Munguti, J. (2016). Aquaculture: A promising solution for food insecurity, poverty and malnutrition in Kenya. *African Journal of Food Agriculture, Nutrition and Development,* 16(4):11331-11350.

Ogello, E. O., Musa, S. M., Aura, C. M., Abwao, J. O., &Munguti, J. M. (2014). An appraisal of the feasibility of tilapia production in ponds using biofloc technology: A review. *International Journal of Aquatic Science*, 5(1):2-3.

Ogello, E. O., Wullur, S., & Hagiwara, A. (2019). Blending fishwastes and chicken manure extract as low-cost and stable diet for mass culture of freshwater zooplankton, optimized for aquaculture. *IOP conference Series: Material Science and Engineering*, 567.

Ogello, E. O., Wullur, S., Sakakura, Y., &Hagiwara, A. (2018). Compositing fishwastes as low-cost and stable diet for culturing BrachionusrotundiformisTschugunoff (Rotifera): Influence on water quality and microbiota. *Aquaculture,* 486: 232-239.

Omasaki, S. K. (2017). Optimization of breeding schemes for Nile tilapia (*Oreochromis niloticus*) in smallholder production systems in Kenya. PhD thesis, Wageningen University, the Netherlands.

Opiyo, M. A., Obiero, K. O., Abwao, J., Awuor, F. J., Kyule, D., Munguti, J. (2020). Comparative Growth Performance of Genetically Male, Sex-Reversed, and Mixed-Sex Nile Tilapia (*Oreochromis niloticus*) Reared in Earthen Ponds in Sagana, Kenya. *Aquaculture studies,* 21(1), 23-30.

Orlando, T., Oliveira, M., Paulino, R., Costa, A., Allaman, B., &Rosa, V. (2017). Reproductive performance of female Nile tilapia (*Oreochromis niloticus*) fed diets with different digestible energy levels. *Brazilian Journal of Animal Science*, Zootec., 46(1):1-7.

Panigrahi, A., Sundaram, M., Saranya, C., Satish Kumar, R., SyamaDayal, J., Saraswathy, R., Otta, S. K., Shyne Anand, P. S., Nila Rekha, P. & Gopal, C. (2018). Influence of differential protein levels of feed on production performance and immune response of pacific white leg shrimp in a biofloc–based system. *Aquaculture*, 503: 118-127.

Patkar, A. R., Manjappa, A. R., & Nayak, H. (2021). Dynamics of plankton in substrate-based fish and prawn culture. *Journal of Entomology and Zoology Studies*, 9(1):175-182.

Perez-Fuentes, J. A., Perez-Rostro, C. I., & Hernandez-Vergara, M. P. (2013). Pond reared Malaysian prawn *Macrobrachiumrosenbergii* with the biofloc system. *Aquaculture,* 400-401: 105-110.

Perez-Fuentez, J. A., Hernandez-Vergara, M. P., Perez-Rostro, C. I., & Fogel, I. (2016). C:N ratios affect nitrogen removal and production of Nile tilapia *Oreochromis niloticus* raised in a biofloc system under high density cultivation. *Aquaculture,* 425:247-251.

Pertiwi, P., Abinawanto, A., &Yimastria, S. (2017). Fertilization Rate of Lukas Fish (*Puntius bramoides*). *Proceedings of the 3rd International Symposium on Current Progress in Mathematics and Sciences*; 2023, 020160-1-020160-4.

Piedrahita, R. H. (2003). Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture* 226: 35-44.

Promthale, P., Pongtippatee, P., Withyachumnarnkul, B., & Wongprasert, K. (2019). Bioflocs substituted fishmeal feed stimulates immune response and protects shrimp from Vibrio parahaemolyticus infection. *Fish and Shellfish Immunology, 93*(June), 1067–1075.

Riche, M., &Garling, D. (2003). Fish: Feed and Nutrition. Feeding Tilapia in Intensive Recirculating Systems. Available at; http://www.hatcheryfeed.com/hf-articles/141/.

Rocha, M. J. (2008). Fish Reproduction. *Science Publisher*. California, USA, pp. 9–37.

Rodde, C., Chatain, B., Vandeputte, M., Trinh, T. Q., Benzie, J. A. H., & de Verdal, H. (2020). Can individual feed conversion ratio at commercial size be predicted from juvenile performance in individually reared Nile tilapia *Oreochromis niloticus*? *Aquaculture reports,* 17, 100349.

Rodriguez, P., Vera, M., & Pizarro, H. (2012). Primary production of phytoplankton and periphyton in two humic lakes of a South American wetland. *Limnology*,13: 10.1007/s10201-012-0373-9.

Ross, L. L. (2002). Environmental physiology and energetic. *Fish and Fisheries Series,* 25:89–128.

Russell, M., Shuke, R., & Samantha, S. (2011). Effects of Conductivity on Survivorship and Weight of Goldfish (*Carassius auratus*). http://departments.juniata.edu/biology/ eco/documents.

Saikia, S. K., Nandi, S., & Majumder, S. (2011). The Upside of Grazer-Periphyton interactions: A Review. In: Zooplankton and Phytoplankton: Types, Characteristics, and Ecology (Ed. GiriKattel) *Nova Science Publishers* Inc, UK p.89-106.

Saikia, S., & Das, D. (2009). Potentiality of periphyton-based aquaculture technology in rice-fish environment. *Journal of Scientific Research,* 30: 427-430.

Sakr, M., Shalaby, M., Wassef, A., El-Sayed, M., &Moneim, I. (2015). Evaluation of periphyton as a food source for Nile tilapia (*Oreochromis niloticus*) juveniles fed reduced protein levels in cages. *Journal of Applied Aquaculture,* 27:50-60.

Samocha, T. M., Lawrence, A. L., Craig, A. C., Frank, C. L., William, A. B., Craig, J. D., Philip, G. L.,& Gary, F. W. (2004). Production of the Pacific white shrimp, *Litoptnaeusvannamei,* in high-density greenhouse-enclosed raceways using low salinity groundwater. *Journal of Applied Aquaculture,* 15(3):1-19.

Sarkar, R., Ghosh, A. R., & Mondal, N. K. (2020). Comparative study on physicochemical status and diversity of macrophytes and zooplanktons of two urban ponds of Chandannagar, WB, India. *Applied Water Science,* 10:63

Sarkar, S., Rekha, P. N., &Panigrahi, A. (2021). Integrated brackishwater farming of red sea weed *Agarophytontenuistipitatum* and Pacific white leg shrimp *Litopenaeusvannamei* (boone) in biofloc system: a production and bioremediation way out. *Aquaculture International,* 154-159.

Schneider, O., Sereti, V., Eding, E. H., &Verreth, J. A. J. (2005). Analysis of nutrient flows in integrated intensive aquaculture systems. *Aquacultural Engineering,* 32(3-4): 379-401.

Schrader K. K., Green, B. W., &Perschbacher, P. W. (2011). Development of phytoplankton communities and common off-flavors in a biofloc technology system used for the culture of channel catfish (*Ictalurus punctatus*). *Aquacultural Engineering,* 45: 118-126.

SDF. (2014). State Department of Fisheries. Fisheries Annual Statistical Bulletin 2014. Nairobi, State Department of Fisheries: p. 59.

SDF. (2016). State Department of Fisheries Kenya. Fish Farming Enterprise Productivity Capacity Assessment and Gap Analysis Report 2016. State Department of Fisheries Nairobi.

Semyalo, R. P. (2009). The effects of cyanobacteria on the growth, survival, and behavior of a tropical fish (Nile Tilapia) and zooplankton (*Daphnia lumholtzi*). *University of Bergen*, PhD Thesis.

Semyalo, R., Rohrlack, T., Kayiira, D., Kizito, Y. S., Byarujali, S., Nyakairu, G., & Larsson. (2011). On the diet of Nile tilapia in two eutrophic tropical lakes containing toxin producing cyanobacteria. *Limnologia*, 41(1): 30-36.

Sharifinia, M., AfshariBahmanbeigloo, Z., Keshavarzifard, M., Khanjani, M. H.,& Lyons, B.P., (2020). Microplastic pollution as a grand challenge in marine research: A closer look at their adverse impacts on the immune and reproductive systems. *Ecotoxicology and Environmental Safety,* 204: 111109.

Shoko, A. P., Limbu, S. M., Mrosso, H. D. J. &Mgaya, Y. D. (2015). Reproductive biology of female Nile tilapia *Oreochromis niloticus* (Linnaeus) reared in monoculture and polyculture with African sharp tooth catfish *Clariusgariepinus* (Burchell). *SpringerPlus*4:275.

Shore, L. S., & Shemesh, M. (2003). Naturally produced steroid hormones and their release into the environment. *Pure and Applied Chemistry,* 75: 1859-1871.

Simoes, M. R., De Fatima, C., Ribeiro, A., Amaral, C., Park, K. J., & Elizabeth, F. (2007). Physicochemical and microbiological composition and yield of thai-style tilapia fillets (*Oreochromis niloticus*).*Food Science and Technology*, 27: 608-613.

Supamattaya, K., Kiriratnikom, S., Boonyaratpalin, M., &Borowitzka, L. (2005). Effect of a Dunaliella extract on growth performance, health condition, immune response and disease resistance in black tiger shrimp (*Penaeus monodon*). *Aquaculture*, 248: 207–216.

Suresh, V., &Bhujel, R. C. (2012). Tilapias.In: Lucas, J.S., Southgate, P.C., editors. Aquaculture: farming aquatic animals and plants. United Kingdom: *Wiley-Blackwell Publishing Company*, Pp.338-364.

Tacon, A.G. (1993). Feed ingredients for warmwater fish, fish meal and other processed feedstuffs. *FAO Fisheries Circular (FAO). no. 856.* Rome, Italy.

Tahoun, A. M., Ibrahim, A. A-R., Hammouda, Y, F., Eid, M. S., Zaki, M. A., &Magouz, F. I. (2008). Effects of age and stocking density on spawning performance of Nile tilapia, *Oreochromis niloticus* (L.) broodstock reared in hapas. 8th International Symposium on Tilapia in Aquaculture.

Tiago, G. G. &Gianesella, S. M. F. (2003). The use of water by aquaculture: Strategies and tools for management implementation. *Boletim do Instituto dePesca*, 29: 1-7.

Timmons, M. B., Ebeling, J. M., Wheaton, F. W., Summerfelt, S. T., & Vinci. B. J. (2002). Recirculating Aquaculture systems, 2nd edition. Northeastern Regional Aquaculture Center. Publication No. 01-002. *Cayuga Aqua Ventures*. Ithaca, NY. 769 pp.

Tinh, T. H., Momoh, T. A., Kokou, F., Hai, T. N., Schrama, J. W., Verreth, J. A. J., &Verdegem, M. C. J. (2021). Effects of carbohydrate addition methods on Pacific white shrimp (*Litopenaeusvannamei*). *Aquaculture,* 543, 736890, 0044-8486.

TNAU (Tamil Nadu Agricultural University) (2008). Water quality management. Accesses at http://www.agritech.tnau.ac.in/fishery/water.html.

Tripathy, P. K., &Adhikary, S. P. (1990). Preliminary studies on the water pollution of river Nandira. *Indian Journal of Environment & Health,* 32(4):363–368.

Troell, M., Vetenskapsakademien, K., & Kautsky, N. (2017). Aquaculture, In Reference Module in Life Sciences. *Elsevier, January*. https://doi.org/10.1016/B978-0-12-809633-8.02007-0

Tucker, C. S., Pote, J. W., Wax, C. L., & Brown, T. W. (2015). Improving water-use efficiency for Ictalurid catfish pond aquaculture in Northwest Mississippi, USA. *Aquaculture Research,* 48:447-458.

Uddin, M. S., Verdegem, M. C. J., Azim, M. E., & Wahab, M. A. (2007). Periphyton-based tilapia-prawn polyculture. New dimension in aquaculture. May support organic status. *Global Aquaculture Advocate*,10(1): 50-53.

Uddin, M.S. (2007). Mixed culture of tilapia (*Oreochromis niloticus*) and freshwater prawn (*Macrobrachiumrosenbergii*) in periphyton-based ponds. Ph.D. Thesis. *Fish Culture and Fisheries Group,* Wageningen University, The Netherlands, 208 pp.

van Dam, A. A., Beveridge, M. C. M., Azim, E. M., &Verdegem, M. C. J. (2002). The potential of fish production based on Periphyton. *Reviews in Fish Biology and Fisheries,* 12: 1-3.

Vazquez, L., Alpuche, J., Maldonado, G., Agundis, C., Morales, P., &Zenteno, E. (2009). Immunity mechanisms in crustaceans. *Innate Immun*ity, 15:179-188.

Verdegem, M. C. J., &Bosma, R. H. (2009). Water withdrawal for brackish and inland aquaculture, and options to produce more fish in ponds with present water use. *Water Policy,* 11:52-68.

Wang, C., Pan, L., Zhang, K., Xu, W., Zhao, D., & Mei, L. (2015). Effects of different carbon sources addition on nutrition composition and extracellular enzymes activity of bioflocs, and digestive enzymes activity and growth performance of *Litopenaeusvannamei* in zero-exchange culture tanks. *Aquaculture Research*, 47(10):1-12.

Wasielesky, W., Atwood, H., Stokes, A., & Craig, L. B. (2006). Effect of natural production in a zero-exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeusvannamei.Aquaculture*, 2258(1-4):396-403.

Withford, L. A. & Schumacher, G. J. (1973). A manual of freshwater algae. Sparks, Raleigh, North Carolina, USA.

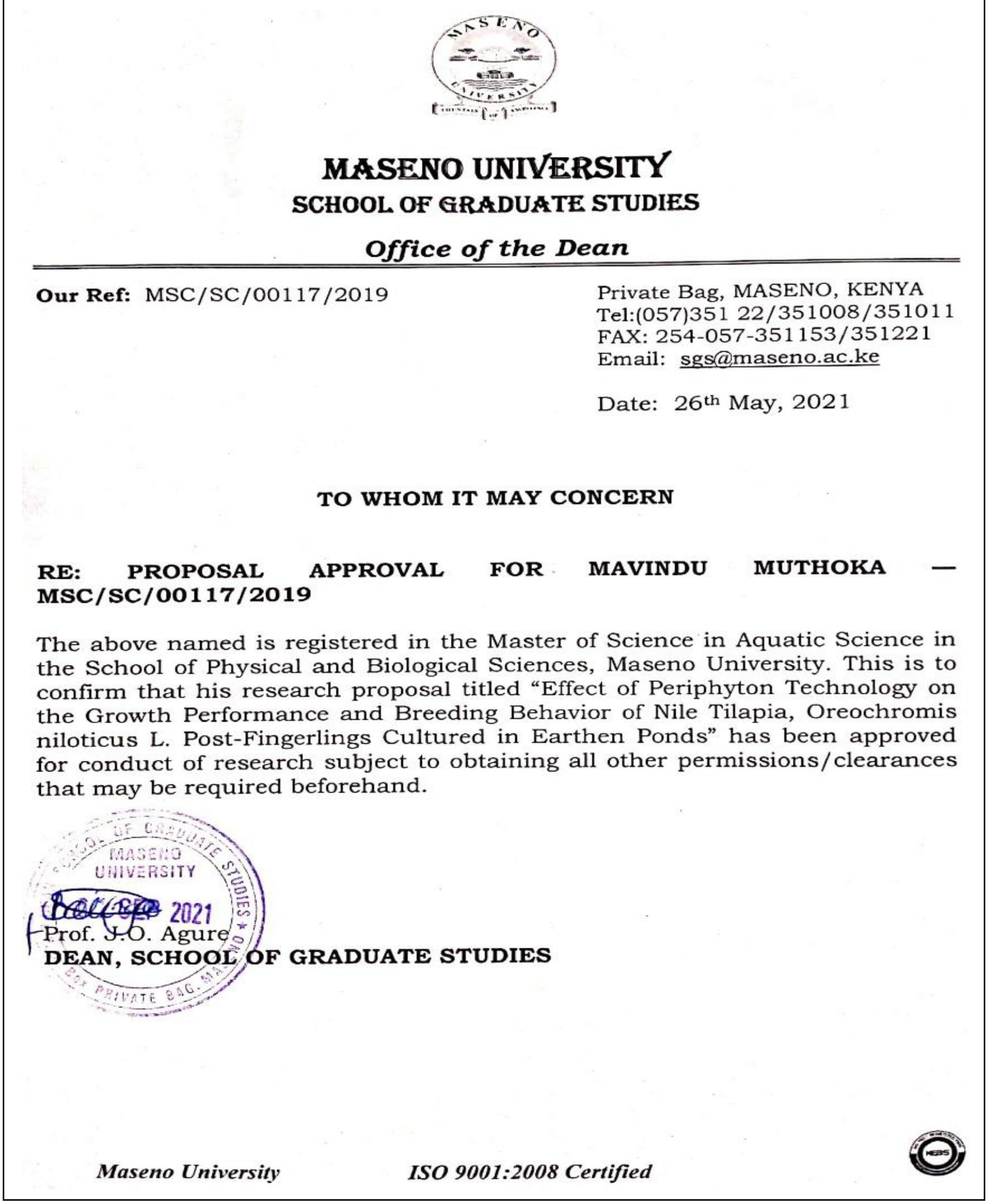
Xu, W. J., & Pan, L. Q. (2013). Enhancement of immune response and antioxidant status of (*Litopenaeusvannamei*) juvenile in biofloc-based culture tanks manipulating high C/N ratio of feed input. *Aquaculture*, 412-413:117-124.

Xu, W. J., Morris, T. C., &Samocha, T. M. (2016). Effects of C/N ratio on biofloc development, water quality and performance of *Litopenaeusvannamei*juveniles in a biofloc-based, outdoor tank system. *Aquaculture*, 453: 169-175.

Yadav, R., Kumar, P., Saini, V. P., & Sharma, B. K. (2017). Importance of Periphyton for aquaculture. *Aqua star*. pp 38-40.

APPENDICES

pendix 1: Maseno University SGS Proposal Approval Letter



MASENO UNIVERSITY
SCHOOL OF GRADUATE STUDIES
Office of the Dean

Our Ref: MSC/SC/00117/2019

Private Bag, MASENO, KENYA
Tel:(057)351 22/351008/351011
FAX: 254-057-351153/351221
Email: sgs@maseno.ac.ke

Date: 26th May, 2021

TO WHOM IT MAY CONCERN

RE: PROPOSAL APPROVAL FOR MAVINDU MUTHOKA — MSC/SC/00117/2019

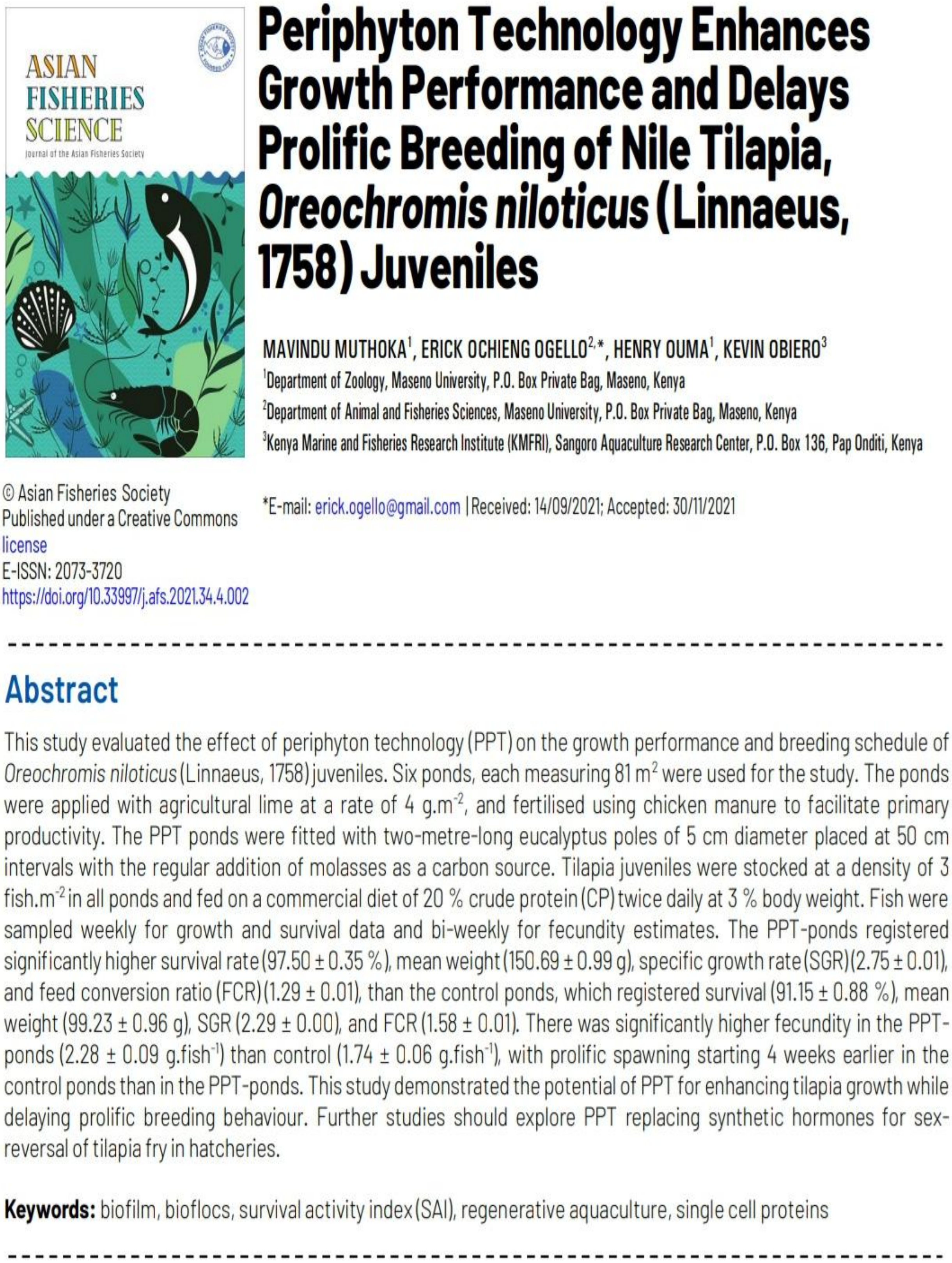
The above named is registered in the Master of Science in Aquatic Science in the School of Physical and Biological Sciences, Maseno University. This is to confirm that his research proposal titled "Effect of Periphyton Technology on the Growth Performance and Breeding Behavior of Nile Tilapia, Oreochromis niloticus L. Post-Fingerlings Cultured in Earthen Ponds" has been approved for conduct of research subject to obtaining all other permissions/clearances that may be required beforehand.

2021

Prof. J.O. Agure
DEAN, SCHOOL OF GRADUATE STUDIES

Maseno University *ISO 9001:2008 Certified*

Appendix 2: Published Research Paper for Work Drawn from the Thesis



Periphyton Technology Enhances Growth Performance and Delays Prolific Breeding of Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758) Juveniles

MAVINDU MUTHOKA[1], ERICK OCHIENG OGELLO[2,*], HENRY OUMA[1], KEVIN OBIERO[3]
[1]Department of Zoology, Maseno University, P.O. Box Private Bag, Maseno, Kenya
[2]Department of Animal and Fisheries Sciences, Maseno University, P.O. Box Private Bag, Maseno, Kenya
[3]Kenya Marine and Fisheries Research Institute (KMFRI), Sangoro Aquaculture Research Center, P.O. Box 136, Pap Onditi, Kenya

E-ISSN: 2073-3720
https://doi.org/10.33997/j.afs.2021.34.4.002

*E-mail: erick.ogello@gmail.com | Received: 14/09/2021; Accepted: 30/11/2021

Abstract

This study evaluated the effect of periphyton technology (PPT) on the growth performance and breeding schedule of *Oreochromis niloticus* (Linnaeus, 1758) juveniles. Six ponds, each measuring 81 m^2 were used for the study. The ponds were applied with agricultural lime at a rate of 4 g.m^{-2}, and fertilised using chicken manure to facilitate primary productivity. The PPT ponds were fitted with two-metre-long eucalyptus poles of 5 cm diameter placed at 50 cm intervals with the regular addition of molasses as a carbon source. Tilapia juveniles were stocked at a density of 3 fish.m^{-2} in all ponds and fed on a commercial diet of 20 % crude protein (CP) twice daily at 3 % body weight. Fish were sampled weekly for growth and survival data and bi-weekly for fecundity estimates. The PPT-ponds registered significantly higher survival rate (97.50 ± 0.35 %), mean weight (150.69 ± 0.99 g), specific growth rate (SGR) (2.75 ± 0.01), and feed conversion ratio (FCR) (1.29 ± 0.01), than the control ponds, which registered survival (91.15 ± 0.88 %), mean weight (99.23 ± 0.96 g), SGR (2.29 ± 0.00), and FCR (1.58 ± 0.01). There was significantly higher fecundity in the PPT-ponds (2.28 ± 0.09 g.fish^{-1}) than control (1.74 ± 0.06 g.fish^{-1}), with prolific spawning starting 4 weeks earlier in the control ponds than in the PPT-ponds. This study demonstrated the potential of PPT for enhancing tilapia growth while delaying prolific breeding behaviour. Further studies should explore PPT replacing synthetic hormones for sex-reversal of tilapia fry in hatcheries.

Keywords: biofilm, bioflocs, survival activity index (SAI), regenerative aquaculture, single cell proteins