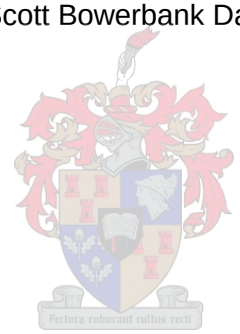
A Growth Comparison Among Three Commercial Tilapia Species in a Biofloc Technology System in South Africa

by

Scott Bowerbank Day



Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in the Faculty of AgriSciences at Stellenbosch University

Supervisor: Dr Khalid Salie

Co-supervisor: Prof Danie Brink

Faculty of AgriSciences

Department of Animal Sciences

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Summary

With environmental conservation playing a growing role in decision making today, the aquaculture industry has sought new innovative ways to reduce the impact on the environment, and maximize efficiency. Much of the focus of such developments has included lower water usage and better feed utilization, with the emergence of biofloc technology systems addressing such needs. Tilapia has been identified as a fish for the future, with its production having already risen to make it an important food fish globally, and an increase in its market share is predicted going forward. Although it is common around the world, the production of tilapia has failed to take off in South Africa, with the reasoning being put forward including; legislative restrictions, unfavourable climate, incorrect species choice and shortage of expertise and supporting industry.

The major aims of this study were to address the main constraints and uncertainties facing the local tilapia industry in terms of species choice, and to investigate the biofloc technology concept under local conditions. Being restricted to the use of the Mozambique tilapia (*Oreochromis mossambicus*), and with the recent changes allowing the use of the Nile tilapia (*Oreochromis niloticus*), the question whether *O. niloticus* will in fact outperform *O. mossambicus* under South African conditions, was raised. With the production of the Three Spot tilapia (*Oreochromis andersonii*) also prevalent in southern Africa, this species was also included in the study. The secondary aim of the study was to create a profile of key water quality parameters during the start-up period of the biofloc technology system prior to fish being introduced, and throughout the course of the trial.

With the biofloc technology concept being expected to play a role in the development of the South African tilapia industry, the study was conducted in a biofloc technology system. Twelve tanks, connected through airlift pumps to homogenize the water quality, were stocked with fish. Each of the three species were stocked randomly into four tanks with each tank being considered an independent replicate. The growth trial was conducted within a greenhouse, and commenced at the start of the summer grow out period for tilapia in South Africa, in October 2014, running for 10 weeks. Fish were sampled fortnightly from the system, with the mass data being used to fit a linear regression. Water quality measurements were taken from the start-up period in mid-September, for 12 weeks.

Profiles for temperature, dissolved oxygen, pH, salinity, electro-conductivity, total dissolved solids, floc volume, total ammonia nitrogen, nitrite, nitrate, orthophosphate, total suspended solids and turbidity were successfully documented over the trial period. Two spikes in total ammonia nitrogen and nitrite were recorded, one during start-up, and one following a water loss event during the trial. A regression fitted to the mass data showed *O. niloticus* to have the highest growth rate with an average daily gain of 0.693±0.018 g/day, followed by *O. mossambicus* of 0.405±0.025 g/day, and then *O. andersonii* of 0.185±0.025 g/day, with significant difference between all three species. The lowest feed conversion ratio was also recorded for *O. niloticus* being 1.00±0.05, followed by *O. mossambicus* of 2.24±0.16, and *O. andersonii* with 2.53±0.28. These results suggest that of the three species investigated, *O. niloticus* performs best in a biofloc technology system in South African conditions. It is recommended that this species should therefore be used in biofloc technology systems as they develop in South Africa.

Opsomming

Omgewingsbewaring speel vandag ‘n toenemend groter rol in besluitnemingsprosesse. In hierdie lig is die akwakultuurbedryf op soek na nuwe, innoverende maniere om impak op die omgewing te verminder en doeltreffendheid te maksimeer. Heelwat van die fokus in sulke ontwikkelinge is op laer waterverbruik en meer effektiewe voerbenutting gebaseer. Met die ontwikkeling van biofloc tegnologie sisteme, kan sulke behoeftes aangespreek word. Tilapia is geidentifiseer as 'n vis vir die toekoms. Die produksie van tilapia is toenemend besig om te styg en is reeds 'n belangrike voedselbron wêreldwyd terwyl 'n verdere verhoging in markaandele voorspel word. Alhoewel die produksie van tilapia regoor die wêreld algemeen voorkom, het produksie nog nie werklik in Suid-Afrika posgevat nie. Die redes hiervoor is onder andere: wetlike beperkings, ongunstige klimaat, verkeerde spesie-keuses, 'n tekort aan kundigheid en gebrekkige ondersteuning van die bedryf.

Die hoofdoelwitte van die studie was om die belangrikste beperkinge en onsekerhede wat die plaaslike tilapia-bedryf in terme van spesie-keuse ervaar te identifiseer en om die biofloc tegnologie-konsep onder plaaslike omstandighede, te ondersoek. Beperk tot die gebruik van die Blou Kurper (*Oreochromis mossambicus*) en met die veranderinge wat die gebruik van die Nyl Kurper (*Oreochromis niloticus*) wettig gemaak het, was die vraag: Watter van of *O. niloticus* of *O. mossambicus* beter onder Suid-Afrikaanse toestande sal presteer. Die Driekol Kurper (*Oreochromis andersonii*), algemeen geboer in suidelike Afrika, was ook vir die studiedoeleindes ingesluit. Die sekondêre doel van die studie was om 'n profiel op te bou van die belangrikste waterkwaliteit-parameters vanaf die begin van die aanvangstydperk van die biofloc tegnologie systeem, voor vis ingesit was, en deurlopend tot aan die einde van die hele studie.

Met die konsep, wat verwag was om 'n belangrike rol te speel in die ontwikkeling van die Suid-Afrikaanse tilapia-bedryf, het die studie in 'n biofloc tegnologie sisteem plaasgevind. Vier herhalingtenks vir elk van die drie spesies (behandelings) was opgestel. Die aanwasproef het binne 'n kweekhuis plaasgevind. Dit het met die aanvang van die somer-uitgroei-tydperk in Oktober 2014 begin, en vir 10 weke geduur. Vismonsters was twee-weekliks geneem en die massadata was gebruik om 'n lineêre regressiekurwe op te stel. Waterkwaliteitmetings was vanaf die aanvangstydperk in middel-September 2014, vir 12 weke geneem.

Profiele vir temperatuur, suurstof, pH, soutgehalte, elektro-konduktiwiteit, opgeloste vastestowwe, floc volume, totale ammoniakstikstof, nitriet, nitraat, ortofosfate, totale gesuspendeerde vastestowwe en troebelrigheid was suksesvol gedokumenteer oor die proeftydperk. Twee uiterstes in totale ammoniakstikstof en nitriet is aangeteken: een aan die begin, tydens die aanvangstydperk, en ‘n ander na 'n waterverlies-geval tydens die proef. *Oreochromis niloticus* het die hoogste groeitempo van 0.693±0.018 g/dag gehad, gevolg deur *O. mossambicus* van 0.405±0.025 g/dag en dan *O. andersonii* van 0.185±0.025 g/dag, met 'n beduidende verskil tussen al drie spesies. Die laagste voeromsettingsverhouding van 1.00±0.05 was aangeteken vir *O. niloticus*, gevolg deur 2.24±0.16 vir *O. mossambicus* en dan 2.53±0.28 vir *O. andersonii*. Die uitslae dui daarop dat *O. niloticus* die beste

presteer in 'n biofloc tegnologie sisteem onder Suid-Afrikaanse toestande. Dit word dus aanbeveel dat *O. niloticus* gebruik moet word in biofloc tegnologie sisteme soos dit verder in Suid-Afrika ontwikkel.

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Dedication

I would like to dedicate this thesis to my father and mother, Philip and Michelle. Thank you for always challenging Michael and I, and doing all in your power to ensure the best for us. Thank you for stepping things up when we outgrew our surroundings. Thank you for an incredible foundation you built for us, and a stable home. You have allowed us to get to where we are today, and I cannot fault the way you brought us up. Thank you for identifying the importance of education, but also guiding me to understand that life is more than that. Thank you for allowing me to get to know God, and to develop a personal relationship with Him, that He may guide me, and use my talents. Thank you for ensuring that the base is set for me to think for myself and be effective in what I do. You have gone far beyond your call of duty to ensure that my life is a success beyond worldly terms, and I am humbled to be living it.

Notes

The language and style used in this thesis are in accordance with the requirements of the *Aquaculture International Journal*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has been unavoidable.

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Symbols and Abbreviations

ºC - Degrees Celsius

> - Larger than

% - Percentage

< - Smaller than

ANOVA – Analysis of Variance

BOD - Biological Oxygen Demand

BFT – Biofloc Technology

C:N - Carbon-to Nitrogen Ratio

CH – Carbohydrate

DO – Dissolved Oxygen

EC – Electro-conductivity

FCR - Feed Conversion Ratio

FV – Floc Volume

GMO - Genetically Modified Organism

HDPE - High Density Polyethylene

IA-N – Ionised Ammonium

LS – Least Squares (Mean)

MCCI - Microbial Community Colour Index

MSY - Maximum Sustainable Yield

Mt - Metric tons

NO_2 - Nitrite

NO_3 – Nitrate

NOB - Nitrite Oxidising Bacteria

NTU - Nephelometric Turbidity Units

P – Probability as a statistically significant limit

PO_4 – Orthophosphate

pp. - Pages

PVC - Polyvinyl Chloride

RAS - Recirculating Aquaculture System

SL – Standard Length

spp. - Species

TAN – Total Ammonia Nitrogen

TDS - Total Dissolved Solids

TL – Total Length

TSS - Total Suspended Solids

UIA-N – Un-Ionised Ammonium

USD – United States Dollars

1 Introduction

1.1 World Aquaculture

With declining wild fish stocks, aquaculture has risen globally to supply an estimated 70,500,000 Mt of food fish in 2013 (FAO 2014). Ninety-two point seven percent of the farmed fish in 2012 was produced in just 15 countries, with about 88 % of the production by volume taking place in Asia (FAO 2014). With this knowledge base gained in such areas, and the many areas with suitable conditions yet undeveloped, there is much room for the expansion of the industry into new regions.

In recent times between 2000 and 2012, the production of food fish by aquaculture has more than doubled (FAO 2014). Over the same period, Africa is the continent which has shown the highest growth rate of aquaculture production being 11.7 % annually (FAO 2014). Much of this growth is attributed to the development of the booming Tilapia industry in Egypt (El-Sayed 2013).

The global production of food fish is dominated by inland, freshwater fish culture, making up 57.9 % of production in 2013 (FAO 2014). Between 2000 and 2010, the mean annual growth rate for production in freshwater aquaculture systems was 7.2 % (FAO 2012). Of the freshwater species cultured, carps make up the major portion of around 72 % of production by volume, followed by tilapias and catfishes (FAO 2012).

1.2 Tilapia Production

Originating from Africa, tilapia has been deemed the fish of the 21st century (El-Sayed 2013), with a rise in its share of global production being imminent. Currently it is the most widely produced species around the world, with production being recorded in 135 countries (FAO 2014). The production systems used for tilapia culture are usually simple semi-intensive systems, with only basic techniques required for successful cultivation. Favouring warmer water, and being an easily produced species (El-Sayed 2013), tilapia shows great promise in the many developing countries located within warmer regions.

The hardy nature of the species is evident in the large range in environmental parameters tolerable by tilapia (Boyd 2004; Jamandre et al. 2011; Celik 2012). This trait is beneficial in the basic culture techniques practised, where there may be a lack in the availability of effective mechanisms for

water quality control and monitoring due to the nature of the systems. Being positioned at a low-trophic level with omnivorous feeding habits (Njiru et al. 2004; Fitzsimmons et al. 2011), tilapia is able to utilize a plant based diet effectively, and produce a versatile product well accepted by consumers (Brown et al. 2014; Andretto et al. 2015).

The role of such freshwater fish farming, identified as the fundamental mechanism for achieving food and protein security (FAO 2014), cements the place of tilapia in the future as a key species in developing countries. With large portions of the world's population living under the poverty line, particularly in developing countries holding potential for tilapia culture, the capacity for employing basic culture methods using tilapia is prevalent, and can potentially play a critical humanitarian role.

1.3 Progression of the Industry

There has been a significant rise in the prevalence of aquaculture since the 1970's when the fish supplied by capture fisheries begun to level off (Figure 1.1). Today, all rise in demand for seafood is supplied by aquaculture, with capture fisheries in a steady to declining state. The early methods used in aquaculture, particularly the salmon industry in Europe in the 1970's and 80's, were often considered highly detrimental to the environment (Tveterås 2002), creating a negative stigma still haunting the aquaculture industry today. The use of fishmeal and medicines, diseases, the escape of farmed fish, the enrichment of water through effluent, long term sustainability and the use of Genetically Modified Organisms (GMO's) are some of the issues which have brought aquaculture under public scrutiny (Hallerman and Kapuscinski 1995; Beveridge et al. 1997; Tovar et al. 2000; Tveterås 2002). Over time, as would be expected with any developing industry, many of these issues have been remedied, with research ongoing into developing more efficient culture mechanisms.

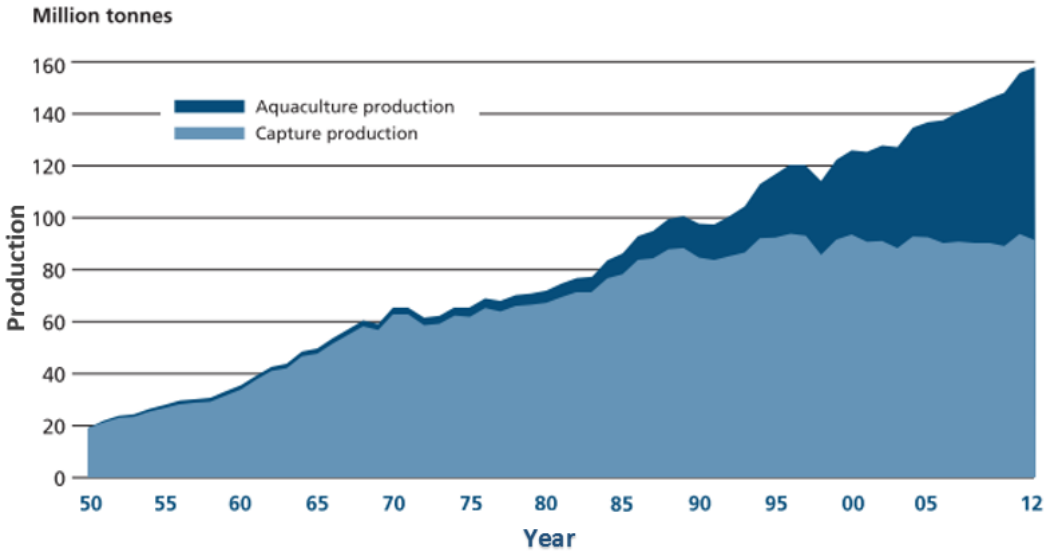

Figure 1.1. World production from capture fisheries and aquaculture over time, modified from *FAO (2014)*.

With the environmental impact of all industries becoming an ever increasing consideration, there has been a worldwide drive to go “green”, and ensure the sustainability of our practises. With aquaculture being a largely technology driven industry, new innovative ways of limiting the harm caused on the environment, are emerging. One such facet under intense investigation is the search for alternative protein sources to the traditionally used fishmeal, which is diminishing in supply (Carter and Hauler 2000; Millamena 2002; Naylor et al. 2009). Being able to replace fishmeal in the manufacture of aquafeeds, aids in relieving pressure on strained stocks supplying the fishmeal, as well as significantly decreasing the high cost of aquafeeds.

An exciting field emerging with a wide application which is ever broadening, is the use of micro-organisms. Due to the sheer diversity, and lack of sufficient knowledge (Zhou et al. 2009), the application of micro-organisms in our everyday lives is a largely unexplored field, virtually untapped. Although the role of micro-organisms has been known, and relied upon in aquaculture for centuries (Moriarty 1997), knowledge on the detailed mechanisms as to how they work, and their enhancement has been limited. It is no surprise that their potential impact on the aquaculture industry could be enormous. Rooted in innovation, aquaculture research has begun to look into developing a better understanding, and incorporating this field with vast potential into the aquaculture industry. The application of using micro-organisms to clean water has therefore emerged, with one facet being that of biofloc technology (BFT).

1.4 Biofloc Technology

Biofloc technology research in the USA and Israel begun in the late 1980's and early 90's with the focus being on limiting water and land usage, as well as reducing the environmental impact of aquaculture practises (Emerenciano et al. 2013; Hargreaves 2013). The concept enhances the action of predominantly heterotrophic bacteria, through raising the carbon to nitrogen ratio (C:N) within the water of the culture environment, by adding an organic carbon source (Avnimelech 2012). The water is well-aerated and vigorously mixed to ensure that heterotrophic bacteria proliferate, with the nitrogenous compounds, excreted by the fish and excess feed, being the limiting factor to the growth of the heterotrophic community (Avnimelech 2012; Hargreaves 2013). The mixing plays an important role in keeping the coagulations of micro-organisms, referred to as bioflocs, in suspension, and therefore prevents anoxic conditions from developing on the pond/tank bottom (Avnimelech 2012; Hargreaves 2013). By successfully implementing a BFT system, the benefit is two pronged. Firstly, dangerous nitrogen species are eliminated from the water *in situ*, therefore avoiding the need for filtration, and allowing much higher stocking densities to be achieved than are observed in extensive systems (Avnimelech 2012; Hargreaves 2013). Secondly, nutrients are recycled from the waste and incorporated into microbial protein by the bioflocs, which can be harvested by filter feeding fish, such as tilapia, and shrimp, significantly reducing their usage of conventional feed, and enhancing their growth rate (Avnimelech 2012; Hargreaves 2013). Water usage is low as the efficiency of the whole system is enhanced, with environmental impact being reduced (Hargreaves 2013). With such benefits, it is no surprise that large scale BFT systems have been set up across Asia, Latin America as well as Central America, with the smaller scale greenhouse systems having an even broader reach (Emerenciano et al. 2013).

1.5 South African Finfish Aquaculture Industry

With catch fisheries having provided the vast majority of fish up to this point, South Africa is entering a phase where this source is reaching its maximum sustainable yield (MSY) (DAFF 2013). If exploitation continues, without catch being limited at the level which is currently exploited, a collapse in the fish stocks will be induced as has been seen extensively around the world. The developing gap between the seafood supplied by wild catch fisheries, and that demanded by consumers, needs to be

filled by aquaculture (DAFF 2013). With aquaculture successfully growing to fill this gap around the world, the same looks probably in South Africa (DAFF 2013).

With the rest of the world having put extensive effort in developing technology and refining species to where they are today, the knowledge transfer and adoption of these modern methods is imperative. The importance of testing such systems in our local conditions and circumstances is equally crucial to prevent failure and ensure their viability (DAFF 2013).

With government realizing that the development of a local aquaculture industry is imminent, recent legislative changes have occurred in order to smooth the path for such development (DAFF 2013). One being changes in the categorization of Nile tilapia, *Oreochromis niloticus*, from being a prohibited species, to allow its use in certain biosecure aquaculture systems (DEA 2014). This comes as a great victory to the emerging local tilapia industry who have struggled with using the indigenous Mozambique tilapia, *Oreochromis mossambicus*, up to this point.

1.6 Rationale

Given the current situation in South Africa, and through extensive research into the current questions being asked by local tilapia industry stakeholders, a study was designed to probe the BFT concept under local conditions, and address a question which is being widely asked: will *O. niloticus* live up to what has been speculated, and actually outperform *O. mossambicus* in our local conditions? This study was intended to be a baseline starting point from which other studies could stem and elaborate on.

Given the current drive for green development in South Africa, and with excessive water use, feed wastage and environmental degradation often being associated with aquaculture practises (Emerenciano et al. 2013), the modern BFT concept shows great potential to be employed at a grass roots level in developing the local tilapia industry. Additionally it is in line with major government and humanitarian obligations, to address hunger, malnutrition and food security in an environmentally friendly way, by providing a good, cheap source of protein to impoverished communities (Celik 2012; FAO 2014). Being a new concept in South Africa, the study aimed to explore and proof test the BFT concept in local conditions, looking predominantly at water quality and growth rates of tilapia. It aimed to give interested parties and future farmers an idea of what to expect when employing the concept, as

well as create a pioneering platform for future research to adjust and customize the concept to better suit our circumstances.

With the culture of the Three Spot tilapia, *Oreochromis andersonii*, gaining traction in its naturally distributed regions in Zambia, and given its availability to the researcher, it was incorporated into the study. Although the three species investigated fill similar niches in their geographically separate natural distributions (Skelton 2001), it is speculated that they may feed at slightly different trophic levels. This speculation is supported by diet differences between the species (Popma and Lovshin 1996; Skelton 2001; Winemiller and Kelso-Winemiller 2003; Njiru et al. 2004). If one species were to be more suited to the utilization of biofloc in the diet, and therefore show higher growth rate and lower feed conversion ratio (FCR) than the other species, it would intuitively be the preferred species for use in such systems. Another dimension added to the frame is that of different levels of selection and breeding experienced between the three species used. It was therefore decided to use what was available to the researcher, and mimic the circumstances which will be faced by a commercial farmer in the same region. For a species which has experienced less strain development for aquaculture purposes to be considered for use in a commercial system, it would need to gain notably more benefit from the biofloc than the most developed species, in order to show higher growth rate and a lower FCR. With no previous work encountered by the researcher on this comparison in a BFT system, the study aimed to investigate which species would indeed be best suited for use in a BFT system in a South African context.

1.7 Aims

- To act as an initial study into tilapia BFT systems in South Africa, aiding in proof testing the concept in local conditions.
- To create a platform from which further research may stem into adjusting and customizing the BFT concept to better suit our local South African conditions.
- To give interested parties and future farmers an idea on what to expect from BFT systems in terms of water quality and tilapia growth rates.
- To map a profile of key water quality parameters over a 10-week period.
- To determine which species of tilapia is best suited for BFT systems in South Africa.

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2 Literature Review

2.1 Global Tilapia Production

Historical Overview

The origin of tilapia culture allegedly lies some 4000 years ago, 1000 years prior to carp culture first being introduced in China (Balarin and Hatton 1979). Apart from depictions in ancient Egyptian tombs and biblical references, very little is known about the culture of tilapia in these early ages (El-Sayed 2006b; Mjoun et al. 2010). In 1924 the first culture of tilapia was seen taking place for scientific purposes in Kenya (Gupta and Acosta 2004). Thereafter Courtenay and Williams (1992) reported the first non-native establishment of tilapia on the Java island of Indonesia in the 1930's, recorded as an aquarium release. Throughout World War II from 1939-1945, introduction of tilapia occurred in numerous pacific islands as it proved to be a convenient, and easily transported source of protein for Japanese soldiers (Popma and Lovshin 1996; Boyd 2004).

This was the start of the widespread development of the industry, and by 1950 seven countries were reported to be producing tilapia (Fishstat.Plus 2004; El-Sayed 2006b). This figure had risen to 12 countries by 1969, with production at 24, 633 Mt, making up 0.76 % of total production by aquaculture (Fishstat.Plus 2004; El-Sayed 2006b). At this time the concept of aquaculture as a food production industry was still unfamiliar in most countries, and throughout the 1960's and 70's international aid and development agencies endorsed aquaculture as a protein production method, promoting food security in developing countries without having the environmental troubles accompanying conventional agriculture (Canonico 2005; El-Sayed 2006b).

There was a continual growth and increased interest in the production of tilapia, and by 1990 global annual production was 383,654 Mt, making up 2.28 % of the total production by aquaculture (Fishstat.Plus 2004; El-Sayed 2006b). From 1970 until 1990, the average growth of tilapia production was 14.2 % annually (Figure 2.1), with the number of countries producing tilapia rising to 78 (Fishstat.Plus 2004; El-Sayed 2006b). This rapid expansion of tilapia production continued through the next decade with new and improved techniques being developed. By 2002 global annual production was initially reported to have risen to 1,505,804 Mt at an annual growth rate close on 12.2 % (Fishstat.Plus 2004; El-Sayed 2006b). This is similar to the 1,499,000 Mt reported by Josupeit 2005. These figures were however changed in 2008 when China revised its production statistics and

subsequently reduced its initial historical figures by around 13 %, which resulted in the figure for global annual production being reduced to 1,418,816 Mt for 2002 (FIGIS 2015a). By 2010 this production had more than doubled and was at 3,200,000 Mt (Fitzsimmons et al. 2011). This estimation for 2010 is however slightly lower than reported by the FAO (2015) at 3,496,165 Mt. Variation in estimation technique and model used to arrive at this global production figure for tilapia may explain this difference. By 2012 this rapid growth of global tilapia production was still ongoing and production was reported to be 4,507,002 Mt (FIGIS 2015a). The global annual production of tilapia from 1991-2013 showed good growth (Figure 2.2). For the decade preceding 2012, global tilapia production by aquaculture had therefore risen at an average rate of 14.5 % per annum. As the quantity produced increased, so did the value of the tilapia aquaculture sector.

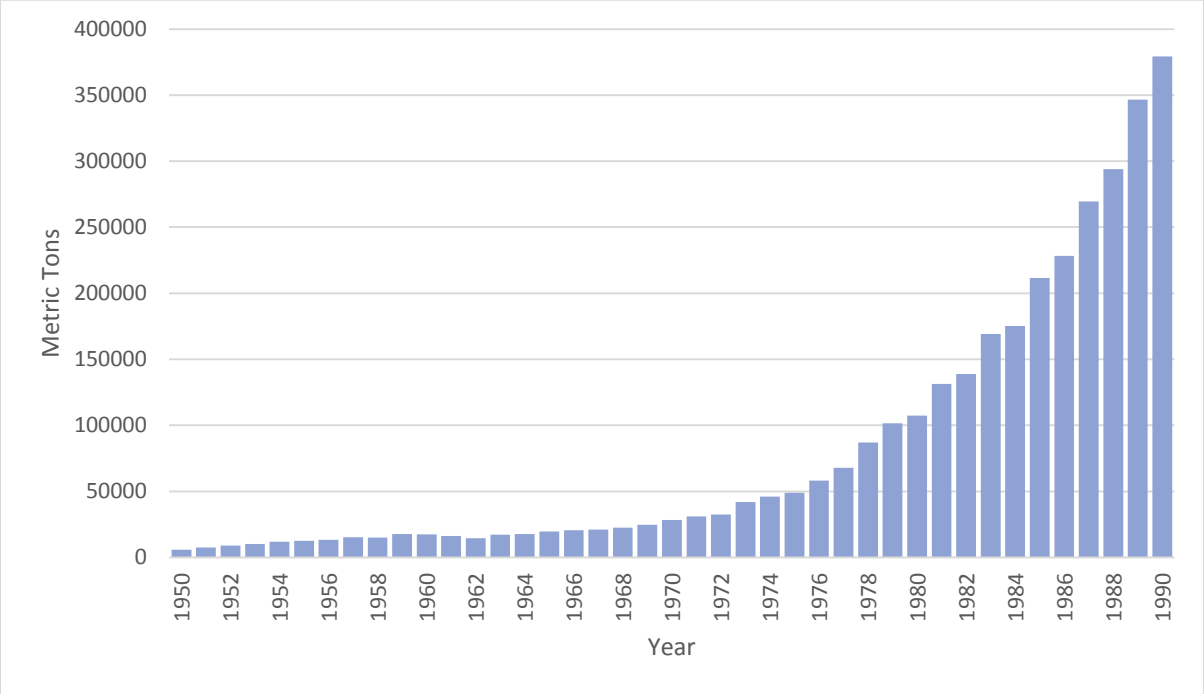

Figure 2.1. The global annual production of tilapia in Metric Tons from 1950-1990 compiled with data obtained from *FAO (2015b*).

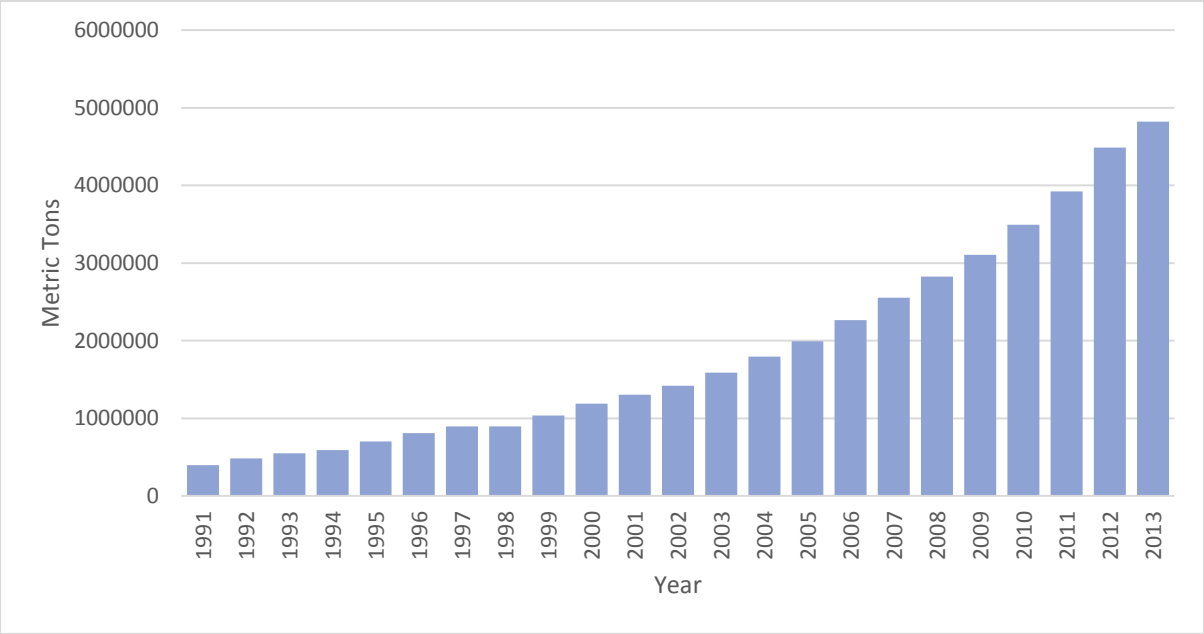

Figure 2.2. The global annual production of tilapia in Metric Tons from 1991-2013 compiled with data obtained from *FAO (2015b*).

Economics and International Trade

From 2002 until 2012, FAO (2015) reported that the value of the global tilapia sector had grown from 1,696,522,000 United States Dollars (USD) in 2002 to 7,656,257,000 USD in 2012. There was significant growth in the value of the global tilapia aquaculture sector from 2002 to 2013 (Figure 2.3). The production from China made up 30 % of this value in 2012, having decreases from holding 36 % in 2002 (FIGIS 2015a).

The majority of tilapia produced is consumed locally within the country in which it is cultured (El-Sayed 2006b). International trade of tilapia products is however growing, with the United States of America (USA) being the largest importer of tilapia (Mjoun et al. 2010). In 2014, the USA imported a total of 230,738 Mt of tilapia, of which 164,992 Mt was in the form of frozen fillets (ERS 2015). Imports into the USA from China totalled 186,349 Mt or 81 % of the total tilapia imported into the USA in 2014 (ERS 2015). The value of tilapia imports into the USA for 2014 stood at 1,114,646,000 USD having risen steadily over the past decade (ERS 2015).

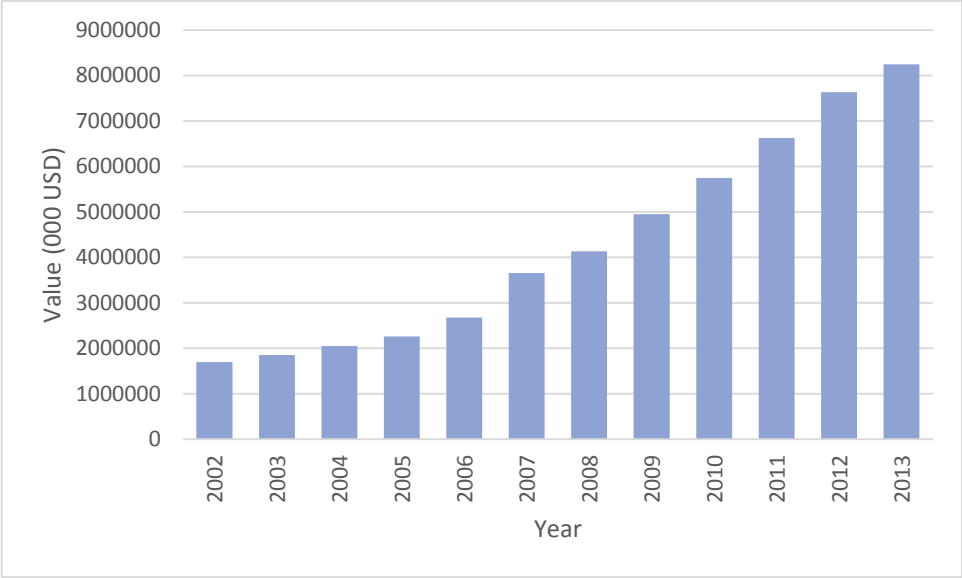

Figure 2.3. Illustrates the value of the global tilapia aquaculture sector from 2002-2013 compiled with data obtained from *FAO (2015b*).

Production by Region

Tilapia has been introduced to many countries around the world because of its important role in aquaculture, with its culture becoming widespread throughout Asia particularly. In 2002, China showed a production of 611,165 Mt, making up 43 % of global tilapia production alone (FIGIS 2015a). Asia as a whole accounted for 1,083,849 Mt in 2002 which equates to 76 % of tilapia produced globally (FIGIS 2015a).

In the decade proceeding 2002, tilapia production had increased in areas outside of Asia, which by 2012 held a decreased 69 % of the global share of tilapia production. The share held by China was also decreased to 34 % in 2012 (FIGIS 2015a). Over the same period, the Americas had increased production of tilapia from 140,302 Mt per annum or 9.9 % to 463,738 Mt, equivalent to 10.3 % of global production in 2012 (FIGIS 2015a). Tilapia production in Africa had also grown substantially over the same period from making up 13.7 % or 193,981 Mt of global production in 2002 to 919,017 Mt equating to 20.4 % in 2012 (FIGIS 2015a). This production is largely credited to Egypt which showed 11.8 % of global tilapia production in 2002 and 17.1 % by 2012 (Figure 2.4) (El-Sayed 2013; FIGIS 2015a).

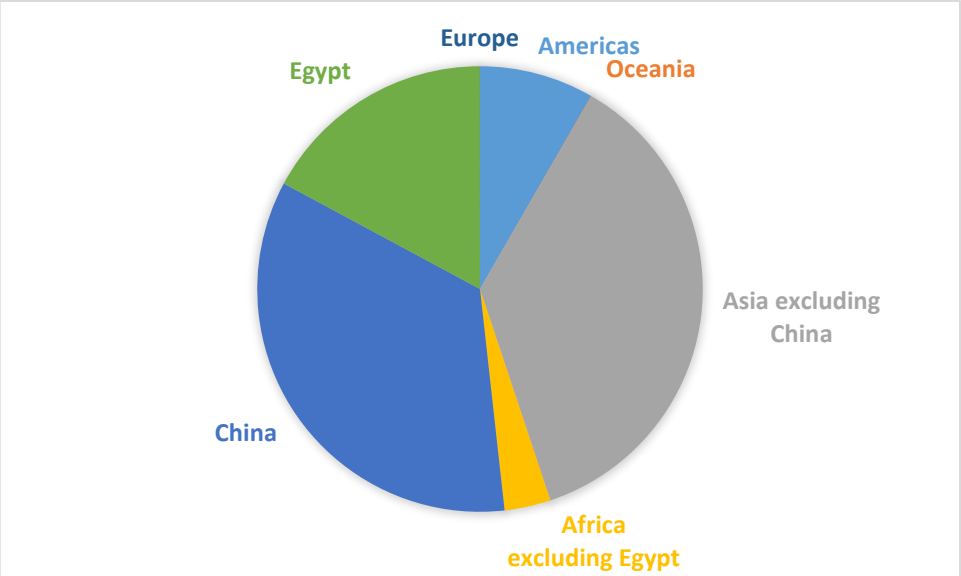

Figure 2.4. Global tilapia production by region for 2012 *(FIGIS 2015b)*.

By 2012, the culture of *Oreochromis niloticus* made up a share of 71 % or 3,197,330 Mt of the 4,507,002 Mt of total tilapia and other cichlids produced (Figure 2.5) (FIGIS 2015a). This share is slightly lower than the 72 % reported by Fitzsimmons (2000) and the 74 % reported by the FAO (2015a) for the year 1995. It shows that over the 17 year period preceding 2012, the share of *O. niloticus* had decreased slightly even after holding a 79 % share in 2002 (FIGIS 2015a). *Oreochromis aureus* showed a total production of 4,995 Mt for the year 2012 (Figure 2.5), having decreased its share of 0.24 % in 2002 to 0.11 % in 2012 (FIGIS 2015a). The development of the *O. niloticus x O. aureus* hybrid and the increase of its use in commercial aquaculture may have played a crucial role in decreasing the share of these two individual species by 2012. *Oreochromis niloticus x O. aureus* depicted a total production of 388,139 Mt for the year 2012, making up a share of 8.6 % of total tilapia production (Figure 2.5) (FIGIS 2015a). *Oreochromis mossambicus* production dropped significantly from its 2002 figures, and was reported to be 24,385 Mt in 2012 making up only 0.54 % of global tilapia production (Figure 2.5) (FIGIS 2015a). Its use in Africa however increased from a low 130 Mt in 2002 to 1,440 Mt in 2012 (FIGIS 2015a). The production of *Oreochromis andersonii* was still relatively low in 2012 recording 4038 Mt produced with a decreased share of 0.090 % of total tilapia production (Figure 2.5) (FIGIS 2015a). Its use in aquaculture had however increased significantly from 2002, and all production recorded in 2012 came from the African continent (FIGIS 2015a).

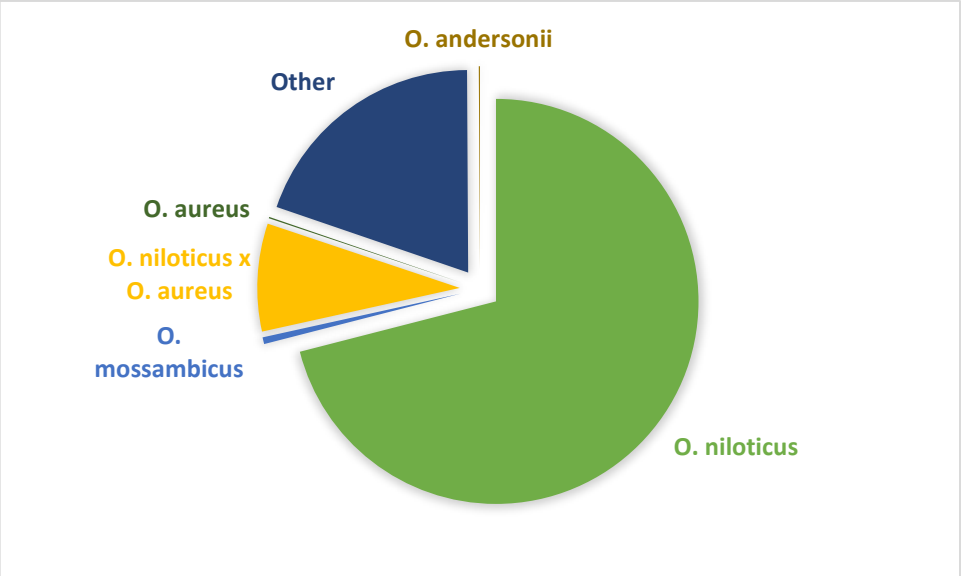

Figure 2.5. The tilapia species comprising total tilapia production by aquaculture in 2012 *(FIGIS 2015a)*.

2.2 Biofloc Technology (BFT)

History

The first research into the use of BFT allegedly started in the early 1970's by Ifremer-COP (French research institute for exploitation of the sea, oceanic centre of pacific) using shrimp, with the concept being likened to an external rumen (Cuzon et al. 2004; Emerenciano et al. 2012b). In 1980 the Ecotron science program was started by Ifremer to probe and better the understanding as to how such systems work (Emerenciano et al. 2013). Research was initiated in the late 1980's and early 1990's by Israel and the USA into the same concept, but using tilapia, with focus on water limitations, land costs and environmental concerns being the driving force (Emerenciano et al. 2013). The first commercial application of the concept is reported in 1988 at the Sopomer farm in Tahiti as well as the Belize Aquaculture farm where much of the expertise for running shrimp biofloc systems was developed (Emerenciano et al. 2013). Today, BFT systems are used on a commercial basis throughout the world, with tilapia and shrimp being the major focus.

Overview

BFT involves an unconventional way of thinking which deviates from the common mind-set that clear water is best for aquaculture (Avnimelech 2012; Hargreaves 2013). It enhances the use of a microbial community to produce a supplementary microbial food source, high protein bioflocs (a coagulation of micro-organisms), which can be utilised by species capable of filter-feeding (Avnimelech

2012). Sufficient aeration and mixing is required to keep the floc in suspension in the water, which allows the water quality to be controlled (Hargreaves 2013). The microbial community, predominantly heterotrophic bacteria within the water act as a biofilter, resulting in nitrogen uptake and reducing ammonia (NH_4) levels faster and more efficiently than the nitrification process (Avnimelech 1999; Crab et al. 2012). Biofloc systems have very low water exchange, and thus have a significantly lower impact on the surrounding environment through lower water requirements and minimal to no effluent water discharge (Avnimelech 2007; Avnimelech 2012; Hargreaves 2013). Degradation of the environment is further reduced by the lower amount of pelleted feed needed and the efficiency in recycling nutrients from the fish waste and uneaten feed (Hargreaves 2013).

Carbon Addition

The carbon to nitrogen ratio (C:N) plays an integral part of BFT systems (Hargreaves 2013). It is this ratio which controls the proliferation of heterotrophic bacteria, which are generally limited by the availability of organic carbon, and nitrifying bacteria which are not (Avnimelech 1999; Michaud et al. 2006; Emerenciano et al. 2013; Hargreaves 2013; Luo et al. 2014). By increasing the C:N ratio, heterotrophic bacteria flourish, and ammonia is taken up from the water and converted into microbial biomass, containing protein (Hargreaves 2013). The critical point of this ratio where heterotrophic bacteria will outcompete nitrifying bacteria is variable with different organic carbon sources (Michaud et al. 2006). Luo et al. (2014) put forward that in a BFT system the C:N ratio should be maintained at >10:1 with Hargreaves (2013) suggesting that it should be closer to 12-15:1 to support the heterotrophic pathway. The lack of consensus on this point is further compounded by Emerenciano et al. (2013) suggesting that the optimal ratio of C:N lies from 15-20:1. It is therefore evident that there is no exact “golden ratio”, and that there is agreement in that the ratio should be elevated by the provision of an additional organic carbohydrate (CH) source.

In the case of emergency where total ammonia nitrogen (TAN) levels are high, Avnimelech (2012) suggests that 20 mg/L CH should be added to reduce TAN levels by one milligram per litre. The addition of CH in BFT systems is closely linked to the feeding rate and protein content, or more specifically nitrogen content of the feed (Hargreaves 2013). Hargreaves (2013) put forward that for every one kilogram feed of 30-38 % protein added to the water, a CH source of 0.5-1 kg should also be added to maintain the high C:N ratio. As protein is comprised on average of 15.5 % N, and fish excrete

as much as 75 % of N from the feed as NH_4 (Avnimelech 2012), the amount of feed can be inserted along with the calculated %N content of the feed expressed as a decimal, into the equation presented by Avnimelech (1999) and Avnimelech (2012) below to calculate the amount of CH addition needed:

$$\Delta CH = \Delta Feed \times \%N \; in \; feed \times \%N \; excretion \div 0.05$$

In practise, the choice of an organic carbon source is dependent on the availability of a cheap carbohydrate near to where the BFT system is located (Emerenciano et al. 2013). Therefore, a range of organic carbon sources such as wheat bran (Emerenciano et al. 2011; Emerenciano et al. 2012a), molasses (Burford et al. 2004; Panjaitan 2004; Samocha et al. 2007), glucose (Crab et al. 2010), cellulose (Avnimelech et al. 1989), cassava meal (Avnimelech and Mokady 1988; Chen et al. 2015), sorghum meal (Avnimelech et al. 1989; López-Elías et al. 2015), wheat flour (Azim and Little 2008; Xu et al. 2012) and corn/maize meal (Milstein et al. 2001; Asaduzzaman et al. 2010; Xu et al. 2012; Kurup 2015) can be used. The organic carbon source is linked to the feeding rate and is usually added to the water once, or twice a day where practically possible, often after feeding has taken place (Avnimelech 1999; Azim and Little 2008; Xu et al. 2012). The practical application and specific routine of adding an organic carbon source is dependent on the nature of the BFT system used.

Production Systems

Tilapia can be cultured in freshwater, brackish-water as well as seawater environments (El-Sayed 2006b). This range of salinities allows for the use of a diverse number of different culture methods to be employed. The majority of production is however in freshwater environments which was the preferred salinity for 83 % of global tilapia production in 2012 (FIGIS 2015a).

As tilapia is a relatively low-value finfish when compared to other cultured species such as salmonids or shrimp, low-cost production is key to financial success in most regions of the world (Edwards 2000; Celik 2012; FAO 2012). The contribution of aquaculture to the livelihoods of impoverished communities is large, as explored by Edwards (2000), and may play an increasing role in rural development in the future. By providing these livelihoods, the importance of tilapia on a subsistence scale to human welfare as well as growing the sector should not be underestimated. These circumstances, coupled with tilapia being a hardy fish, largely resistant to stress and tolerant of a wide

spectrum of environmental conditions, lead towards very basic culture methods being most effective (El-Sayed 2006b; Celik 2012; El-Sayed 2013).

Semi-intensive culture systems are the most common for small scale producers in developing countries (Celik 2012). The prevalence of this intensity for the production of tilapia in China was also reported by El-Sayed (2006). Additionally it was noted that as global production rises, a shift to become ever more intensive in our culture practises is evident (El-Sayed 2006b). This shift does however demand more hands-on management and increasing the use of innovative technology in aquaculture systems, but does not necessarily hold the key to the further development of the tilapia industry within developing countries in particular (El-Sayed 2013). Semi-intensive culture makes use of both the natural productivity of the pond as well as supplementary feed, positioning itself midway between the two extremes of intensive and extensive culture methods (El-Sayed 2006b).

Biofloc Technology Systems

The semi-intensive application of commercial scale BFT operations means that they are mainly exposed to light and employ only a few system types, namely; plastic lined ponds or tanks, and raceway systems in greenhouses (Hargreaves 2013). Pond sizes are generally 0.5-1.5 ha in size and are based on specifications used in early research at the Waddell mariculture centre, being lined with up to a 1 mm thick high density polyethylene (HDPE) plastic (Gupta and Acosta 2004; Emerenciano et al. 2013; Hargreaves 2013; Green et al. 2014). In commercial BFT ponds, Hargreaves (2013) suggested that aeration and mixing is mainly conducted by paddlewheel aerators at a rate of 28-32 hp/ha (20.9-23.9 kW/ha). This is slightly less than the 50 hp/ha (37.3 kW/ha) used by Burford et al. 2004 in Belize. Avnimelech (2012) suggested that aeration can range from 6.7 hp/ha (5 kW/ha) to more than 134 hp/ha (100 kW/ha) with an effective approximate being one kilowatt per 500 kg of fish or shrimp. The positioning and rotation of aerators within a pond is important to prevent sludge collecting in calm areas where anaerobic conditions may develop (Avnimelech 2007; Avnimelech 2012; Hargreaves 2013). Commercial BFT systems may have zero or minimal water exchange taking place, where sludge is drained (Burford et al. 2003; Avnimelech 2007; Widanarni et al. 2012; Emerenciano et al. 2013).

Research systems tend to encompass a wider range of smaller systems both outdoors and indoors, and in cases where the system is indoors, temperatures can be more controlled and the system not exposed to light (Azim and Little 2008; Hargreaves 2013). These systems are more easily monitored

and controlled, often making use of a compressor/blower to ensure aeration and sufficient mixing through air diffusers (Azim et al. 2008; Azim and Little 2008; Green et al. 2014).

As BFT systems are often situated in areas of water scarcity, the water used in these systems is limited, and biosecurity issues have driven the minimal to zero water exchange approach (Hargreaves 2013). BFT has therefore been seen as an environmentally friendly aquaculture technique, with sustainability at its core (Widanarni et al. 2012). Before stocking, BFT systems are usually prepared for a number of weeks where the water may be seeded with biofloc, and is fertilized with organic matter to allow the biofloc time to establish in the water (Avnimelech 2012; Hargreaves 2013). Ekasari et al. (2014) reported a three week period of preparation until total suspended solids (TSS) exceeded 500 mg/L and the trials commenced. This is in line with a few weeks suggested by Avnimelech (2012). Water quality parameters fluctuate throughout this establishment period and through the grow-out phase of the cultured species.

Water Quality

The importance and monitoring of water quality is well known in aquaculture, and no different in BFT systems (Azim and Little 2008; Prajith and Madhusoodana 2011; Widanarni et al. 2012; Crab et al. 2012; Liu et al. 2014). As well as being of interest to other researchers, a profile of different water parameters in a BFT system within a particular region can serve as a guideline to farmers in the area in what to expect in their systems. With the concept of BFT being new in South Africa, and no commercial BFT facilities yet, it is research such as this which will aid the development and understanding of the BFT concept and its implementation. A range of different parameters are measured and recorded over different time frames. The measurement of some parameters may be time consuming, depending on the method used, and needs to be weighed up against the importance of the parameter, in order to find out how often it should be measured. Azim and Little (2008) reported measuring temperature, dissolved oxygen (DO) and pH twice weekly in their BFT research system. These parameters were measured more frequently by Widanarni et al. (2012) and Liu et al. (2014) who took measurements once a day before feeding in the morning. These regimes are similar to the DO, Temperature and floc volume (FV) measurements taken daily by Avnimelech (2007). There is however evidence to suggest that there is fluctuation in these parameters over the course of a day (Neori et al. 1989). Crab et al. (2009) looked into this by taking DO and temperature readings every three hours,

and found that the readings taken at 7am were representative of the average DO and temperature throughout the 24 h period. Other parameters such as TAN, NO_2, NO_3 and TSS are often monitored weekly (Azim and Little 2008; Liu et al. 2014), or bi-weekly (Prajith and Madhusoodana 2011; Widanarni et al. 2012). As water quality measurements in BFT research systems are usually taken to ensure the wellbeing and safety of the fish, it would be of value to create a "typical" water quality profile or band for each parameter over the grow-out cycle, and give farmers an indication of where their water quality should be at any particular time.

Crab et al. (2009) reported that temperature is optimal for tilapia growth from 25-28 ºC, which is within the range of 25-30 ºC reported by El-Sayed (2006b) who also indicated that normal growth is experienced down to 20 ºC. Temperature in a BFT system cannot be easily controlled due to the outdoor nature, making BFT systems dependent on climatic variables in the chosen area (De Schryver et al. 2012). With minimal water exchange and large volumes in BFT systems, heat can be relatively well conserved within the water body and act to buffer the air temperature (Crab et al. 2009).

Popma and Lovshin (1996) along with Ebeling et al. (2006) and Avnimelech (2012) identified DO levels as the major limiting factor in increasing production for intensive aquaculture systems. It is therefore a fundamental need for organisms being cultured, and should be efficiently supplied and constantly monitored. It is common knowledge that oxygen is more soluble in cooler water and DO levels are therefore generally higher when water is cooler (Swann 1997; El-Sayed 2006b; Avnimelech 2012). It is intuitive that DO levels are also affected by the biological load within the water body which consumes DO through respiration (Hargreaves 2013). As DO levels are fundamental to respiration of both the cultured species and the micro-organisms making up the suspended floc, the demand for DO is elevated in BFT systems to as high as 5-8 mg O_2/L/h (Hargreaves 2013). The response time in the event of system failure is thus often less than one hour (Hargreaves 2013). Popma and Lovshin (1996) indicated that tilapia show a higher tolerance to low DO levels than other cultured species, and are able to withstand DO levels lower than 0.5 mg O_2/L. El-Sayed (2006b) claimed that they can even tolerate levels lower than this, and make use of surface air when DO is at zero mg O_2/L. It is however best to keep levels higher than 2-3 mg O_2/L to limit stress on the tilapia (Popma and Lovshin 1996). Avnimelech (2012) suggested that DO levels are best at above four milligrams O_2 per litre for BFT systems.

Oreochromis niloticus can tolerate a pH range from 4-11 (Balarin and Hatton 1979; Wangead et al. 1988). The best growth in tilapia is seen at pH levels which are neutral or slightly alkaline (Popma and Lovshin 1996). Acidic conditions tend to reduce the natural productivity of a pond, and would therefore have an effect on the growth rate of tilapia (Popma and Lovshin 1996). Widanarni et al. (2012) found that pH was relatively stable from 6.3-7.5 for BFT tanks, unlike the fluctuations observed in their control tanks without biofloc. The pH range observed by Azim and Little (2008) in BFT trials was greater and spanned from 5.0-8.5. Avnimelech (2012) put forward that the optimal pH range for BFT systems is 7-9. pH is related to nitrification and fluctuations are observed throughout a day (Avnimelech 2012).

FV is measured through the use of an Imhoff Cone, where one litre of water is left to stand for a set amount of time, usually 10-20 minutes, before the floc volume settled towards the apex of the cone is measured in mL/L (Hargreaves 2013). This simple method gives a good indication of the level of biofloc in the water. Hargreaves (2013) suggested that FV for tilapia should be maintained at 25-50 mL/L, which is lower than the level of up to 100 mL/L put forward by Avnimelech (2012).

Electro-conductivity (EC) is a measure of the amount of nutrient dissolved in the water and is linked to the pH, hardness, salinity as well as the total dissolved solids (TDS) (Roets et al. 1999; De Schryver et al. 2008; Rahman 2010). A study conducted by Iqbal et al. (2012) showed significant correlation between EC and the growth of *O. niloticus,* although it may be more closely linked to pH. Fluctuations in EC levels are often as a result of water loss/change, liming to raise pH, and higher feeding rate which therefore often elevated the EC throughout a growout period (Roets et al. 1999). Considerably higher levels on EC were recorded in a BFT system by Rahman (2010) when compared to other intensive systems, due to low water exchange and elevated total suspended solids (TSS) levels.

El-Sayed (2006b) included dissolved solid metabolites among the most important factors which determine the success for intensive cultivation of tilapia. TDS is generated by the bacterial degradation of faecal materials as well as leaching from feed (Rafiee and Saad 2005). It is this organic matter as well as the microbial community degrading it which raises the TDS as well as TSS measurement (Rafiee and Saad 2005). Lack of water exchange also leads to elevated TDS levels in aquaculture, which can be potentially toxic to certain cultured species when >2000 mg/L (Rafiee and Saad 2005). TDS levels have not been well documented in BFT systems, and it is speculated that it would be kept within

tolerable levels due to the harvesting of biofloc by the cultured species. Boyd (2000) stated that there is a strong correlation between TDS and salinity which share very similar concentrations in natural waters.

Tilapia, and particularly *O. mossambicus,* are able to handle highly saline waters (Popma and Lovshin 1996). Much of the tilapia cultured around the world is done in brackish water (Legendre and Ecoutin 1989; Suresh and Lin 1992). BFT is practised in a range of water salinities, with studies concluding that the salinity level has no major effect on the bacterial growth or other nutritional parameters (fatty acid profile, crude protein, crude lipid, ash) which were similar at different salinities (Del Giorgio and Cole 1998; Nielsen et al. 2003).

Nitrogen species are commonly measured in aquaculture, and they are often considered to be the primary limiting factor to the survival of the cultured organism (Avnimelech 1999; Tseng and Chen 2004; Crab et al. 2007; Barbieri 2010; Xian et al. 2011; Santacruz-Reyes and Chien 2012). Ammonia originates from uneaten feed, along with the majority coming from the cultured organism, as it is a final product of protein metabolism (Avnimelech 1999; El-Sayed 2006b; Crab et al. 2007). Ionised ammonium (NH_4^+ or NH_4^+–N or IA–N) and un-ionised ammonia (NH_3 or NH_3–N or UIA-N) levels are in equilibrium, being reliant on the pH and temperature of the water (Timmons et al. 2002). These two forms together make up TAN, possibly the most critical inorganic nitrogenous species of interest to the aquaculture industry. It is however the UIA–N which is the most dangerous of the two to cultured organisms (El-Shafai et al. 2004; El-Sayed 2006b). In most aquaculture systems, IA–N is oxidised by nitrifying bacteria to the highly toxic nitrite (NO_2) and then on to the much less toxic nitrate (NO_3) species (Avnimelech 1999). In a closed intensive aquaculture system, these nitrogen compound levels can accumulate until toxic levels are reached, and mortalities occur, as there are few effective mechanisms for their removal (Avnimelech 1999). The nitrification process with an end product of the less toxic NO_3 is therefore facilitated, along with water exchange to remove the enriched water (Avnimelech 1999; El-Sayed 2006b). In a BFT system, an alternative pathway is encouraged for the removal of dangerous nitrogen compounds from the water, and makes use of the action of predominantly heterotrophic bacteria (Figure 2.10). Uptake by algae, as well as nitrification, do still play variable roles in the immobilization of TAN from the water body, but their action is overshadowed by that of the heterotrophic bacteria (Hargreaves 2013). At low pH, the majority of the TAN is stored in the water as IA–N, which is

therefore less toxic to the fish (Avnimelech 2012). The level of toxicity of TAN is also elevated at low DO levels (Avnimelech 2012). TAN, which is commonly measured, can be used to calculate the UIA–N level described by El-Shafai et al. (2004) using the general equation of bases illustrated by Albert (1973):

$$\frac{[NH_3 + NH_4^+]}{[1 + 10^{(pK_a - pH)}]}$$

With pKa in freshwater being calculated by the formula presented by Emerson et al. 1975:

$$pK_a = 0.09018 + \frac{2729.92}{T}$$

where T is measured in Kelvin which is 273 + T ºC.

Tilapia can tolerate UIA–N up to two milligrams per litre (El-Shafai et al. 2004; Avnimelech 2012). Other reports however suggest that the 48 hour median lethal concentration (LC50) is much higher, with Ça and Köksal (2005) reporting LC50 of 7.40 mg/L for *O. niloticus* fingerlings, and Daud et al. (1988) finding LC50 to be 6.6 mg/L for *O. mossambicus x O. niloticus*. Although higher levels can be tolerated, this tolerance is coupled to a certain exposure time period and compromised growth, which would therefore direct tilapia farmers to keep UIA–N well below this level. The toxic level for UIA–N is as low as 0.14 mg/L and it should be maintained below 0.1 mg/L (El-Shafai et al. 2004). NO_2 is an intermediatary product of the nitrification process between TAN and NO_3, and highly toxic to aquatic life (El-Sayed 2006b; Avnimelech 2012; Hargreaves 2013). NO_2 spikes are often observed during the start-up of the nitrification process, and if insufficient aeration is taking place (Avnimelech 2012). The 96 hour LC50 of NO_2 for tilapia has been extensively researched between different fish sizes and chloride concentrations, with reports ranging from 44.67 mg/L at high chloride levels (Wang et al. 2006), to 81 mg/L for small O. niloticus (Atwood et al. 2001). The addition of salt (NaCl) can therefore be used to lower the NO_2 toxcicity in culture systems when needed (Durborow et al. 1997). Rakocy (1989) reported that tilapia usually begin to die at NO_2 concentrations above five milligrams per litre. It is however good practise to constantly monitor and maintain NO_2 concentrations below one milligram per litre for optimal production (Stone and Thomforde 2004). As the toxicity of NO_3 is greatly reduced from that of NO_2, and seldom has an effect on the wellbeing of fish, it should be monitored accordingly to keep track of the prevalence of the nitrification process taking place in a BFT system.

Reactive phosphorus/orthophosphate (PO_4^{3-} or PO_4–P) originates from the feed, and is an important measurement in water quality monitoring, as increased levels, along with NO_3 can result in algae blooms (Macintosh and Little 1995; Stone and Thomforde 2004). This mechanism is however useful when fertilizing ponds to stimulate autotrophs and heterotrophs, and the process of using animal litter to supply these nutrients has been practised for centuries (Macintosh and Little 1995; Nguenga et al. 1997; Stone and Thomforde 2004; El-Sayed 2006b). Luo et al. (2014) found the level of PO_4 to be considerably lower in BFT tanks when compared to the levels found in recirculating aquaculture systems (RAS). This is similar to results by Ray et al. (2011) who found lower PO_4 levels in BFT raceways with high solids.This may be due to the extensive utilization of the PO_4 which takes place by the outsized community of autotrophs and heterotrophs within the BFT system, and thus keeps levels lower (Luo et al. 2014).

Turbidity is a measure of the degree to which the transparency of water is lost due to suspended solids in the water, and it is linked to the FV, TSS and therefore the level of biofloc (Ray et al. 2011; Hargreaves 2013). High levels of turbidity have reportedly lead to lower production in ponds, due to less light penetration into the water, which hampers photosynthesis (Chandler 1942; Ledgerwood et al. 1978; Kirk 1985; Green et al. 1990). This is also linked to reduced artificial feed intake which has been observed for tilapia when turbidity is high (Ardjosoediro and Ramnarine 2002; Azim and Little 2008). There are however some desirable observations, and lower aggression and cannibalism levels in fish, have been attributed to high turbidity levels (Hecht and Pienaar 1993; Popma and Lovshin 1996). There is however uncertainty in the recommended turbidity level in biofloc system, with Ray et al. (2010a) attempting to maintain levels below 30 nephelometric turbidity units (NTU), Hargreaves (2013) suggesting that normal levels range from 75-150 NTU and Rakocy et al. (2008) as well as Ray et al. (2011) experiencing levels far higher. With the nature of a BFT system, variation in turbidity levels are expected, with Azim and Little (2008) rationalizing the difficulty in maintaining constant turbidity and TSS levels.

TSS is an indication of biofloc level, being a measurement of the amount of solid particles suspended in a water body, with high levels being discouraged in intensive aquaculture systems where clear water has been preferred (El-Sayed 2006b). However, BFT encourages higher levels of TSS as long as the pond mixing system can keep solids suspended, to prevent accumulation on the bottom of

the pond leading to anaerobic conditions (Avnimelech 2012). Although Avnimelech (2012) estimated that intervention should take place above a level of 500 mg/L, each farm is unique, with levels rising up to a point of 2100 mg/L in a study by Green et al. (2014). However, there appears to be an optimal band of TSS levels in BFT systems, with studies by Green et al. (2014) as well as Ray et al. (2010b) concluding that the removal of solids above a certain level, resulted in higher yield and lower feed conversion ratio (FCR) of Channel catfish (*Ictalurus punctatus*) and Whiteleg shrimp (*Litopenaeus vannamei*) respectively. The consumption of biofloc by tilapia was measured as a reduction in TSS by Ekasari et al. (2014) who determined that consumption was at a level of 117 g TSS/kg fish, regardless of the floc size. It is therefore evident that the TSS level is very dependent on the stocking density and feeding rate within a BFT system, however little is known about optimal levels for these variables, particularly with tilapia (Azim and Little 2008; Avnimelech 2012; Emerenciano et al. 2013; Green et al. 2014).

Different fish species have different effects on the water quality of an aquaculture system, and depends on factors such as the feeding behaviour, its ability to change feeding habits, and the trophic level at which it operates (Bosma and Verdegem 2011).

Environmental Requirements

Most parts of South Africa have been regarded by many as an unfavourable environment for the culture of tilapia, with this being put forward as reasoning for the inability of the local tilapia aquaculture sector to develop. Tilapia are considered a hardy fish, and can be cultured under a large range of environmental conditions (Boyd 2004; Jamandre et al. 2011). Generally, tilapia are able to handle poor water quality with low dissolved oxygen (DO) content and high ammonia levels (Shelton 2002). They are able to adapt to a range of culture conditions at varying stocking densities, with different species and being fed different food types (Shelton 2002). Salinities of varying levels can be tolerated by tilapia, with culture being able to take place in seawater which opens up the scope to even more diverse habitats (Potts et al. 1967; Heijden et al. 1997; Shelton 2002; de Oca et al. 2015). Temperatures from 10-40ºC can be tolerated by tilapia (Josupeit 2005; Jinliang 2011). It must be noted that although tilapia can tolerate a diverse range of environmental parameters, growth will not be optimized throughout the range, and will decrease outside of the ideal conditions.

2.3 Tilapia Biology

General Classification

The phylum Chordata is a large group of organisms possessing a notochord within the animal kingdom including Mammalia, Aves, Reptilia, Amphibia and Pisces among other classes. Within the class Pisces, the order Perciformes accommodates the Cichlidae family which is the largest fish family in Africa, with around 900 described species (Skelton 2001). Tilapia is a broad name given to three genera within the Cichlidae family, namely: *Oreochromis, Tilapia* and *Sarotherodon* (Kocher et al. 1998; Canonico et al. 2005). It is within the maternal mouth-brooding *Oreochromis* genus where we find the most prevalent tilapia species' in the aquaculture industry (El-Sayed 2006b; D'Amato et al. 2007).

Oreochromis niloticus is the most widely produced of the *Oreochromis* genus and has been introduced around the world for its use in aquaculture where production has been recorded in more than 135 countries (Figueredo and Giani 2005; FAO 2014). Of the individual tilapia species used in aquaculture globally, the production of *Oreochromis mossambicus* is second only to *O. niloticus* (El-Sayed 2006b; FIGIS 2015a). However the use of hybrid *O. niloticus x O. aureus* has grown in popularity and proved a successful alternative to *O. niloticus* in certain conditions, positioning itself above O. *mossambicus* on the production scale (FAO 2015). Within Africa, the *O. andersonii* is still highly regarded as a viable option, particularly within its natural distribution areas where it is cultured (Gopalakrishnan 1988; Kefi et al. 2012). Although unrefined, and still very much undeveloped, its potential for aquaculture is being examined (Gopalakrishnan 1988; Prein 1993; Musuka and Musonda 2012).

Distribution and Habitat

Today tilapia is mainly cultured in developing countries located in areas exhibiting a tropical or subtropical climate (Boyd 2004; Figueredo and Giani 2005). Within these climatic regions, tilapia thrive in disturbed habitats, and are able to survive in almost all aquatic environments, posing major threats to the biodiversity and indigenous organisms of the water body (Kaufman 1992; Fitzsimmons 2001; Boyd 2004; Canonico et al. 2005). They have established in virtually all of the water bodies in which they are cultured, or in which they have managed to gain access, including estuarine systems, making

them the most widely distributed group of exotic fish around the world (Courtenay 1997; Costa-Pierce 2003; FAO 2014).

Oreochromis niloticus is indigenous to parts of central and northern Africa, including the Nile basin and lakes of the Rift Valley (Figure 2.6) (Skelton 2001). Pickering (2015) established a Global Mapping tool for depicting the occurrence of a chosen species based on numerous sources. Although present outside of the tropical and subtropical regions, it is clear from that the species is more concentrated in the central band consisting of warmer climates (Figure 2.6). *Oreochromis niloticus* shows a preference to well vegetated, shallow, stagnant water (Picker and Griffiths 2013). First being introduced to South Africa in 1959, as a food fish for Bass (*Micropterus spp*.) in the Kwa-Zulu Natal and Western Cape province, *O. niloticus* escaped into river systems and formed well established populations in the Limpopo and Incomati systems (van Schoor 1966; Picker and Griffiths 2013).

Oreochromis mossambicus is indigenous to southern Africa with its distribution stretching in the eastward flowing rivers from the lower Zambezi system, down the east coast of the continent until the Bushman's river system in the Eastern Cape province of South Africa (Skelton 2001; Russell et al. 2012). In the southern reaches of its natural distribution, *O. mossambicus* occurred only in estuaries and the coastal reaches of rivers (Skelton 2001). *Oreochromis mossambicus,* as with *O. niloticus,* has been introduced to many countries around the world for it various uses which are similar to those of *O. niloticus* (Figure 2.7) (Fitzsimmons 2000; Skelton 2001; Omar et al. 2014). Its introduction outside of its native range is reported to have taken place before *O. niloticus,* as it was possibly mistaken to be the best tilapia species for use in aquaculture before *O. niloticus* became the dominant species (Fitzsimmons 2000; Canonico et al. 2005). Although not being indigenous to all parts of South Africa, *O. mossambicus* has been extensively introduced to many water bodies throughout South Africa in which it has successfully established (Skelton 2001).

Oreochromis andersonii is naturally found in the Cunene, Upper Zambezi, Kafue and Okavango river systems in southern Africa (Trewavas 1983; Skelton 2001). It has not experienced the same degree of commercialization as seen with *O. niloticus* and *O. mossambicus,* but was identified as an aquaculture candidate in Zambia as early as 1980 (Gopalakrishnan 1988). *Oreochromis andersonii* has not been widely introduced outside of its natural range (Figure 2.8). An interesting study by Økland et

al. (2007) found that adult *O. andersonii* did not seem to be specialists in any habitat type with a lot of individual variation and flexible movement patterns being observed. 51 % of the tagged fish preferred to reside in vegetative areas, with 11 % being near vegetation, and 39 % away from vegetation (Økland et al. 2007). This relatively high percentage away from vegetation coincides with the 39 % observed to reside in the main stream of the river, where the flowing water often prevents the growth of vegetation (Økland et al. 2007). This habitat selection of different fish species is directly linked to their behaviour, with certain traits being beneficial and others detrimental when cultured in a confined system.

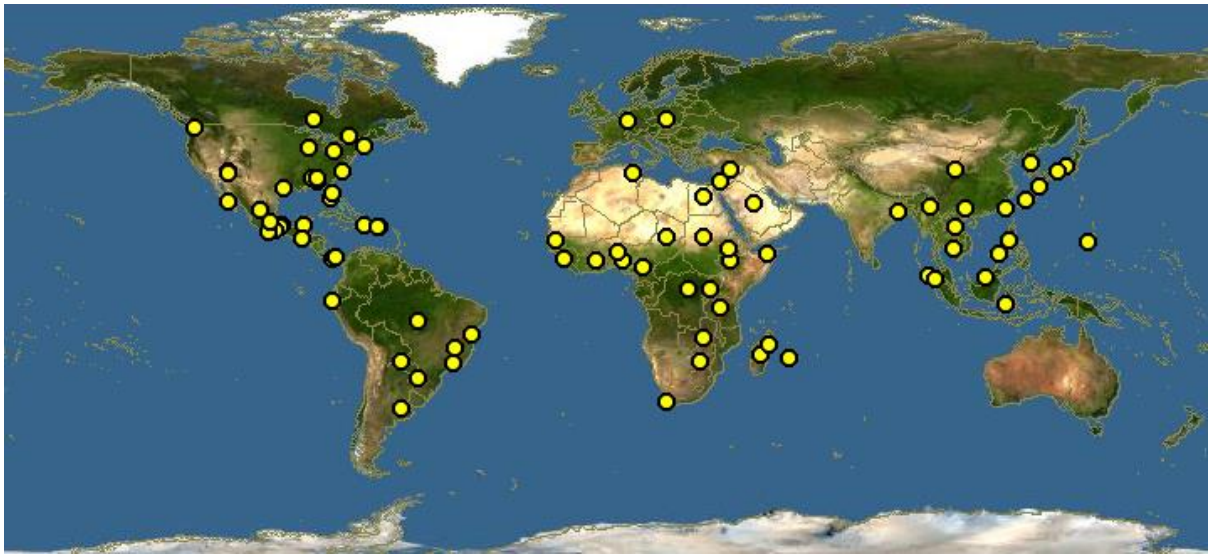

Figure 2.6. Global distribution of Oreochromis niloticus (Global.Mapper 2015).

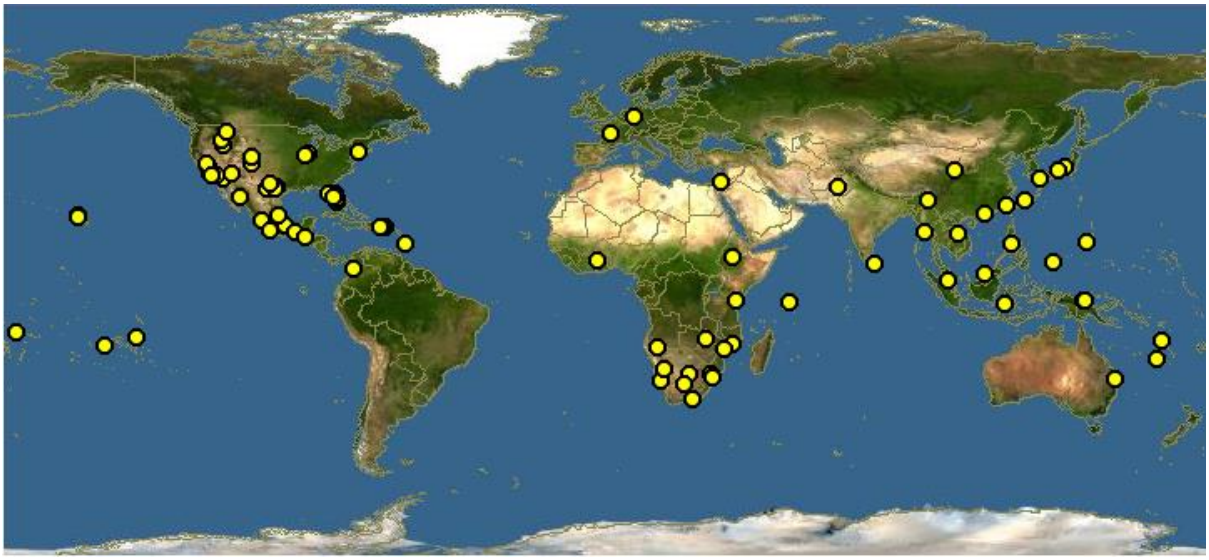

Figure 2.7. Global distribution of Oreochromis mossambicus (Global.Mapper 2015).

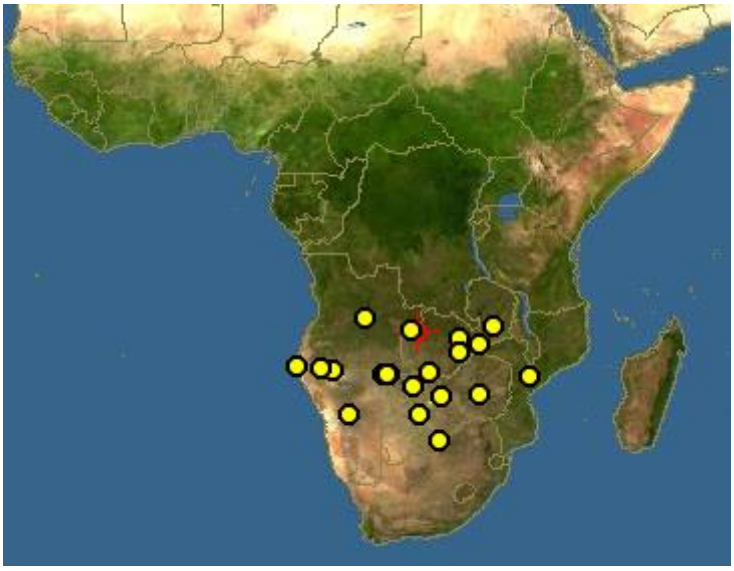

Figure 2.8. Global distribution of Oreochromis andersonii (Global.Mapper 2015).

Behaviour

Fish behaviour and the response to feeding play a crucial role in monitoring the wellbeing of fish as well as increases efficiency and profit margins in any aquaculture system. Although difficult to quantify and measure, behavioural changes observed by the feeder can save feed costs and reduce environmental degradation by reducing feed wastage (Alanärä et al. 2001; El-Sayed 2006b). Observing fish behaviour can also give an indication of various diseases and water quality problems from which the fish may be suffering (Toguyeni et al. 1997; El-Sayed 2006b). The importance of understanding the specific behaviour of the fish species being cultured is no secret to successful aquaculture, and forms the fundamental bases of husbandry practises.

El-Sayed (2006b) indicated the importance of human contact in small scale tilapia aquaculture, as there are benefits of hand feeding tilapia rather than using other feeding methods. This labour intensive practise does however become difficult in large-scale production, and is suggested to be used in combination with other feeding methods (Alanärä et al. 2001; El-Sayed 2006b). With the continuous feeding behaviour exhibited by tilapia, it is appropriate to adapt the feeding regime accordingly to achieve maximum efficiency (Boyd 2004; El-Sayed 2006b).

Tilapia have been found to feed predominantly at night (Fortes-Silva et al. 2010). This is contrary to the findings by Toguyeni et al. (1997) who found that O. niloticus fry were more inclined to feed from self feeders during the light period. The importance of this is however contextualized by the

results from Madrid et al. (2001) who specified that the circadian rhythm is not as imperative in fish as in higher vertebrates. The level at which fish feed in the water column is also a significant behavioural trait which is important to fish farmers. Although earlier studies suggested *O. andersonii* to be a bottom feeder, Gopalakrishnan (1988) discovered that they actually feed at all levels. Regardless of the light period and level at which tilapia feed, biofloc is always available to them. Dempster et al. (1993) suggested that quantitative aspects of ingestion have not been extensively explored in tilapia. This is however a topic of interest as it ultimately has influence on the diet and feeding behaviour of the fish. With tilapia predominantly being regarded as omnivorous, and differences in feeding behaviour between species of tilapia being likely, it is probable that different species will lie at different trophic levels or points of a scale between herbivore and carnivore.

In tilapia grow-out systems, aggression is considered a negative trait and should be mitigated as much as possible (Watanabe et al. 1988). With aggression being shown, less energy is expended on growth with injuries as well as cannibalism being a reality (Pantastico et al. 1988; El-Sayed and Kawanna 2004; El-Sayed 2006b). The social stress associated with aggression can also lead to decreased disease resistance (Volpato et al. 2003). Watanabe et al. (1988) found that by increasing the salinity of the water, the territorial aggression of Florida Red tilapia was reduced. Stocking of predominantly male tilapia into grow-out systems is also known to mitigate territorial aggression associated with breeding. It is through mitigating this aggression that we are able to increase efficiency in tilapia aquaculture. Falter (1996) reported a difference in the level of aggression between *Oreochromis macrochir* and *O. niloticus* even though interbreeding was observed, and suggested mapping species on an aggression gradient with overlapping zones which are areas where interbreeding may take place. It is also conveyed by Falter (1996) that the risk of hybridization is elevated where species of the same genus interact as the level of aggression is often similar within a genus.

Anatomy

The Cichlidae family of fish can be distinguished from other bony fishes by the break in the lateral line, running lower down on the posterior part of the flank, and higher up closer to the head (Popma and Lovshin 1996). Fish of the *Oreochromis* genus are generally deep bodied fish, laterally compressed, and can grow to large sizes in comparison to other cichlids (Skelton 2001; El-Sayed

2006b). The skin of tilapia is covered with cycloid scales which are large in size (Macintosh and Little 1995; El-Sayed 2006b). For commercial strains, most reports of fillet yield range from 30-38 % (Boyd 2004; Josupeit 2005; Netto et al. 2014). Tilapia produce white flesh with a good taste, used in many restaurants around the world (Josupeit 2005; Stickney 2006; Celik 2012). Females of *O. mossambicus* have a larger buccal cavity than males, due to the preopercular and interopercular bones being larger, which is in line with their responsibility of mouth-brooding (Oliveira and Almada 1995). Within the mouth, tilapia have pharyngeal teeth which are used in the grinding of vegetable matter, and it is also suggested that these teeth play a role in tilapia not being well suited for capturing large prey (Trewavas 1983; Popma and Lovshin 1996). The long intestine of tilapia, usually six times the total length of the fish, allows for efficient utilization of plant material (Popma and Lovshin 1996; Boyd 2004). Judging by a shorter intestine length, it was also suggested by Popma and Lovshin (1996) that *O. mossambicus* is less efficient at making use of planktonic algae than what *O. niloticus* and *O. aureus* are.

Distinguishing between different species of the *Oreochromis* genus can be a difficult task due to the diverse phenotypic variation found within species. Using the count of gill rakers on the lower limb of the first gill arch is a common method used to identify fish species, along with the fin ray formula and a lateral scale count (Ginsburg 1945; Randall 1958; Elst and Wallace 1976; Lindsey 1981; McDowall 2001; El-Sayed 2006b; Randall and Page 2014). Common anatomical differences and characteristics are useful to distinguish between the three *Oreochromis* species of interest to this study (Table 2.1).

Table 2.1. Illustrates differences to distinguish between the three Oreochromis species of interest[1]

Structure	**Species**		
	O. andersonii	***O. mossambicus***	***O. niloticus***
Mouth	enlarged jaw	duck-bill like	not enlarged
Gill rakers	21-27	16-20	20-26
Lateral line scales	31-35	30-32	30-34
Caudal fin	no vertical lines	no vertical lines	vertical lines
Dorsal spines	16-18	15-17	16-18
Dorsal branched rays	11-14	10-13	12-14
Anal spines	3	3	3
Anal branched rays	11-13	9-12	9-11
Colour pattern	3 spots on flank	mid-lateral blotches	banded
Breeding colours	blue-purplish head	black with white	dark, reddish head

[1] Content depicted in table sourced from (Trewavas 1983; Skelton 2001; Lamboj 2004)

2.4 Feeding

Natural Diet

The anatomy of fish in the *Oreochromis* genus is well suited to the grazing of algae, diatoms and detritus (Skelton 2001). A high number of gill rakers, a long intestine, fine pharyngeal teeth and a few rows of fine teeth on the jaws make *Oreochromis spp.* good grazers as well as filter feeders (Popma and Lovshin 1996; Skelton 2001; Figueredo and Giani 2005).

Popma and Lovshin (1996) reiterated the favourable ability of tilapia to obtain nutritional benefit from bacteria and plant material. They put it down to two key mechanisms, namely the grinding of material between the fine teeth on the phanyngeal plates, and the low stomach pH of below two, which ruptures cell walls resulting in the ability of tilapia to utilise 30-60 % of protein in algae (Popma and Lovshin 1996).

Oreochromis niloticus, originally known to be herbivorous (Getachew and Fernando 1989; Njiru et al. 2004), is now described as a typical omnivore, consuming insects, fish, algae, planktonic organisms, and plant material (Popma and Lovshin 1996; Njiru et al. 2004; Figueredo and Giani 2005; Gominho-Rosa et al. 2015; Pedrotti et al. 2015). Juveniles <50 mm tend to utilise zooplankton as their main food source (Njiru et al. 2004).

Naturally, *O. mossambicus* feeds on insects, algae, diatoms, invertebrates and detritus (Skelton 2001). A study by Whitfield and Blaber (1978) in Lake St. Lucia in northern Kwa-Zulu Natal, South Africa revealed a decrease in the dependence on animal material in the diet of *O. mossambicus* with age. This shift towards the utilization of more plant material is interesting and may be linked to the development of the gut with age.

Oreochromis andersonii feed on zooplankton, detritus and diatoms (Skelton 2001). Winemiller and Kelso-Winemiller (2003) found similar feeding preferences on vegetative detritus and diatoms as well as invertebrates and algae being consumed. Juveniles were found to have consumed more invertebrates than adult *O. andersonii,* similar to the findings for *O. mossambicus* (Winemiller and Kelso-Winemiller 2003).

Oreochromis niloticus, O. andersonii and *O. mossambicus* exhibit diet overlap (Skelton 2001), but may feed at slightly different trophic levels, as was found between *O. niloticus, Oreochromis*

esculentus and *Oreochromis variabilis* in a study by Njiru et al. (2004). This possible difference may allow the three species of interest to utilise certain food resources better than the respective other species. If this is the case, aquafeeds developed for a particular species may be utilised best if they were customized for that species. With there being no overlap in their natural distribution range, it is likely that these three species may have fitted into a similar position in the foodweb of their natural ecosystems, exhibiting niche complementarity when under culture conditions (Skelton 2001; Winemiller and Kelso-Winemiller 2003). Findings by Oso et al. (2006) suggest a high degree of trophic flexibility for *O. niloticus*, but this may be due to the small variety of natural foods reported in the reservoire. Zambrano et al. (2006) advocate that *O. niloticus* often alter the trophic structure within an ecosystem by competing with other fish as well as preying on juvenile fish and amphibians, which is supportive of trophic flexibility of the species (Kolding 1989; Morgan et al. 2004).

Aquaculture Feeding

Fish can respond to their nutritional requirements and select a diet which will fulfil their nutrient need (Sánchez-Vázquez et al. 1998; Sánchez--Vázquez et al. 1999; Yamamoto et al. 2000; Vivas et al. 2006). Fortes-Silva et al. (2010) illustrated the advantageous use of a self-selective feeding method to better understand the nutritional requirements and therefore play a role in feed development.

In more extensive tilapia aquaculture practises, the natural food produced in the water body is solely relied on to feed the fish (El-Sayed 2006b). Fertilizing the pond with organic or inorganic fertilizers stimulates the primary productivity of the pond, which is the growth of autotrophic and heterotrophic organisms on which the fish feed (Celik 2012).

Semi-intensive systems combine the natural productivity of the pond with a supplementary feeding regime and are therefore positioned between extensive and intensive systems (De Silva 1995). Interactions between supplementary feeding and natural productivity are very complex and not well understood (De Silva 1995). Fish in semi-intensive systems have been documented to select a narrower range of food items, and the full utilization of the food variety available may be questionable (Spataru et al. 1980). Supplementary feed needs to be well balanced, taking into account the quantity and type of natural food available to ensure that the total food consumed by the fish is of the correct balance (De Silva 1995; Hooley et al. 2014).

Intensive systems consist of a feeding regime whereby all the food consumed by the fish is formulated, usually in the form of pellets. Celik (2012) reiterated the dependence of intensive tilapia culture on high quality commercial feeds.

Protein is arguably the most important ingredient in aquafeed. It makes up the greatest cost in feed, and it is important to feed the correct level of protein in the diet to different life stages of tilapia, taking into account the energy content of the diet as well as the protein source (Wu et al. 1994; Wu et al. 1995; El-Sayed 2006b; Loum et al. 2013). Adult tilapia are generally fed a diet with a protein content of 20-30 %. (El-Sayed 2004; El-Sayed 2006b).

Fishmeal has been used extensively in the manufacture of aquafeeds for use in aquaculture around the world (El-Sayed 2006b; Tacon and Metian 2008; Singh and Muthukumarappan 2014). High protein content, good digestibility and good composition of essential amino acids and essential fatty acids have led to fishmeal being deemed the “gold standard” protein source for carnivorous fish by Lunger et al. (2006). Global shortage of fishmeal, along with much ethical protest of its use has resulted in prices sky rocketing, and a search for alternative proteins to replace fishmeal (Lunger et al. 2006; Naylor et al. 2009; Hardy 2010; Singh and Muthukumarappan 2014).

Soyabean meal offers the closest known plant protein alternative to fishmeal, with its high protein content and essential amino acid composition (El-Sayed 2006b; Singh and Muthukumarappan 2014). Much research has been done on the replacement of fishmeal at various levels by soybean meal, which can supply between 67 and 100 % of the protein requirements of tilapia (Shiau et al. 1990; Fontainhas-Fernandes et al. 1999; El-Sayed et al. 2000; El-Saidy and Gaber 2002; Trosvik et al. 2013). A study by Lin and Luo (2011) indicated that above a level of 75 % of fishmeal replaced by soybean meal, adverse effects were encountered in terms of growth rate, feed utilization and serum lysozyme levels. There are however some additional drawbacks with the use of soybean meal, namely the undersupply of sulphur containing amino acids, the disabling effect of heating which is required when using soybean meal, and the presence of endogenous anti nutrients (El-Sayed et al. 2000; El-Sayed 2006b).

In recent times, researches have looked into the use of maggot meal to take the pressure off of fishmeal as a protein source in animal feeds, particularly for monogastrics (Téguia et al. 2002;

Awoniyi et al. 2003; Ajani et al. 2004; Sing et al. 2014; Charlton et al. 2015). Being of animal origin, and grown on animal waste, the prospects for cheap production are promising, with studies on tilapia showing good growth and encouraging results as an effective, sustainable substitute (Ogunji et al. 2007; Ogunji et al. 2008; Sing et al. 2014).

Intuitive thinking has led to another alternative protein source. The use of microbial protein by ruminants as their main protein source, through internal action by the rumen, is well documented (Stern and Hoover 1979; Clark et al. 1992; Firkins 1996; Zhu et al. 2013). The external production of microbial protein is now being explored to aid in replacing fishmeal used in the manufacture of animal feeds, and in particular feeds used in aquaculture (Azim et al. 2008). Microbial protein is able to be generated from waste products produced by other industries such as food processing, by enhancing the use of micro-organisms (Browdy et al. 2010). Through nutrient recycling by micro-organisms, the efficiency of systems can be improved and a valuable protein source can be produced. With aquaculture firmly embedded in efficiency, the use of microbial protein has been explored with one school of thought being that of biofloc technology (BFT) which is applicable to filter feeding organisms such as tilapia.

Microbial Community Composition

Biofloc systems may be indoors or outdoors, with outdoor system being more commercialized and showing increased share of photoautotrophs due to the presence of light (Ray et al. 2009; Avnimelech 2012; Baloi et al. 2013). In the development of an outdoor BFT system, the water shifts from a greenwater algal system to a brownwater bacterial system with increased feeding, and can be measured on the Microbial Community Colour Index (MCCI) (Hargreaves 2013). This shift also indicates a shift from algae as the main mechanism for ammonia control to the more efficient bacteria, which also require an increase in aeration (Hargreaves 2013).

A single floc was described by Emerenciano et al. (2013) as a macroaggregate (Figure 2.9). BFT systems are made up of flocs, typically consisting of organic matter, physical substrate, phytoplankton, free and attached bacteria and zooplankton grazers (Ray et al. 2010b; Emerenciano et al. 2013). The interactions and ratios in abundance between the micro-organisms are however complex and vary considerably from case to case (Emerenciano et al. 2013). A study by Avnimelech and Kochba (2009) determined that organisms in the biofloc have a typical residence time of 10 hours, indicating a

speedy turnover rate through the harvesting by fish and microbial degradation, which leads to a consistency of mainly young and active cells (Avnimelech 2012).

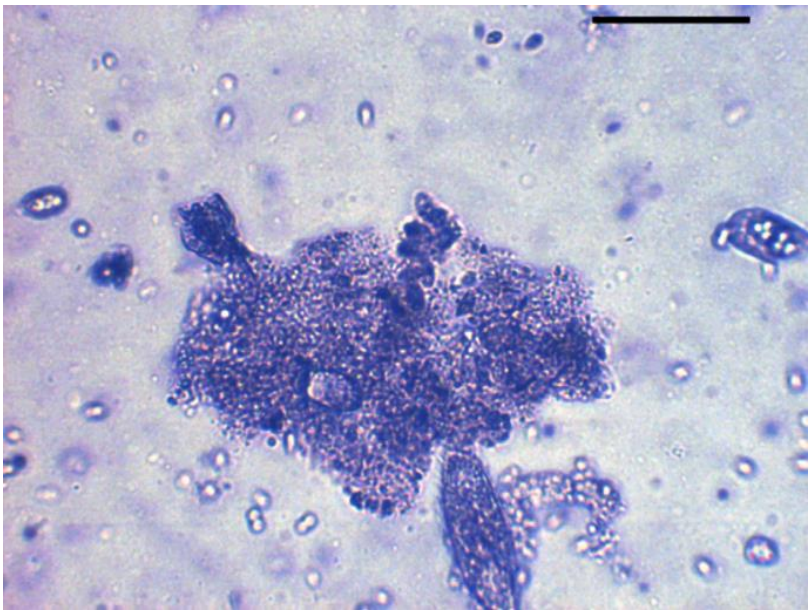

Figure 2.9. A single biofloc, a coagulation of micro-organisms with the scale bar being 100 microns *(Hargreaves 2013)*.

Within a BFT system, it is bacteria which is the most fundamentally important micro-organism in providing a microbial food source and controlling water quality (Avnimelech 2012). There are two functional groups of bacteria which are relied upon to control the water quality, namely chemoautotrophic nitrifying bacteria, and heterotrophic ammonia assimilating bacteria (Ebeling et al. 2006; Ray et al. 2010b). The action of these functional groups results in high oxygen use and lowered alkalinity, which both require supplementation (Ray et al. 2010b).

The pathway of nitrifying bacteria in aquaculture systems is well understood and has been extensively studied (Tal et al. 2003; Michaud et al. 2006; Crab et al. 2007). It works by TAN being converted by oxidation reactions to the highly toxic nitrite species, and is in turn converted to nitrate which poses a much reduced danger to the cultured fish (Peng and Zhu 2006; Ray et al. 2010b; Hargreaves 2013).

On the other hand, heterotrophic bacteria removes TAN from the water and incorporates it into cellular protein, which can be consumed by the cultured species (Figure 2.10) (Ebeling et al. 2006; Ray et al. 2010b; Celik 2012). Additionally, heterotrophic bacteria have a growth rate up to ten times higher than nitrifying bacteria in the presence of adequate amounts of an organic carbon supply, which results

in a much more rapid reduction of TAN levels and increase in microbial biomass, holding nutritional potential (Hargreaves 2006; Crab et al. 2012; Hargreaves 2013).

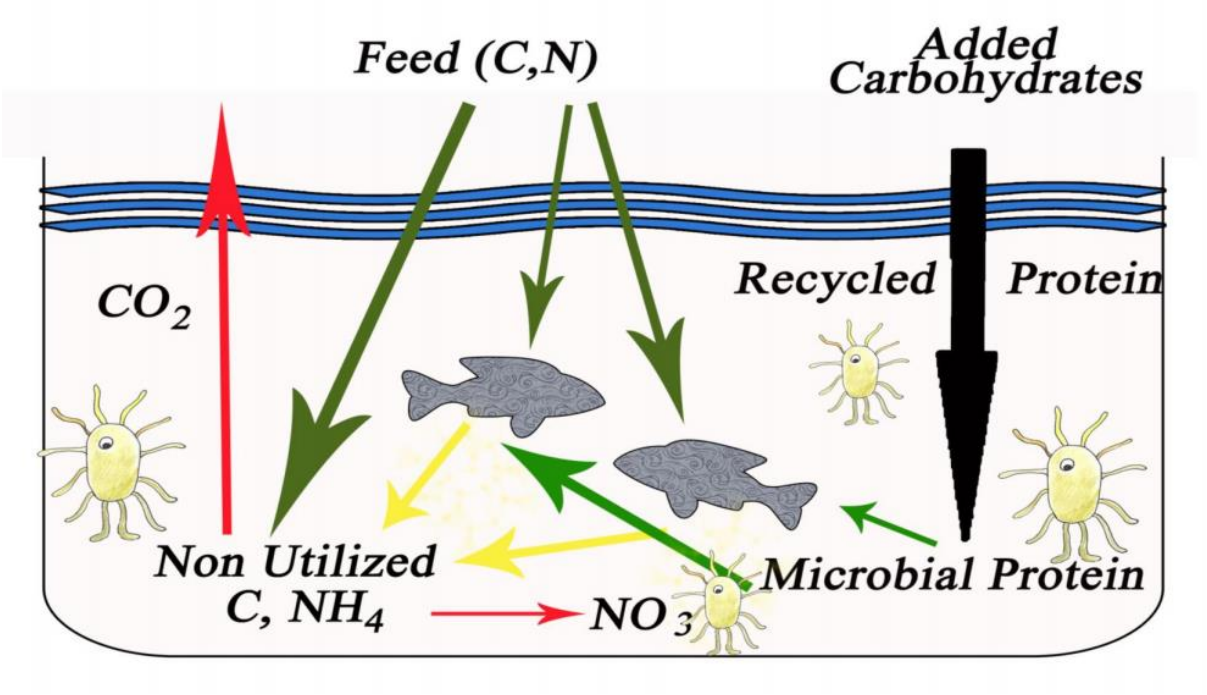

Figure 2.10. The heterotrophic pathway exhibited in a biofloc technology (BFT) system *(Avnimelech 2012)*.

Nutritional Properties

Crab et al. 2010 put forward that biofloc consists of sufficient levels of protein, lipid, carbohydrate and ash to comprise a complete aquafeed. Bioflocs offer good nutritional properties, but due to the nature of BFT systems, there is varying consistency in different culture environments, as the biological composition of the biofloc varies (Ju et al. 2008; Hargreaves 2013). Widanarni et al. (2012) found a crude protein content of between 39 and 48 % for biofloc collected from Red tilapia trials of varying stocking density. This is consistent with the 25-50 % range reported by Hargreaves (2013). However, Ekasari et al. (2014) presented 17.2-27.8 % protein for biofloc harvested from shrimp culture systems, with Crab et al. (2010) recording 58 %, which is testimony of the varying consistency between production environments. The lipid content of 6-7.5 % found by Ekasari et al. (2014) was within the typical range of 0.5-15 % reported by Hargreaves (2013). The elevated lipid content of up to 24 % found by Widanarni et al. (2012) is not encompassed by the range put forward by Hargreaves (2013), or in line with other findings of 2-5 % by Azim et al. (2008); Azim and Little (2008) and Crab et al. (2010). This high lipid level reported by Widanarni et al. (2012) may be partly explained by the high level of

diatoms reportedly found in the biofloc investigated, which can contain lipid levels of up to 25 % (Shifrin and Chisholm 1981). The amino acid and fatty acid composition of biofloc is not well documented, with contradictory results requiring more research in the field in order to better understand this facet (Crab et al. 2012; Hargreaves 2013).

Feeding in a Biofloc System

Tilapia can be fed with biofloc in two different ways, namely harvesting of biofloc and using it to create a pelleted feed, and the *in situ* production and consumption of biofloc within the pond in which the fish are grown (Avnimelech 2012; Widanarni et al. 2012; Emerenciano et al. 2013). The former may offer value as not only an aquafeed, but as a feed for other animals in the form of a biofloc meal (Emerenciano et al. 2013). The processing of the biofloc for this product is however costly, and the nutritional qualities of the biofloc may be altered through the processing, but nevertheless it is gaining more attention and the focus of much research (Kuhn et al. 2009; Kuhn et al. 2010; Bauer et al. 2012; Emerenciano et al. 2013; Hussain et al. 2014; Kurup 2015; Valle et al. 2015). *In situ* utilization requires a filter feeding species to harvest the biofloc directly from the water in which it is cultured, and is the major application of BFT in the aquaculture industry today (Avnimelech 2012; Crab et al. 2012; Hargreaves 2013; Ekasari et al. 2014).

There are a number of ways in which the gain experienced in BFT systems is expressed. Hargreaves (2013) suggested that shrimp or tilapia in biofloc systems are able to derive 20-30 % of their growth from feeding on this available microbial protein. A study by Avnimelech (2007) put forward that the biofloc consumed by tilapia makes up around 50 % of the usual amount of feed which is fed to the fish. Widanarni et al. (2012) found that Red tilapia fed on biofloc required up to 73 % less feed than the control treatments. The gains experienced by Luo et al. (2014) were depicted in a number of different ways. The mass of individual fish at harvest was 22 % higher in BFT treatments with FCR being 18 % lower as opposed to RAS treatments (Luo et al. 2014). Total mass gained was 128 % higher in the BFT treatment with the specific growth rate being 112 % higher than the RAS treatment (Luo et al. 2014). No difference in growth was observed by Azim and Little (2008) for tilapia in BFT tanks fed pelleted feed of 35 % and 24 % protein respectively, although both treatments exhibited higher growth than the control without biofloc and fed the 35 % protein diet.

It is therefore evident that within BFT systems following the conventional, low-cost, *in situ* approach, the administering of pelleted feed is able to be significantly reduced, as the cultured organism is able to feed on the biofloc *ad lib* (Avnimelech 2007; Azim and Little 2008; Avnimelech 2012; Celik 2012; Widanarni et al. 2012). It is well known in aquaculture that by increasing the frequency of feeding, higher efficiency and better growth are realised (Popma and Lovshin 1996; El-Sayed 2006a; Fortes-Silva et al. 2010; Celik 2012). The benefit from the *ad lib* availability of biofloc in these systems is therefore evident.

Growth

Mass and length have formed the basis of measurements for recording fish growth in fishery science and aquaculture (Pelletier et al. 1995; Jones et al. 1999; Richter et al. 2000; Cade et al. 2008).

The growth rate and efficiency of growth is of utmost importance to tilapia farmers around the world. A number of indicators may be calculated to measure efficiency; FCR is one, and is an indication of the amount of dry feed given to the fish divided by the wet mass gained (Stadtlander et al. 2011). This is however more challenging to calculate in more extensive types of systems, as the fish utilise natural feed which is difficult to quantify. Fish grown extensively tend to grow slower than those grown more intensively where feeding is more meticulous. Within a semi-intensive biofloc technology (BFT) system, tilapia are able to feed continuously on the natural food source produced in the form of bioflocs within the water body, and in addition, are fed a pelleted feed. Being able to feed continuously should therefore result in better growth than if just fed at set times (Emerenciano et al. 2013).

Feeding more frequently has also proven to be more efficient with fish showing decreased FCR when fed frequently (El-Sayed 2006b). Feed conversion ratio values of below 1:1 have been reported for *O. niloticus*, with FCR varying throughout the life cycle of the fish (De Silva and Anderson 1995; Ogunji et al. 2008).

The protein type and content of the feed are also of importance to the growth rate and ability of the fish to utilise feed (Tacon and Hassan 2007). Protein which shows a higher bioavailability will often yield better growth, but are usually more costly. It is up to the tilapia farmer to weigh up the cost against the benefit gained from the feed, and work out which is best for him.

With the two pronged approach of traditional animal selection and genetic improvement taking place, faster growing strains of tilapia are being developed, with the effort being accelerated in recent times (Lutz et al. 2006; Ansah et al. 2014). This comes as no surprise, as genetic improvement has been earmarked to be one of the cheapest and influential ways of improving the efficiency of aquaculture (Ponzoni et al. 2007). The genetic improvement of farmed tilapia (GIFT) project has been a significant advancement in recent times, and the technology and method used in developing the GIFT strain of *O. niloticus* has been replicated elsewhere (Attipoe et al. 2013).

Much of the effort put into genetic improvement has been focussed on *O. niloticus* (Mair et al. 1997; Bentsen et al. 1998; Eknath et al. 2007; Tanamati et al. 2015). This is largely due to its extensive use throughout the globe as it has been regarded as the best tilapia species for aquaculture. The usage of *O. mossambicus* globally, has also resulted in this species experiencing a fair amount of genetic improvement itself (Ch'ang 1971; Cnaani et al. 2000). Having been introduced internationally in the 1930's, the time consuming practise of selective breeding programs have had time to better the aquaculture potential of this species (Ch'ang 1971; Agresti et al. 2000). On the other hand, *O. andersonii* has experienced very little genetic improvement, with Gopalakrishnan (1988) identifying the need for the development of high quality seed.

As genetic improvement has inevitably been focussed on certain species more than others (Attipoe et al. 2013), it adds complication when comparing the growth rates between species. Additionally to being difficult to quantify, genetic improvement of tilapia species has used different wild strains and taken place independently in some cases, but also replicated in different countries around the world in other cases (Attipoe et al. 2013). It would be implausible to assume that one could obtain strains with the same level of genetic improvement and should rather be focussed on what is available and likely to be used in commercial aquaculture at the location at which the researcher is applying the research.

Species

Species chosen to be cultured in a BFT system should be able to tolerate poorer water quality with high solids, as well as obtain nutrition from the biofloc through filter feeding (Crab et al. 2012; Hargreaves 2013). It is also suggested by Crab et al. (2012) that the focus should be on lower trophic species which exhibit a herbivorous diet. Hargreaves (2013) indicated the use of either Shrimp, Carp

or tilapia in almost all BFT systems worldwide, with Widanarni et al. (2012) pointing out the additional use of Sturgeon an Snook in a study by Serfling (2006). Although research is also looking into the incorporation of various Catfish species (Green et al. 2014), the inability to handle high solid content, as well as not possessing the adaptation to gain nutritional benefit from biofloc, are limiting factors (Hargreaves 2013). It is suggested by Avnimelech (2012) that Shrimp and tilapia are the major species cultured in BFT systems, with Ekasari et al. (2013) ear-marking tilapia as one of the most promising.

Tilapia are a warm water fish species, and therefore their commercial use in BFT systems is predominantly done in countries which possess adequate climatic conditions to successfully culture this species. Although research has been conducted in controlled microclimates outside of these preferred areas (Azim and Little 2008; De Schryver and Verstraete 2009; Ekasari et al. 2010), the commercial application in these areas would be questionable with high costs of maintaining suitable temperatures.

In BFT systems, tilapia are grown at a stocking density of up to 50 kg/m^3 or 500 ton/ha with daily feeding rates being up to one kilogram per metre cubed (Avnimelech 2012). Tilapia can make use of the biofloc as they are efficient filter feeders, having the physiological ability to harvest biofloc from the water (Hargreaves 2013). Considered to occupy a low trophic level, tilapia is foreseen to play an increasingly important role into the future where lower trophic species are being nominated for intensive focus on the way forward (Fitzsimmons 2000; Shelton 2002; Tacon et al. 2009; Swaminathan 2012; Crab et al. 2012).

The three tilapia species in focus, *O. niloticus, O. mossambicus* and *O. andersonii* differ in biological aspects from anatomy to natural diet and feeding behaviour. As these are different species of tilapia utilised in aquaculture, there is reason to believe that they may feed at slightly different trophic levels and occupy niches which somewhat differ from one another. If a particular species could fill the required niche better than another species in an aquaculture system, efficiency would increase without having to rely on the overlapping niche of the less preferred species. By selecting the preferred species to fill this required niche, the species would be more compatible in that position in the food-web, and would show increased production in an aquaculture facility. Although all three of these tilapia species can utilise biofloc, comparisons on their compatibility to a BFT system in terms of growth have not been investigated. It would therefore be of value to conduct a study into which of these species shows the best utilization of biofloc and therefore the highest growth rate in a BFT system.

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3 A Growth Comparison Among Three Commercial Tilapia Species in a Biofloc System

3.1 Abstract

Despite a worldwide boom in tilapia aquaculture, South Africa has failed to follow suit, resulting in a small, very much undeveloped local industry. Much of the justification for this reality lies on the inadequacy of the species used and the stringent legislation preventing the use of the Nile tilapia, *Oreochromis niloticus.* With momentum gained in recent years, the local industry lobbying for the legalisation of the *O. niloticus* has made great progress and its use in certain systems has recently been facilitated. This study was conducted to compare the growth performance between the indigenous Mozambique tilapia, *Oreochromis mossambicus,* another African alternative gaining momentum in Zambia, the Three Spot tilapia, *Oreochromis andersonii,* and the globally reputable *O. niloticus*. The study was conducted in a biofloc technology (BFT) system, showing great potential as an environmentally friendly technology. The trial was run for 10 weeks with 60 fish/tank stocked into four repeats tanks per species. Fish were sampled on six occasions with a regression fitted to the mass data. *Oreochromis niloticus* showed a significantly higher growth rate with an average daily gain of 0.693±0.018 g/day and the lowest feed conversion ratio (FCR) of 1.01±0.05, followed by *O. mossambicus* with average daily gain of 0.405±0.025 g/day and FCR of 2.24±0.16, and then *O. andersonii* with an average daily gain of 0.185±0.025 g/day and an FCR of 2.53±0.28 respectively. From this study, *O. niloticus* therefore appears to be the most adequate species for use in BFT systems in South Africa.

3.2 Introduction

Tilapia is a general name referring to three genera within the Cichlidae family, namely: *Oreochromis, Tilapia* and *Sarotherodon* (Kocher et al. 1998; Canonico et al. 2005). Despite a large number of tilapia species, it is within the *Oreochromis* genus where we find the species' most extensively used in aquaculture today (El-Sayed 2006; D'Amato et al. 2007).

Global tilapia production has risen to supply 4,823,312 Mt in 2013, with the industry being valued at 8,248,933,000 USD (FIGIS 2015). In Asia, 75.9 % of the world's tilapia production takes place, with China alone making up 34.4 % (FIGIS 2015).

Being a low-valued finfish, low cost production of tilapia dominates, with basic culture methods being most effective (El-Sayed 2006; Celik 2012; El-Sayed 2013). This offers potential for developing countries in particular, where it can play a critical role in job creation and food security, boosting the income of low earning households (Edwards 2000; Musuka and Musonda 2013; Arita and Leung 2014; Avadí and Fréon 2014; Belton et al. 2014; Dygert et al. 2014; Eltholth et al. 2015).

Within South Africa, marine catch fisheries supply the bulk of the seafood produced, with aquaculture producing an estimated 6010 Mt in 2013 (FIGIS 2015). Freshwater fishes make up 250 Mt of this production (FIGIS 2015). Strict and poorly understood environmental regulations, unfavourable climate, poor strains and sufficient cheap supply by marine catch fisheries have been put forward as justification for the inability of the local aquaculture sector to advance as it has worldwide (DAFF 2013).

The commercial production of tilapia as a food fish in South Africa has up until recently been restricted to only indigenous species, predominantly *O. mossambicus,* with little strain development and extension support services being implemented. Conversely, work in Zambia involving their indigenous *O. andersonii* has been ongoing since the 1980's (Gopalakrishnan 1988; Kefi et al. 2012; Musuka and Musonda 2012), with Cayron-Thomas (2007) prospecting it to be the most adequate tilapia species for use in Zambia. This is however contrary to suggestions by Simataa and Musuka (2013) indicating poorer compatibility of *O. andersonii* to culture systems when compared to *O. niloticus,* with particular reference to the superior ability of *O. niloticus* to utilise plankton as a food source and therefore incur lower feeding costs than *O. andersonii.* The culture of *O. niloticus* is widely practised and well established worldwide (El-Sayed 2006), with recent changes in its categorization in South African legislation permitting its use in certain aquaculture systems under regulation (DEA 2014).

Biofloc technology (BFT) has emerged as an innovative technique to increase the efficiency, lower the water usage, recycle nutrients, and lower the running costs of aquaculture systems, while reducing the adverse effects inflicted on the environment (Azim and Little 2008; Avnimelech 2012; Hargreaves 2013; Luo et al. 2014). Through utilising a microbial community, dominated by heterotrophic bacteria, BFT systems act as an *in situ* biofilter removing dangerous nitrogen compounds from the water (Avnimelech 1999; Crab et al. 2012). By raising the carbon to nitrogen ratio (C:N) through the addition of a carbohydrate source, nitrogen is then incorporated into microbial protein in the form of bioflocs, a

coagulation of micro-organisms, which are harvested by the filter feeding cultured species such as tilapia (Avnimelech 2012; Widanarni et al. 2012).

With insufficient information available on growth performance comparisons between these three tilapia species, and given the current controversy and species dilemma facing South Africa, the current study aims to provide information around the issue, and aid decision makers with scientific backing to validate decisions on which tilapia species to endorse. It also aims to explore the field of BFT in South Africa, opening up doors for future research in this alternative approach, which may hold great potential and assist in the development of the tilapia industry both locally and worldwide.

3.3 Material and Methods

Experimental system

The experiment was conducted on the Welgevallen experimental farm near Stellenbosch, South Africa. A single greenhouse was used to house the experiment, and fitted with removable sides in order to aid in temperature regulation and mimic commercial BFT systems in more temperate environments. Twelve cylindrical tanks with a diameter of 620 mm, each with a volume of 250 L as used by Azim and Little (2008), were fitted with a central 40 mm drain at the bottom in order to effectively drain the sludge when necessary. Tanks were elevated on building blocks and placed in a 6x2 formation (Figure 3.1).

Figure 3.1. The experimental setup displaying the 250 L tanks used as well as the main 40 mm aeration ring.

Aeration and circulation

A 1.1 kw side channel blower (FPZ, Concorezzo, Italy) was used, and ran continuously throughout the duration of the experiment. It fed into a ring of 40 mm black irrigation piping, which aided to even the pressure and ensure that each individual tank received even aeration and mixing. From the 40 mm ring, a 15 mm black irrigation pipe was run into the bottom of each tank, and attached to the aeration ring. This aeration ring consisted of a 1940 mm long piece of 15 mm irrigation pipe bent into a ring, with individual holes made with a pin every 25 mm along the length of the pipe. This ring fitted snugly around the inner perimeter of the tank, producing a ring of bubbles additionally aiding in mixing, and was anchored in place with two stones.

To ensure that the water quality was uniform and homogenised throughout the tanks, 12 airlift pumps were constructed using 15 mm polyvinyl chloride (PVC) pipes, with air fed from the 40 mm air ring using micro tubing commonly used in drip irrigation systems. These air lift pumps were inter-leading from one tank to the next, and had an output of six litres per minute each. In anticipation for the case of a blockage of an airlift pump, a connection between adjacent tanks was made using 32 mm flexible pipe, just above the 250 L water level of the tanks, in order to even out the water and prevent tanks from overflowing.

Fish Stocking

All procedures throughout the trial were in line with the ethical protocol (SU-ACUD15-00005) approved by the research ethics committee for animal care and use at Stellenbosch University. Fish were housed in a recirculating aquaculture system (RAS) on the Welgevallen premises, and fed the pelleted feed used in the experiment for two weeks prior to the commencement of the trial, in order to accustom them to the feed. The three fish species were stocked at a rate of 60 mixed sex fish/tank, into an already established BFT system on 6 October 2014, once temperatures had risen sufficiently after the winter, and removed 10 weeks later on 15 December 2014. The 12 tanks allowed for four repeat tanks per species as done by Azim and Little (2008), with completely random allocation. *Oreochromis andersonii* of average mass 19.30±0.41 g were stocked into tanks one, two, seven and 10. *Oreochromis niloticus* of average mass 36.82±0.59 g, similar to that used by Hooley (2012), were stocked into tanks 3,4,8 and 12, with *O. mossambicus* of average mass 36.53±0.45 g being stocked in tanks 5,6,9 and 11 respectively. Differences in starting mass were as a result of the limited availability of all species of

similar size at the time when the experiment commenced. *Oreochromis andersonii* used were the offspring of wild caught fish from the Kaprivi region in Namibia. *Oreochromis niloticus* were of the GIFT strain, and imported as fry from Thailand, being grown to the size at which they were stocked into the system. The red strain of *O. mossambicus* used in the trial came from stock at Stellenbosch University which has been the result of selective breeding strategies at this respective institution.

Tank Management

The system was checked at least four times a day, with feeding taking place twice a day, at 08h00 and at 16h00. Special effort was made to individually observe each tank, and check for signs of unconventional behaviour indicating distress, in order to ensure the well-being of the fish and pick up any problems. The afternoon feeding did take place slightly later at 17:00 in the beginning of the experiment, but a high biological oxygen demand (BOD) in the water under low light conditions resulted in dissolved oxygen (DO) levels falling into the evening. It was therefore brought forward in order to allow the autotrophs within the microbial community to still utilise sunlight and aid in replenishing DO levels. As is conventionally done, temperature, DO, pH, floc volume (FV), salinity, total dissolved solids (TDS) and electro-conductivity (EC) were monitored twice daily, with total ammonia nitrogen (TAN), nitrite (NO_2), nitrate (NO_3), un-ionized ammonia nitrogen (UIA-N), orthophosphate (PO_4), total suspended solids (TSS) and turbidity being monitored weekly. All parameters were kept within desirable levels for the greater part of the experiment. Sludge was drained weekly, and replaced with fresh water from a seperate backup biofloc tank containing no fish. Each day at the evening feeding event, a Secchi disk was used to record the visibility in each tank, and give an idea of how homogenized the water was. An alarm system was installed on day nine of the trial following water loss, in order to trigger when such an event was taking place.

Mortalities

Mortalities were removed from the respective tanks, without being replaced. Measurements of the mortalities were taken as was done with the sampling of the fish, along with the date and tank of origin being recorded.

Feeding

Fish were fed a three millimetre pelleted feed which is commercially available (AVI feeds, tilapia grower, protein: 35% lipid: 5%) (Table 3.1). Feeding rates were in line with the amount fed by Azim and

Little (2008) with 1.5 % of body mass fed daily and being split evenly between the two feeding events with some adjustments down to 1.0 %, 0.5 % or no feed when required to improve water quality. A carbohydrate source in the form of maize meal (Table 3.1), was added to the water directly after each feeding at a rate of 80 % of the mass of the feed given, in accordance with the suggestion by Hargreaves (2013) and calculation using the following equation developed by Avnimelech (2012):

$$\Delta CH = \Delta Feed \times \%N \text{ in } feed \times \%N \text{ excretion} \div 0.05$$

This was increased when required to improve water quality with feeding and carbohydrate addition being adjusted fortnightly after sampling of the fish took place.

Table 3.1. The nutrient composition of the three millimetre pelleted feed and the carbohydrate added.

Nutrient	**% composition in:**	
	Feed	**Maize Meal**
protein	35	6,6
lysine	2	-
fat	5	1,2
fibre	5	3,7
Ca	3	-
P	0,7	-
moisture	10	-
sodium	-	0,018
Glycaemic carbohydrate	-	74

Feed Conversion Ratio

Cumulative FCR's were calculated for each tank over the period of the trial. This was done using the recorded amount of feed given to each tank daily, along with the mass increase per tank which was extrapolated from the sampled fish at each sampling event. This was done using the following formula:

$$FCR = amount\ of\ feed\ fed\ (g) \div total\ mass\ increase\ of\ fish\ from\ tank\ (g)$$

The maize meal used to stimulate the biofloc was not included as feed, as it was not directly consumed by the fish. In tanks where mortalities occurred, a commercial standpoint was taken and it was viewed to decrease the total mass from each tank, and they were therefore not excluded from the calculation.

Sampling

Fish were sampled from each tank on six occasions throughout the experiment, at the start of the trial and fortnightly thereafter until the end of the trial. Every two weeks, all the fish in each tank were netted using a specially constructed circular net. Twenty fish/tank were randomly selected and

placed in a mild solution of clove oil (0.1 mL/10 L water) for a period of 30 seconds in order to sedate them. Mass, total length (TL) and standard length (SL) measurements were taken, after which the fish were placed into a well-aerated recovery bucket with clean water, and then returned to the tank once fully recovered. Special care was taken to ensure that the sampling process was fast and efficient, and would impose the least possible stress on the fish. Furthermore, feeding did not take place on sampling days in order to reduce the stress.

Statistical Analysis

Data were analysed using SAS for windows version 9.3. Average daily weight gain was calculated by means of fitting a simple linear regression for each species, then comparing the species using PROC GLM. Due to the larger difference in starting mass of the *O. andersonii*, the intercept was included as a covariate and the Bonferroni least squares means were calculated for all variables. For the FCR's calculated, one way analysis of variance (ANOVA) was used to analyse the data as well as Bonferroni multiple comparison tests, where $P < 0.05$ was considered significant. The assumptions of normality and homogeneity for the analyses were tested using the Shapiro-Wilk and Levene's test respectively.

3.4 Results

Daily Water Quality

Throughout the trial, the mean water temperature recorded in the morning was 22.5±0.2ºC with the afternoon reading being 27.5±0.2ºC (Table 3.2). On the two occasions early on in the trial where the water temperature dropped below 20ºC in the morning, feeding was postponed until after the temperature had risen above 20ºC in accordance with normal growth recommendations by El-Sayed (2006). DO levels had a mean of 6.5±0.1 mg O_2/L throughout the trial with pH averaging 6.28±0.04 (Table 3.2). Total dissolved solids (TDS) levels averaged 1.493±0.063 g/L with floc volume (FV) having a mean of 48±4 mL/L (Table 3.2). Secchi disk readings were the same throughout each tank on each day, indicating that the water was well homogenized.

Table 3.2. The mean values for the respective water quality parameters measured throughout the trial.

Frequency	**Parameter**	**Unit**	**Mean Value**
Daily	Temperature am	ºC	22,5±0.2
	Temperature pm	ºC	27,5±0.2
	DO	mg/L	6,5±0.1
	pH	-	6,28±0.04
	EC	mS/cm	2.309±0.099
	salinity	ppt	1,2±0.1
	TDS	mg/L	1,493±0.063
	FV	mlL/L	48±4
Weekly	TAN	mg/L	3,8±0.4
	NO_2	mg/L	3,510±0.799
	NO_3	mg/L	5,6±0.9
	UIA-N	mg/L	0,008±0.002
	PO_4	mg/L	6,4±1.6
	Turbidity	FAU	458±51
	TSS	mg/L	520±55

Weekly Water Quality

Mean values for the parameters measured weekly were calculated (Table 3.2). High levels of NO_2 were recorded from day 13 to day 18 of the experiment, following an unplanned water loss event due to system failure, which took place on day nine and a cold spell on days 12 and 13. This resulted in the mortality of 32 *O. andersonii*, and 39 *O. mossambicus* with none for *O. niloticus*. Un-ionised ammonia nitrogen (UIA-N) was calculated as described by El-Shafai et al. (2004) (Table 3.2), with the value never exceeding 0.042 mg/L throughout the course of the trial.

Fish Mortality

Between days 13 and 18, a total of 32 *O. andersonii*, and 39 *O. mossambicus* mortalities were observed, with none being observed for *O. niloticus.*

Feeding

The different parameters related to feeding are displayed as an average per tank of 60 fish for each species (Table 3.3). In tanks where the fish grew faster and therefore had a higher body mass used to adjust feeding rates, total feeding was intuitively higher. The end stocking density was highest in the *O. niloticus* tanks, having increased by 123 % over the course of the trial as opposed to the 39 % increase for both the *O. mossambicus and the O. andersonii* respectively. These values also

correspond to the percentage mass gain for the respective species over the 10 week trial. Mortalities were highest, and occurred first in the *O. mossambicus* tanks when NO_2 levels rose. An average of 12.25 mortalities/tank were recorded for *O. mossambicus*, with 9.5 /tank documented for *O. andersonii* over the whole course of the trial. Very few mortalities were recorded for *O. niloticus* (1.75 /tank), with most of them being as a result of jumping out of the tanks. This was as a result of the vigorous feeding behaviour displayed by *O. niloticus* as opposed to the less intensive but observed behaviour by *O. mossambicus,* and no surface response by *O. andersonii,* which were not observed on the surface at all throughout the course of the trial. *Oreochromis niloticus* displayed the lowest FCR of average 1.00±0.05 which was significantly different to the 2.24±0.16 and 2.53±0.28 for *O. mossambicus* and *O. andersonii* respectively. There was however no significant difference in the FCR's of *O. mossambicus* and *O. andersonii.*

Table 3.3. Showing the mean growth and feeding related parameters ± standard error per tank for the four repeats of the three species.

Parameter	O. andersonii	O. mossambicus	O. niloticus
Total feed over 10 weeks (g)	1074.74±56.64	1869.58±57.60	2721.70±109.01
Start stocking density (kg/m³)	5.15±0.12	9.74±0.10	9.82±0.54
End stocking density (kg/m³)	7.14±0.45	13.53±0.33	21.88±0.70
Start mass (g)	1157.79±27.72	2191.80±21.93	2209.34±121.46
End mass (g)	1607.19±102.01	3044.80±74.31	4923.63±156.72
Mass gain (g)	449.41±75.61	853.00±87.64	2714.28±85.26
FCR	2.53±0.28	2.24±0.16	1.01±0.05

The results from the Shapiro-Wilk test for normality and the Levene's test for homogeneity suggest, with a $p>0.05$, that the assumptions of normality and homogeneity of the data were met

Table 3.4. The results from the Shapiro-Wilk test for normality performed on the feed conversion ratio data.

Shapiro-Wilk test				
Test		Statistic		p Value
Shapiro-Wilk	W	0.970954	Pr < W	0.9205

Table 3.5. The results from the Levene's test for homogeneity performed ion the feed conversion ratio data.

Levene's test

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Species	2	0.0200	0.0100	1.49	0.2816
Error	8	0.0537	0.00671		

Growth Rates

A linear regression was fitted to the mass data of each tank (Figure 3.2). There were significant differences ($p < 0.05$) between the growth rates for each species, indicated by the mean mass increase over the period of the experiment. *Oreochromis andersonii* showed the lowest growth rate with an average daily gain of 0.185±0.025 g/day (R^2=0.752) as compared to *O. mossambicus* of 0.405±0.025 g/day (R^2=0.919) (Figure 3.2). *Oreochromis niloticus* showed the highest growth rate with an average daily gain of 0.693±0.018 g/day (R^2=0.918) (Figure 3.2).

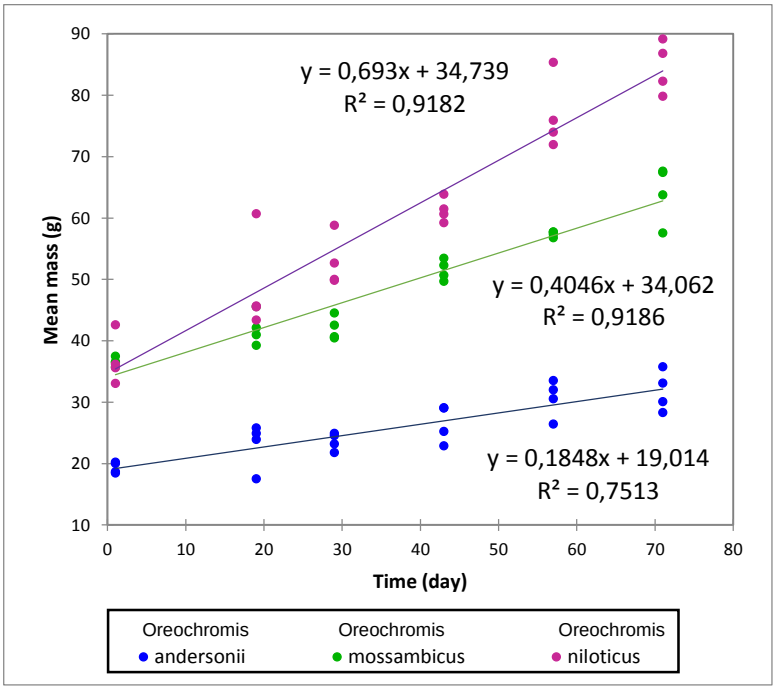

Figure 3.2. A regression for each species fitted on the rate of mean mass increase.

The results from the Shapiro-Wilk test for normality and the Levene's test for homogeneity suggest, with a $p>0.05$, that the assumptions of normality and homogeneity of the data were met (Table 3.6 and Table 3.7)

Table 3.6. The results from the Shapiro-Wilk test for normality of the average daily mass gain data.

Shapiro-Wilk test

Test		Statistic		p Value
Shapiro-Wilk	**W**	0.882528	**Pr < W**	0.0944

Table 3.7. The results from the Levene's test for homogeneity of the average daily mass gain data.

Levene's Test

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Species	2	2.149×10^{-6}	1.074×10^{-6}	0.26	0.7773

3.5 Discussion

Water Temperature

The mean morning (22.5±0.2 ºC) and afternoon (27.5±0.2 ºC) water temperature readings were both within the range of 20-35 ºC given by El-Sayed (2006) for the normal growth of tilapia. Temperatures in the morning were however outside the general consensus of an optimal range, where estimations are within the 25-30 ºC bracket (El-Sayed 2006; Crab et al. 2009). This optimal temperature range for the fish is however higher than the range of 20-25 ºC put forward by De Schryver et al. (2008) as the ideal temperature for a stable BFT system. It is therefore difficult to optimize both simultaneously, and with the daily temperature changes expected in commercial scale BFT systems, it is likely for temperatures to vary between both brackets at different times of the day.

Water Loss

Water loss was recorded on day nine after an airlift pump became blocked. As the system was filled up with clean water following this event, and the occurrence of a cold spell on days 12 and 13, it resulted in a considerable decrease in the concentration of biofloc in the system. This reduced level of biofloc was therefore not capable of controlling the high nitrogen input by feeding, and lethal concentrations of NO_2 and TAN levels were measured, resulting in mortalities. These spikes are similar to those reported by Avnimelech (2012) in the establishment of new BFT systems. This event highlighted the fine balance of the system and the importance of an adequate level of biofloc required in order to control the level of dangerous nitrogenous compounds. It also emphasized the importance of having backup water with biofloc established for research systems, and the significance of an alarm system to notify the relevant personnel when water is being lost in such systems. To help prevent the high levels of toxic nitrogen compounds in such an event, feeding should also be cut with an increase in the level of the carbohydrate source added to the water in order to allow the effective recovery and

re-establishment of the biofloc. Feeding should then be slowly increased to the required level as the level of biofloc increases accordingly.

As a result of this event, corrective measures implemented, and mortalities, growth rates were significantly reduced between the sampling events on days 19 and 29. *Oreochromis andersonii* showed an average increase in mass per fish from 23.00±1.14 g to 23.57±0.97 g, with *O. mossambicus* increasing from 41.96±1.14 g to 42.01±0.97 g respectively. *Oreochromis niloticus* however, showed a higher growth rate with the average mass per fish increasing from 48.78±1.14 g to 52.79±0.97 g. This, coupled with no mortalities induced by poor water quality, and a constant response to feeding when the other species did not feed, advocates the superior resilience of *O. niloticus* in poor water quality conditions. It is resilience such as this which is sought after when selecting a species for use in aquaculture (Boyd 2004; Jamandre et al. 2011). Due to the control mechanism used for dangerous nitrogen compounds in BFT, and its dynamic nature, a hardy species which can handle these levels if needed will be an advantage.

Feed Conversion Ratio

During the period from day 19 to 29, and being substantially influenced by the number of mortalities, the overall mass in seven tanks (three *O. mossambicus,* three *O. andersonii* and one *O. niloticus*) decreased and therefore rendered negative FCR values for this period. Negative FCR have been documented in studies by Cotton et al. (2003) as well as Jalali et al. (2013) and are often linked to mortalities where although the individual fish mass may not decrease, the total mass per tank decreases.

Over the 10 week period of the trial, *O. niloticus* showed a superior FCR of 1.01±0.05 as opposed to the 2.24±0.16 for *O. mossambicus* and the 2.53±0.28 for *O. andersonii*. This is similar to findings by Gopalakrishnan (1988) and Siddiqui and Al-Harbi (1995) on the better efficiency performance of *O. niloticus* when compared to the other concerned species in earthen ponds and RAS respectively. Although the number of mortalities recorded for *O. mossambicus* and *O. andersonii* did effect this FCR, these finding suggests that the superior hardiness and efficiency of *O. niloticus* is consistent in BFT systems as well. As mortalities are considered a negative factor from a commercial standpoint, a decrease in fish biomass due to mortality is decreasing the total mass potentially harvested from each tank. To keep FCR linked to this application, and credit species showing the

desirable hardiness, tanks with fewer mortalities would have a better chance of reflecting this in their FCR data as the total mass from each tank would decrease less due to mortality. Even though this approach was used, the FCR's obtained may partially relate to the suggestion by Popma and Lovshin (1996) that the shortened intestine as recorded for *O. mossambicus,* may inhibit its ability to utilize planktonic algae, and thus better use of this food source is observed for *O. niloticus.*

Growth

The growth rate of *O. niloticus* was significantly higher ($p < 0.0001$) than that of *O. mossambicus* which was significantly higher ($p = 0.0106$) than that of *O. andersonii* over the entire period of the trial. This ranking was also in sequence with the amount of selective breeding and strain development attributed to the three species investigated, and highlights the importance of such strategies in raising the performance of cultured organisms. Even though the stocking density in kg/m^3 was significantly lower in the *O. andersonii* tanks due to the smaller starting size, this did not appear to be a limiting factor for the *O. niloticus* or *O. mossambicus* tanks, which still showed superior growth when compared to the *O. andersonii*.

As these species occupy slightly different niches and feed at different trophic levels in their segregated natural habitats (Skelton 2001), it was speculate that one species may be able to better utilise biofloc and therefore grow better in a BFT system. The FCR and growth results suggest that this is not the case for *O. mossambicus* and *O. andersonii* respectively, as they cannot derive significantly higher benefit from the consumption of biofloc in order to compete with the low FCR and high growth rate of *O. niloticus.*

3.6 Conclusion

BFT systems can operate successfully in local South African conditions. The study could serve to give interested parties an idea on tilapia growth rate when utilizing BFT systems in South Africa. *Oreochromis niloticus* showed superior growth rates and FCR to *O. mossambicus* which was superior to *O. andersonii*. With this documented in a BFT system under South African conditions, *O. niloticus* was the most compatible candidate of the three species investigated to proceed with in such systems in South Africa. The industry should therefore advise farmers on the benefits of using this species in BFT systems instead of the other tilapia species investigated, and pave the way by providing farmers with adequate accessibility to *O. niloticus* fingerlings. There is much scope for further research in more

extensive periods of the life cycle, as well as the seasonal viability of BFT, given our local climate. Studies into different mechanisms of customizing BFT to local South African conditions would be of value as well as looking at the correlation between intestinal length, and the efficiency to utilize biofloc between species. Economic viability comparisons between BFT and other aquaculture systems used for the cultivation of tilapia would also be beneficial to the growing industry in South Africa and worldwide.

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4 A Description of Key Water Quality Parameters in a Tilapia Biofloc System

4.1 Abstract

Biofloc technology (BFT) is envisaged to play an increasing role in fish production, with research into the mechanisms and dynamics being of paramount importance. Water quality plays a key role in the performance of the cultured species, therefore it is intuitive to document this facet over time in a BFT system. This study was aimed at building a profile of key water quality parameters during the initial 15 day start-up phase, and once tilapia (*Oreochromis spp*) were introduced to a BFT system. Furthermore it looked at the effects of the addition of salt (NaCl) on a variety of water quality parameters. The trial was initiated in September 2014 at the start of the summer growing season for tilapia in South Africa. Temperature, dissolved oxygen (DO), pH, floc volume (FV), salinity, total dissolved solids (TDS) and electro-conductivity (EC) were measured twice daily, with total ammonia nitrogen (TAN), nitrite (NO_2), nitrate (NO_3), un-ionized ammonia nitrogen (UIA-N), orthophosphate (PO_4), total suspended solids (TSS) and turbidity being measured weekly over the 87 day trial. Salt was added to the system 31 days into the trial. Nitrogenous compounds reached critical levels on two occasions, once during start-up and the other with fish present, following a water loss event. TAN, TSS and turbidity showed a steady increase as the trial progressed. It was found to be difficult to maintain all parameters within optimal levels for tilapia for the entire duration of the trial in the small research system used. Salt had an effect on increasing EC and TDS while decreasing NO_2 levels, and may have had an indirect effect on decreasing DO levels. It was apparent that salt was a minor contributing factor to these changes with other primary contributing factors playing a larger role. This trial will prove valuable in what water quality changes to expect during start-up and following a water loss event in a BFT system.

4.2 Introduction

Water quality is widely accepted to be of importance to aquaculture, with extensive work being done to develop a better understanding of this eminent aspect (Boyd and Tucker 1992; El-Sayed 2006; Cole et al. 2009; Auffret et al. 2013; Amal et al. 2015). Although a number of parameters can influence water quality, the measurement of a few key parameters is practical and common practise in commercial systems to maintain good water quality (Boyd and Pillai 1985; El-Sayed 2006).

Biofloc Technology

Biofloc technology (BFT) employs an alternative approach to maintaining good water quality through the *in situ* action of micro-organisms within the water, predominantly heterotrophic bacteria (Avnimelech 1999; Avnimelech 2012; Hargreaves 2013). The concept of BFT requires the manipulation of the C:N ratio of inputs through the addition of a carbohydrate source, as well as sufficient mixing and aeration in order to keep the biofloc (aggregation of micro-organisms and organic matter) in suspension and active (Avnimelech 1999; Crab et al. 2012). Heterotrophic bacteria operate at up to a factor 10 higher growth rate than nitrifying bacteria, and therefore rapidly remove TAN from the water and incorporate it into microbial protein (Hargreaves 2006; Avnimelech 2012; Crab et al. 2012). This microbial protein housed in the suspended biofloc, is then available to be harvested by cultured species which are capable of filter feeding (Hargreaves 2013).

Originating from studies on water conservation, land costs and environmental concerns, BFT was developed to make more efficient use of the inputs available (Emerenciano et al. 2013). The recycling of nutrients within a BFT system allows feeding with pelleted feed to be significantly reduced, as feeding efficiency is enhanced (Avnimelech 2007; Widanarni et al. 2012; Hargreaves 2013; Luo et al. 2014). With water being constantly ridded of nitrogen compounds, and low to zero water exchange in BFT systems, the costs associated with purchasing and running external filters are eliminated, with the environmental cost of high water usage and effluent release being minimised (Avnimelech 2007; Avnimelech 2012; Emerenciano et al. 2012; Hargreaves 2013). Furthermore, the low water usage in BFT systems means that there is no need to be constantly pumping water into the pond or through a filtration system, and therefore the high costs associated with pumping are reduced. With sustainability and efficiency at its core, BFT has been labelled as an environmentally friendly aquaculture technique, and therefore shows potential for future application (Emerenciano et al. 2011; Widanarni et al. 2012; Emerenciano et al. 2013).

Tilapia

With tilapia being identified as the fish of the 21st century (Fitzsimmons 2000; Ridha 2006; El-Sayed 2013), capable of filter feeding and withstanding a high total suspended solids (TSS) level (El-Sayed 2006; Avnimelech 2012), its increased use in BFT shows positive potential. The stocking density of tilapia in BFT systems can also be far greater than that of shrimp, reaching up to 50 kg/m^3 or 500

tons/ha (Avnimelech 2012). At such high stocking densities, the constant monitoring of water quality is essential to ensure the well-being of the fish at all times.

Water Quality Monitoring

In BFT research and commercial systems it is common practise to have two groups of water parameters which are measured, as was done by Azim and Little (2008); Widanarni et al. (2012) and Liu et al. (2014). The first is easier to measure and usually performed daily, or twice a day, in the morning and evening. It includes parameters such as temperature, dissolved oxygen (DO), pH, floc volume (FV), salinity, electro-conductivity (EC) and total dissolved solids (TDS). The importance of these individual parameters to the proper functioning of a BFT system varies, with the major focus being on temperature, DO, pH and FV (Avnimelech 2007; Widanarni et al. 2012; Liu et al. 2014). The interrelatedness of these parameters is also evident, and would aid in the justification of only measuring certain ones.

The second and less frequently measured group is typically evaluated weekly or bi-weekly, and includes total ammonia nitrogen (TAN), nitrite (NO_2), nitrate (NO_3), orthophosphate (PO_4), alkalinity, turbidity and TSS (Azim and Little 2008; Prajith and Madhusoodana 2011; Widanarni et al. 2012; Liu et al. 2014). Nitrogen parameters have been regarded worldwide to be the foremost limiting factor to the survival of organisms grown in aquaculture (Tseng and Chen 2004; Crab et al. 2007; Barbieri 2010; Xian et al. 2011; Santacruz-Reyes and Chien 2012). The dangerous nitrogen levels are often displayed as un-ionised ammonia nitrogen (UIA-N), NH_3–N or NH_3 which is in equilibrium with the far less toxic ionized ammonia nitrogen (IA-N), NH_4^+ or NH_4^+–N, together making up TAN.

The importance of monitoring water quality in BFT systems should not be underestimated. Being intricately balanced and totally reliant on the microbial community for good water quality, it is important to consistently monitor water quality in order to better understand this poorly documented component of a BFT system. By describing a profile of the water quality in BFT systems, and the collective effort of many such studies combined, we can compile a standard profile of the different parameters. This will not only aid in enhancing existing BFT systems, but also create a starting point for market entrants on what to expect. Being faced with high feed costs, water restrictions, little market development, and a know-how shortage, the infant tilapia sector in South Africa may gain benefit from a combination of such studies on the BFT concept where such issues are addressed.

4.3 Material and Methods

Experimental System

The experiment took place from 20 September 2014 until 15 December 2014 in a greenhouse, on the Welgevallen experimental farm near Stellenbosch, South Africa. The experiment commenced once water temperatures had risen satisfactorily to be consistently above 20ºC after the winter, and would coincide with the start of the summer growing season. The same system as described in section 3.3 of chapter three was used in this trial.

Biofloc Establishment

Matured tank water from the recirculating aquaculture system (RAS) system housing the fish prior to the trial was used to fill the newly constructed experimental system on 20 September 2014, sixteen days prior to fish being introduced to the system. It was intended that this water would provide a base level inoculation of some of the microbial community required in a biofloc system, and therefore speed up the establishment of the microbial community, as opposed to using fresh water. The system was left to run for two days to observe the functionality of the new system, after which the addition of feed and a carbon source in the form of maize meal commenced, to stimulate the progression into a biofloc system.

A commercially available three millimetre extruded, pelleted feed, the same as would be used to feed the fish in the trial was utilized to fertilize the experimental system prior to the trial. The feed contained five percent lipids and 35 % protein. The amount added per day was based on a calculation of the amount of uneaten feed which would be entering the system if the fish were present, assuming 25 % nitrogen assimilation by the fish (Hargreaves 2013). Maize meal was added to the inoculated system based on the calculation from the equation below, presented by Avnimelech (1999) and Avnimelech (2012).

$$\Delta CH = \Delta Feed \times \%N \; in \; feed \times \%N \; excretion \div 0.05$$

Fish Management

Three available species of tilapia; *Oreochromis niloticus, Oreochromis mossambicus* and *Oreochromis andersonii* of average mass 36.82±0.49 g, 36.53±0.49 g and 19.30±0.49 g respectively, were separately stocked by random allocation into four tanks each. Sixty fish were stocked per tank to

ensure that densities did not become a limiting factor by the end of the trial, with all processes being compliant with the ethical protocol (SU-ACUD15-00005) approved by the research ethics committee for animal care and use at Stellenbosch University. Fish were fed a pelleted commercial diet equivalent to 1.5 % of their body mass daily, through two feeding events. Feeding rates were adjusted fortnightly following growth sampling of the fish, where a sample was used to estimate the total mass of fish in each tank.

Water Monitoring

Temperature, DO, pH, EC, salinity, TDS and FV were measured in the system twice daily before feeding events at 08h00 and 16h00. Temperature and DO were measured more frequently using an oxygen meter (YSI, Pro ODO, Yellow Springs, USA) each time the tanks were checked in order to ensure the well-being of the fish, with measurements only being recorded twice daily. pH was measured using a pH and temperature meter (YSI, 60/FT, Yellow Springs, USA) with a conductivity meter (YSI, EC300, Yellow Springs, USA) being used to measure the EC, salinity and TDS. The FV was measured using an Imhoff cone by allowing one litre of tank water to settle for 15 minutes while the other parameters were measured, before reading off the settled level.

The second list of parameters was measured weekly, with more frequent measurements taking place at some intervals when elevated levels were a concern. All of the following measurements were done using a colorimeter (Hach, DR/850, Loveland, USA) with repeat measurements from two randomly chosen tanks. Turbidity was measured using the absorptiometric method with the photometric method being followed to measure TSS. PO_4 was measured using the ascorbic acid method. Nitrogenous compounds, namely TAN and NO_2 were closely monitored when their levels were a concern, using the salicylate and diazotization methods respectively. NO_3 was measured accordingly using the cadmium reduction method. The toxic UIA-N level was calculated as described by El-Shafai et al. (2004) using the TAN, pH and temperature levels as inputs into the general equation of bases presented by Albert (1973):

$$\frac{[NH_3 + NH_4^+]}{[1 + 10^{(pK_a - pH)}]}$$

With pKa in freshwater being calculated by the formula presented by Emerson et al. (1975):

$$pK_a = 0.09018 + \frac{2729.92}{T}$$

where T is measured in Kelvin which is 273 + TºC. In the case where the levels of nitrogen compounds were deemed to be hazardously high, salt (NaCl) was added, feeding rates were reduced or cut, with maize meal addition being increased in order to stimulate the microbial community to reduce the high levels. Sludge, with an approximate volume of three litres was drained weekly from each tank, was also removed more frequently during these critical times. When pH levels were below 6.0, one kilogram of limestone was added per tank in perforated bags. This was intended to help with raising the pH along with adding to the buffering capacity of the system.

Correlation Analysis

The mean values of each of the water parameters measured were compared for the two periods from 15 days before the stocking event to day 15 of the trial (before salt addition) and days 16-71 (after salt addition) using one way analysis of variance (ANOVA) coupled with Bonferroni multiple comparison tests. Differences were considered statistically significant where $P < 0.05$.

4.4 Results and Discussion

Temperature

Water temperatures increased accordingly over the 15 days prior to fish being introduced to the system, from 12.8ºC on day -15 to 23.9ºC on day one (Figure 4.1). This was in line with the start of the seasonal growing period for tilapia in more temperate areas, where fish would be introduced after temperatures had risen sufficiently after the winter (Dan and Little 2000). As expected, temperature readings were higher in the afternoon due to the solar heating received during the daylight hours. Over the course of the trial, the mean morning and afternoon temperatures were 22.5±0.2ºC and 27.5±0.2ºC respectively.

It was reported by De Schryver et al. (2008) that temperatures from 20-25ºC are optimal for maintaining a stable BFT system at an intermediate FV. This requirement needs to also be balanced with the optimal temperature requirement of the cultured species, reported between 25 and 30ºC for tilapia (El-Sayed 2006; Crab et al. 2009). Due to the nature of a semi-intensive BFT system, it would be impractical to maintain temperatures within one of these ranges for the duration of the culture period, and in most cases temperatures will vary through both ranges.

Due to the large surface area to volume ratio of the small research system used in the trial, heat loss and temperature fluctuations were observed. These fluctuations would otherwise be buffered in large scale commercial systems, where a smaller surface area to volume ratio results in less heat loss per unit volume. In order to prevent water temperatures from dropping below 20ºC for long periods, heaters were placed in each tank and used on four occasions throughout the course of the trial when temperatures were below 20ºC. On warm days when air temperatures where high, the tunnel housing the system was opened up on the ends to allow ventilation, with the lids of tanks being removed and the air intake being drawn through an ice box in extreme heat, in order to prevent water temperatures from exceeding 30ºC for extended periods.

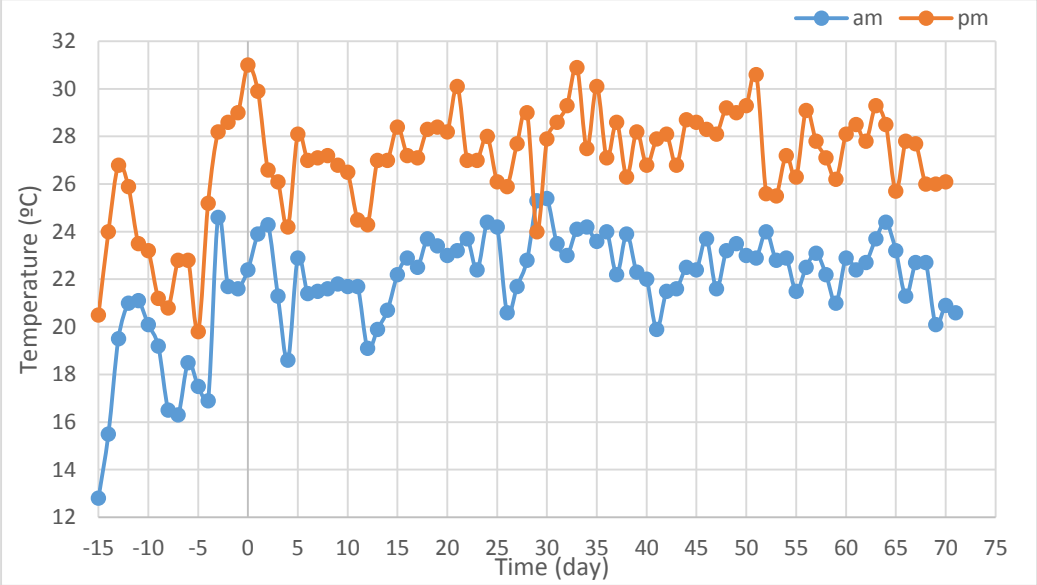

Figure 4.1. Morning and afternoon water temperatures through the course of the trial, measured twice daily at 08h00 (am) and 16h00 (pm) in the system.

Dissolved Oxygen

As water temperature increases, DO levels decrease (El-Sayed 2006; Avnimelech 2012). Thus, from days -15 until day zero, the decrease in DO level (Figure 4.2), corresponds to the increase in the water temperature (Figure 4.1). The decrease in DO levels over this initial period of the trial is also consistent with the development and growth of biofloc in the system, increasing the Biological Oxygen Demand (BOD) of the system. With a high BOD being characteristic of a BFT system, it was expected that DO levels may become hazardously low for tilapia at higher temperatures experienced during the early afternoon (Figure 4.2). This may be further exacerbated at high stocking densities of the cultured

organism as well as at high biofloc levels revealed in FV, turbidity and TSS readings. DO levels steadily decreased from day 40 until the end of the trial (Figure 4.2), consistent with the growing BOD on the system driven by the increase in stocking density (kg/m³) caused by the growth of the fish, rather than by temperature, which was relatively constant (Stickney 2009; Widanarni et al. 2012).

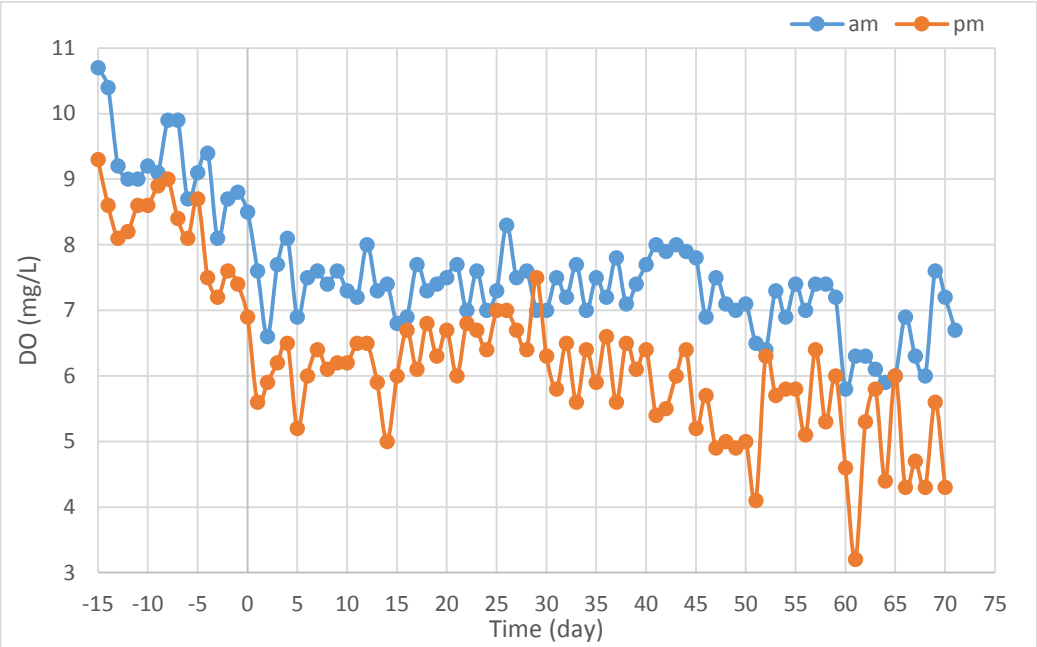

Figure 4.2. Morning and afternoon dissolved oxygen (DO) levels of the tank water through the course of the trial, measured twice daily at 08h00 (am) and 16h00 (pm).

Salinity, Total Dissolved Solids and Electro-conductivity

The solubility of oxygen is lower in more saline conditions (Boyd and Pillai 1985), therefore DO decrease as salinity increases. EC, salinity and TDS are closely related (Figure 4.3), with some researchers regarding TDS measurements as a measure of salinity (Grattan 2002). Although this may be the case in clean water, there are however other organic solutes contributing to TDS apart from just salts, which make up the salinity reading. The measurements for TDS and salinity are derived from EC as this easily measured parameter is directly related to the ion concentration in the water, with this relationship being clearly evident (Figure 4.3) These EC related parameters are used as an early indication of significant changes in the water quality through the presence of dissolved solutes which could be of detriment to the aquatic life. The drop in EC, salinity and TDS seen on day 52 was as a result of separation strategies and sludge drainage incited by high FV and low DO readings, indicative of an excessive level of biofloc.

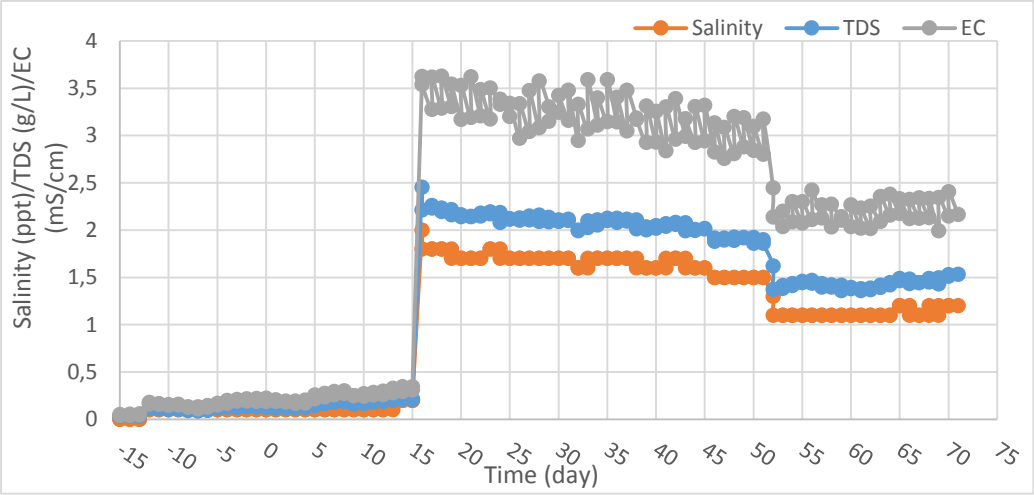

Figure 4.3. EC, salinity and total dissolved solid (TDS) levels through the course of the trial, measured twice daily at 08h00 (am) and 16h00 (pm) in the system.

Orthophosphate

High levels of PO_4 in water has been linked to the growth of autotrophs as well as heterotrophs (Macintosh and Little 1995; Stone and Thomforde 2004). This can be beneficial in BFT systems, up to a point where the FV or TSS is too high and additional proliferation of the microbial community is discouraged. As the micro-organisms constituting the biofloc utilise and thus incorporate PO_4 into their cells which are harvested by the cultured organism, levels of PO_4 have been found to be lower in BFT systems as opposed to RAS (Luo et al. 2014). Although there were some peaks in the PO_4 levels during the trial, levels were well below those reported in a BFT system by Luo et al. (2014) (Figure 4.4). The removal of solids can significantly reduce the PO_4 levels observed in aquaculture systems (Cole et al. 1997; Ray et al. 2010). However, the sludge removal events throughout this trial period did not appear to coincide with the reduction of PO_4 levels, possibly indicating that the increased mixing of a BFT system inhibits the settlement of PO_4 into the sludge.

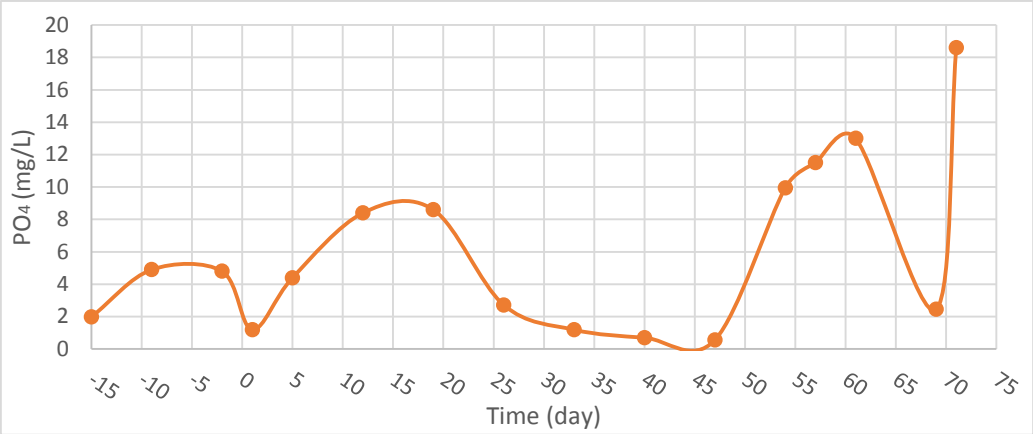

Figure 4.4. Orthophosphate (PO_4) levels measured in the tanks through the course of the trial.

Turbidity and Total Suspended Solids

Turbidity and TSS are closely related, as was seen with their profiles over the course of the trial (Figure 4.5). The levels of both parameters rose relatively consistently over the period of the trial, similar to findings by Liu et al. (2014), with two events leading to short term reductions in their levels. The first was the stocking of fish on day one of the trial. The consumption of biofloc by the fish, meant that the levels of turbidity (253 FAU) and TSS (322 mg/L) recorded on day minus two of the trial dropped, only to be surpassed on day 26. This decrease in TSS levels with stocking, is similar to findings by Avnimelech (2007) with Azim and Little (2008) also pointing out a link to stocking density and suggesting further research into finding the optimum TSS level at different stocking densities. Achieving a balance at which TSS levels are relatively constant at a certain stocking density without separation or settlement strategies would be of significant value, and is a major challenge. The practicality of achieving this on a large scale system may be questionable at this stage, but may have prospects in the future. The second decrease in turbidity and TSS seen from days 50-55 (Figure 4.5), was as a result of settlement strategies implemented by allowing biofloc to settle, and then being drained from tank water in order to bring down the biological load in the system. This was done following low DO levels and suggestion by Avnimelech 2012 to maintain TSS below 500 mg/L.

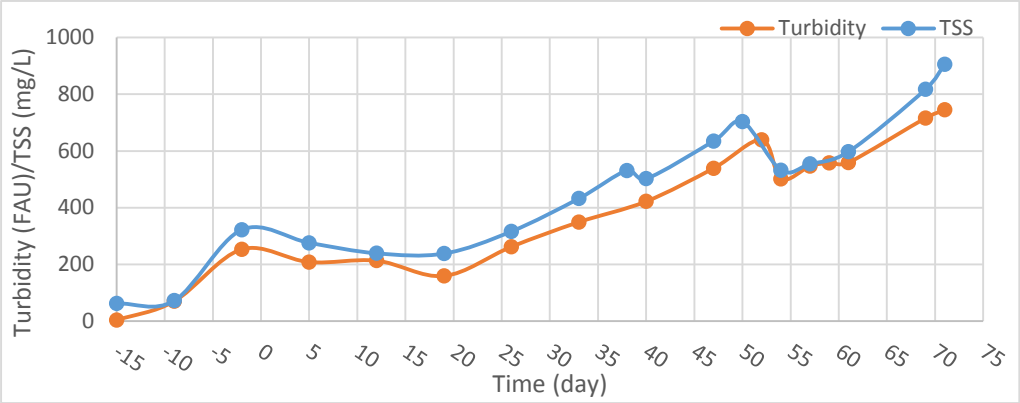

Figure 4.5. Turbidity measured in formazin attenuation units (FAU) and total suspended solid (TSS) levels measured in the tanks through the course of the trial.

Floc Volume

The levels of FV varied between zero and 240 mL/L through the period of the trial, showing two major peaks (Figure 4.6). Being closely related, these peaks are in similar positions to those of TSS and turbidity (Figure 4.5), with the levels of FV dropping off for similar reasons, namely the stocking of fish and settlement strategies. As the trial progressed with FV readings being taken twice daily, scepticism arose around the standardization by using a standardized time when measuring FV. Although a standard settlement time of 15 minutes was used throughout the trial, it was noticed that the amount of time taken for the floc plug to settle changed through the course of the trial. In the beginning of the trial, all the floc in the sampled water appeared to be settled out in 15 minutes and if left for 20 minutes, would re-suspend. From day 59 to the end of the trial, this was not the case, and if left for an extra five to ten minutes, the FV reading would significantly increase. This seems to point towards different densities of the biofloc at different stages in the culture cycle, and did not appear to be linked to the addition of salt which occurred on day 15 of the trial. There was no significant difference between the mean FV values from before (40±5 mL/L) and after (50±7 mL/L) this day, with p=0.280 (Table 4.1).

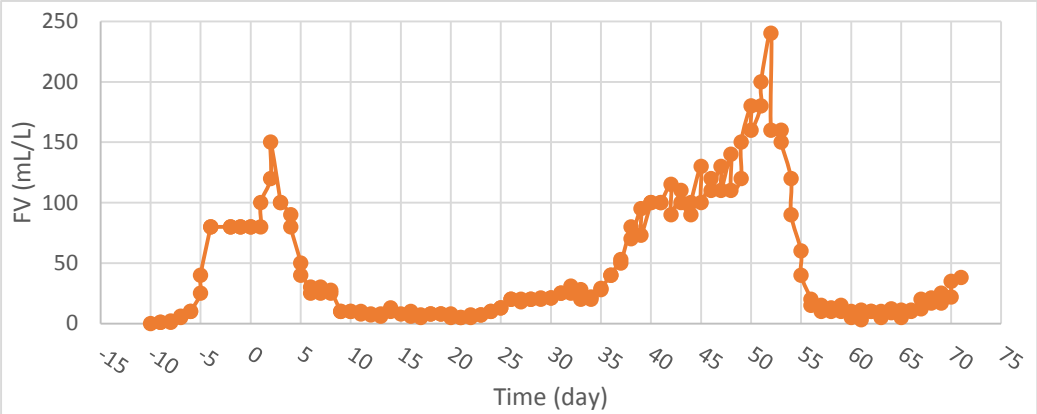

Figure 4.6. Floc volume (FV) levels through the course of the trial, measured twice daily in the system.

pH

pH levels showed great fluctuation throughout the period of the trial (Figure 4.7). Limestone was added to the system on day four in an attempt to raise the critically low pH levels. Morning readings were generally higher than afternoon readings, consistent with findings by Samocha et al. (2007). Declining FV appeared to be associated with decreasing pH. This may be due to the action of bacteria being reduced by the decreasing pH level (Hargreaves 2013). The input of acid to the system through the nitrification process appears to have been a major component of the pH fluctuations observed, as correlation between the various nitrogenous compounds associated with nitrification and the pH is evident.

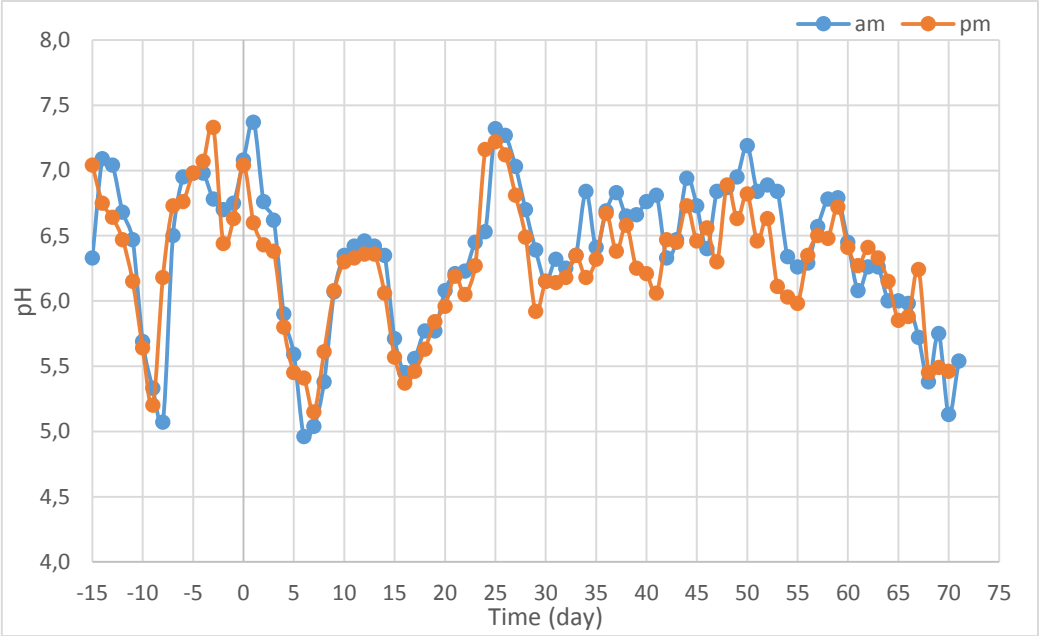

Figure 4.7. Morning and afternoon pH levels through the course of the trial, measured twice daily in the system.

Nitrogenous Compounds

At low pH, the toxicity of TAN is reduced, as the equilibrium between UIA-N and IA-N favours the less toxic IA-N species (Avnimelech 2012). TAN levels showed an upward trend through the trial period, with various peaks and a maximum value of 7.3 mg/L being recorded on day 57 (Figure 4.8a). This was less than the maximum values observed in BFT systems of eight milligrams per litre and 60 mg/L by Azim and Little (2008) as well as Luo et al. (2014) respectively.

The UIA-N levels were calculated over the course of the trial (Figure 4.8c). Partly due to low pH values shifting the equilibrium, UIA-N levels were kept below the 0.1 mg/L recommended level for the majority of the trial, and well within the tolerance levels of tilapia throughout the entire trial period (El-Shafai et al. 2004; Avnimelech 2012).

Two major spikes in NO_2 levels were observed just prior to the fish stocking event, and from days 13-18 (Figure 4.8b). As in the first instance, spikes are expected in the establishment of a new BFT system during the start-up period prior to fish being stocked, as the microbial community controlling the nitrogen assimilation has not yet established (Avnimelech 2012). Spikes in this dangerous compound can lead to mortalities, as observed in the second instance. The second spike appears to have been induced by system failure causing a water loss event to take place on day nine of the trial whereby the sudden loss of biofloc caused the FV reading to decrease from 27.5 mL/L on day eight to

10 mL/L on day nine. This, coupled with a cold period on days 12 and 13, possibly inhibiting the efficient re-establishment of the microbial community, may have caused a shift away from the heterotrophic pathway of nitrogen control to a more nitrification centred pathway. With this sudden shift, the action of the nitrite oxidising bacteria (NOB), tasked with converting NO_2 into NO_3, did not have the immediate capacity of handling such high levels, causing partial nitrification where NO_2 levels rose to toxic levels, as is often observed in start-up (Hargreaves 2013). Such events should be avoided at all costs in BFT systems, and mechanisms such as alarm systems and back-up biofloc containing tanks should be installed in order to minimize the risk of such events.

The presence of NO_3, being the end product of the nitrification process, indicates that this process was still taking place in the experimental system, even though it may not have been the dominant mechanism of nitrogen control for much of the trial period. High levels of NO_3 (Figure 4.8d), although not toxic, were observed, following peaks in TAN levels (Figure 4.8a), indicative of the two step nitrification process taking place. There did not appear to be an accumulation of NO_3 over the course of the trial as has been obseved by Azim and Little (2008) and Luo et al. (2014) (Figure 4.8d), and may be due to the weekly sludge draining acting as an output for this compound.

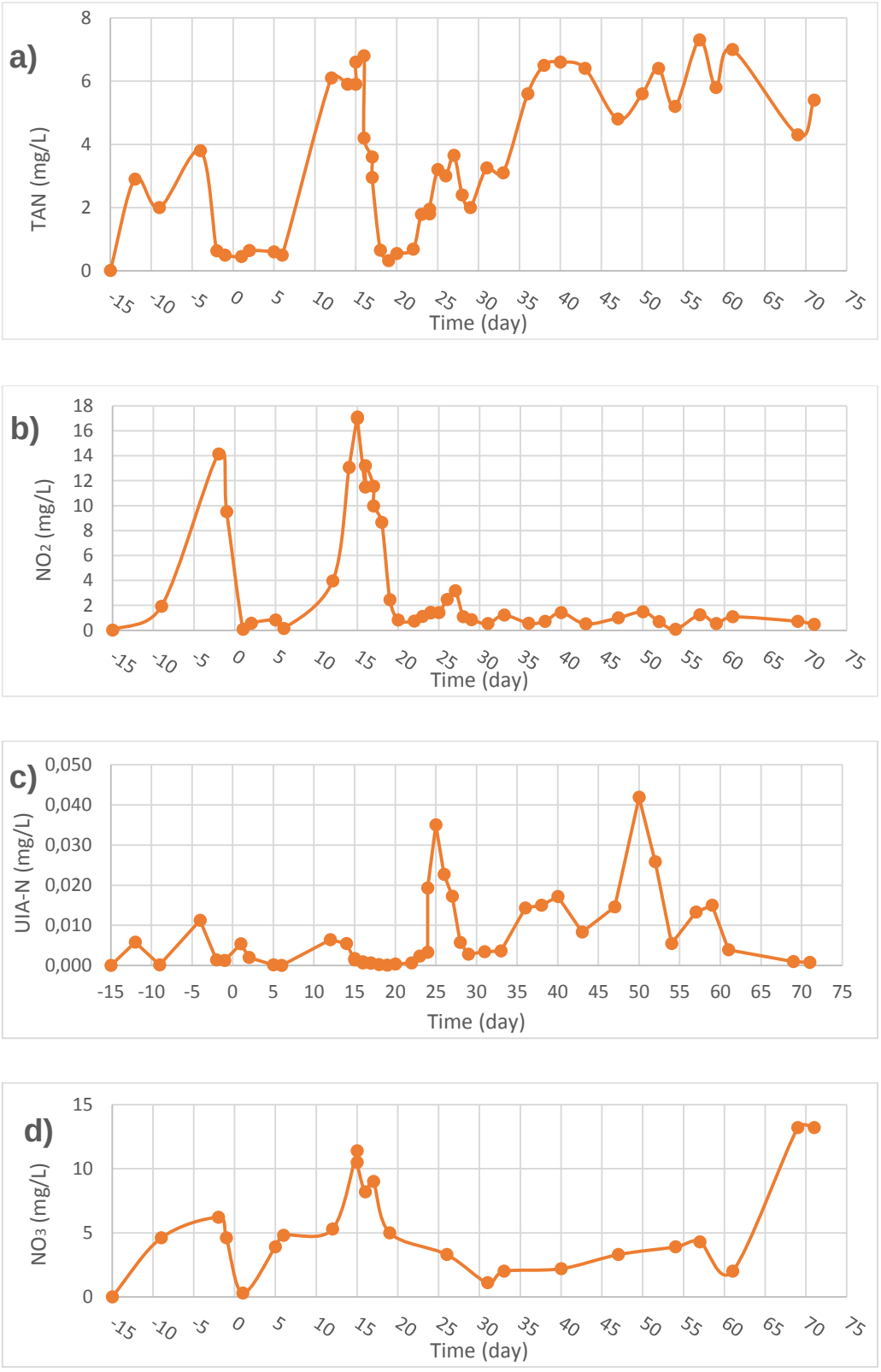

Figure 4.8. The profiles of nitrogenous compounds, a) total ammonia nitrogen (TAN), b) nitrite (NO_2), c) un-ionised ammonia nitrogen (UIA-N), and d) nitrate (NO_3) measured from tank water over the course of the trial.

Salt Addition

Salt was added to the research system on day 15 of the trial following high NO_2 levels, to prevent NO_2 toxicity as was demonstrated by Wang et al. (2006); Wuertz et al. (2013) and Luo et al. (2014).

The temperature of water was significantly different before and after the addition of salt (Table 4.1). Due to the changing of seasons, and with the trial running into the summer period, this is as a result of changing climatic conditions, and shouldn't be correlated to the presence of salt in the water. The addition of salt appeared to have a significant ($P < 0.0001$) effect on the reduction of DO levels from 7.7±0.2 mg/L to 6.5±0.1 mg/L. As DO levels are largely dependent on water temperature, this rise is likely to be as an indirect result of the progression into the summer heat. As DO levels are also largely dependent on the bioload of the system, this significant difference should not be entirely attributed to the rising temperatures, as the addition of salt may in fact increase the system's bioload and therefore indirectly influence DO levels.

Table 4.1. Showing the mean values for various parameters as well as whether there is significant difference before salt was added to the system and with salt present (p values from Bonferroni multiple comparison tests).

Parameter	**Unit**	**No Salt**	**Salt**	**P value**	**Significant**
Temperature	°C	22.9±0.5	25.2±0.3	<0.0001	Yes
DO	mg/L	7.7±0.2	6.5±0.1	<0.0001	Yes
pH	-	6.30±0.08	6.34±0.04	0.647	No
EC	mS/cm	0.198±0.010	2.865±0.050	<0.0001	Yes
salinity	ppt	0.1±0.005	1.5±0.026	<0.0001	Yes
TDS	mg/L	0.132±0.006	1.851±0.030	<0.0001	Yes
FV	mL/L	40±5	50±7	0.280	No
TAN	mg/L	2.6±0.7	4.0±0.4	0.071	No
NO_2	mg/L	6.529±2.042	2.713±0.680	0.027	Yes
NO_3	mg/L	5.2±1.2	5.4±1.2	0.869	No
PO_4	mg/L	4.3±1.0	6.9±2.0	0.347	No
Turbidity	FAU	149±48	500±51	0.001	Yes
TSS	mg/L	194±54	564±55	0.001	Yes
UIA-N	mg/L	0.003±0.001	0.010±0.002	0.034	Yes

Mean salinity levels rose significantly from 0.1±0.005 ppt before the event to 1.5±0.026 ppt after the addition of salt (Table 4.1). Mean TDS (0.132±0.006 g/L) and EC (0.198±0.010 mS/cm) levels before, were significantly different to those recorded after the salt addition event, where means were

1.851±0.030 g/L and 2.865±0.050 mS/cm respectively. As expected, the addition of salt affected these closely related parameters accordingly.

Despite TAN and NO_3 showing an increase, but no significant difference between the two periods (Table 4.1), NO_2 levels were significantly reduced from 6.529±2.042 mg/L to 2.713±0.680 mg/L when salt was added. It should be noted that the addition of salt is often employed in aquaculture to reduce the toxicity of NO_2 rather than decrease its level (Durborow et al. 1997). This significant difference observed, may be as a result of the first period including the two peaks in the NO_2 levels which prompted the addition of salt to the system. As these peaks were classified as extreme events, the addition of salt may not be totally responsible for the lower NO_2 levels. Salt did however appeared to have a stabilizing effect on the NO_2 levels (Figure 4.8b), and thus would be a recommended addition to aid in stabilizing a system. UIA-N levels were significantly higher after salt was added to the system, rising from 0.003±0.001 mg/L to 0.010±0.002 mg/L. As this calculated level is dependent on TAN, pH and temperature, all of which showed an increase, it may have be expected to have increased. However, being at such a low level even after the increase, it is improbable that the level of UIA-N would have had a significant effect on the BFT system.

With the levels of turbidity and TSS consistently rising over the period of the trial (Figure 4.5), it is no surprise that there is significant difference in the mean values of both these parameters before and after the addition of salt to the system. As the rise was relatively stable, it is unlikely that the addition of salt was a primary contributing factor to these elevated levels.

4.5 Conclusions

Although water quality parameters may have been kept within optimal levels for tilapia for the majority of the trial, maintaining these optimal conditions for 100 % of the time proved to be difficult. Due to the small size of many BFT research systems, similar problems were documented in other studies (Azim and Little 2008; Luo et al. 2014). Using larger water bodies in larger commercial style systems may have its benefits, but also comes at a significantly higher cost. The profiles of various parameters documented over time, along with those of other such studies may serve as valuable information to farmers entering or already involved in BFT, giving an idea of what to expect in terms of water quality. Other such studies on water quality in South African conditions can aid in filling the information gap and aiding farmers to better understand their water quality profiles. Documenting the

profiles of these parameters over an entire grow out season in certain conditions would be highly beneficial. Although the mean values for Temperature, DO, EC, TDS, NO_2, turbidity, TSS and UIA-N showed significant difference for the periods before and after the addition of salt, it is unlikely that this was the primary contributing factor to these differences. The addition of salt may however have played a lesser role in these differences. Adding salt to the system did appear to have had a stabilizing effect on the NO_2 levels, and should thus be used as a tool in such instances. More comprehensive studies are needed on the effects of salt addition, where multiple repeats can further credit the findings. Further research is needed into strategies of curbing NO_2 spikes following water loss or other events causing system imbalances. A more standardized field method for quantifying the levels of biofloc, and determining and maintaining the optimal level of biofloc for tilapia should be investigated.

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5 General Conclusion

This study set out to pioneer the concept of biofloc technology (BFT) under South African conditions with special focus on tilapia species, namely *Oreochromis niloticus*, *Oreochromis mossambicus*, and *Oreochromis andersonii*. It was intended to act as an initial investigation for the BFT concept, from which future research could stem. It aimed to address the species dilemma currently facing South Africa, and shed light on the performance of different tilapia species under BFT conditions. It was conducted at a crucial and exciting time, with the freshwater aquaculture industry's emergence from its infant stage imminent, due to its rapid development being prioritized and streamlined by government.

A 10-week growth comparison trial was conducted in a BFT system between three tilapia species; *O. niloticus, O. mossambicus* and *O. andersonii,* with a regression being fitted to the mass data. The average growth rate of *O. niloticus* shown as an average daily gain of 0.693±0.018 g/day, was significantly ($p < 0.05$) higher than the 0.405±0.025 g/day of *O. mossambicus*, which was significantly higher than the 0.185±0.025 g/day shown by *O. andersonii.* Additionally, the lowest feed conversion ratio (FCR) was recorded for *O. niloticus*, being 1.01±0.05, followed by *O. mossambicus* and then *O. andersonii*, being 2.24±0.16 and 2.53±0.28 respectively. Mortalities and reduced feeding were observed in *O. mossambicus* and *O. andersonii*, tanks during periods of poor water quality, which was not the case for *O. niloticus* tanks. This indicates the higher tolerance of the latter species to poor water quality, being beneficial to the application in dynamic BFT systems.

A number of water quality parameters were successfully monitored throughout the trial period, and were used to create a profile of the various parameters over time, which may be useful to researchers and farmers alike. Temperature, dissolved oxygen (DO), pH, floc volume (FV), salinity, total dissolved solids (TDS), electro-conductivity (EC) were monitored twice daily with total ammonia nitrogen (TAN), nitrite (NO_2), nitrate (NO_3), un-ionized ammonia nitrogen (UIA-N), orthophosphate (PO_4), total suspended solids (TSS) and turbidity being measured weekly. Two spikes in the levels of dangerous nitrogen compounds were recorded during start-up and due to a water loss event. The first

spike was expected during the start-up phase, with the second coming as a result of a sudden decrease in the bioload in the water, induced by filling up the system with clean water not containing biofloc. This resulted in the inability to effectively remove TAN through the heterotrophic pathway, and stimulated the nitrification pathway to initiate, resulting in the initial accumulation of NO_2. This unplanned event illustrated the importance of preventing major water loss in BFT systems, as the lag in recovery of the heterotrophic community can result in the levels of TAN and NO_2 rising to lethal concentrations. The water quality parameters were however maintained within optimal levels for tilapia throughout the majority of the trial, although it was evident that keeping levels constantly within these limits did prove difficult in this dynamic system.

The study provided valuable information, and was a successful introduction into research on BFT systems in South Africa. It achieved its objective of being a baseline study, with many prospects being opened for further research. It has provided an idea of what to expect, and shed light on some vital current issues facing the South African tilapia industry. It is intended to be of value to researchers and the aquaculture sector in South Africa alike, and aid in enhancing the progress of the local industry. The results from this study suggest that BFT can indeed work in South Africa, and *O. niloticus* appears to be the most compatible species for use in such systems.

5.1 Limitations and Future Recommendations

Through the research conducted, and the experience gained, a number of areas for future research have become apparent:

Due to time constraints, the duration of the trial was 10 weeks, simulating the beginning of the grow-out season. It would be of value for future research to look at the whole grow out period within BFT systems, from stocking until harvest.

Four repeats were used per species. It could be beneficial to incorporate additional repeats in future research.

Water loss occurred on day nine of the trial due to an airlift pump becoming blocked. After this event an alarm system was installed using a mobile phone to trigger when water was being lost. The water loss caused an imbalance in the system, resulting in the levels of dangerous nitrogenous

compounds rising substantially. Water loss should be prevented at all costs in future research in BFT systems, through the use of alarm systems from the outset.

The time it took for the settlement of the biofloc when taking floc volume (FV) readings appeared to differ at different times of the trial. This concept could be further explored to provide reasons for this, as well as devise a better measurement method which can be easily performed in practice.

The use of a larger water body would better simulate commercial conditions. This would be beneficial in buffering the temperature fluctuations observed in smaller systems with a large surface area to volume ratio. It is likely that commercial facilities will be available within South Africa in the near future, and could be mutually beneficial if research were conducted in these systems.

As South Africa has often been regarded as being too cold for year round production of tilapia, research into the seasonal variability in biofloc levels and the growth rates of tilapia would be valuable.

As there is variation within species, the use of additional strains from each particular species could aid in refining the preferred species to a preferred strain of that species within a BFT system. Further research could look at single sex culture, common in commercial application. As this trial was run in the Western Cape, additional trials can be conducted in other provinces of South Africa, and provide a better indication of the performance of tilapia or other species under the specific conditions experienced in those areas.

A variable which was not explored in this thesis, but is of high interest to the tilapia industry in South Africa, is the comparison of BFT against recirculating aquaculture systems (RAS). This could be looked at in economic, feeding inputs, as well as yield/production terms. A lifecycle assessment of tilapia between these respective systems may also be valuable.