FACULTY OF BIOSCIENCE ENGINEERING

COMPARISON OF GROWTH RATES OF TILAPIA SPECIES (*OREOCHROMIS MOSSAMBICUS* AND *OREOCHROMIS NILOTICUS*) RAISED IN A BIOFLOC AND A STANDARD RECIRCULATING AQUACULTURE (RAS) SYSTEM

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DEDICATION

This thesis is dedicated to

My Lord and Saviour, Jesus Christ

My beloved husband, Cornel Verster

My parents, Johan and Gretel Olivier

My mother in law, Hanlie Verster and my late father in law, Bertie Verster

My brother and sister, Francois and Renea Olivier

My fellow Ghentenaars, Marina Albertyn, Louise Van der Nest, Julian Bunge, Lene Oosthuizen, Anna-Sophia Froeberg, Camngna Mda and Elena Baldi

All my teachers

For their support and patience

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LIST OF ABBREVIATIONS

˚C	Degrees Celsius
€	Euro
BFT	Biofloc technology
BOD	Biological oxygen demand
DM	Dry matter
DO	Dissolved oxygen
EC	Electro-conductivity
FCE	Feed conversion efficiency
FCR	Feed conversion ratio
FV	Floc volume
FW	Freshwater
NFE	nitrogen free extract
PVC	Polyvinyl chloride
RAS	Recirculating aquaculture system
SCFA	Short chain fatty acid
SD	Standard deviation
SGR	Specific growth rate
SL	Standard length
SW	Seawater
TA	Total ammonia
TAN	Total ammonia nitrogen
TL	Total length
UIA	Unionized ammonia
UV	Ultraviolet
WW	Wet weight
ZAR	South African rand

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ABSTRACT

The study was conducted to investigate the effects of raising juvenile Nile tilapia (*Oreochromis niloticus*) and Mozambique tilapia (*Oreochromis mossambicus)* in a biofloc technology (BFT) system and a recirculation aquaculture system (RAS) on water quality, fish robustness, productivity, growth performance, feed conversion and the cost-effectiveness of production. The study consisted of two simultaneous growth trials, during which feeding rates of 6% and 2.5% tank biomass were delivered, twice-daily, for the RAS and BFT systems, respectively. The BFT system received an external carbon source, in the form of maize meal, to attain a C: N ratio of 14.6. No significant differences in survival were observed between species or rearing system type. Average temperature recorded in the RAS (26.4±3.3˚C) was significantly higher than that recorded in the BFT system (21.8±2.8˚C). Average DO was higher in the RAS (8.5±0.9 mg/L) than in the BFT system (7.8±1.4 mg/L). Average pH, electro-conductivity and salinity were significantly higher in the BFT system. Average total ammonia and nitrite concentrations were significantly higher in the RAS (2.89±1.33 mg/L and 0.81±0.28 mg/L, respectively) than in the BFT system (2.29±1.30 mg/L and 0.08±0.07 mg/L, respectively) while UIA concentration was significantly higher in the BFT system (0.009±0.014 mg/L) than in the RAS (0.001±0.00 mg/L). No significant difference in average nitrate concentration between the two system types was observed. Average floc volume in the BFT system was recorded as 47.8±1.1 mg/L. The average wet weight (WW) gain and specific growth rate (SGR) of *O. niloticus* were, respectively, 86.6% and 46.3% higher in the RAS fish than in the BFT fish. The average WW gain and SGR of *O. mossambicus* were, respectively, 15.3% and 41.1% higher in the RAS fish than in the BFT fish. Both average WW gain and SGR were significantly higher for *O. niloticus* than for *O. mossambicus.* Average tank FCR for *O. niloticus* and *O. mossambicus* was 30.3% and 34.1%, respectively, lower in the BFT system than in the RAS and was significantly lower for *O. niloticus* than *O. mossambicus*. Analysis of the cost-effectiveness of operations under laboratory conditions revealed that the BFT model has lower fixed and operational costs, but ultimately demonstrates a lower cost-efficiency (€/kg) due to the low productivity observed in this system in the present study.

CHAPTER 1

INTRODUCTION

The profitability of intensive tilapia farming is restricted to a large degree by the cost of a formulated feed. A study by Losordo and Westerman (1994) revealed that reduction in feed costs and a decrease in the feed conversion ratio are the operational variables which cause the most significant reduction in production cost of tilapia in a small recirculating production system. The appeal of using a recirculating aquaculture system (RAS) for intensive tilapia culture lies in the ability to reuse water after circulation through water treatment infrastructures, thereby reducing water requirements. This water treatment gives producers a mechanism to stabilize and control water quality conditions which enhances fish welfare and subsequently productivity when a nutritionally complete formulated diet can be delivered. Biofloc technology (BFT) in indoor tanks has been proposed as a potential alternative to recirculating aquaculture systems (RAS) for intensive tilapia culture (Azim and Little, 2008). BFT used in aquaculture has demonstrated potential to achieve high productive yields along with a level of control over water quality and bacterial infections (Crab *et al.*, 2007; Little *et al.*, 2008; Luo *et al.*, 2014). The potential of biofloc systems to produce fish at a lower artificial feed requirement than general RAS has also been identified, particularly due to feed protein recycling (Avnimelech, 2007). This has potential implications for the financial feasibility of tilapia aquaculture in South Africa.

An improvement in growth rate above that which is achieved in general RAS due to the application of BFT can decrease production costs and increase the economic return per unit of production due to a shorter production cycle. Additionally, the successful application of BFT in tilapia culture may affect investment cost since this technology does not require costly biological and mechanical filtration components as are necessary for maintaining suitable water quality in RAS. On the other hand, if BFT systems cannot sustain commercially viable stocking densities due to a relatively lower waste conversion capacity or oxygen deficits, it may not be suitable for commercial application. The practical disadvantages of implementing a BFT system to culture fish includes the additional requirement of organic carbon delivery to maintain a C:N ratio above 10 and relatively high energy costs associated with intense mixing and aeration to prevent active bioflocs from settling out of suspension and to meet the additional biological oxygen demand (BOD) caused by elevated microbial respiration (Hargreaves, 2013; Avnimelech, 2015). Excessive suspended solid concentration in the rearing environment can also clog the gills of fish, resulting in growth and welfare depression (Luo *et al.*, 2014).

How RAS and BFT systems perform relative to each other in terms of growth performance for *Oreochromis mossambicus* (Mozambique tilapia) and *Oreochromis niloticus* (Nile tilapia) has not been described in local (South African) conditions. A quantitative comparison of the productive capacity of the two systems in terms of the growth performance, survival, feed conversion ratio and biomass yield which can be supported in both systems will give an indication of whether BFT is a technically and commercially viable alternative to RAS. A comparison of the growth rate of two tilapia species relevant to the South African industry, namely *O. mossambicus* and *O. niloticus* on an inter- and intra- species level in the two culture systems will give additional insight into the suitability of each species to the culture environments offered by BFT and RAS.

The most important aims of the study are:

- To evaluate the suitability of water quality parameters of interest to fish growth and welfare present in the two systems.
- To evaluate the effects of system-specific characteristics or processes on water quality.
- To investigate the growth performance, feed conversion ratio and survival of two tilapia species housed in different production systems (BFT and RAS) at an inter-species level. The results will give a good indication of the technical viability of biofloc aquaculture systems compared to general RAS for the respective tilapia species.
- To investigate the productive capacity of a BFT system relative to RAS for tilapia culture by stocking all tanks at an equivalent density and evaluating performances.
- To evaluate the contribution of biofloc to the growth and production of two tilapia aquaculture species.
- To determine relevant fixed and operational costs for both system types and to compare cost-effectiveness of using RAS and BFT systems for tilapia aquaculture.

CHAPTER 2

LITERATURE REVIEW

2.1 SUSTAINABLE AQUACULTURE DEVELOPMENT

2.1.1 Status and trends in global aquaculture production

Aquaculture has been recognized as a crucial contributing sector to food fish supply. While production from capture fisheries has remained relatively stagnant since the 1990's, aquaculture production has increased dramatically over the past five decades to reach 73.8 million tonnes of food fish harvested in 2014 **(Figure 2.1)** (FAO, 2016). This is comprised of 49.8 million tonnes of finfish, 16.1 million tonnes of molluscs and 6.9 million tonnes of crustaceans and 7.3 million tonnes of other aquatic animals (FAO, 2016). The average annual growth rate of aquaculture has slowed from 9.5% between 1990 and 2000 to 5.8% between 2005 and 2014 (FAO, 2014). The highest annual growth rate of aquaculture production in the past decade has taken place in Africa (FAO, 2014), presumably as a result of the development of tilapia aquaculture in Egypt (Liu *et al.*, 2013). The aquaculture sector is diverse and dominated by freshwater (FW) fish which are utilizing the natural productivity of their culture environment entirely, or at least partly (Bostock *et al.*, 2010). In this category, carps are the most important contributors, making up 72% of cultured FW species production by volume, followed by tilapias and catfishes (FAO, 2012).

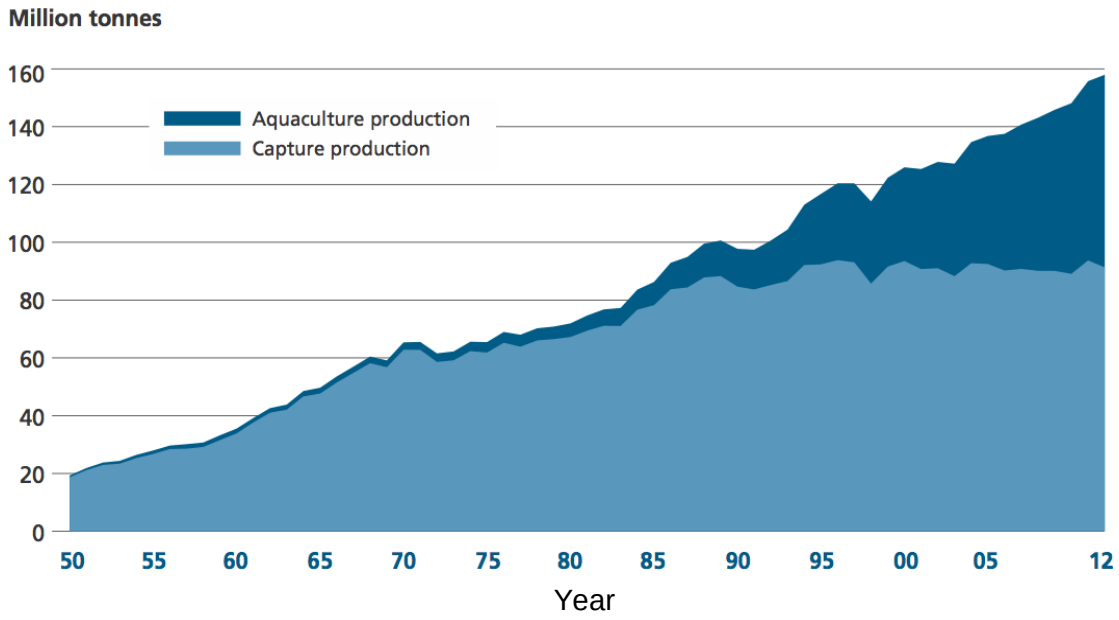


Figure 2.1 Global contribution of capture fisheries and aquaculture production (excluding aquatic plants) (FAO 2014).

Aquatic food production has made a shift from being almost solely based on capture of wild aquatic species to being predominantly obtained from farmed species. A milestone in this transition was reached in 2014, during which the contribution from the aquaculture sector towards fish and shellfish for human consumption was greater than the supply from the capture fishery sector **(Figure 2.2)** (FAO, 2016). Fish supply from capture fisheries has stagnated since the late 1980s while the supply from aquaculture has more than tripled in the same time frame. A large proportion of this growth can be attributed to developments in China which currently

contributes in excess of 60% of global aquaculture production (FAO, 2016). The large production figures in this region can be attributed to well-developed existing practices, a relaxed legislative environment, economic and population growth and increasing exports (Bostock *et al.*, 2010). The global consumption of fish (including shellfish) per capita has also increased from 9.9 kg in 1960 to 19.7 kg in 2013 with further growth to approximately 20 kg predicted. Despite a steady rise in consumption of human food from an aquatic environment in developing and low-income food deficit countries, consumption of fish and shellfish remains higher in more developed regions, with a sizeable share of this arising from imports. The increase in global consumption is attributed to the rise in production, better distribution and international trade, population growth and higher incomes (FAO, 2016). Timmons and Ebeling (2007) also suggested that consumer trends such as increased numbers of meals being eaten away from home in higher income countries increases the demand for a reliable, year-round seafood supply to restaurants. This can rarely be provided by natural fisheries, thereby boosting the demand for aquaculture products. The demand increase accompanied by population growth warrants further increase in aquaculture production in the future, despite increasing land and water scarcity. At present, 92.7% of total production is derived from aquaculture activities in only 15 countries (FAO, 2014), with many undeveloped, suitable regions offering room for expansion of the industry.

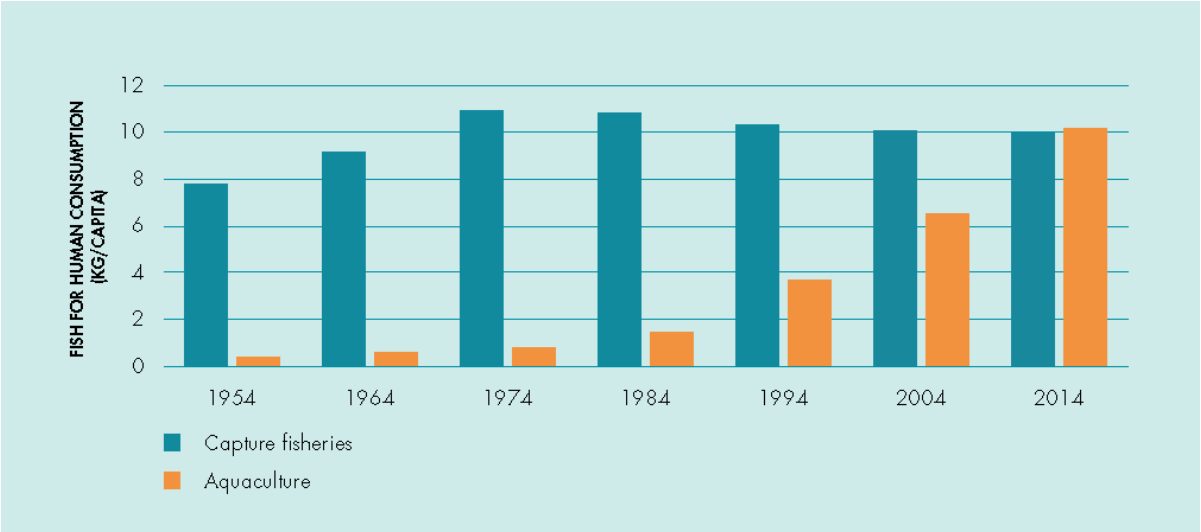


Figure 2.2 Relative contribution of aquaculture and capture fisheries to fish for human consumption (FAO 2016).

More stringent regulation of water supplies, effluent discharge and health-related factors related to the food production industry is contributing to the costs of aquaculture production (Timmons and Ebeling, 2007). In addition, public concerns regarding environmental issues like pollution, visual “pollution” and competition for coastal sites for both industrial and recreational activities is causing shifts from the more traditional extensive, open culture systems in ponds or cages to more intensive, closed culture systems where the producer can exert more control over the culture environment and waste discharges, but which is typically associated with higher operational and capital costs (Timmons and Ebeling, 2007). These costs may be recovered by the increased growth rates and better feed conversion as well as more efficient use of labour, which can potentially be achieved in intensive aquaculture systems.

2.1.2 Effects of intensive aquaculture on water quality

Intensive aquaculture systems aim to minimize space and water inputs by maintaining high stocking densities, consequently requiring high feed or nutrient delivery per unit of area (Ekasari, 2014). Intensive aquaculture generates a relatively higher concentration of waste in the culture system since fish retain only a fraction of fed nutrients (Boyd and Pillai, 1985; Avnimelech and Ritvo, 2003), while the remainder accumulates and ultimately causes a deterioration of the water quality. This has a direct impact on the cultured animals such as growth and health impairment (Kautsky *et al.*, 2000), but may also contribute to eutrophication of natural water bodies if the effluents from these aquaculture systems are not treated. This nutrient enrichment and waste generation could threaten the long-term success of producers and impede other activities related to the affected water bodies.

Nitrogen is a nutritional requirement included in the feed of aquaculture organisms, generally in the form of protein. Following feed transit through the gastrointestinal tract (GIT), protein may be either excreted with the faeces as indigestible nucleic acids or protein, or may be hydrolysed to amino acids which can then be assimilated in the form of protein or nucleic acids, converted to carbohydrates or fatty acids or catabolized to yield energy (Wilson, 2002; Holmer *et al.*, 2008). These uses of absorbed amino acids within the tissues of fish involve deamination, yielding ammonia: a highly toxic compound which must be kept at a low concentration to avoid mortalities, growth impairment or immune suppression of the cultured species. Ammonia constitutes the bulk of nitrogenous waste (70-90%) while a small proportion (5-15%) is excreted as urea (Wilson, 2002). The exact partitioning is related to the species and stage of ontogeny of the cultured organism (Wilson, 2002). In fish, ammonia is excreted from the blood to the surrounding water body by diffusion or via active Na^+/NH_4^+ exchange at the gill surface (Wilson, 2002). The retention of dietary nitrogen for most fish species is in the range of 28 – 66%, with the remaining fraction being released into the surrounding aquatic environment (Cho and Bureau, 2001).

Phosphorus is another nutrient required by all organisms as it is bound to molecules like DNA, RNA, nucleic acids, ATP and phospholipids. Phosphates are essential for metabolism and act as a buffer in body tissues (Lall, 2002; Holmer *et al.*, 2008). Dietary phosphorus may be degraded to inorganic phosphate in the GIT or excreted with the faeces if not required. The quantity of phosphorus which will be degraded during GIT transit is related to the phosphate concentration in the plasma (Bureau and Cho, 1999). If plasma phosphate levels get too high, phosphate may be excreted with the urine (Bureau and Cho, 1999; Roy and Lall, 2004). Retained dietary phosphorus is between 13 - 64% for most aquaculture animals. Phosphorus in the form of bone-phosphorus from animal origin has a much higher digestibility than the alternative phytate-phosphorus from plant origin, which is almost indigestible by fish. Excreted phosphorus is mainly released into the aquatic environment with the faeces (38-50%) but a small proportion exits the fish via the urine or over the gills (10-20%) (Holmer *et al.*, 2008). Phosphorus is considered a limiting nutrient in most natural water bodies (Bureau and Cho, 1999).

Accumulation of nutrients like nitrogen and phosphorus in the surrounding aquatic environment may lead to eutrophication of the effluent receiving water body if no treatment is performed prior to discharge. Notable potential negative impact of the release of nutrient rich water from aquaculture is the development of harmful algal blooms and alterations in the species and phytoplankton composition which affects the healthy functioning of the receiving environment (Gowen, 1994; Bonsdorff *et al.*, 1997; Smith, Tilman and Nekola, 1999; Herbeck and Unger, 2013). The high production levels attained by intensive aquaculture make waste accumulation

especially applicable to this production method. Nutrient waste also presents financial implications for the producer in the form of costly feed losses and taxes payable per unit of nutrient discharged as prescribed by legislation (Bergheim and Brinker, 2003; Read and Fernandes, 2003; Tacon and Forster, 2003).

2.1.3 Recirculating aquaculture

The use of recirculating aquaculture systems (RAS) has been identified as a solution to environmental constraints such as limited land space and FW availability as well as concerns regarding pollution caused by aquaculture related activities since water is reused and not continually discharged into the environment (Badiola, Mendiola and Bostock, 2012). RAS has been shown to decrease water consumption by 90-99% and land area use by more than 99% relative to conventional, extensive systems (Timmons and Ebeling, 2007). The concept of RAS allows for the manipulation of environmental and water quality parameters to ensure that fish are cultured in near optimal conditions year-round (Heinen, Hankins and Adler, 1996), permitting predictable growth rates and, therefore, harvesting schedules (Timmons and Ebeling, 2007) as well as locating production activities in close proximity to final markets. Production in near optimal conditions also reduces stress, thereby decreasing susceptibility of fish to disease. However, it is associated with high operational costs and costly water treatment components, limitations in disease treatment and requires skilled management and a complete diet (O Schneider *et al.*, 2006). The mechanical sophistication and biological complexity of RAS systems has also resulted in common problems such as deterioration of water quality due to component failure, potentially resulting in stress, poor growth and disease. The spread of disease may also be facilitated by the design of a RAS if culture water is allowed to circulate between rearing tanks.

The design of a RAS should ensure that the critical parameters are properly balanced, that flow rates of water and air are uniform between rearing tanks, that adequate water supply of an appropriate quality is available to fill tanks in a reasonable time and allow for emergency or routine flushing and that operations can continue uninterrupted during the production cycle. A reduction of flow variation can be achieved by ensuring that pipes are not constricted by biological growth by scouring, using oversized and/or short pipes and ensuring that screens between rearing tanks are clean and an appropriate mesh size. To ensure uninterrupted operation, a backup power source should be included in a producer's inventory and programmed to start automatically in the event of power failure. A well-designed RAS should also facilitate a high rate of water reuse thereby reducing the volume of discharge water which requires treatment as well as energy requirements for heating or cooling the culture water.

Critical parameters typically monitored and affected by treatment components of RAS systems include temperature, pH, alkalinity, suspended solids, ammonia, nitrite, dissolved oxygen and carbon dioxide (CO_2) (Timmons and Ebeling, 2007). These parameters exert an influence on the growth and health of the cultured species both individually and via, sometimes complex, interactive effects. An example of such an interactive effect is the relationship between pH, dissolved CO_2 levels and the toxicity of ammonia. The toxicity of the un-ionized fraction of ammonia (NH_3) is significantly higher than the ionized form (NH_4^+) due to its ability to cross cell membranes, and the proportion of un-ionized ammonia increases as the pH increases and is also influenced by salinity and temperature. pH levels, in turn are affected by dissolved CO_2 levels, with pH increasing as dissolved CO_2 levels decrease.

Temperature needs to be maintained in the optimum range of the species being cultured which promotes efficient feed conversion and fast growth and at a suitable level for the proliferation

and functionality of nitrifying bacteria. Regulation of temperature can be done by incorporating heat exchanging or chilling components into the RAS design or with the use of immersion heaters (Masser, Rakocy and Losordo, 1999).

A pH range of 6 to 9.5 is appropriate for most fish species. The nitrifying bacteria of the biofilter are inhibited at a pH below 6.8 (Masser, Rakocy and Losordo, 1999). High stocking densities, and therefore high respiration rates, characteristic of RAS aquaculture tend to decrease pH because of carbon dioxide production and subsequent conversion to carbonic acid. The pH decrease is a result of the conversion of carbon dioxide in the water to carbonic acid. Rapid pH fluctuations can be avoided by the addition of alkaline buffers such as calcium carbonate or sodium bicarbonate or the less commonly used and more caustic calcium hydroxide, sodium hydroxide and calcium oxide to the circulated water. pH levels should be measured daily and adjusted as required. The addition of alkaline buffers is generally sufficient to also maintain the alkalinity of the system at adequate levels of approximately 50 mg/L (Masser, Rakocy and Losordo, 1999).

The removal of suspended solids, consisting predominantly of uneaten feed and faeces, is depicted in **Figure 2.3** as “Primary Clarification”. This particulate matter should be removed to prevent oxygen consumption and ammonia or toxic gas production during its decomposition, as well as to prevent excessive growth of microorganisms which consume oxygen and may produce compounds which may cause an off-flavour in the fish delivered to consumers. Sterilization is occasionally also incorporated into the RAS design to decrease the microbial load of the system in an additional attempt to minimize these effects as well as to minimize the presence of parasites, pathogens or opportunistic pathogens. Suspended solids can be removed via three primary methods – filtration, flotation or gravity separation (sedimentation). Filters should be kept clean and the collected sludge generated by these removal processes must be disposed of in an environmentally responsible manner, either on wet or dry basis (Masser, Rakocy and Losordo, 1999).

Toxic ammonia, arising predominantly from the digestion of protein, and nitrite can be removed from culture water by the action of a biofilter which consists of actively growing nitrifying bacteria growing on a surface. Ammonia, existing in water in two forms: ionized (NH_4^+) and un-ionized ammonia (UIA) (NH_3), is oxidized by these bacteria to nitrite which is subsequently oxidized to nitrate. The activation of such a biofilter involves the development of a healthy population of nitrifiers with the capacity to remove ammonia at a rate which maintains suitable ammonia and nitrite levels during normal feed application. This process of biofilter activation requires at least one month, and stocking and feeding levels must be reduced below commercial levels during this time (Masser, Rakocy and Losordo, 1999). Accumulation of the relatively nontoxic end-product of nitrification, nitrate, can be prevented by ensuring some (5-10 percent) daily water exchange combined with some level of denitrification which takes place in most RAS. Denitrification entails the conversion of nitrate to nitrogen gas by bacteria (Masser, Rakocy and Losordo, 1999).

Dissolved oxygen levels can be manipulated by the supply or restriction of supply of air or oxygen by aeration systems. This supply needs to be high enough to satisfy the oxygen demands of the fish and microorganisms in the system. Typical oxygen stress signs exhibited by fish, such as gathering at the surface or around the aeration device output, should motivate actions such as employing a supplemental aeration system, mixing supersaturated water with culture water or reducing feeding rate (Masser, Rakocy and Losordo, 1999). Carbon dioxide

accumulation can be prevented by the incorporation of aeration or degassing components in a RAS (Masser, Rakocy and Losordo, 1999).

Biosecurity is an important aspect of RAS due to the narrow association of rearing tanks to one another and to treatment components. Diseases may enter the system via incoming water or the introduction of fish and can be spread by equipment which is used between tanks without intermediate sterilization or assignment of equipment to specific tanks or if water from different rearing tanks are mixed in common water treatment components (Masser, Rakocy and Losordo, 1999). The RAS design should allow for as much separation of water of individual rearing tanks and treatments components as possible to minimize disease spread in the case of outbreak.

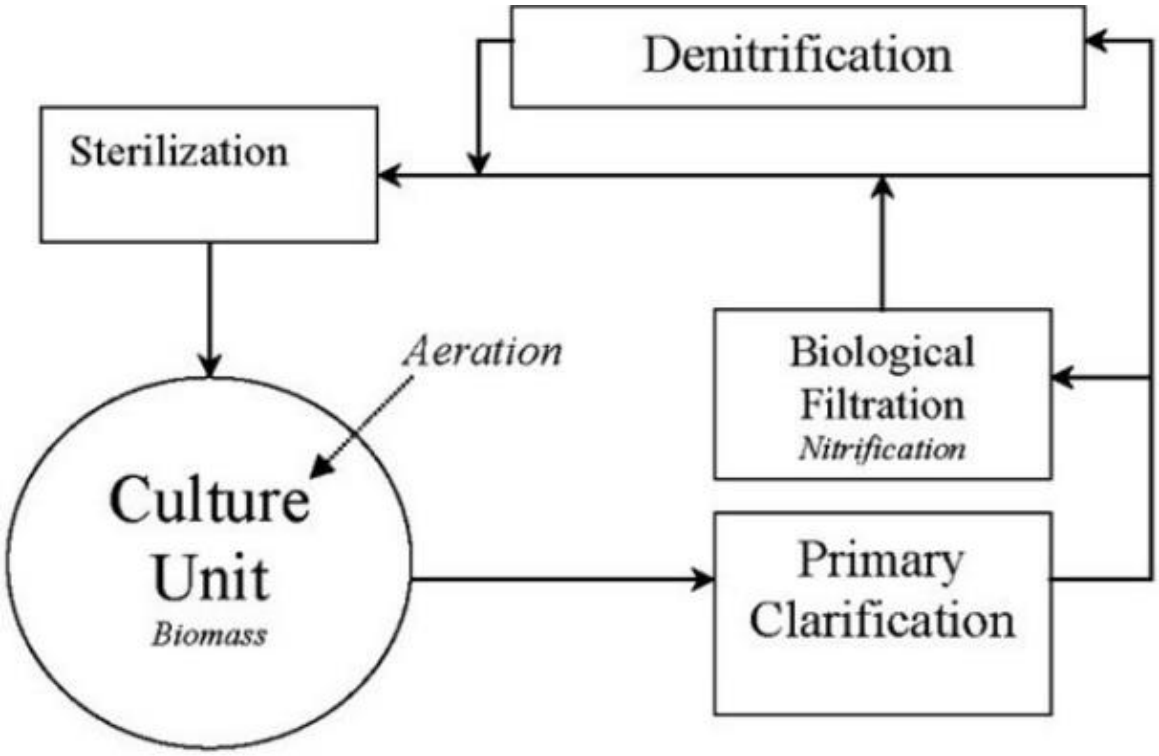


Figure 2.3 Schematic flow diagram indicating the categories and stages of filtration commonly found in recirculating aquaculture systems (Steicke, Jegatheesan and Zeng, 2009).

2.2 BIOFLOC TECHNOLOGY

2.2.1 Principles of Biofloc Technology (BFT) setup and management

BFT has been investigated as a possible alternative to semi-extensive pond culture and intensive RAS since the early 1980's in the USA and Israel, as a system to culture aquaculture species in high densities while minimizing land and water inputs and environmental degradation (Avnimelech *et al.*, 1986). The goal is for the producer to exert more control over the microbial activity in aquaculture set-ups, particularly in intensive systems which are well aerated and have zero or low water exchange. Growth of heterotrophic bacteria is selectively stimulated by the addition of organic carbon as substrate. BFT is based on the understanding that fish production is not an isolated element, but rather a constituent of a broader eco-system with several components such as the physical features of the production system, chemical components, a rich biota and the cultured species as well as the interactions within and between each component (Avnimelech, 2015). This awareness has resulted in increased efforts towards manipulating each component of the cultured animal's environment contributing to its ultimate health and growth performance, including the microbial community.

The benefit of BFT is that water quality is enhanced *in situ* since organic waste and ammonium is retained by being incorporated into microbial biomass suspended in the culture system (Azim and Little, 2008) when the balance between carbon and nitrogen is appropriate (O Schneider *et al.*, 2006). The formation of biofloc in fertilized and/or fed systems is achieved by adding an external carbon source or increasing the feed carbon content to act as an organic substrate for aerobic decomposition by heterotrophic bacteria and by applying constant aeration and agitation of the water column to keep an active floc in suspension (Avnimelech *et al.*, 1986). Oxygen is a limiting factor in aquatic environments and demand is dependent on several biological, physical and chemical factors (Piedrahita, 1991). Low oxygen levels may result in slow growth rates, poor feed utilization efficiency, stress disease and potentially mortality in tilapia (Avnimelech, 2015). Sufficient aeration is an important managerial component for successful biofloc systems due to the additional oxygen consumption by aerobic heterotrophs. An aeration system must have the capacity to cover oxygen consumption, mix the water and the sediment, allow even distribution of oxygen and minimize the extent of sludge accumulation sites (Avnimelech, 2015). Benefits of successful application of a BFT system include the conversion of toxic inorganic nitrogen species such as ammonium to microbial biomass (Hargreaves, 2013) and degradation of accumulating organic residues (Avnimelech *et al.*, 1986), thereby avoiding the need for waste treatment infrastructure while maintaining stocking densities higher than what is possible in extensive production systems. BFT is a simple technology which results in a more economical use of water and space while providing a potential proteinaceous feed source for the cultured species (Hargreaves, 2006; Crab *et al.*, 2012).

Additional benefits of BFT have been identified, including the contribution of exogenous digestive enzymes (Xu and Pan, 2012; Xu *et al.*, 2012), control of potential pathogens (Crab *et al.*, 2010) and stimulation of the immune response (Ekasari *et al.*, 2014; Xu and Pan, 2014). Potential challenges associated with this technology include the requirement of reliable mixing and aeration systems, the build-up of microbial biomass (Ray, Dillon and Lotz, 2011) and an increased CO_2 release from the biofloc organisms (Hu *et al.*, 2014). Besides the production of aquatic species for consumers despite land and water scarcity, additional pressure on aquaculture comes in the form of environmental regulations which prohibit the release of water enriched by nutrients and feed. Farmers also need to achieve profitability to achieve economic

sustainability in a competitive, capital-intensive industry with costly inputs. Each of these challenges is addressed in some way by the application of BFT.

2.2.2 Biofloc morphology and composition

An understanding of the biological aspects involved in bioflocculation can facilitate manipulation of the nutritional composition, morphology and level of biofloc production in a system (Avnimelech, 2015). The constituents of microbial flocs include bacteria, fungi, microalgae, organic polymers, particles, dead cells, colloids, cations and microbial grazers such as nematodes, ciliates and flagellates in an irregularly shaped, heterogenous mixture up to 1000 µm in size **(Figure 2.4)** (De Schryver *et al.*, 2008). Cohesion of the flocculant is made possible by mucus derived from bacterial secretions, binding by filamentous microorganisms or electrostatic interactions (Hargreaves, 2013). Bioflocs are generally between 50 and 200 µm in size and the majority are microscopic, although particularly large flocs are macroscopic (Hargreaves, 2013).

Typical attributes of bioflocs include high porosity resulting in high permeability and a wide range of particle sizes (Avnimelech, 2015). The biological composition of microbial flocs can be influenced by the organic carbon source delivered (Crab *et al.*, 2010) as well as the quantity delivered (De Schryver *et al.*, 2008), dissolved oxygen levels (De Schryver *et al.*, 2008), rate of floc consumption or mechanical removal (Ray *et al.*, 2010), addition of microalgae or probiotics (Zhao *et al.*, 2012), and salinity (Maicá, de Borba and Wasielesky, 2012). The physical nature of the flocs can be affected by operational parameters such as dissolved oxygen levels, mixing intensity, temperature and pH.

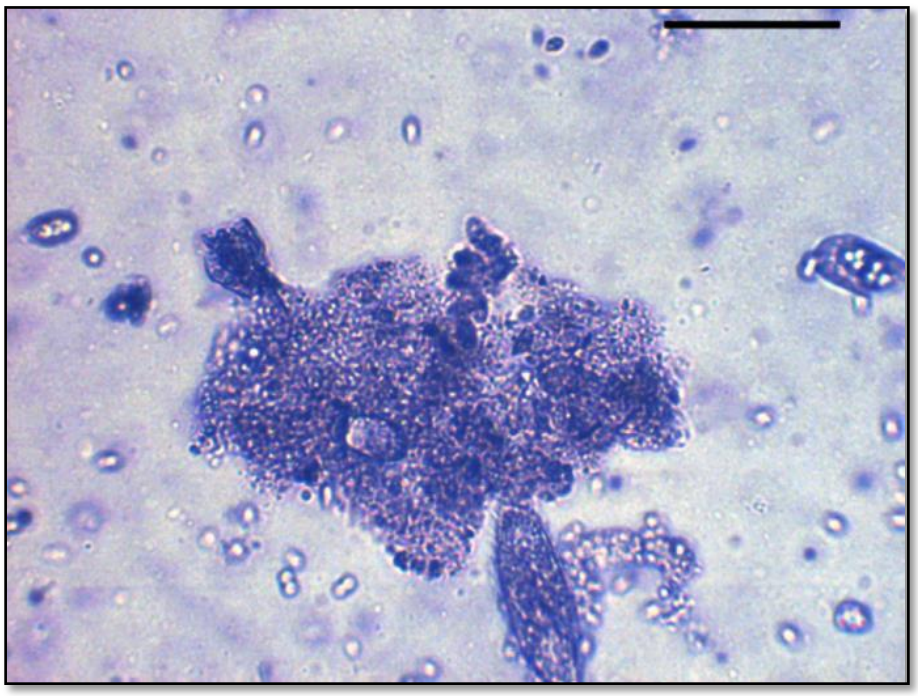


Figure 2.4 An individual biofloc. (scale: 100 µm) (Hargreaves, 2013).

Microorganisms benefit from aggregation since they have an increased capacity to settle, thereby escaping grazers, escaping the impact of light such as potential harmful ultraviolet (UV) exposure and the resulting inhibition of heterotrophic bacterial activity (Alonso-Sáez *et al.*, 2006) or light induced decay (McCambridge and McMeekin, 1981), and they may have

access to more nutrients in the sediment due to settling of nutrient-rich faeces and uneaten feed. Microorganisms in bioflocs which remain in suspension also attain a nutritious advantage due to the fact that a mixed water flow through a porous microbial floc results in a higher quantity of nutrients supplied to the microbes in comparison with the quantity supplied via laminar flow to dispersed, individual cells in the water column (Avnimelech, 2015). There is thus increased substrate availability due to flocculation.

2.2.3 Using BFT to enhance water quality

Waste in aquaculture systems is present either as dissolved or solid waste (Bureau and Hua, 2010). The solid fraction is derived primarily from uneaten feed and faeces, while the dissolved fraction is derived primarily from excretion products and includes compounds such as ammonia and orthophosphate. Solid waste is eventually decomposed to form part of the dissolved waste (Crab *et al.*, 2007).

Besides water exchange, nutrients are predominantly removed from water by metabolic processes involving microorganisms (Ekasari, 2014). For example, inorganic nitrogen accumulation may be controlled in an aquaculture system by photoautotrophic removal by algae, heterotrophic conversion or photoautotrophic oxidation by nitrifying bacteria (Ebeling, Timmons and Bisogni, 2006; Crab *et al.*, 2007). Heterotrophic microorganisms fed with carbon substrates assimilate nitrogen from the culture water to produce their constitutive proteins during cell growth and multiplication. In this way, these organisms effectively reduce the amount of inorganic nitrogen, especially total ammonia nitrogen (TAN), in the system. The amount of inorganic nitrogen removed can therefore be precisely controlled based on the addition of organic carbon substrate at prescribed levels (Avnimelech, 1999; Crab *et al.*, 2007) if the microbial conversion efficiency is known for a system. The reduction of ammonium concentration by heterotrophic bacteria occurs faster than what can be achieved by nitrifying bacteria since the growth rate of heterotrophs is significantly higher (Hargreaves, 2006).

If microorganisms involved with nutrient removal are in turn consumed by the fish, the nutrients are recycled and this improves the overall efficiency of use of nutrients (Ekasari, 2014). In a biofloc system, the heterotrophic conversion is stimulated by the addition of an external carbon source or by increasing the carbohydrate content of the delivered feed (Avnimelech, 1999) and is expected to be the main vector of nitrogen removal, but nitrification and photoautotrophic nitrogen removal also contribute (Burford *et al.*, 2003; Ebeling, Timmons and Bisogni, 2006; Hargreaves, 2006; Azim and Little, 2008). An alteration of the carbon to nitrogen (C: N) ratio facilitates manipulation of the ratio between nitrification and nitrogen immobilization. The stoichiometry involved with the various conversion processes determines their effects on relevant water quality parameters, e.g. heterotrophic conversion involves a higher dissolved oxygen (DO) consumption but produces more CO_2 and a higher microbial biomass in comparison with nitrification (Ebeling, Timmons and Bisogni, 2006) while photoautotrophic nitrogen removal and nitrification requires a higher alkalinity in comparison with heterotrophic conversion (Ekasari, 2014). It was also shown that a relatively high density of denitrifying bacteria may be present in biofloc-based systems (Gao *et al.*, 2012). The presence of heterotrophic denitrifiers is thought to be a result of high organic carbon and nitrate availability (Ekasari, 2014) while anoxic denitrification is facilitated by micro-niches that develop within the flocs (Schramm *et al.*, 2000). Additional evidence of denitrification in biofloc systems include observed nitrate reduction (Hu *et al.*, 2014) and nitrogen loss (Ray, Dillon and Lotz, 2011; Luo *et al.*, 2014).

2.2.4 Biofloc as a feed source

Biofloc was initially developed as a solution to water quality deterioration, but a by-product of this was the production of microbial protein within the culture environment. Feeds are primarily composed of the organic macronutrients carbohydrates, lipids and proteins. In contrast to plant cells, proteins and lipids are the main constituents of animal cell walls, resulting in low deposition of fed carbohydrates in the structural materials representative of somatic growth in animals (Houlihan, Boujard and Jobling, 2008). Considering that 65-85% dry matter (DM) of a fish carcass comprises of protein (Jauncey and Ross, 1982), production parameters important in aquaculture, such as wet weight (WW) gain over time, can therefore potentially be enhanced by the availability of a high-protein diet. However, protein sources are generally the most costly feed ingredients, usually making up more than 50% of the total feed cost (Jauncey and Ross, 1982). Protein content is not in itself considered a direct assessment of protein quality, as this is dependent on the "amino acid composition, the availability of amino acids to the animal, and upon their physiological utilisation following digestion and absorption" (Houlihan, Boujard and Jobling, 2008). In turn, the physiological utilisation of protein is affected by the physiological state of the animal (weight, age, maturity), the energy content of the diet, feed intake and water quality (Jauncey and Ross, 1982), all of which therefore influence the ideal protein level which yields maximum growth in the culture species. Provided that the *in situ* microbial biomass generated by BFT can be absorbed and assimilated by fish cultured in the biofloc rich water, this microbial biomass may act as a high value feed with the potential of decreasing the producer's dependence on a costly, protein-rich formulated feed (Avnimelech and Schroeder, 1989).

The nutritional composition of bioflocs has been shown to be variable and affected by light exposure, carbon source, salinity, nutrient loading and microbial composition (Crab *et al.*, 2012; Maicá, de Borba and Wasielesky, 2012; Ekasari, 2014). Although the levels of respective nutrients vary between studies, there is a general consensus that bioflocs contain noteworthy levels of fatty acids, essential amino acids, protein, lipid and carotenoids (Crab *et al.*, 2010; Kuhn *et al.*, 2010). Ekasari (2014) reported that the levels of most essential nutrients in microbial flocs were comparable to the requirements of fish but that the lipid levels and essential fatty acids were somewhat lacking. Protein content is between 25-50%, lipid content from 0.5-15% and vitamin and mineral levels are appropriate for most fish species (Hargreaves, 2013).

Whether the bioflocs in a system will be consumed by fish depends on the biofloc intake ability of the fish (e.g. species, feeding behaviour, size, activity level, gill structure, amount of water filtration etc.) and biofloc properties (e.g. concentration, size, composition, density, surface properties etc.) (Avnimelech, 2007). The contribution of the microbial biomass to the fish's feed intake also depends on the concentration of individual flocs per volume of water which in turn is dependent on the amount of available organic substrates which may be derived from external sources (i.e. the rate of delivery of formulated feed to the system and its digestibility) or by the excretion of un-utilized nutrients by fish. The rate at which the floc is biodegraded also affects its concentration and is related to the associated microbial community (Avnimelech, 2007). All of these processes are influenced by the operational and environmental parameters such as the aeration and mixing intensity, temperature, rate of water exchange, salinity etc. (Avnimelech, 2007).

Some aquaculture organisms, such as cichlids, cyprinids and penaeids are able to utilize the generated microbial flocs *in situ* (Azim and Little, 2008; Crab *et al.*, 2012; Ekasari and Maryam, 2012; Luo *et al.*, 2014), whereas these bioflocs may also be processed and incorporated into

formulated feeds as a feed ingredient (Kuhn *et al.*, 2010; Anand *et al.*, 2013). Avnimelech (2007) determined that microbial flocs in BFT systems are effectively taken up by tilapia and were shown to contribute approximately 50% of the protein requirement of the fish. In contrast to delivered, artificial feeds, bioflocs are available 24 hours per day. It is therefore feasible that feed inputs can be reduced in BFT systems up to the feed equivalence level of the resident flocs and that this reduction by consumption may even be a necessary managerial aspect to prevent excess build-up of microbial biomass. The potential feed reservoir contained in the culture environment can be quantified by measuring the floc volume (FV), representing the volume of settled flocs contained in 1 L of biofloc-rich water (Avnimelech, 2007).

2.2.5 BFT effects on fish disease control

The high stocking densities intrinsic to intensive aquaculture are associated with an increased risk of disease outbreaks and spreading of infection is facilitated by close interaction between healthy and infected animals. High densities may also cause conditions which cause stress, a precursor of disease outbreak. Abrupt fluctuations in water quality parameters are potentially stress inducing in cultured fish. The presence of bioflocs has been shown to increase the stability of water quality parameters such as DO and pH (Avnimelech, 2015). Increased water quality stability observed in BFT systems is a definite advantage over more extensive, pond-based approaches to aquaculture, but less so over RAS systems where parameters are somewhat controlled.

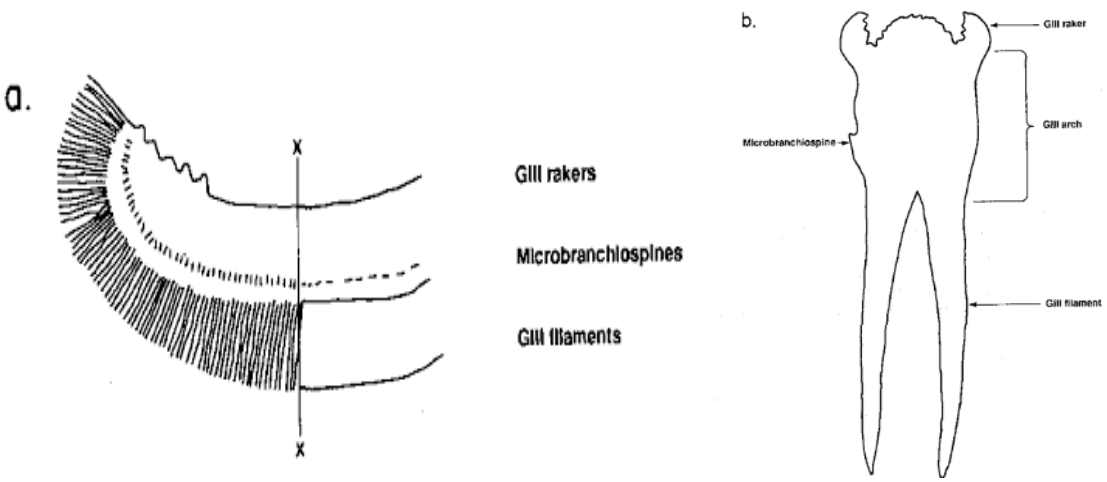
A possible solution to the introduction of pathogens via water, is the application of a zero or minimal water exchange system such as what can be achieved with BFT. Incoming water may be sterilized or filtered to enhance biosecurity, especially in cases when introduction of a disease via the incoming water is suspected (Avnimelech, 2015). Fish cultured in BFT systems have been shown to be less susceptible to disease, indicating that BFT may improve the immunity of fish. De Schryver et al. (2008) reported the potential of bioflocs as bio-control agents via the release of short chain fatty acids (SCFA) and poly-β-hydroxybutyrate which contributes to protection against *Vibrio* infections and thus acts as a probiotic. Anti-inflammatory effects exerted by bioflocs were documented by Sinha et al. (2008). The probiotic effect of bioflocs is possibly a result of several of the potential modes of action acting simultaneously. In addition, the dense heterotrophic microbial population competes with opportunistic pathogens in the culture water for both nutrients and microbial adherence to the cultured fish, decreasing the possibility of pathogenicity (Avnimelech, 2015).

2.3 TILAPIA

2.3.1 Biology and feeding behaviour

Tilapia is the common name assigned to three genera within the family Cichlidae occurring in FW, including macrophagous, substrate spawning *Tilapia* and microphagous, mouthbrooding *Oreochromis* and *Sarotherodon* (Kocher *et al.*, 1998). Tilapia are indigenous to tropical and subtropical regions of Africa and the Middle East, but have been distributed to every continent with the exception of Antarctica (Watanabe *et al.*, 2002). Sexual maturation is reached early in life, generally before the age of six months and female tilapia within the mouthbrooding genera exhibit high levels of maternal care. Fry have a large yolk sac at hatching and are omnivorous at the start of exogenous feeding (Watanabe *et al.*, 2002).

Tilapia in the genera *Oreochromis* and *Sarotherodon* are primarily omnivorous, feeding on periphyton, phytoplankton and detritus (Fitzsimmons, 1997) while members of the genera *Tilapia* typically consume macrophytes (Jauncey and Ross, 1982). Tilapia species therefore feed at a low trophic level. Consequently, less refined protein sources can be converted to high quality protein suitable for human consumption in these animals (Jauncey and Ross, 1982).The natural feeding ecology of adult tilapia characteristically includes feeding on plant matter or detritus of plant origin, including macrophytes, diatoms, amorphous detritus and algae. A small proportion of intake may consist of animal material (Bowen, 1982). Juveniles typically feed on phytoplankton and small invertebrates (Jauncey and Ross, 1982). The thickness, length and spacing of gill rakers are indicative of the feeding habits of tilapia species, where few, large gill rakers suggest consumption of larger feed particles and numerous, narrow gill rakers suggest consumption of plankton (Bardach, 1972). Filter feeding, however, is not linked to the spacing or number of gill rakers since particles in suspension have been shown, rather, to be entrapped by mucus **(Figure 2.5)** (Northcott and Beveridge, 1988). Filter feeding in juvenile tilapia has been demonstrated in *Oreochromis mossambicus* (Mozambique tilapia) (de Moor et al., 1986) and *Oreochromis niloticus* (Nile tilapia) *(Trewavas, 1983, Northcoss et al., 1991)*. A study by Dempster, Baird and Beveridge (1995) evaluated the extent of algal uptake by filter feeding in tilapias and reported that intake is limited by saturation of the filter feeding apparatus and intake is positively correlated to particle size. The authors concluded that the nutritional needs of tilapia could not be fulfilled by filter feeding alone.



(a) General view of gill arch bearing gill rakers, row of microbranchiospines and gill filament.
(b) Cross-section of gill arch taken at x – x

Figure 2.5 Gill structure in *O. niloticus* (Beveridge *et al.*, 1988)

Feeding activity is thought to be predominantly controlled by light and takes place essentially during light hours, but a small proportion (less than 20%) takes place during dark hours (Toguyeni *et al.*, 1997). Two feeding activity peaks have been reported by Toguyeni et al. (1997): at dawn and at dusk. Besides the two peaks, tilapia exhibits an almost constant feeding activity during daylight hours. A high feeding frequency has been shown to result in a more regular nutrient supply and an enhanced digestive efficiency and nutrient utilization (Siraj et al. 1988; Wang et al. 1998; Riche et al. 2004).

Tilapia display distinct sex-related phenotypes. Males grow faster and to a greater maximum size compared to females (Toguyeni *et al.*, 1997). The differential growth between males and females is attributed to a higher proportion of energy being invested in reproduction in females, anabolic effects of androgens (Higgs *et al.*, 1977; Matty and Lone, 1979; Ufodike and Madu, 1986) and behavioural patterns associated with sex (Toguyeni *et al.*, 1997). Voluntary feed intake in females is significantly reduced during the period after spawning while eggs are incubated in the mouth of the female and during fry care (Toguyeni *et al.*, 1997). However, there is no difference in feed intake between sexes in periods where no reproductive activities are taking place, although growth performance in males remains superior, suggesting that males have a higher metabolic capacity (Toguyeni *et al.*, 1997). Mixed sex populations display a higher feed consumption and lower growth rate compared to monosex populations cultured in the same conditions, presumably due to increased social activity resulting in higher energy channelled away from growth (Toguyeni *et al.*, 1997). Social interactions may also be stress-inducing, affecting the feed conversion efficiency (Toguyeni *et al.*, 1997).

2.3.2 Tilapia in aquaculture

Tilapia has emerged from obscurity to become “one of the most productive and internationally traded food fish in the world” (Gupta and Acosta, 2004). Reflecting its tropical origin, the optimal temperature for most species of tilapia is 25-30˚C (Cnaani, Gall and Hulata, 2000) with a lethal lower limit of 10˚C and a lethal upper limit of 40˚C. Commercial culture of tilapia in a variety of scales and production system types has seen significant development in the past three decades, mostly outside their habitats of origin (Gupta and Acosta, 2004). Tilapia is a highly adaptable fish and can survive in almost all aquatic environments (Kaufman, 1992; Boyd, 2004; Hannelly, 2009). This attribute has resulted in establishment of tilapia populations in most of the water bodies in which tilapia aquaculture has been practiced or to which tilapia were introduced (Watanabe *et al.*, 2002; Hung *et al.*, 2011; Esselman, Schmitter-Soto and Allan, 2013). These fish have a high tolerance for poor water quality frequently associated with high densities in aquaculture such as high ammonia concentration (2.4 to 3.4 mg/L unionized) and low dissolved oxygen (1 g/L) and can survive in a wide range of pH (5-11) and salinity (depending on the strain, varying from FW to full strength seawater (SW) (Watanabe *et al.*, 2002) making them very successful aquaculture species.

Although approximately 70 species of *Tilapia* and *Oreochromis* have been identified, the most important tilapia species in the aquaculture industry belongs to the *Oreochromis* genus, namely *mossambicus*, *niloticus* and *aureus* (El-Sayed, 2006b) while *Tilapia rendalli* and *Tilapia zillii* have also been used in practical culture. *O. niloticus* boasts the largest production figures and is currently produced in more than 135 countries (FAO, 2014). Besides *O. niloticus,* two species, namely *Oreochromis aureus* and *O. mossambicus* dominate global production albeit with a significantly smaller share of total tilapia production in relation to *O. niloticus.* Hybrid *O. niloticus* x *O. aureus* has gained popularity for use in aquaculture due to the comparatively

high growth rate, cold tolerance, coloration and sex ratio it exhibits relative to several tested hybrids and currently boasts significant production levels (Hulata, Wohlfarth and Halevy, 1988). *Oreochromis andersonii* is also cultured, especially within its endemic distribution in Northern and Western Africa and the Middle East and the potential of this species for aquaculture is under examination (Gopalakrishnan, 1988; Prein, Hulata and Pauly, 1993; Kefi *et al.*, 2012; Musuka and Musonda, 2012).

The farming of tilapia in South Africa began with the endemic species *O. mossambicus*, but most commercial endeavours were met with failure due to the undesirable characteristics of this species such as slow growth rate, and a higher feed conversion relative to *O. niloticus* (Day, 2015). This resulted in national tilapia production halving, from 160 tonnes to 80 tonnes between 2003 and 2006 (Shipton and Britz, 2007) and it has remained at that level until 2012. Commercial production of the invasive species, *O. niloticus*, has been legalised in South Africa for permit holders since 2014. This change in legislation has sparked renewed interest in expanding the local tilapia industry. However, the ideal temperature profile of tilapia has necessitated some level of water temperature control to maintain productivity despite ambient temperature decreases in winter experienced in most regions of South Africa. This phenomenon has led to a mainly intensive approach, with production in a variation of high-density cage or raceway culture systems in which formulated feeds must all nutritional demands of tilapia. These systems are generally situated in greenhouses for cost effective heat retention in colder months.

2.4 GROWTH TRIALS

The “quality” of a feedstuff is generally evaluated based on how efficiently it is retained as growth. Growth, in turn, is influenced by a variety of extraneous factors which warrants the inclusion of a description of test conditions such as water quality, feeding rate, stocking density, size and sex of test animals and stage of reproductive cycle when reporting growth data (Houlihan, Boujard and Jobling, 2008). Data concerning consumption and growth, thus output and input, are usually collected to calculate the feed conversion efficiency (FCE) or WW gain per unit feed consumed. Commercial aquaculture generally uses the reciprocal of feed efficiency, known as the feed conversion ratio (FCR) to express the amount of feed which is necessary to produce 1 kg wet WW gain in the culture organism as an indicator of feed quality in a particular environment for a given culture species.

CHAPTER 3

MATERIALS & METHODS

3.1 EXPERIMENTAL LOCATION AND FACILITIES

3.1.1 Research location and timing

The overall study consisted of two simultaneous growth trials investigating the effect of different rearing environments present in two separate experimental system types, namely BFT and RAS, on the specific growth rate (SGR), feed conversion ratio (FCR), survival, condition factor and biomass yield of two tilapia species, namely *O. niloticus* and *O. mossambicus.* The growth trials were carried out in two culture system types, glass indoor aquaria (120 L each) for the RAS and fiberglass indoor tanks (250 L each) for the BFT system at the Aquaculture Research

Section on the Welgevallen Experimental Farm, Stellenbosch University, South Africa. The experiments commenced on 28 March 2017 and continued for 30-days. This coincided with the end of the summer growing season and a decline in ambient temperature over the course of the experimental period.

3.1.2 Fish

Juvenile, all-male *O. niloticus* (4.46±0.96 g, mean±SD) and *O. mossambicus* (3.17±1.09 g, mean±SD) were obtained from Rivendell Hatchery, Grahamstown, South Africa. Upon arrival at the Welgevallen experimental farm on 23 March 2017, they were acclimated to laboratory conditions over five days in four, 120 L glass aquaria (two aquaria stocked with *O. niloticus* and two with *O. mossambicus*) in a RAS **(Figure 3.1)**. Of these four aquaria, two adjacent tanks were stocked with *O. niloticus* and the remaining two adjacent tanks were stocked with *O. mossambicus*. During acclimation, they were fed the commercial diet (Aquagem, Tilapia grower, 2mm) which would be used for feeding over the course of the experimental period.

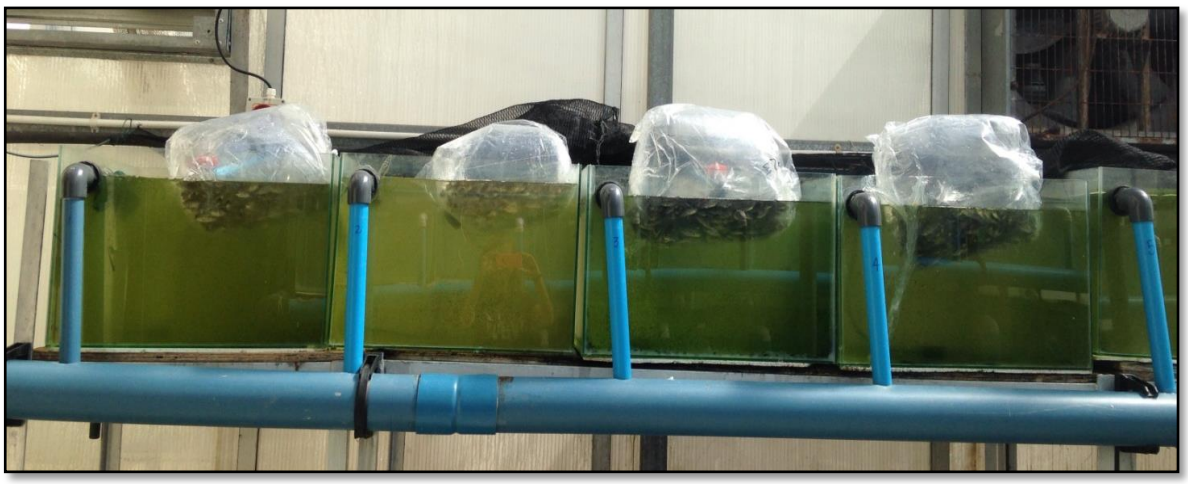


Figure 3.1 *O. mossambicus* (left 2 tanks) and *O. niloticus* (right 2 tanks) juveniles upon arrival at the Welgevallen experimental farm, Stellenbosch.

A total of twelve tanks constituted the BFT system and ten tanks constituted the RAS system. Of the total twelve BFT tanks, ten would eventually be stocked with fish and two would contain biofloc rich water only, serving as backup BFT tanks. At the beginning of the experimental period, *O. niloticus* and *O. mossambicus* were stocked separately at a rate of thirty fish per tank in both the BFT and RAS culture system types. Five replicates were performed per species per rearing system type, thus a total of twenty tanks; ten BFT and ten RAS with completely random allocation. Of the ten RAS and ten BFT tanks, five tanks in each system were stocked with *O. niloticus* and the remaining five were stocked with *O. mossambicus.*

For the BFT system, *O. mossambicus* (3.72±2.93 g, mean±SD) were stocked into tanks 3, 6, 8, 10 and 12 and *O. niloticus* (4.50±0.96 g mean±SD) were stocked into tanks 1, 2, 4, 9 and 11. This left tank 5 and 7 devoid of fish, serving as backup biofloc tanks. For the RAS, *O. mossambicus* (2.62±1.09 g, mean±SD) were stocked into tanks 1, 3, 6, 9 and 10 and *O. niloticus* (4.43±0.86 g, mean±SD) were stocked into tanks 2, 4, 5, 7 and 8. This translates to an average stocking density of 0.73 kg/m^3 and 0.90 kg/m^3 for *O. mossambicus* and *O. niloticus* respectively, stocked into the BFT system and 0.66 kg/m^3 and 1.11 kg/m^3 for *O. mossambicus* and *O. niloticus* respectively, stocked into the RAS system.

3.1.3 Experimental systems

3.1.3.1 Housing structures

The BFT and RAS experimental systems were housed in two greenhouses **(Figure 3.2)**. The greenhouse housing the BFT system was fitted with removable sides, which could be hoisted or lowered in response to changes in ambient temperature to keep fluctuations to a minimum and the water temperature close to the optimal range of 25-28˚C. In comparison, the greenhouse housing the RAS system was fitted with immovable sides anchored by a permanent, concrete structure.

Figure 3.2 The housing structures for the BFT (left) and RAS (right) culture systems.

3.1.3.2 Biofloc technology

Rearing tanks

As done by Azim & Little (2008) and Day (2015), a total of twelve cylindrical tanks, each with a diameter of 620 mm and a volume of 250 L, were fitted with a central 40 mm drain at the base of the tank which could be used to drain any accumulated sludge when necessary **(Figure 3.3)**. Tanks were elevated on concrete bricks and organized in a 6x2 formation. Water level was maintained at 150 L per tank throughout the duration of the trial.

Figure 3.3 The experimental setup displaying the BFT tilapia rearing tanks.

Aeration and circulation

A 0.55 KW channel blower (CFW, model ZxB 310) fed into 40 mm piping which was organized into a ring that encircled the rearing tanks, ensuring that an even pressure was maintained. This uniform pressure minimized variations in aeration and mixing intensity between tanks. From the main aeration ring, pipes channelled air to an aeration ring which fit into the inner perimeter at the base of each rearing tank. Holes were pierced into the aeration ring at 25 mm intervals. Each aeration ring was anchored at the base of its respective tank with two stones.

To improve homogeneity and uniformity of water quality between rearing tanks, twelve airlift pumps were constructed using 15 mm polyvinyl chloride (PVC) pipes fed with air from the main aeration ring via micro tubing. These airlift pumps pumped water from each tank to the adjacent tank at a rate of 6 L per minute. As an added measure to prevent overflow in the case of blockage of an airlift pump, adjacent tanks were connected with a piece of 32 mm flexible pipe above the 250 L water level.

Biofloc development and enhancement

Biofloc rich water was developed in the BFT rearing tanks over a period of 21 days prior to the introduction of fish. At the start of the biofloc development stage, a suspension of natural phytoplankton (green water) from a RAS system was transferred to the empty BFT rearing tanks. Green water was used due to the assumption that biofloc would develop more rapidly if a base level inoculation of a microbial community was present relative to the comparatively lower microbial count of filtered (clear) water. To fertilize the limited water exchange BFT system over the biofloc development stage, pelleted feed (Aquagem, Tilapia grower, 2mm) and a carbon source in the form of maize meal was delivered to supply the required nutrients for microbial assimilation and nitrogen uptake.

Pelleted feed was delivered at a rate of 10 g per day per BFT tank. Assuming a C:N ratio of 4 in microbial biomass (Gaudy and Gaudy, 1981), a microbial conversion coefficient, E, of 40% (Avnimelech, 1999) and that the carbohydrate source, maize meal, has a carbon content of 57.8% (with the underlying assumption that the added carbon content of carbohydrate is 66.7% according to the general molecular formula of carbohydrates (CH_2O)) (Table 3.1). the amount of carbohydrate addition (ΔCH) needed to reduce nitrogen can be determined by the equation (Avnimelech, 1999):

$$\Delta CH = \Delta N \div 0.057 \quad (1)$$

The amount of nitrogen addition from the feed (ΔN) can be determined with the following equation:

$$\Delta N = feed \times \%Nfeed \quad (2)$$

At a feeding rate of 10g per day with a pelleted feed which was shown by proximate analysis to have a crude nitrogen content of 5.77% (Table 3.1), 0.58 g of nitrogen was introduced per tank per day. According to equation (1), the feed having 5.77% nitrogen should be amended by an additional daily portion of 11.6 g made of carbohydrates with no protein. The maize meal used was shown to have a carbohydrate content of 86.7% (Table 3.1), thus maize meal should be adjusted to a rate of 13.4 g per day. In turn, the maize meal was shown to have a crude nitrogen content of 1.32% (Table 3.1) which warrants the addition of an additional 4.8 g of maize meal per day to remove the nitrogen introduced with maize meal delivery, thus a total

of 18.2 g maize meal per day per tank (or 182% of daily feed delivery). The nitrogen content of the delivered feed and maize meal should accordingly be:

$$\boldsymbol{nitrogen\ content\ delivered} = (\mathbf{5.77\%} \times \Delta\boldsymbol{Feed}) + (\mathbf{1.32\%} \times \Delta\boldsymbol{CH}) \div \Delta\boldsymbol{Feed} + \Delta\boldsymbol{CH} \quad (3)$$

Where: Δfeed = daily feed addition (g)
ΔCH = daily carbohydrate addition (g)

Thus, at a daily feed addition of 10 g:

$$\boldsymbol{nitrogen\ content\ delivered} = (\mathbf{5.77\%} \times \mathbf{10\ g}) + (\mathbf{1.32\%} \times \mathbf{18.2\ g}) \div \mathbf{28.2g} = \mathbf{2.9\%} \quad (4)$$

The carbon content of the delivered feed and maize meal can be determined by the equation:

$$\boldsymbol{carbon\ content} = \%\boldsymbol{carbohydrate} \times \%\boldsymbol{Ccarbohydrate} \quad (5)$$

Assuming the carbon content of carbohydrate is 66.7% based on the general molecular formula of carbohydrates (CH_2O) and with known carbohydrate contents of 51.9% in the pelleted feed and 86.7% in the maize meal (Table 3.1), solving equation 4 yields a carbon content of 57.8% in maize meal and 34.6% in the pelleted feed. The carbon content of the delivered feed and maize meal combination delivered should accordingly be:

$$\boldsymbol{carbon\ content\ delivered} = (\mathbf{34.6\%} \times \Delta\boldsymbol{Feed}) + (\mathbf{57.8\%} \times \Delta\boldsymbol{CH}) \div \Delta\boldsymbol{Feed} + \Delta\boldsymbol{CH} \quad (6)$$

Where: Δfeed = daily feed addition (g)
ΔCH = daily carbohydrate addition (g)

Thus, at a daily feed addition of 10 g:

$$\boldsymbol{carbon\ content\ delivered} = (\mathbf{34.6\%} \times \mathbf{10g}) + (\mathbf{57.8\%} \times \mathbf{18.2\ g}) \div \mathbf{28.2\ g} = \mathbf{49.6\%} \quad (7)$$

This yielded a C: N ratio of 17.1 delivered daily to each BFT tank over the biofloc development stage, before fish were introduced to the system.

3.1.3.3 Recirculating aquaculture system

Rearing tanks

The RAS consisted out of ten 120 L glass aquaria in series, each with an aeration supply **(Figure 3.4)**. The tanks were covered by a shade net to prevent escapees. Mechanical and biological water filtration components (Ultra Zap, low pressure biological filter) were incorporated into the circuit **(Figure 3.5)**. A constant water flow rate of 12.2 ± 1.3 L per second per tank was maintained for the duration of the trial. Aeration was supplied by 1.1 KW side channel blower (FPZ, model KO4 MS 1P) and water was circulated by a 0.2 KW pump (AquaDrive 390, model 6452L TL-A12X).

Figure 3.4 The experimental setup of the RAS rearing tanks.

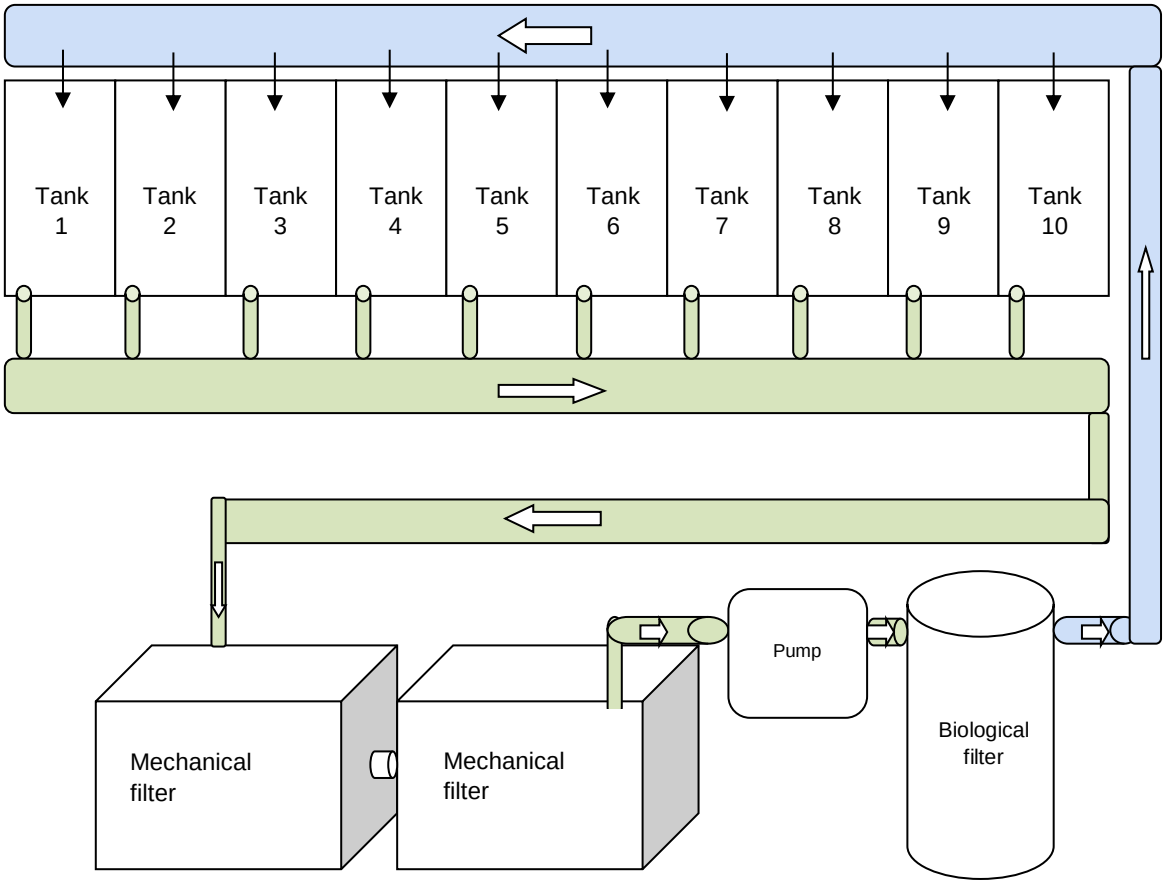


Figure 3.5 Layout of the recirculating aquaculture system.

3.2 LIVE MEASUREMENTS

Every stocked fish from each tank in both culture system types was sampled initially and every ten days thereafter over the experimental period of 30-days, thus a total of four sampling events. Before sampling, fish were sedated by being placed in a low dosage of Tricaine MS222 for approximately 30 seconds.

Individual body mass was recorded by drying the fish slightly on a hand towel before the wet weight was measured using an electronic scale (UWE, model HGS-300). Standard length (SL) was recorded by using a measuring board, measuring every animal from the tip of the snout to the tip of the caudal peduncle. Total length (TL) was measured from the tip of the snout to the tip of the caudal fin (Skelton, 2001). These parameters, in combination with feeding data, were used to calculate the overall SGR and FCR for each tank and individual condition factors for each fish over the trial period as follows:

a) Feed conversion ratio

The FCR for each tank over the trial period was calculated using the total mass of feed delivered and the recorded fish wet weight increase per tank according to the formula:

$$FCR = amount\ of\ feed\ fed\ (g) \div total\ mass\ increase\ of\ fish\ from\ tank\ (g) \quad (8)$$

The addition of maize meal was excluded from the FCR calculation since it was not a direct feed source for the test animals. Mortalities were included in the calculation by adding the recorded wet weight of the dead animals to the final tank biomass (W_f).

b) Specific growth rate

The SGR in each tank was calculated as the average percentage of body WG per day according to the formula:

$$\mathbf{SGR} = \frac{100 \times (\ln W_f - \ln W_i)}{\text{time in days}} \quad (9)$$

Where: W_f = final biomass (g)
W_i = initial biomass (g)

c) Biomass yield

The biomass yields for the RAS and BFT systems were represented by both production (total kg WG over the culture period) and the derived productivity (total kg WG per m^3 over the culture period). Production was converted to productivity in the BFT system by:

$$Productivity = Production\ (kg) \times \left(1000 \frac{L}{m^3} \div 150\ L\right) \quad (10)$$

And in the RAS by:

$$Productivity = Production\ (kg) \times \left(1000 \frac{L}{m^3} \div 120\ L\right) \quad (11)$$

d) Condition Factor

The condition factor (K) for each fish was calculated at the time of the final sampling event as follows:

$$K = \frac{100 \times W}{L^3} \quad (12)$$

Where: W = Fish wet weight (g)
L = Total length (cm)

After being weighed and measured, sampled fish were placed in a recovery bath with clean, well-aerated water before being returned to the tank from which they originated. Feeding was

restricted to only one feeding event at 16:00 h on sampling days. The feed delivered on sampling days was calculated based on the actual tank biomass recorded during sampling.

3.3 TANK MANAGEMENT AND WATER QUALITY MONITORING

The functionality of both systems was checked a minimum of four times per day. Two feeding events took place daily, at 8:00 h and 16:00 h. Behaviour and feeding activity of the fish were monitored at each feeding event, while external clinical signs indicative of disease or stress such as fin deterioration, skin ulceration or haemorrhaging, irregular body shape and unusual colouring were monitored during the sampling events described in Section 3.2. Temperature, pH, dissolved oxygen (DO), salinity and electro-conductivity (EC) were monitored twice daily before feeding events and floc volume (FV) was monitored once a day prior to the 8:00 h feeding. Temperature and DO were measured using an oxygen probe (YSI, Pro ODO, Yellow Springs, USA), pH was measured using a portable pH meter (Hach, sension 1, Loveland, USA) and salinity and EC were measured using a combo pH/conductivity/salinity/DO meter (IP67 Water Quality Meter). The FV in mg/L was measured using an Imhoff cone by letting 1 L of culture water settle for 15 min and recording the settled volume.

Total ammonia (TA), nitrite (NO_2^-), nitrate (NO_3^-), un-ionized ammonia (UIA), orthophosphate (PO_4^{3-}) and turbidity were monitored weekly. These parameters were measured using a colorimeter (Hach, DR/850, Loveland, USA) with random repeat measurements from two tanks. TA and NO_2^- were periodically measured more frequently when high levels were detected or suspected and were measured using the salicylate and diazotization methods, respectively. NO_3^- was measured using the cadmium reduction method. PO_4^{3-} was measured using the ascorbic acid method and the absorptometric method was applied to measure turbidity. As described by El-Shafai *et al.* (2004), toxic UIA could be determined by incorporating TA, pH and temperature levels into the general equation of bases put forth by Albert (1973):

$$UIA = \frac{[TAN]}{[1+10^{(pK_a - pH)}]} \quad (13)$$

The determination of pK_a in FW was based on the formula developed by Emerson *et al.* (1975):

$$pK_a = 0.09018 + \frac{2729.92}{273.2+T} \quad (14)$$

Where: T = temperature (˚C)

Sludge was drained once a week from the BFT rearing tanks, where after the removed water was replenished from the two backup BFT tanks. All mortalities were recorded daily for the RAS rearing tanks and feeding levels were adjusted for the following day. The turbidity of the BFT tanks prevented daily visual confirmation of mortalities, so the reduction in number of fish per tank between sampling events was considered the mortality rate and feeding levels were adjusted at each sampling event for the following ten days. The terminal number of animals remaining in each tank on day 30 was used to calculate the overall survival rate for each tank as follows:

$$Survival\ rate\ (\%) = \frac{Final\ number\ of\ fish \times 100}{Initial\ number\ of\ fish} \quad (15)$$

When high levels of critical nitrogenous compounds such as UIA and NO_2^- were detected, feeding rates were reduced, the input of maize meal was increased and sludge was drained more frequently.

3.4 FEEDING

Fish in both the RAS and BFT system were fed a commercial tilapia feed (Aquagem, Tilapia grower, 2mm).

3.4.1 Recirculating aquaculture system

Following the guidelines of Chowdhury (2011), a daily feeding level of 6% of tank biomass was delivered to juvenile tilapia in RAS tanks. Daily growth between sampling events was predicted using the formula:

$$W_f = \sqrt[3]{W_i} + (g \times t)^3 \quad (16)$$

Where: W_f = final biomass per tank
W_i = initial biomass per tank
g = linear regression of the growth curve
t = time in days

Between day 1 and 10, 'g' for each tank in the RAS system was taken to be 0.1. After the sampling event on day 10, the realized 'g' values for *O. mossambicus* and *O. niloticus* were determined based on the observed growth across all tanks between day 0 and 10 and these values were incorporated into the above formula to estimate, albeit retrospectively, the daily increase in biomass for the following ten days (day 11-20) as a measure to ensure that feeding levels remained realistic throughout the interval between sampling events. This was repeated to calculate 'g' over day 11-20, and incorporated into the above formula to estimate daily growth for the period day 21-30. The daily feed ration for each tank was split evenly over two feeding events, one at 8:00 h and the other at 16:00 h.

3.4.2 Biofloc technology system

Following the guidelines of Chowdhury (2011), a daily feeding level of 2.5% of tank biomass was delivered to juvenile tilapia in BFT tanks. Daily growth was predicted in the same way as was done for RAS tanks (see section 3.4.1).

In accordance with a study conducted by Hargreaves (2013), the carbohydrate source, maize meal, was delivered immediately after feeding at a rate which was sufficient to remove the nitrogen introduced by the pelleted feed. Assuming that the microbial biomass has a C:N ratio of 4 (Gaudy and Gaudy, 1981), the microbial conversion coefficient, E, was 40% (Avnimelech, 1999), and that the carbohydrate source, maize meal, has a carbon content of 57.8% (Table 3.1) with the underlying assumption that the added carbon content of carbohydrate is 66.7% according to the general molecular formula of carbohydrates (CH_2O), the appropriate daily carbohydrate delivery rate per tank could be determined by the equation developed by Avnimelech (1999):

$$\Delta CH = \Delta Feed \times \%N\ in\ feed \times \%N\ excretion \div 0.057 \quad (17)$$

Where: ΔCH = carbohydrate addition (g)
Δfeed = feed addition (g)

Percentage nitrogen in feed was determined to be 5.77% by proximate analysis (Table 3.1) and percentage nitrogen excretion was assumed to be 75% under the assumption that 25% of the nitrogen added in the feed was assimilated by the fish (Avnimelech and Lacher, 1979; Boyd, 1985; Muthuwani and Lin, 1996). Thus:

$$\Delta CH = \Delta Feed \times 0.0577 \times 0.75 \div 0.057 = \Delta Feed \times 0.759 \quad (18)$$

The maize meal delivered was shown to have a nitrogen free extract (NFE) or carbohydrate content of 86.7% (Table 3.1), thus maize meal should be adjusted to a daily rate of:

$$\Delta CH = \Delta Feed \times 0.759 \div 0.867 = \Delta Feed \times 0.876 \quad (19)$$

In turn, the maize meal was shown to have a crude nitrogen content of 1.32% (Table 3.1), thus for each 1 g of feed delivered an additional 0.012 g of nitrogen was delivered with the accompanying maize meal.

Thus:

$$\Delta CH = \Delta Feed \times 0.012 \times 1 \div 0.057 = \Delta Feed \times 0.211 \quad (20)$$

Adjusted to compensate for the carbohydrate content of 86.7% (Table 3.1) to:

$$\Delta CH = \Delta Feed \times 0.211 \div 0.867 = \Delta Feed \times 0.243 \quad (21)$$

Additional maize meal to the level of 24.3% of daily feed delivery should therefore have been delivered per day to remove the additional nitrogen entering the tank via the maize meal itself, assuming that none of the nitrogen delivered with the maize meal was assimilated by the fish. Repeating this exercise until each subsequent nitrogen addition introduced with additional maize meal was removed - a total of Δfeed x 1.21 (or 121% of daily feed delivery) maize meal per day per tank was required.

The C: N ratio delivered over the experimental period could be calculated in the same way as was done in section 3.1.3.2 under the subsection *Biofloc development and enhancement*. Substituting the relationship between daily feed and maize meal addition calculated for the biofloc development stage (ΔCH = Δfeed x 1.82) with that calculated for the experimental period (ΔCH = Δfeed x 1.21) into equation (3) and (6), this yielded a C: N ratio of 14.6 delivered daily to each BFT tank over the experimental period.

Samples of the pelleted feed, maize meal and biofloc were collected and prepared by drying in an oven at 60˚C for 24 hours before being transferred to a vacuum bag until later analysis. The biofloc sample was collected by allowing 5 L from each experimental BFT tank to settle for 10 hours and collecting the settled sludge. Biofloc samples collected from each tank were pooled to constitute the sample which was analysed. Proximate analyses were performed on these three samples according to the methods described by the AOAC (1997) (Table 3.1).

Table 3.1. Proximate parameters of the pelleted feed, maize meal (carbohydrate source) and biofloc.

Composition (% DM)	**Feed**	**Maize meal**	**Biofloc**
Nitrogen	5.77	1.32	2.96
Crude protein	36.06	8.25	18.5
Crude fat	2.64	2.00	1.08
Crude fibre	4.55	0.79	3.63
Ash	7.61	0.46	5.26
Moisture	1.76	2.56	14.94
Nitrogen-free extract (NFE) (carbohydrate)	51.9	86.7	60.2

3.5 ECONOMIC ANALYSIS

Costs of production and relative cost efficiencies of the two systems were calculated based on tilapia production performance indicators (productivity and biomass yield) which were realized over the culture period and all realized operational costs in the form of energy, feed and labour expenses incurred over the same period.

The components and estimations of their currency value which would represent fixed costs relevant to each system type, including the housing structure, circulation and aeration pumps, rearing tanks, plumbing, water treatment units, supporting structures and accessories were described to illustrate potential differences in capital requirements for the two systems. The currency value estimations assigned to these components were not included in the final cost efficiency determination due to the speculative nature of the estimations which were based on current market value rather than realized past expenditure of the Aquaculture Division of Stellenbosch University. The tilapia market price was based on the "farm gate" price realized by a commercial *O. niloticus* producer in Johannesburg, South Africa in May 2017.

The reported cost efficiency (kg/€) represents the kg of whole wet weight tilapia which can be produced per euro. The conversion of currency was based on the exchange rate of 1 € (euro) = 14.5 ZAR (South African rand).

3.5 STATISTICAL ANALYSES

All statistical analyses were performed using RStudio version R 3.4.0 software for windows. Differences were considered significant at $p<0.05$. The effects of system type on water quality parameters measured daily (temperature, dissolved oxygen, pH, electro-conductivity and salinity) were analysed using a Welch two-sample t-test, and comparing all values recorded in the BFT system to that of the RAS. Morning and afternoon readings of these water quality parameters taken in a single system type were analysed using a paired t-test, and comparing all values obtained at the morning readings to that obtained at the evening readings. The effects of system type on water quality parameters determined weekly (TA, nitrite, nitrate, UIA, orthophosphate and turbidity) were analysed using Welch Two Sample t-tests. The assumptions of normality and homogeneity of variances were tested using the Shapiro-Wilk Levene's test, respectively. When normality could not be assumed for paired data, the Wilcoxon signed rank test was used, while the Wilcoxon rank-sum test was used when normality could not be assumed for unpaired data.

The effects of system type/species combinations on fish survival, wet weight, FCR, SGR, condition factor and 'g' values were analysed using one-way analysis of variance (ANOVA). Differences within each system type/species combination group between sampling events were analysed using one-way analysis of variance (ANOVA). When differences were considered significant, Tukey's test was used to identify differences between system types. The assumptions of normality and homogeneity of variance for the analyses were tested using the Shapiro-Wilk and Levene's test, respectively. If normality could not be assumed, a non-parametric test (Kruskal Wallis test) was applied.

The results are presented as averages and standard deviations (SD).

CHAPTER 4

RESULTS

4.1 WATER QUALITY

4.1.1 Temperature

Daily water temperatures (average±SD, total 29 days of measurement) recorded in RAS and BFT systems were 26.4±3.3˚C and 21.8±2.8˚C, respectively, over the trial period. Outside air temperature was 20.6±3.1˚C. **Table 4.1** shows that water temperature differed significantly between production system types ($p<0.05$) and between the RAS and the outside air temperature. Water temperature in the BFT system and outside air temperature did not differ significantly ($p=0.07$). Water temperatures did not increase or decrease significantly over the experimental period in either system type nor in the ambient outside temperature when comparing the overall average temperature recorded twice daily over consecutive ten-day intervals **(Figure 4.1)**. The average water temperature increase above outside temperature was significantly higher in RAS at 5.8±2.3˚C relative to the BFT system at 1.2±2.1˚C.

Table 4.1 The average (± SD) air temperature recorded twice daily and water temperature (˚C) recorded twice daily over 10 replicate tanks in each culture system type over three ten-day intervals.

System type	Period after stocking			Overall
	Day 1-10	Day 11-20	Day 21-30	Day 0-30
RAS	26.1±3.1^{b} (n=200)	25.9±3.5^{b} (n=200)	27.2±3.3^{b} (n=200)	26.4±3.3^{b} (n=600)
BFT	22.3±2.4^{a} (n=200)	21.8±3.2^{a} (n=200)	21.3±2.7^{a} (n=200)	21.8±2.8^{a} (n=600)
Air	20.5±2.3^{a} (n=20)	20.1±3.8^{a} (n=20)	21.2±3.4^{a} (n=20)	20.6±3.1^{a} (n=60)

Values reported with the same superscript letter in the same row or column are not significantly different.

Correlation analysis revealed a positive linear relationship between air temperature and water temperature in both the RAS (r=0.74) and BFT system (r=0.77) **(Figure 4.1)**.

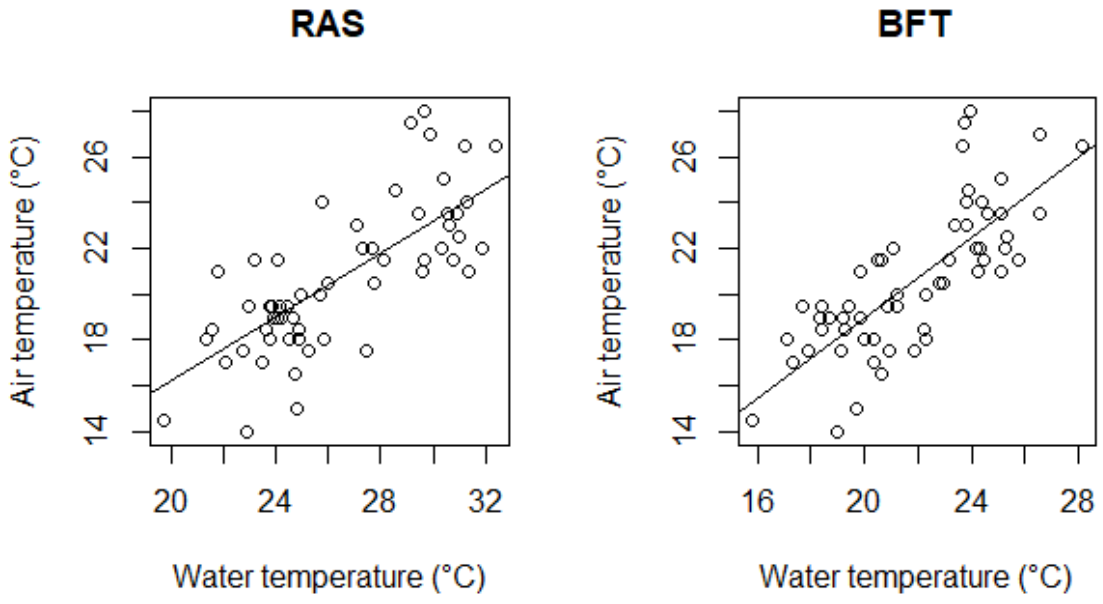


Figure 4.1 Scatter plots illustrating a positive linear relationship between air temperature and water temperature for the RAS (left) and BFT (right) systems.

Temperature readings for both systems were significantly higher at the 16:00 h reading relative to the 8:00 h reading ($p<0.05$). Average (± SD) morning temperatures of 19.8±1.8˚C and 23.8±1.6˚C and average afternoon temperatures of 23.8±2.1˚C and 29.0±2.4˚C were determined for BFT and RAS systems, respectively. The fluctuations in daily average water temperature recorded in the two systems at the morning and afternoon readings are reflected in **Figure 4.2**.

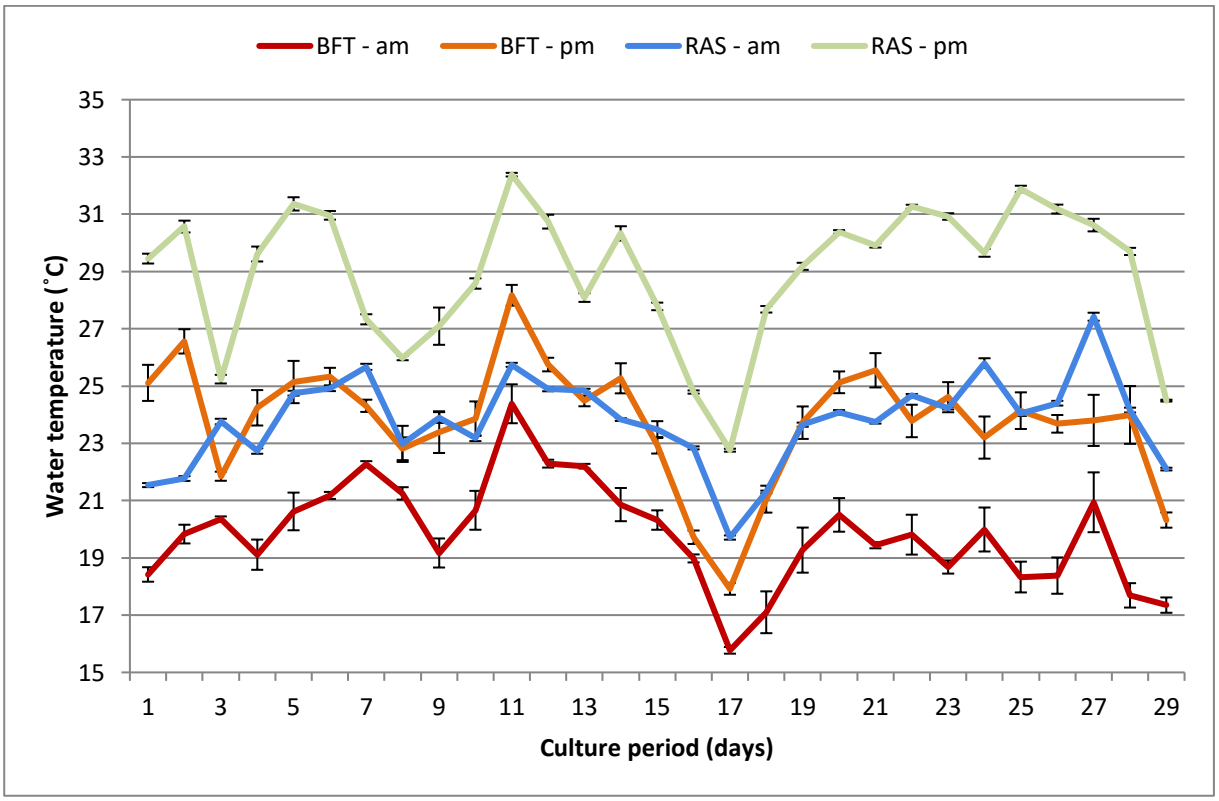


Figure 4.2 Evolution of average water temperature of ten replicate tanks in BFT and RAS systems measured at 8:00 h and 16:00 h daily over a 30-day culture period. Error bars represent SD of ten replicates.

Minimum and maximum average temperatures recorded in the BFT system over the duration of the trial were 15.8˚C and 28.2˚C, respectively. In the RAS, they were recorded as 19.7˚C and 32.4˚C, respectively. For both system types, the minimum average temperature was recorded on day 17 at the 8:00 h reading and the maximum was recorded on day 11 at the 16:00 h reading. Average morning temperatures below 20˚C were recorded for a total of 12 days in the BFT system and 1 day in the RAS system over the trial period. Average afternoon temperatures dropped below 20˚C on only one occasion on day 17 in the BFT system but never once occurred in the RAS.

4.1.2 Dissolved oxygen

Overall average (± SD) DO content was significantly lower in the BFT system at 7.8±1.4 mg/L relative to that measured in the RAS system at 8.5±0.9 mg/L ($p<0.05$). Average DO levels were significantly higher at the 8:00 h readings relative to the 16:00 h readings for both BFT and RAS systems ($p<0.05$). Average (± SD) morning DO levels were 8.8±0.9 mg/L and 9.2±0.5

mg/L for BFT and RAS systems, respectively. Average (± SD) afternoon DO levels were 6.8±1.1 mg/L and 7.7±0.6 for BFT and RAS systems, respectively. The fluctuations in daily average DO levels recorded in the two systems at the morning and afternoon readings are reflected in **Figure 4.3**.

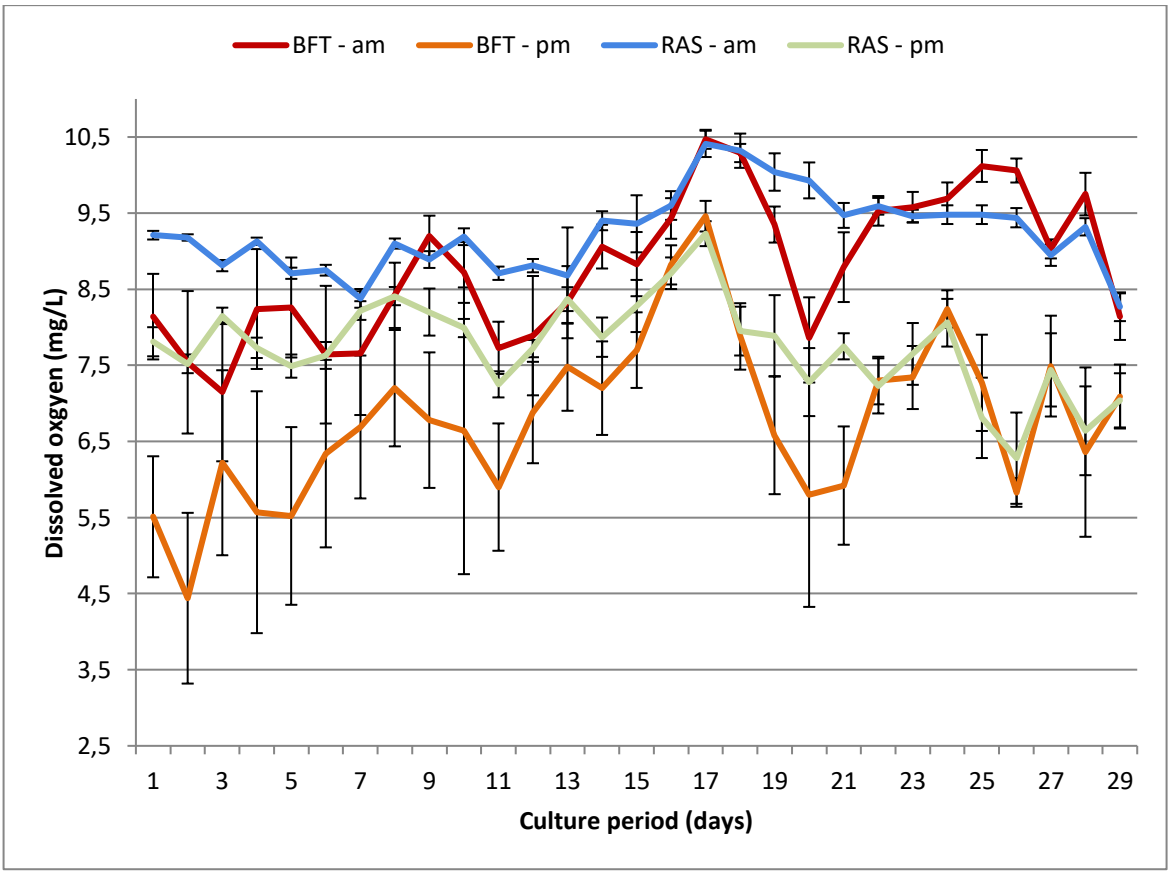


Figure 4.3 Evolution of average dissolved oxygen (DO) levels of ten replicate tanks in BFT and RAS systems measured at 8:00 h and 16:00 h daily over a 30-day culture period. Error bars represent SD of ten replicates.

An inverse correlation could be observed between DO levels and water temperature in both the BFT (r=-0.75) and RAS systems (r=-0.84) **(Figure 4.4)**.

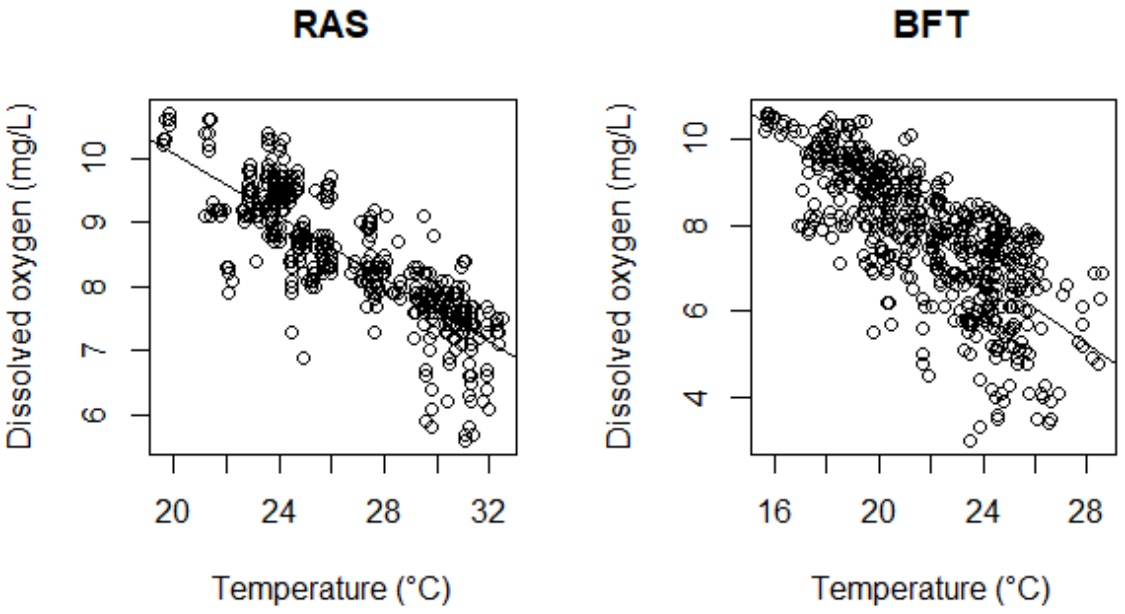


Figure 4.4 Scatter plots illustrating an inverse relationship between temperature and dissolved oxygen levels for the RAS (left) and BFT (right) systems.

Minimum and maximum average DO levels in the BFT system were recorded as 4.4 mg/L and 10.5 mg/L, respectively. In the RAS, they were recorded as 6.3 mg/L and 10.4 mg/L, respectively. The minimum DO level was recorded on day 2 at the 16:00 h reading for the BFT system and on day 26 at the 16:00 h reading for the RAS system. The maximum DO level was recorded on day 17 at the 8:00 h reading for both the BFT and RAS systems, corresponding to the minimum average temperatures recorded for both systems.

4.1.3 pH

Average (± SD) pH levels were shown to be significantly higher in the BFT system relative to the RAS system ($p<0.05$) at 6.72±0.37 and 6.00±1.10 for the BFT and RAS systems, respectively. For each system, 8:00 h readings were significantly higher relative to 16:00 h readings ($p<0.05$). Average (± SD) 8:00 h readings were 6.81±0.37 and 6.12±0.35 for the BFT and RAS systems, respectively. Average (± SD) 16:00 h pH readings were 6.64±0.35 and 5.89±0.27 for the BFT and RAS systems, respectively. The fluctuations in daily average pH recorded in the two systems at the morning and afternoon readings are reflected in **Figure 4.5**.

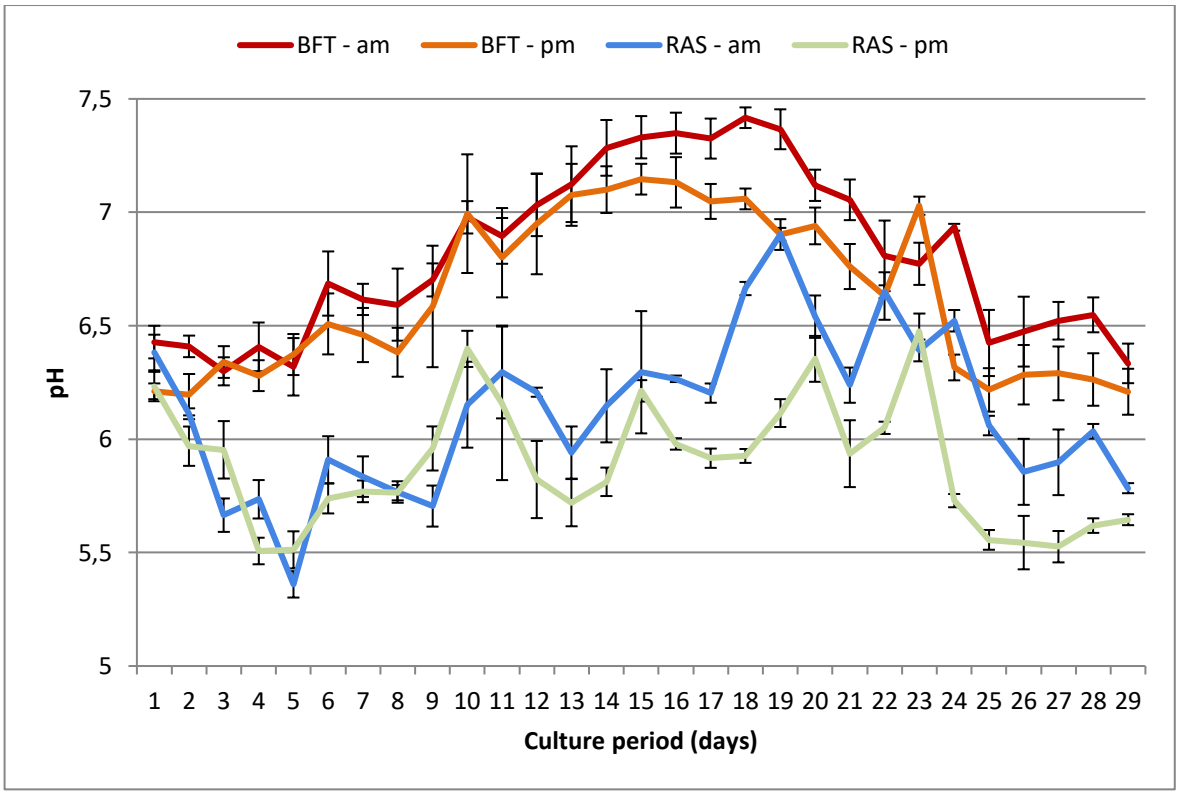


Figure 4.5 Evolution of average pH levels of ten replicate tanks in BFT and RAS systems measured at 8:00 h and 16:00 h daily over a 30-day culture period. Error bars represent SD of ten replicates.

4.1.4 Electro-conductivity and salinity

Both average electro-conductivity (EC) and salinity differed significantly between BFT and RAS systems ($p<0.05$). Average (± SD) EC was consistently higher in the BFT system at 249±37 µS relative to the 146±18 µS measured in the RAS system. 8:00 h and 16:00 h readings were not significantly different in either the BFT or RAS system ($p=0.12$ and $p=0.08$, respectively). Average (± SD) EC recorded at 8:00 h was 247±39 µS and 145±18 µS for the BFT and RAS systems, respectively and 251±36 µS and 147±18 µS at the 16:00 h reading for BFT and RAS systems, respectively. The fluctuations in daily average EC recorded in the two systems at the morning and afternoon readings are reflected in **Figure 4.6**.

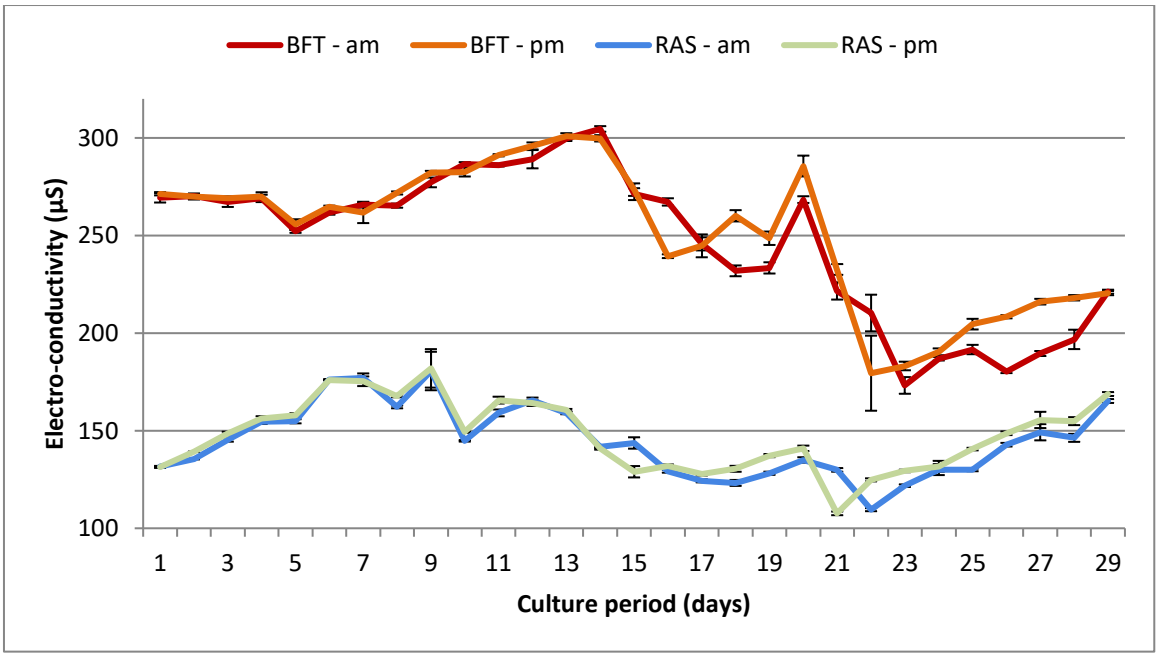


Figure 4.6 Evolution of average electro-conductivity of ten replicate tanks in BFT and RAS systems measured at 8:00 h and 16:00 h daily over a 30-day culture period. Error bars represent SD of ten replicates.

Average (± SD) salinity was significantly higher in the BFT system at 0.12±0.02 g/L relative to the 0.07±0.01 g/L measured in the RAS system. Morning and afternoon readings differed significantly in the RAS system ($p<0.05$) but not in the BFT system ($p=0.24$). Average (± SD) salinity recorded at 8:00 h was 0.12±0.02 g/L and 0.07±0.01 g/L for the BFT and RAS systems, respectively and 0.12±0.02 g/L and 0.07±0.01 g/L at the 16:00 h reading for BFT and RAS systems, respectively. The fluctuations in daily average salinity recorded in the two systems at the morning and afternoon readings are reflected in **Figure 4.7**.

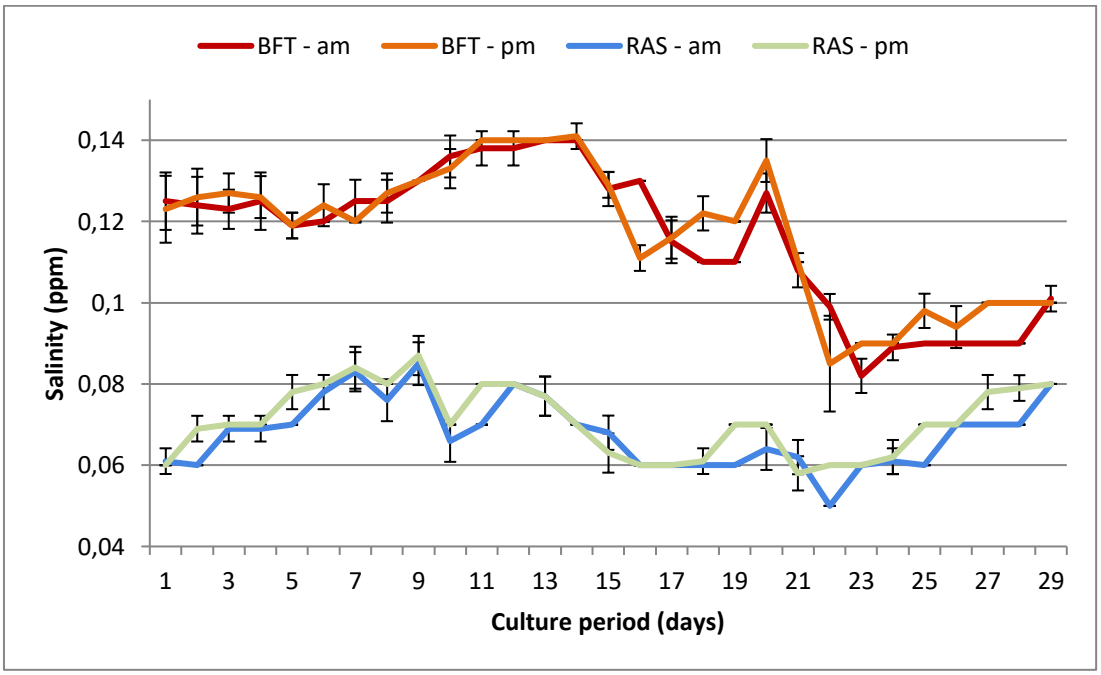


Figure 4.7 Evolution of average salinity of ten replicate tanks in BFT and RAS systems measured at 8:00 h and 16:00 h daily over a 30-day culture period. Error bars represent SD of ten replicates.

Correlation analysis revealed a positive linear relationship between electro-conductivity and salinity in both the RAS (r=0.90) and BFT system (r=0.97) **(Figure 4.8)**.

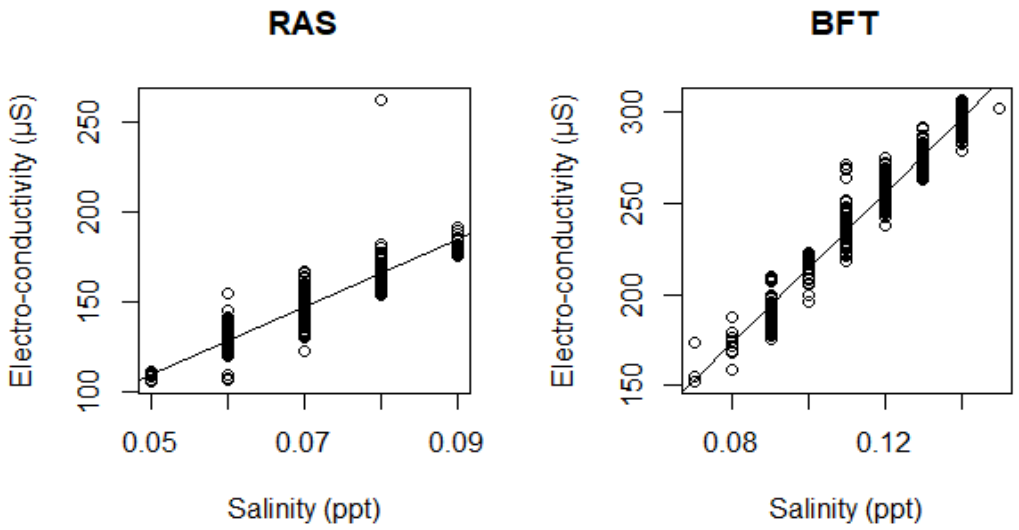


Figure 4.8 Scatter plots illustrating a positive relationship between electro-conductivity and salinity levels for the RAS (left) and BFT (right) systems.

4.1.5 Floc volume

Floc volume (FV) was recorded once a day (8:00 h) only in the BFT system rearing tanks. The average (± SD) over the experimental period was 47.75±1.08 mg/L and FV readings ranged between a minimum of 45.7 mg/L on day 22 and a maximum of 49.6 mg/L on day 13. The fluctuations in daily average floc volume recorded over the culture period are reflected in **Figure 4.9**. An initial upward trend in average FV between day 1 and 13 could be observed, followed by a downward trend between day 13 and day 22 and a subsequent upward trend between day 22 and day 29.

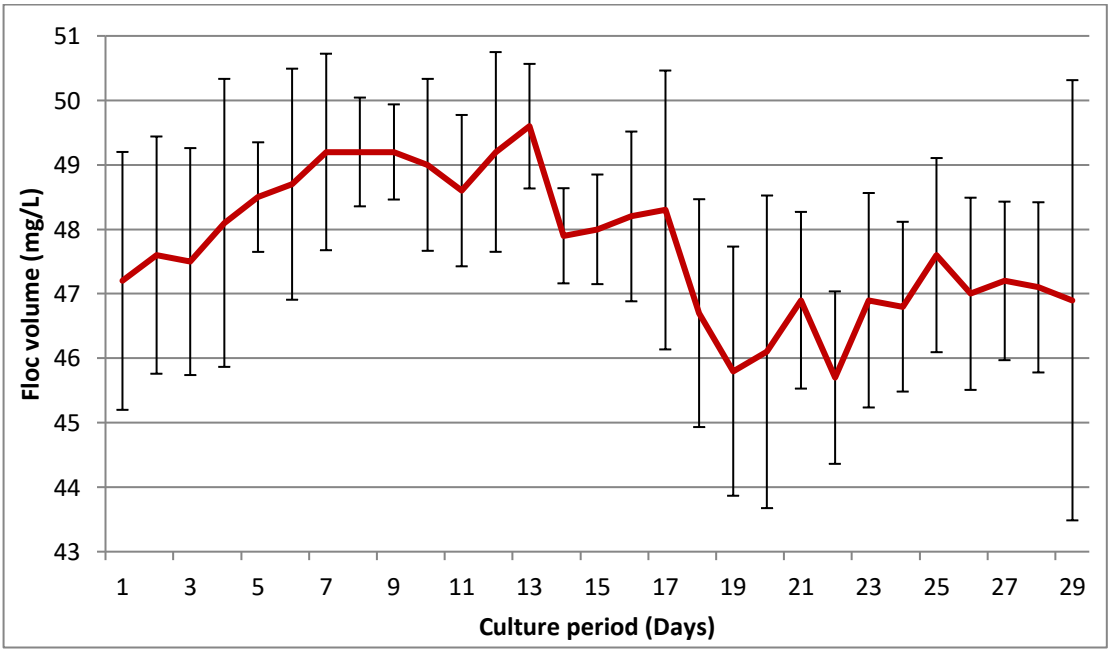


Figure 4.9 Evolution of average floc volume in the BFT system measured daily over a 30-day culture period. Error bars represent SD of ten replicates.

4.1.6 Dissolved inorganic nitrogen

There were significant differences observed for total ammonia (TA), nitrite, and unionized ammonia (UIA) concentrations between production system types ($p<0.05$). No significant difference was observed for nitrate levels between the RAS and BFT system ($p=0.16$).

Average (± SD) TA concentration was significantly higher in the RAS at 2.89±1.33 mg/L compared to that measured in the BFT system at 2.29±1.30 mg/L over the experimental period. TA content readings in the BFT system showed weekly increase between day 0 and 21, before it declined between day 21 and day 31. TA content in the RAS system increased sharply initially between day 0 and 7, declined slightly between day 7 and 14 before again increasing slightly between day 14 and 28 and sharply between day 28 and day 31 **(Figure 4.10A)**.

Average (± SD) UIA concentration was significantly higher in the BFT system at 0.009±0.014 mg/L relative to that recorded in the RAS at 0.001±0.00 mg/L. UIA content in the RAS system remained relatively constant, showing no major deviation from the average over the experimental period. UIA content in the BFT system demonstrated a sharp upward spike on day 14 before gradually declining to its previous level between day 14 and day 28 **(Figure 4.10D)**.

Overall average (± SD) nitrite concentration was significantly higher in the RAS at 0.81±0.28 mg/L relative to that measured in the BFT system at 0.08±0.07 mg/L. Nitrite content in the BFT system remained relatively constant over the culture period, demonstrating only a slight increase between day 14 and 21 and a subsequent slight decrease between day 21 and day 28. Nitrite content in the RAS system demonstrated a sharp decline between day 0 and 7, a sharp increase between day 7 and day 14 before declining after day 14 until the end of the culture period on day 31 **(Figure 4.10B)**.

Average (± SD) nitrate concentration recorded in the BFT system was slightly, but not significantly higher at 21.67±7.42 mg/L relative to that recorded in the RAS at 19.96±4.80 mg/L. Nitrate levels in the RAS remained relatively stable over the culture period, demonstrating slight increases in nitrate levels between day 0 and 7 as well as between day 21 and 31. Nitrate levels in the BFT system increased between day 0 and day 21, before declining sharply between day 21 and day 31 **(Figure 4.10C)**.

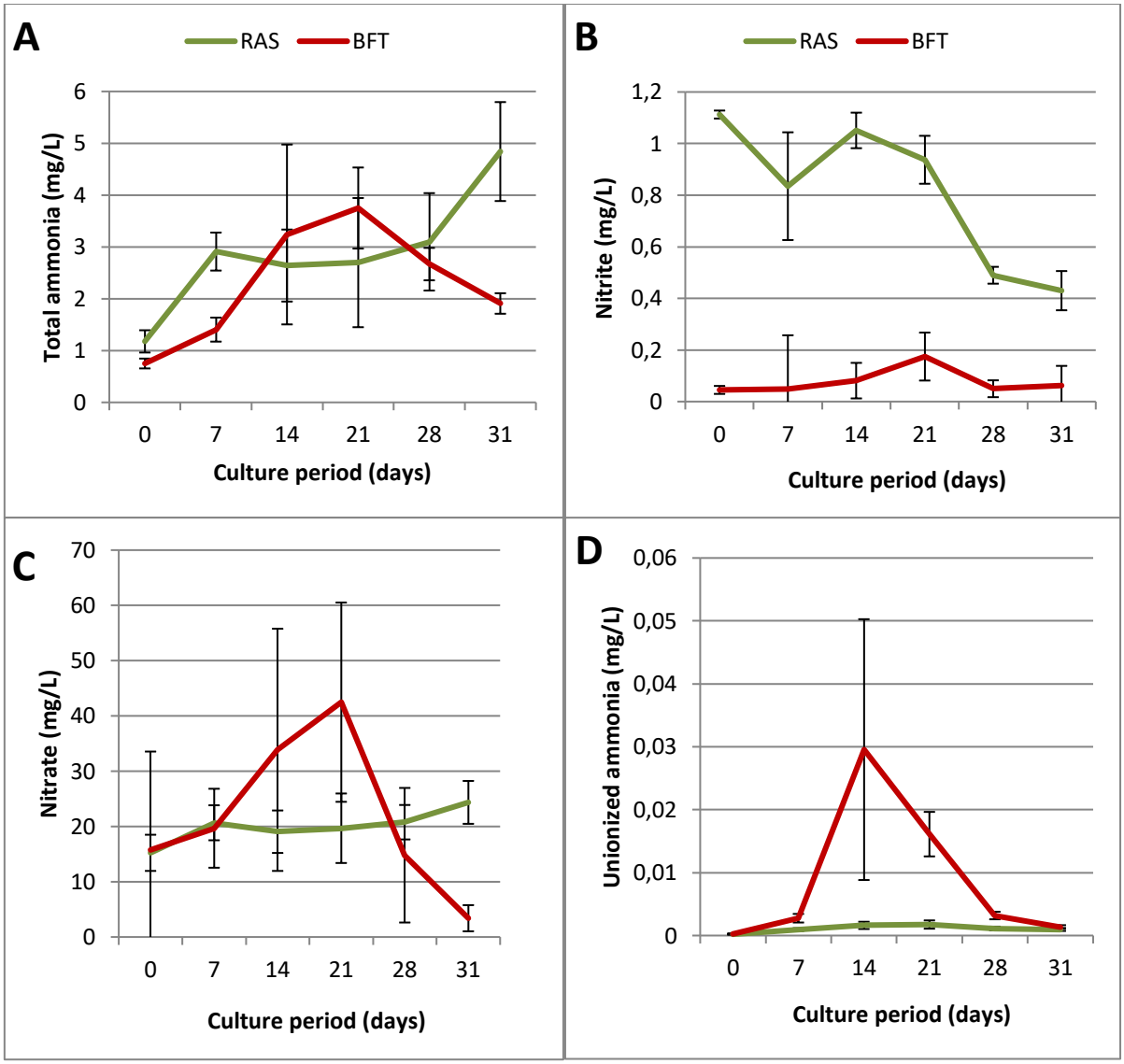


Figure 4.10 Average values of dissolved inorganic nitrogen concentration in the rearing water of BFT and RAS systems measured at seven-day intervals over a 30-day culture period and one day after the termination of the experiment; (A) Total ammonia, (B) Nitrite, (C) Nitrate and (D) Unionized ammonia. Error bars represent SD of ten replicates.

4.1.7 Orthophosphate

The orthophosphate (PO_4^{3-}) levels differed significantly between the RAS and BFT system ($p<0.05$). Average (± SD) orthophosphate levels were significantly higher in the RAS at 15.74±17.78 mg/L compared to that recorded in the BFT system at 3.28±7.81 mg/L. Orthophosphate levels in the RAS system demonstrated a positive slope over the culture period while the levels of orthophosphate in the BFT system remained relatively constant between day 0 and day 28 but increased between day 28 and 31 **(Figure 4.11)**.

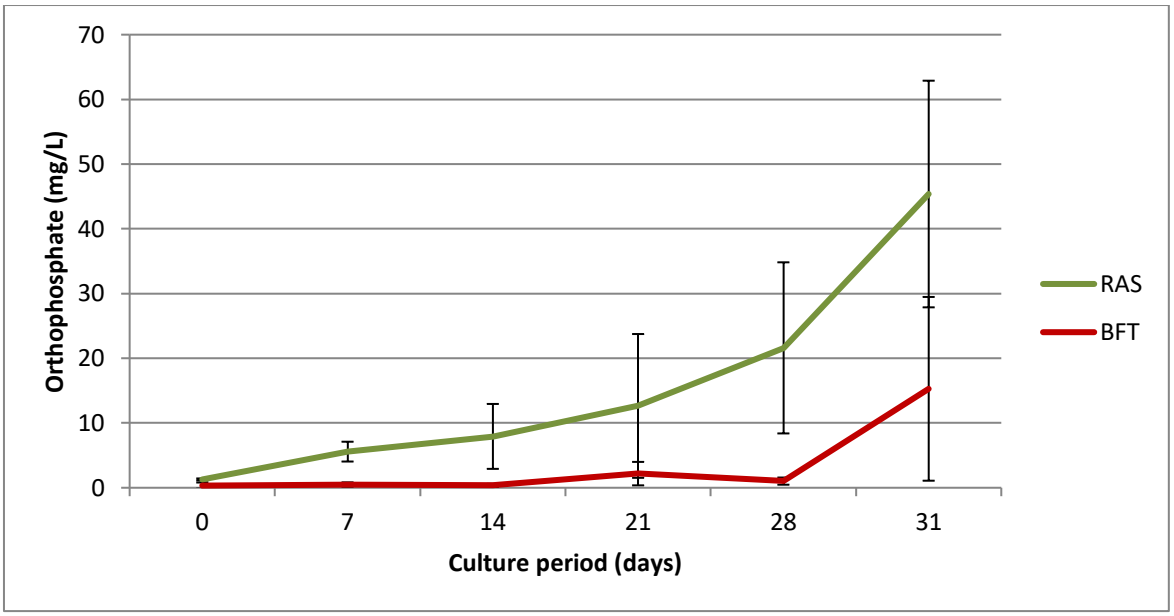


Figure 4.11 Evolution of average orthophosphate levels in BFT and RAS systems measured at seven-day intervals over a 30-day culture period and one day after the termination of the experiment. Error bars represent SD of ten replicates.

4.1.8 Turbidity

The average turbidity in the RAS and BFT system differed significantly ($p<0.05$). Overall average (± SD) turbidity was significantly higher in the BFT system at 308.77±133.75 compared to that measured in the RAS at 119.10±95.97. Average turbidity in the BFT system increased between day 0 and 14 after the introduction of fish to the system, slightly in the first 7 days and sharply in the subsequent 7 days. After day 14, turbidity in the BFT declined gradually and slightly until the end of the experimental period. Average turbidity in the RAS system increased over the experimental period, slightly between day 0 and 7 and between day 28 and 31, while a sharper increase was observed between day 7 and 28 **(Figure 4.12)**.

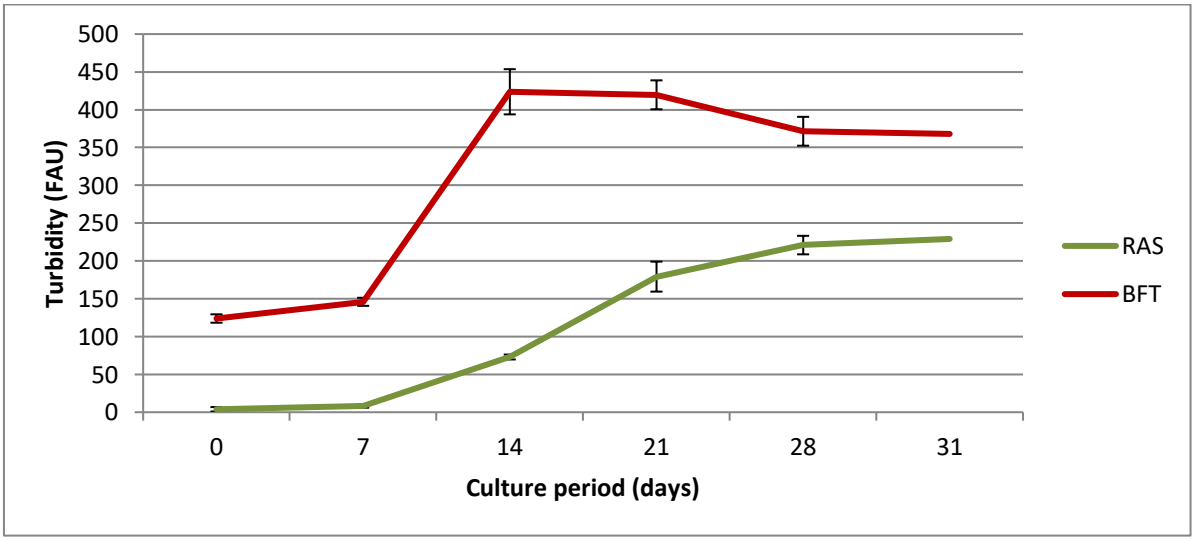


Figure 4.12 Evolution of average turbidity in BFT and RAS systems measured at seven-day intervals over a 30-day culture period and one day after the termination of the experiment. Error bars represent SD of ten replicates.

4.2 FISH PERFORMANCE

4.2.1 Survival

As shown in **Table 4.2** the average survival rates of *O. niloticus* in both system types exceeded that observed for *O. mossambicus.* No significant differences were observed between system types, species or between sampling events. The highest average survival rate was recorded for *O. niloticus* in the BFT system while the lowest was recorded for *O. mossambicus* in the BFT system, but these values did not differ significantly.

Table 4.2 The average (± SD) survival (%) of 5 replicate tanks for each tilapia species in two culture system types over a culture period of 30-days.

Species	System type	Period after stocking		
		Day 10	Day 20	Day 30
O. mossambicus	RAS	89.7±3.0	83.3±11.5	78.0±15.0
O. mossambicus	BFT	88.7±10.7	80.0±10.3	70.7±11.
O. niloticus	RAS	90.0±11.8	85.3±13.0	84.0±14.8
O. niloticus	BFT	96.0±2.8	92.7±2.8	90.7±4.9

4.2.2 Fish wet weight

The average wet weight of fish in all tanks increased as the culture period progressed **(Table 4.3)**. For *O. niloticus*, from day 20 onwards, the RAS resulted in significantly bigger animals than the BFT. Contrary to this, *O. mossambicus* initially stocked in the BFT system were significantly smaller than those stocked in the RAS, but by the end of the experiment the wet weights of animals stocked in the RAS were not significantly different from those stocked in the BFT, suggesting faster growth rates of animals stocked in the RAS. Except for *O. mossambicus* in BFT between sampling events on day 10 and day 20, time had a significant effect on both tilapia species in both production system types, resulting in significant increases in average wet weight between sampling events for each species/system type combination.

Table 4.3 The average (± SD) wet weight (g) of surviving fish (n), housed in 5 replicate tanks, initially and at ten-day interval sampling periods over a culture period of 30-days.

Species	System type	Period after stocking			
		Initial	Day 10	Day 20	Day 30
O. mossambicus	RAS	2.6±1.1^{a} (n=150)	3.6±1.5^{b} (n=148)	4.7±2.0^{e} (n=125)	6.7±2.9^{g} (n=117)
O. mossambicus	BFT	3.7±2.9^{c} (n=150)	4.9±3.4^{f} (n=133)	6.0±3.9^{f} (n=120)	7.3±4.1^{g} (n=106)
O. niloticus	RAS	4.4±0.9^{d} (n=150)	7.3±1.7^{g} (n=135)	11.3±3.1^{i} (n=128)	19.2±6.3^{k} (n=126)
O. niloticus	BFT	4.5±0.96^{d} (n=150)	6.7±1.8^{g} (n = 144)	9.5±2.6^{h} (n=140)	12.4±3.3^{j} (n=136)

Values reported with the same superscript letter in the same row or column are not significantly different.

4.2.3 Biomass yield

O. niloticus demonstrated superior total biomass yields over the experimental period in terms of both production and productivity compared to *O. mossambicus* in both culture system types **(Table 4.4)**, In comparison with biomass yields achieved in BFT, RAS performed better with higher production and productivity for both tilapia species.

Table 4.4 The total yields in terms of production (kg) and productivity ($kg.m^{-3}$) obtained from 5 replicate tanks for each tilapia species in each culture system type over a culture period of 30-days.

Species	System type	Period	Yield	
		(days)	Production (kg)	Productivity ($kg.m^{-3}$)
O. mossambicus	RAS	30	0.411	0.70
O. mossambicus	BFT	30	0.211	0.27
O. niloticus	RAS	30	1.757	2.99
O. niloticus	BFT	30	1.014	1.32

4.2.4 Feed conversion ratio

The feed conversion ratio (FCR) of both species of tilapia in each culture system type over the experimental period is reflected in **Table 4.5**. The FCR's observed in the BFT system were significantly lower than those realized in the RAS system for both species of tilapia. *O. niloticus* performed better in terms of FCR than *O. mossambicus* in both system types. The lowest overall FCR realized over the experimental period is therefore that of *O. niloticus* in the BFT system.

No significant differences in average FCR were observed between the first two ten-day intervals in average FCR for either tilapia species in the BFT system while a significant increase could be seen between that calculated over the period day 11-20 and that calculated over day 21-30 for both tilapia species in this system. Average FCR of *O. mossambicus* in the RAS increased significantly between the first and second ten-day intervals and decreased between the second and third ten-day interval. Average FCR of *O. niloticus* did not significantly differ between the first two ten-day intervals, but a significant decrease was observed between the day 11-20 interval and the day 21-30 interval **(Table 4.5).**

Table 4.5 The average (± SD) feed conversion ratio (FCR) of 5 replicate tanks for each tilapia species in each culture system type over a culture period of 30-days (n=5)

Species	System type	Period after stocking			Overall
		Day 0-10	Day 11-20	Day 21-30	Day 0-30
O. mossambicus	RAS	2.12 ± 0.18^{g}	2.47 ± 0.28^{h}	1.65 ± 0.18^{f}	2.17 ± 0.20^{gh}
O. mossambicus	BFT	1.18 ± 0.12^{e}	1.29 ± 0.16^{e}	1.61 ± 0.21^{d}	1.43 ± 0.11^{ed}
O. niloticus	RAS	1.28 ± 0.16^{de}	1.40 ± 0.03^{e}	1.11 ± 0.12^{d}	1.22 ± 0.09^{d}
O. niloticus	BFT	0.71 ± 0.07^{a}	0.74 ± 0.06^{a}	1.03 ± 0.07^{c}	0.85 ± 0.06^{b}

Values reported with the same superscript letter in the same row or column are not significantly different.

4.2.5 Specific growth rate

As shown in **Table 4.6**, the percentage SGR per day of body WW (SGR % d^{-1}) was significantly higher in the RAS system for both tilapia species for each ten-day interval as well as for the overall culture period, except for the interval day 0-10 for *O. mossambicus*. The SGR calculated for *O. niloticus* was significantly higher than that of *O. mossambicus* in both system types for each ten-day interval and the overall culture period. The highest overall SGR was observed for *O. niloticus* in the RAS system and the lowest was observed for *O. mossambicus* in the BFT system.

A significant decrease in average SGR was observed between the day 0-10 and day 21-30 intervals for *O. mossambicus* in the BFT system with no significant differences observed between the day 0-10 and day 11-20 intervals or the day 11-20 and day 21-30 intervals. A significant downward trend could be seen for *O. niloticus* in the BFT system over the three ten-day intervals. A significant decrease in average SGR was observed for *O. mossambicus* in the RAS system between the day 0-10 and day 11-20 intervals, followed by an increase between the day 11-20 and day 21-30 intervals. No significant difference was observed for *O. niloticus* in the RAS system between the first two ten-day intervals while a significant increase was subsequently observed between the day 11-20 and day 21-30 intervals **(Table 4.6)**.

Table 4.6 The average (± SD) specific growth rate (SGR %d^{-1} of body wet weight) of 5 replicate tanks for each tilapia species in each culture system type over a culture period of 30-days.

Species	System type	Period after stocking			Overall
		Day 0-10	Day 11-20	Day 21-30	Day 0-30
O. mossambicus	RAS	3.30±0.22^{c}	2.69±0.19^{d}	3.50±0.40^{c}	3.16±0.15^{c}
O. mossambicus	BFT	2.76±0.20^{c}	2.14±0.24^{ac}	1.80±0.30^{a}	2.24±0.11^{b}
O. niloticus	RAS	4.98±0.63^{gh}	4.42±0.28^{g}	5.38±0.47^{h}	4.93±0.31^{gh}
O. niloticus	BFT	4.00±0.13^{f}	3.47±0.30^{e}	2.66±0.08^{d}	3.37±0.14^{e}

Values reported with the same superscript letter in the same row or column are not significantly different.

4.2.6 Condition factor

The average condition factors (K) generally did not differ significantly between RAS and BFT systems for either species, except for *O. niloticus* which demonstrated a significantly higher average condition factor in the RAS on day 30. Average condition factors were significantly higher for *O. niloticus* in the BFT system compared to *O. mossambicus* in either system at each sampling event. For *O. niloticus* in the RAS, average condition factors were only significantly higher than those of *O. mossambicus* at stocking and on day 30. For all species and system type combinations, the average condition factors recorded on day 10 were significantly higher than at stocking. Thereafter, no significant increase was observed at subsequent sampling events, except for *O. niloticus* in RAS which increased significantly between day 20 and 30.

Table 4.7 The average (± SD) condition factor (K) of surviving fish (n), housed in 5 replicate tanks, initially and at ten-day interval sampling periods over a culture period of 30-days.

Species	System type	Period after stocking			
		Initial	Day 10	Day 20	Day 30
O. mossambicus	RAS	1.7x10^{-3}±2.3x10^{-4a} (n=150)	1.9x10^{-3}±2.3x10^{-4c} (n=148)	1.9x10^{-3}±2.4x10^{-4c} (n=125)	1.9x10^{-3}±2.8x10^{-4c} (n=117)

O. mossambicus	BFT	$1.6x10^{-3}\pm3.1x10^{-4a}$ (n=150)	$1.9x10^{-3}\pm2.8x10^{-4c}$ (n=133)	$1.8x10^{-3}\pm2.2x10^{-4c}$ (n=120)	$1.8x10^{-3}\pm2.8x10^{-4c}$ (n=106)
O. niloticus	RAS	$1.8x10^{-3}\pm1.7x10^{-4b}$ (n=150)	$1.9x10^{-3}\pm2.5x10^{-4cd}$ (n=135)	$1.9x10^{-3}\pm1.4x10^{-4cd}$ (n=128)	$2.1x10^{-3}\pm1.9x10^{-4f}$ (n=126)
O. niloticus	BFT	$1.9x10^{-3}\pm1.8x10^{-4b}$ (n=150)	$2.0x10^{-3}\pm2.3x10^{-4d}$ (n = 144)	$1.9x10^{-3}\pm2.0x10^{-4d}$ (n=140)	$1.9x10^{-3}\pm2.1x10^{-4cd}$ (n=136)

Values reported with the same superscript letter in the same row or column are not significantly different.

4.2.7 Linear regression of the growth curve (g)

As described in section 3.4, a growth prediction model was applied to predict daily biomass gain to maintain appropriate feeding levels between sampling events conducted at ten-day intervals. **Table 4.8** reflects the realized 'g' values, representing the linear regression of the growth curve found in each of the three ten-day periods. The 'g' values realized for *O. niloticus* were significantly higher than those observed for *O. mossambicus* in both system types, with significantly higher values observed in the RAS for *O. niloticus* relative to the BFT system for the overall average SGR's as well as over each of the three ten-day intervals. No significant differences were observed between the RAS and BFT system for *O. mossambicus* except over the day 21-30 interval, where the average SGR calculated in the RAS was significantly higher than that calculated in the BFT system.

No significant differences in average 'g' values were observed for either tilapia species in the RAS between the day 0-10 and day 11-20 intervals. Both species subsequently demonstrated a significant increase in 'g' values between the day 11-20 and day 21-30 intervals. No significant differences were observed for either tilapia species in the BFT system over the experimental period.

Table 4.8 The average (± SD) realized 'g' values of 5 replicate tanks for each tilapia species in each culture system type over a culture period of 30-days.

Species	**System type**	**Period after stocking**			**Overall**
		Day 0-10	**Day 11-20**	**Day 21-30**	**Day 0-30**
O. mossambicus	RAS	0.031 ± 0.004^{a}	0.035 ± 0.005^{a}	0.066 ± 0.008^{b}	0.132 ± 0.009^{d}
O. mossambicus	BFT	0.041 ± 0.010^{a}	0.038 ± 0.007^{a}	0.040 ± 0.006^{a}	0.138 ± 0.038^{d}
O. niloticus	RAS	0.096 ± 0.015^{d}	0.135 ± 0.016^{d}	0.270 ± 0.038^{e}	0.501 ± 0.060^{f}
O. niloticus	BFT	0.074 ± 0.004^{c}	0.093 ± 0.012^{c}	0.097 ± 0.009^{c}	0.264 ± 0.024^{e}

'g' being the linear regression of the growth curve: $W_f = \sqrt[3]{W_i} + (g \times t)^3$ Values reported with the same superscript letter in the same row or column are not significantly different.

4.3 ECONOMIC ANALYSIS4.3.1 Fixed costsThe fixed costs excluded the cost of land. For both the BFT system and RAS, the highest fixed cost incurred was the construction of the greenhouse. Due to the nature of the greenhouse structures, the cost of construction of the greenhouse housing the RAS system was considerably higher than that of the greenhouse housing the RAS system. To better illustrate differences in the costs of components of the two systems, **Table 4.9** and **Table 4.10** give two totals (for the BFT and RAS system, respectively) - one excluding and one including the costs of the greenhouses. The costs of the greenhouses cannot be omitted, however, due to the obvious effect of the different structures on the water temperature shown in section 4.1.1, and therefore growth and productivity of the fish.

In the case of the RAS, a more substantial investment in a sturdier greenhouse delivers higher water temperatures and therefore higher SGRs **(Table 4.6)**, thereby contributing to the productivity of the system. The costs of the aeration pumps and fish tanks represented substantial fixed costs in both systems, while plumbing represented a substantial cost in the RAS but not in the BFT system. The cost of components of the RAS system were approximately double that of the BFT system due to the additional costs of a supporting structure and various water treatment components as well as the comparatively higher cost of plumbing and the aeration pump.

Table 4.9 The relevant fixed costs of the BFT system.

Item	Description	Cost (€)
Fish holding tanks	12 x 250 L JoJo tanks	661.24
Plumbing	Full setup	137.93
Concrete blocks	12 x	82.76
0.55 KW Aeration pump	CFW, model ZxB 310	668.97
Total excluding greenhouse		**1 476.43**
Greenhouse	Full setup	3 450.29
Total including greenhouse		**4 926.72**

Based on an exchange rate of 1 € = 14.50 ZAR

Table 4.10 The relevant fixed costs of the RAS system.

Item	Description	Cost (€)
Fish holding tanks	10 x 120 L aquaria	586.21
Sump tanks	2 x 300 L aquaria	220.69
1.1 KW Aeration pump	FPZ, model KO4 MS 1P	775.2
0.2 KW Circulation pump	AquaDrive 390, model 6452L TL-A12X	172.41
Biofilter	5 L UltraZap + bio balls	65.51
Plumbing	Full setup	848.27
Shade net	8 x 8 meters	22.07
Supporting frame	Welded steel	241.38
Total excluding greenhouse		**2 931.74**
Greenhouse	Full setup	8 580.56
Total including greenhouse		**11 512.30**

Based on an exchange rate of 1 € = 14.50 ZAR

4.3.2 Operational costs

The biggest contributor to total operational costs for both systems was labour **(Table 4.11 and Table 4.12)**. Labour accounted for approximately 87.3% for the BFT system and 71.7% for the RAS of the total operational costs incurred over the culture period, but did not differ between system types. The cost of electricity in the RAS was substantially higher than that of the BFT system, accounting for approximately 27.7% of total operational costs in the RAS and only 12.0% in the BFT system. However, the BFT system had the additional, though small, cost of maize meal. The low cost observed for feed can be attributed to the small size of the fish and relatively low densities applied in this experiment. Feed costs are expected to rise as the biomass of the stocked fish increase with the progression of the production cycle.

Table 4.11 The relevant operational costs of the BFT system over the 30-day culture period.

Item	Description	Cost (€)
Tilapia feed	€1.10 per kg (1.47 kg delivered to ten tanks)	1.62
Labour	Salary per person €462 per month	462
Maize meal	€0.69 per kg (2.11 kg delivered to ten tanks)	1.46
Electricity	€0.16 per kWh (Aeration = 720 hours at 0.55 kw = 396 kWh)	63.63
Total		**528.71**

Based on an exchange rate of 1 € = 14.50 ZAR

Table 4.12 The relevant operational costs of the RAS system over the 30-day culture period.

Item	Description	Cost (€)
Tilapia feed	€1.10 per kg (3.32 kg delivered to ten tanks)	3.65
Labour	Salary per person €462 per month	462
Electricity	€0.16 per kWh (Circulation = 720 hours at 0.2 kW = 396 kWh) (Aeration = 720 hours at 1.1 kW = 721.1 kWh)	178.74
Total		**644.39**

Based on an exchange rate of 1 € = 14.50 ZAR

4.3.3 Cost efficiency analysis

The cost efficiency of each tilapia species in each production system type reflected in **Table 4.13**, allows for a comparison of the realized operational inputs and outputs of each system type over the experimental period. The scale and density of production as well as the early life stage of the experimental animals does not allow for economic viability in a commercial sense, rather the aim of the cost efficiency analysis was to determine the performance of both the tilapia species and culture systems relative to each other, in terms of the productive yield and all associated operational costs thereof, achieved in this particular set-up. This cost efficiency, therefore, disregards the speculative and somewhat circumstantial higher potential capital costs associated with RAS (Avnimelech, 2015). **Table 4.13** reveals that the best cost efficiency was achieved by *O. niloticus* in the RAS and the worst was achieved by *O. mossambicus* in the BFT system. Culture of *O. niloticus* demonstrated higher cost-effectiveness than *O. mossambicus* in both culture system types and both species exhibited comparatively better cost efficiencies in the RAS than in the BFT system.

Table 4.13 The inputs (costs), outputs (yield) and overall cost efficiency of two tilapia species in two culture system types over a culture period of 30-days.

Item	**BFT O. nil**	**BFT O. mos**	**RAS O. nil**	**RAS O. mos**
Productivity ($kg.m^{-3}.month^{-1}$)	1.32	0.27	2.99	0.70
Production unit (m^{-3})	0.75 m^3	0.75 m^3	0.6 m^3	0.6 m^3
Biomass yield ($kg.month^{-1}$)	0.99	0.20	1.79	0.42
Total operational cost (€.$month^{-1}$)	528.71	528.71	644.39	644.39
Cost efficiency (kg/€)	**0.002**	**0.0004**	**0.003**	**0.0007**

O. nil = *O. niloticus*, O. mos = *O. mossambicus.*

CHAPTER 5

DISCUSSION

Simultaneous growth trials involving two tilapia species, *O. niloticus* and *O. mossambicus*, were performed in two production system types, RAS and BFT. The study wanted to evaluate whether, and to what extent, the system type exerts an influence on water quality parameters and what the implications of these differences are on fish growth and welfare. In addition, the study aimed to compare the production performance between species in the same system type, between a single species housed in different system types as well as the ultimate cost-effectiveness of production in the relevant systems. Cost-effectiveness was calculated as a function of all incurred operational costs (€) and the overall productivity (kg) of each species over the 30-day culture period. RAS and BFT systems were compared since they have been identified as two alternatives for intensive tilapia aquaculture in temperate regions, each with cited advantages and disadvantages.

5.1 WATER QUALITY IMPLICATIONS FOR TILAPIA PERFORMANCE

5.1.1 Temperature

Temperature exerts a pronounced effect on metabolism and growth of tilapia (El-Sayed, 2006a). De Schryver *et al.* (2008) reported that the optimal water temperature for a stable BFT system is in the range 20 - 25˚C. This range was proposed due to observed deflocculation of flocs at low temperatures and bulking of sludge at high temperatures because of increased extracellular polysaccharide production (De Schryver *et al.*, 2008). This requirement needs to be balanced with the optimal temperature range of tilapia, reported as 25 - 30˚C (Cnaani, Gall and Hulata, 2000; El-Sayed, 2006a; Crab *et al.*, 2009) while normal growth of tilapia can be supported in the range 20 - 35˚C (El-Sayed, 2006a). It is not possible to optimize biofloc stability and tilapia production simultaneously. The best alternative is to manage a BFT system in a way so that daily fluctuations vary between the two optima. Recorded water temperatures in the BFT system over the course of the trial ranged between 15.8˚C - 28.2˚C with an average of 21.8±2.8˚C, dropping below the temperature range which supports normal growth on several occasions, but remaining above the lower lethal limit of 8˚C (Chervinski, 1982). The average temperature recorded in the BFT system was therefore below that which is optimal for tilapia culture, but mostly within the optimal range necessary for maintenance of a stable BFT system. The temperature profile of the RAS system was significantly higher and more suitable to tilapia culture, considering that the recorded daily average temperature range was between 19.7 - 32.4˚C and an overall average temperature of 26.4±3.3˚C was observed over the experimental period.

The effects of the observed deviations from the required temperature range on tilapia growth performance are expected to be substantial, as demonstrated by the results of a study by El-Sayed and Kawanna (2008) which evaluated the effects of water temperatures (24, 28 and 32˚C) on the growth of *O. niloticus* fry in an indoor RAS and found that growth almost doubled at 28˚C relative to that recorded at 24˚C and 32˚C, which were not significantly different. From these results, major deviations in growth performance can be expected even within the temperature range supporting normal growth of tilapia. In addition, Balarin and Haller (1982) reported that feeding is substantially reduced at temperatures below 20˚C and halts at approximately 16˚C.

The impact of cold stress on growth for tilapia is also dependent on the species and strain, with strains inhabiting water bodies geographically further from the equator generally demonstrating higher cold tolerance, presumably due to the higher selective pressure for this trait and conditioning of tilapia at lower temperatures in more temperate areas (Cnaani, Gall and Hulata, 2000; Sifa *et al.*, 2002). Subtle differences in temperature tolerance profiles have been described between *O. mossambicus* and *O. niloticus*. Disregarding slight variations between strains, *O. mossambicus* has been shown to have a slightly higher cold tolerance than O. niloticus and has a lower lethal limit of 8 - 9.5˚C (Chervinski, 1982; Shafland and Pestrak, 1982) and an optimum of 28 - 30˚C (Job, 1969) while *O. niloticus* has been shown to have a lower lethal limit of 8.4 - 11˚C (Sifa *et al.*, 2002) and an optimum of 28 - 32˚C (El Gamal, 1988). Recorded water temperatures were on average lower than these reported optima for both species in both system types, but never reached the lower lethal limits. Tilapia exhibit relatively higher tolerance to high water temperatures than low water temperatures. The upper lethal temperature varies between species, but is generally between 40 - 42˚C (Kirk, 1972; Balarin and Haller, 1982; El-Sayed, 2006a; Azaza, Dhraïef and Kraïem, 2008).

The response of *O. niloticus* to water temperature is dependent on the size of the fish, with an increased susceptibility to cold temperatures observed in smaller fish (Hofer and Watts, 2002; Atwood *et al.*, 2003), but a study by Cnaani, Gall and Hulata (2000) demonstrated no correlation between fish size and cold tolerance in *O. mossambicus* (in the range of 2.3 – 10.5 cm SL). Initial size (SL) of *O. mossambicus* at the time of stocking was significantly smaller than *O. niloticus,* but the SLs of the stocked Mozambique tilapia were within the range which was shown not to be correlated with cold tolerance (>2.3 cm). Thus, the effect of temperature on the growth performance of the smaller *O. mossambicus* is expected to be less substantial than this effect on *O. niloticus*.

Although the experimental period coincided with mid-autumn and the end of the seasonal growing period for tilapia in temperate areas with expected declining temperatures, Table 4.1 demonstrates that average outside air and water temperature demonstrated no general upward or downward trend over the experimental period. However, some dramatic day-to-day variation, and particularly significant differences between morning and afternoon readings in both air and water temperature could be observed in **Figure 4.2**. These dramatic fluctuations over the short term are unfavourable for fish growth. Both systems possessed a large surface area to volume ratio, facilitating higher rates of heat loss than what would be observed in large scale systems where culture tanks occupy higher volumes and therefore demonstrate less heat loss per unit volume (Day, 2015). It can also be observed in **Figure 4.2** that daily fluctuations in morning and afternoon readings for the two systems followed the same pattern, indicating that the water temperature of both systems was affected by ambient air temperatures, which was confirmed by correlation analysis between the daily ambient outside air temperature and water temperatures recorded in the RAS and BFT system. The correlation coefficient was higher for the BFT system than the RAS, indicating a closer association between the water temperature in the BFT and outside air.

Approaches to reducing the effects of over-wintering, such as the application of electrical heating implements or use of geothermal water as influent has constraints such as high energy costs and the requirement of access to a high volume, warm water source (Kirk, 1972; Cruz and Ridha, 1995; Gelegenis, Dalabakis and Ilias, 2006). The use of insulated greenhouses, as was applied in this study, has been identified as a practical approach to overcome these constraints to cultivating tilapia in colder seasons. Temperature is therefore an important consideration when supporting infrastructure for rearing systems are designed, particularly in temperate regions which are characterized by seasonal fluctuations in ambient and water

temperatures. In many temperate regions, these fluctuations limit the grow-out period to approximately 6 – 8 months (Hofer and Watts, 2002). In these regions, the production system and/or housing design should facilitate heat retention or generation in colder months to allow longer productive cycles.

5.1.2 Dissolved Oxygen

Tilapia are renowned for tolerance of low DO levels, up to 0.1-0.5 mg/L and even as low as 0 mg/L if access to surface air is allowed (Abdel Magid and Babiker, 1975; Tsadik and Kutty, 1987), but tilapia rearing tanks should be managed to maintain DO levels above 1 mg/L to prevent growth, metabolism and disease resistance depression (Popma and Masser, 1999). In the present study, average DO levels were significantly higher in the RAS. The lowest level of dissolved oxygen recorded was 4.4 mg/L in the BFT tanks and 6.3 mg/L in the RAS tanks. Metabolic rate limiting DO levels were therefore not experienced over the duration of the trial in either system type. Tilapia also exhibit high tolerance to oxygen supersaturation, up to approximately 40 mg/L (Morgan, 1972). Observed DO levels in this study did not increase above 10.5 mg/L in the BFT system or 10.4 mg/L in the RAS and thus remained within the range which supports normal growth of tilapia for the duration of the trial period.

Fluctuations in DO in an aquatic environment are largely a result of the relative contributions of photosynthesis and respiration, water temperature and mixing/aerating intensity in an aquaculture context. Water temperature affects both the solubility of oxygen and the metabolic rate of microbial and culture species, in turn affecting the tissue oxygen demand. Job (1969) found that DO levels determine metabolic rate of *O. mossambicus* only at saturation levels below 2.5 mg/L in a temperature range of 15 - 30˚C. Low DO levels affect fish feeding and assimilation efficiency (Tsadik and Kutty, 1987). However, Teichert-Coddington and Green (1993) suggested that both the DO level and the length of exposure to hypoxic conditions determine the effect low DO levels have on the metabolic performance of fish.

The inverse relationship which was shown to exists between water temperature and DO levels in this study is consistent with what is described in literature (El-Sayed, 2006a; Avnimelech, 2015). In accordance with this finding, the average DO concentration recorded at the 16:00 reading was significantly lower than that of the 8:00 reading for both system types, while the average water temperature recorded at the 16:00 reading was significantly higher than that of the 8:00 reading. The decline in DO levels during daylight hours and relatively high DO levels at the 8:00 reading, shortly after sunrise, suggests that the combined effects of DO reducing activities, such as respiration and increasing water temperature, are more substantial than that of DO enhancing activities, such as photosynthesis and mixing activities during this period.

In contrast to the negative impact that excessively low or high DO content has on net production of cultured fish in either production system type, relatively low DO in a BFT system can be advantageous in that it causes dominance of filamentous bacteria in microbial flocs which, in turn, results in a higher floc volume index (FVI) and poorer settling properties (De Schryver *et al.*, 2008), thereby decreasing the proportion of flocs that sediment before aquaculture organisms can filter them from suspension.

5.1.3 pH

Fluctuations in pH are known stressors with the potential to manifest as aberrant physiological functioning (De Schryver *et al.*, 2008). In the absence of other stressors, tilapia have been shown to tolerate pH down to 4.0 and up to 11 without an adverse physiological reaction (Balarin and Haller, 1982; Wangead, Geater and Tansakul, 1988; van Ginneken *et al.*, 1997), but both pH and rate of acidification determine the severity of fish growth and health consequences. Nile tilapia die in a pH range of 2-3 if exposure continues for 1-3 days, with adult fish showing higher resistance, and therefore survival at low pH (Wangead, Geater and Tansakul, 1988). In the case of rapid pH decline to 4, skin damage and necrosis of the integumental epithelium have been documented as consequences (Wendelaar Bonga, Flik and Balm, 1987). Gradually declining pH in the aquatic environment of *O. mossambicus* has been shown to decrease metabolic rate and oxygen consumption (Van Dijk, Van Den Thillart and Wendelaar Bonga, 1993). Accordingly, the survival rates of O. niloticus fingerlings at pH 4, 5 and 7 were 57.8, 82.2 and 84.5%, respectively, thus decreased as pH decreased in this range (Wangead, Geater and Tansakul, 1988). In cases where fish are slowly acclimated to low pH levels, long term exposure to pH levels as low as 4 can be tolerated with no significant effect on survival rate (van Ginneken *et al.*, 1997) and maintenance of ionic balance. With regards to ionic balance, it has been observed that *O. mossambicus* has a greater ability to maintain plasma Na^+ in acidic water (pH 3.5) when compared to *O. niloticus* (Yada and Ito, 1997). The observed average pH levels observed in this study were 6.72±0.37 and 6.00±1.10 in the BFT and RAS system, respectively and were both within acceptable range for intensive tilapia aquaculture.

pH also exerts an indirect effect on fish welfare because of the interaction between pH and ammonia toxicity. Ammonia toxicity is determined by pH and temperature, and is enhanced when a higher proportion of un-ionized ammonia (UIA, or NH_3) is present relative to ionized ammonia (NH_4^+). UIA is substantially (at least two orders of magnitude) more toxic to fish than ionized ammonia, even at low levels and the proportion of UIA relative to ionized ammonia is increased as pH increases (Eshchar *et al.*, 2006). For this reason, relatively low levels of pH values in intensive systems presents the possibility of operating the system at high TAN levels without exceeding the UIA concentration which causes decreased growth and survival in fish.

Consistent with the findings of Samocha *et al.* (2007), average morning readings of pH in both systems were significantly higher than afternoon readings. This may be as a result of the increased metabolic rate of microbes and culture species as water temperature increases due to solar heating, resulting in increased respiration rates and CO_2 excretion, thereby lowering the pH (Eshchar, Mozes and Fediuk, 2003). With regards to the BFT system, biofloc stability is affected by changes in pH (Mikkelsen, Gotfredsen and Agerbxk, 1996), with an increase in pH causing improved stability. This finding suggests that biofloc stability decreased as time during daylight hours progressed and increased during the night.

5.1.4 Electro-conductivity and salinity

Electro-conductivity is a measure of the ability of water to conduct electrical flow, derived from the concentration of ions arising from dissolved salts as well as inorganic materials (Shirokova, Forkutsa and Sharafutdinova, 2000). Salinity represents the sum of all ions in water (Küçük *et al.*, 2013) and is therefore closely correlated to electro-conductivity – as was demonstrated over the course of this study, with a slightly higher correlation coefficient determined in the BFT system.

Tilapia are FW fish which are believed to have evolved from marine ancestors and are tolerant of a wide range of water salinity (El-Sayed, 2006a). *O. niloticus* is less salt tolerant than *O. mossambicus* and can tolerate salinities ranging from 0-36 g/L with an optimum limit of 15 g/L (Al-Amoudi, 1987). *O. mossambicus* can tolerate salinities between 0 and 120 g/L (Whitfield and Blaber, 1979) and grows well at salinities approaching or at full strength SW with an optimum limit of 17.5 g/L (Canagaratnam, 1966). Guisheng, Juan and Qiumei (2016) found that, when comparing *O. niloticus* and *O. mossambicus* at four salinities (0, 10, 20 and 30 g/L), *O. mossambicus* showed higher growth rates as salinity increased while *O. niloticus* showed lower growth rates as salinity increased in this range. The near-FW salinity recorded in both systems was therefore more suitable for comparatively high growth rates of *O. niloticus*.

The effect of salinity on tilapia growth and welfare is related to the inverse relationship which exists between salinity and oxygen solubility in water and the energy required for osmoregulation in the presence of an osmotic gradient (Boyd and Pillai, 1985). An osmotic gradient exists at salinities above or below the isosmotic salinity for tilapia of approximately 11.6 g/L. In this study, salinities in both systems remained below 0.15 g/L for the duration of the experiment, thus tilapia were reared in a hypotonic environment, requiring continuous energetically expensive osmoregulation, usually approximately 25-50% of metabolic output (Cnaani, Velan and Hulata, 2011). The significantly higher average salinity observed in the BFT system at 0.12±0.02 g/L relative to that in the RAS system at 0.07±0.01 may result in a marginally lower energy expenditure on osmoregulation activities for culture species housed in the BFT system, and therefore a higher growth capacity, but the salinity difference, though significant, is small and not expected to substantially alter energetic cost or manifest as a noticeable SGR improvement, especially considering the substantial temperature difference between the systems which is expected to offset any small growth improvements as a result of the salinity difference.

5.1.5 Floc volume

FV represents the volume of settleable solids in the water column and can serve as an indication of both floc physical characteristics and abundance in a BFT-based system and was therefore relevant only to the BFT treatment tanks. Avnimelech (2015) recommended that FV remain within the range 2 – 200 mL/L for biofloc systems concerned with fish aquaculture while Hargreaves (2013) recommended a narrower range of 25 – 50 mL/L for good functionality in BFT systems for tilapia. FV in this study was within both recommended ranges and remained at a level which could satisfy at least a proportion of the feed requirements of the resident tilapia, while maintaining ammonia levels at a nontoxic level. At the same time, FV did not exceed levels which would result in DO depletion below tolerable levels or require excessive aeration and mixing energy inputs.

The initial gradual incline in FV over the first ten days seems to indicate that floc generation rate is higher than consumption rate (in combination with losses due to settling), over this period. The subsequent decline may be a result of the increasing size and therefore metabolic rate and biofloc consumption of the fish. The surface over which biofloc is filtered for consumption also increases, possibly increasing their harvesting capacity, thereby driving up the rate of consumption.

5.1.6 Dissolved inorganic nitrogen

In intensive aquaculture, where high stocking densities and little water exchange is applied, ammonia build-up from feed metabolism is generally the second limiting factor to increase production after DO, provided that water temperature is in a tolerable range (Ebeling, Timmons and Bisogni, 2006). Ammonia concentration in aquaculture systems is affected primarily by the rate of ammonia excretion by fish in combination with sediment diffusion (Hargreaves, 1997). El-Shafai *et al.* (2004) reported a no-observable effect concentration (concentration where toxicant exerts no effect on the growth or survival of the test organism) of 0.068 mg/L UIA-N for *O. niloticus* and suggested that 0.1 mg/L UIA-N should be considered the safe level threshold for juvenile Nile tilapia. Feed intake was also not reported to decrease at UIA-N levels below 0.434 mg/L. Despite the peak observed in the BFT system, UIA concentrations in both systems were not suspected to be growth or feed intake limiting.

The presence of the products of nitrification, nitrite and nitrate, in both systems is evidence that nitrification is occurring to some extent in both the RAS and BFT systems. Most nitrifying activities is expected to take place outside the culture unit in the biofilter component of the RAS system whereas nitrification in the BFT system can only take place in the rearing tanks, thus the immediate environment of the cultured tilapia.

As expected, decreasing nitrite concentrations between sampling events in the RAS corresponds to increasing nitrate concentration, evidence of nitrite oxidation to nitrate by nitrifying bacteria. The sharp increase in TA levels between sampling events on day 28 and 31 suggests that the ammonia conversion rate in the biofilter was lower than the rate of ammonia generation by the increasing metabolic outputs in the rearing tanks as fish density increased. In addition, the lack of substantial nitrate accumulation and relatively high nitrite levels coupled with increasing TA levels over the culture period indicates that the biological filter was not functioning very effectively in the experimental RAS. This may be due to inhibition of nitrifying bacteria of the biofilter at a pH below 6.8 (Masser, Rakocy and Losordo, 1999).

The observation that overall average TA in the BFT system was significantly lower than that of the RAS system, despite lower nitrification product concentrations (nitrite and nitrate) suggests that ammonia uptake by an alternative mechanism to nitrification is occurring in this system, most likely nitrogen assimilation by heterotrophic bacteria (Crab *et al.*, 2007). For the first twenty days of the trial, nitrate accumulation was evident in the BFT application system, followed by a sharp decline until the end of the experiment. This decline corresponds to a decline in TA levels in the BFT system, and may be a result of nitrate uptake by heterotrophs and phytoplankton when available TA concentration is lowered (Hargreaves, 1998; Kirchman and Wheeler, 1998; Luque-Almagro, Gates and Moreno-Vivián, 2011). Denitrification is also expected to contribute somewhat to diminishing nitrate levels between day 21 and 31 (Hu *et al.,* 2014) by reducing accumulated nitrate to ultimately produce dinitrogen (N_2) gas which is lost from the water (Ekasari, 2014). Contrary to the findings of Azim and Little (2008) and Luo *et al.* (2014), nitrate concentration did not accumulate over the course of the trial, possibly because of weekly sludge removal.

5.1.7 Orthophosphate

A study by Barak *et al.* (2003) has shown that a large fraction of phosphorus introduced to FW aquaculture systems by feed delivery is not utilized and that the majority thereof (80-90%) is egested with the faeces, urine or over the gills and released into the culture environment, contributing to orthophosphate accumulation. This excreted phosphorus is generally in soluble or particulate form, with orthophosphate and organic phosphor making up the soluble fraction which directly influences water quality (Lall, 2002). A large fraction (30-64%) of total phosphorus waste is in particulate form of which approximately 80% accumulates in the sediment in semi-intensive culture systems (Funge-Smith and Briggs, 1998; Lall, 2002; Ekasari, 2014) and is therefore excluded from phosphorus readings in the present study. Orthophosphate levels in the RAS system demonstrated a positive slope between sampling events over the culture period, possibly due to both accumulation, and delivery of increasing feed quantities as tank biomass increased. Although phosphate is a notable pollutant, toxicity to fish is minimal even at high levels (Iwama, 1991; Tal *et al.*, 2009).

5.1.8 Turbidity

Turbidity measurements serve as a simple way to index suspended solids concentration (Hargreaves, 2013) and, like FV, gives an indication of whether microbial biomass accumulation is within a range which does not compromise the functionality of a BFT system as a biofilter while oxygen demand is kept below levels which might precipitate system failure associated with DO depletion. An excessive microbial biomass accumulation which can be detected by high turbidity readings, heightens the risk of gill blockage by suspended solids (Ebeling, Timmons and Bisogni, 2006; Hargreaves, 2006; Ray *et al.*, 2010; Ray, Dillon and Lotz, 2011). Excessive turbidity also exerts a shading effect which influences primary productivity, decreasing the light incidence in the water column, thereby favouring heterotrophic growth and limiting phototrophic growth.

The lag followed by a sharp increase in turbidity after stocking observed in the BFT system may be caused by the low initial stocking density in combination with fertilization in the form of uneaten feed and nitrogenous metabolic waste excretion by the tilapia. Similar to the findings of Liu *et al.* (2014), weekly turbidity readings in the BFT system generally followed the FV profile. The decrease in turbidity observed between readings on day 21 and 28 may be a result of the FW top-up applied on day 22 in combination with the increased harvesting capacity and intake of tilapia as the experimental period progresses.

5.2 EFFECT OF SYSTEM TYPE ON WATER QUALITY

The significant difference in water temperature between production system types in this experiment was probably due to the structural differences between the greenhouses housing the systems, and not due to inherent characteristics or processes which are generally occurring in RAS or BFT systems. The significantly higher temperatures recorded in RAS tanks indicated that the greenhouse housing the RAS provided superior heat retention compared to the greenhouse housing the BFT system, resulting in a significant water temperature increase in the RAS over outside air temperature. The observation that there was no significant difference between average outside air temperature and the average water temperature in the BFT rearing tanks indicates that the structure housing the BFT system contributed negligibly to heat retention, or that air ventilation via the openings counteracted heat retention.

The significantly lower average DO levels in the BFT system may be attributed to BFT-related characteristics, such as enhanced mixing intensity generally associated with BFT systems coupled with a higher BOD due to additional microbial respiration. De Schryver *et al.* (2008) stated that altering the mixing intensity in an aquaculture system, either by altering the electrical power input or the device, has a direct effect on the DO levels. In this study, the production systems were designed so that the mixing intensity in the BFT system was more intense than in the RAS. Temperature was also significantly lower in the BFT system. Both of these observations are expected to contribute towards a higher DO concentration in the BFT system, but the realized average DO level recorded in the BFT system was significantly lower than that of the RAS, indicating that the biological oxygen demand (BOD) of the additional microbial load constituting the biofloc counteracted these factors and decreased DO concentration to levels below those observed in the RAS.

The significantly lower and less stable pH observed in the RAS system can be explained by the RAS-associated higher rate of nitrification than what is generally taking place in BFT systems. pH levels in the RAS demonstrated a higher SD, and therefore lower stability between culture units as well as bi-daily sampling events. These fluctuations are likely to indirectly be caused by nitrification and photosynthesis processes, which in turn alter the buffering capacity and CO_2 content of water (Ebeling, Timmons and Bisogni, 2006; Ekasari and Maryam, 2012). With regards to alkalinity consumption, Ebeling, Timmons and Bisogni (2006) suggested that nitrogen uptake by heterotrophic bacteria in a BFT system consumes approximately half of that consumed by the process of nitrification, resulting in an relatively higher buffering capacity of the BFT system. The BFT system can therefore buffer the high CO_2 introduction from microbial and fish respiration, thus preventing acidification. The acidification observed in the BFT system between day 20 and 24 may be a result of nitrification, considering that products of nitrification, nitrite and nitrate, levels in the BFT system peak on day 21, suggesting high rates of nitrification.

The significantly higher salinity observed in the BFT system was most likely a result of higher salinity of the water used to fill the tanks, initially. The differences in electro-conductivity and salinity are therefore not caused by system-related properties, but rather a circumstantial discrepancy. The drop in average salinity observed on day 22 was a result of topping up the biofloc tanks with FW from a reservoir, the same which was used to initially fill the RAS system tanks.

The significantly higher TA and UIA levels observed in the RAS are caused by system-specific properties such as the higher daily dietary protein content delivered and a higher contribution of autotrophic nitrifiers to ammonia uptake relative to heterotrophic assimilation. The rate of

ammonia excretion by fish is under the influence of the dietary protein levels, and therefore the amount of nitrogen introduced with the diet (Brunty *et al.*, 1997; Chakraborty and Chakraborty, 1998). Lower TA levels in BFT rearing tanks can therefore, at least partially, be attributed to lower protein content of biofloc, constituting a significant proportion of dietary intake of tilapia stocked in the BFT system, relative to the pelleted feed which constituted the entirety of dietary intake of tilapia stocked in the RAS system.

The significantly higher UIA and lower nitrite content observed in the BFT system can be explained by the relatively lower rates of nitrification and subsequent higher alkalinity and pH levels. Ammonia toxicity was elevated at the UIA peak in the BFT system on day 14, most likely precipitated by the simultaneous high pH and reasonably high temperature recorded in this system. The subsequent decline in UIA levels on day 21 and further decline on day 28 correspond to declining pH while temperature remains relatively stable, indicating that the UIA fluctuations are primarily influenced by pH fluctuations. Significantly lower and more stable UIA levels in the RAS may be ascribed to the significantly lower pH observed in this system. Nitrite content in the BFT system remains relatively stable in the BFT system while this parameter in the RAS system demonstrated a significantly higher concentration and more volatility, most likely due to a higher nitrification rate in the RAS system. This is expected since nitrification is the primary mechanism for ammonia removal in this system type in the absence of heterotrophic bacteria proliferation stimulation by external carbon addition.

Studies by Kirchman (1994) and Schneider, Sereti, *et al.* (2006) have reported that heterotrophic bacteria have the potential to convert phosphorus. The significantly lower orthophosphate levels recorded in the BFT system in this study is similar to the findings of a study by Luo *et al.* (2014) which reported orthophosphate levels in a BFT system housing tilapia ten factors lower than that in RAS. The proposed reason for the disparity was the assimilation of accumulating phosphorus by biofloc microorganisms in BFT-based systems (Luo *et al.*, 2014) while the experimental RAS possesses no targeted mechanism for the removal of phosphorus. These findings suggest that the application of BFT improves phosphorus recycling and utilization efficiency and has the potential to decrease water quality deterioration in intensive aquaculture systems as well as eutrophication impacts imposed on effluent receiving water bodies. Accordingly, Ekasari (2014b) reported that a higher level of phosphorus recovery in tilapia is achieved in the presence of biofloc. This observation may be explained by the suggestion made by Luo *et al.* (2014) that phosphorus assimilated by biofloc and subsequently consumed by tilapia is more readily assimilated by tilapia than phosphorus in pelleted feed, due to higher bioavailability. The low digestibility of phosphorus introduced with pelleted feed is attributed to the fact that the most common phosphorus-containing feed ingredients, fishmeal and plant-based ingredients, introduces dietary phosphorus in largely indigestible forms, such as bone-phosphorus and phytate-phosphorus (Lall, 2002). Biofloc, therefore, contributes toward converting indigestible phosphorus into more digestible phosphorus.

Average turbidity in the BFT was significantly higher since it was influenced by BFT-specific proliferation of heterotrophic bacteria. Turbidity was also recorded in the RAS to allow for comparison between the estimated suspended solids and microbial loads of the two systems. The observation of slight, gradual turbidity increase in the RAS as the trial progresses is expected, since no sterilization component was incorporated. In the presence of sufficient aeration and feed nutrients in the RAS system tanks, it is expected that some level of autotrophic and heterotrophic microbial community development takes place, albeit at lower growth rates than that in BFT due to the absence of an external carbon input.

5.3 PRODUCTION PERFORMANCE OF TILAPIA SPECIES

5.3.1 Survival, growth and yield

Although survival did not differ significantly between species or system type, some slight variations could be observed. Overall survival of *O. mossambicus* in both systems were lower than *O. niloticus.* This corresponds to the findings of Day (2015) in BFT. It is interesting to note that the difference in survival rates between species in the RAS system is substantially smaller than the difference between these two species in the BFT system. The effect of species type on survival was therefore larger in the BFT system than the RAS. This might partially be explained by the lower pH profile recorded in the RAS, and the observation by Yada and Ito (1997) that *O. mossambicus* has the advantage of superior maintenance of ionic balance in acidic water compared to *O. niloticus.* This advantage in low pH conditions may have somewhat compensated for the genetically inferior robustness of *O. mossambicus*, resulting in lower growth depression in the BFT than in the RAS.

The average survival rates for *O. niloticus* in the BFT system was higher than any other species/system type combination, including *O. niloticus* in the RAS, despite the significantly lower temperature recorded in the BFT system. This indicates that the significantly lower temperature did not affect survival of *O. niloticus.* This observation is not surprising, since the lower temperatures recorded in the BFT system were below the range for optimal growth, so some level of growth depression was expected, but remained above the lethal limit. Mortalities are, therefore, not expected as a direct result of temperature, but may be a secondary result of stress caused by a suboptimal temperature profile. If survival was somewhat affected by the temperature disparity, increased mortalities may have been masked by improved survival due to extraneous variables such as increased disease resistance through the action of bioflocs as bio-control agents or competition for pathogens, and lower stress due to higher pH stability over the culture period relative to the RAS. On the other hand, *O. mossambicus* demonstrated better survival in the RAS than the BFT. This may indicate that the compensatory effects of BFT benefits for survival were not as substantial for this species as for *O. niloticus* or that the lower temperature profile had a more pronounced effect on survival of *O. mossambicus*. This is, however, not likely since most *O. mossambicus* strains exhibit a slightly higher cold tolerance than *O. niloticus* (Chervinski, 1982; Sifa *et al.*, 2002). It may also be a result of lower tolerance of *O. mossambicus* of the significantly higher UIA recorded in the BFT system. The mortality rates in all cases were highest between consecutive samplings on day 0 and 10. This might be explained by the residual stress of transportation and transfer into new rearing environments and the accompanying changes in water quality of their immediate environment at stocking.

Wet weight increased between sampling events at varying rates in accordance with the SGR calculated for each tilapia species in each system type. This is expected since the SGR is calculated as a function of wet weight increase. Growth is a complex process determined by many, often interactive, metabolic processes which are, in turn, under the influence of behavioural, physiological, nutritional and environmental factors. Of these, behavioural and physiological factors are related to the culture species while nutritional and environmental factors are related to management decisions and the production system design. The latter two factors were better defined in the present study than behavioural and physiological factors. Nutritional, environmental and physiological factors affect production performance indirectly by contributing to the efficiency with which feed is utilized as well as affecting intake, whereas behavioural factors predominantly affect the rate at which food is consumed.

A major nutritional difference between the two systems was that the crude protein content of the biofloc collected from the BFT rearing tanks was considerably lower than that of the pelleted feed. This resulted in an overall lower protein content of the total diet consumed by tilapia in the BFT system. It has been demonstrated that for all sizes of *O. niloticus*, there is a progressive growth increase with increasing dietary protein from 20% to 30% (Siddiqui, Howlader and Adam, 1988; Al Hafedh, 1999). For *O. niloticus* fry (0.838 g) slightly smaller than the ones used in this study, the best growth was achieved at a dietary protein content of 40% while young *O. niloticus* (40.0 g) achieved the best growth performance at a dietary protein content of 30% (Siddiqui, Howlader and Adam, 1988). The juvenile tilapia used in this study were intermediate between these tested size categories, so the crude protein content of the pelleted feed (36.06%) was appropriate for optimal growth. The reduced protein content of the diet consumed in the BFT system was below the optimal level and some level of growth depression due to insufficient protein is expected to contribute to the significantly lower SGR observed in the BFT system.

Environmental differences between the two systems which are suspected of contributing to the observed higher average SGR in the RAS, include the relatively higher average temperature and DO in combination with the lower average UIA concentration recorded in the RAS. The observation that the average SGR over consecutive 10-day intervals in the BFT system declines gradually as the culture period progresses for both species differs from the pattern observed in the RAS, where the average SGR decreases over the first 20 days before increasing to levels above what it was initially. These fluctuations correspond to the fluctuation patterns observed for average temperature over the same time intervals. This suggests that temperature is the main environmental determinant of SGR.

One of the most influential abiotic factors affecting growth in fish is water temperature (Weatherley and Gill, 1983; Cincotta and Stauffer, 1984; Herzig and Winkler, 1986; Martinez-Palacios, Chavez-Sanchez and Ross, 1996). This justifies attributing the consistent disparity in wet weight increase between production system types mainly to the significantly higher average temperature recorded in RAS system.

When comparing the magnitude of system-related differences in fish performance, it was apparent that *O. niloticus* demonstrated a higher SGR and biomass yield depression in BFT relative to RAS than *O. mossambicus*, indicating that the BFT system was a less suitable alternative to RAS for this species than for *O. mossambicus.* This observation may, at least partially, be explained by the slightly lower cold tolerance of *O. niloticus*, rather than inherent system-specific differences. For *O. niloticus*, growth depression in the BFT is coupled with slightly higher survival in this system, indicating both the robustness of this species despite overall poorer water quality, as well as potential disease-prevention characteristics of the BFT system.

The difference in average wet weight at stocking between *O. mossambicus* and *O. niloticus*, makes a comparison of average increase in wet weight for each species/system type combination more informative than simply comparing the differences in final wet weights. In terms of average wet weight gain, *O. niloticus* outperformed *O. mossambicus* in both systems by a factor of approximately 3 in RAS and 2 in BFT. When considering only *O. mossambicus*, the average increase in wet weight over 30-days was slightly higher in the RAS system (4.1±1.0 g in RAS versus 3.5±1.7 g in BFT system). The same was observed for *O. niloticus*,

with a significantly higher final weight in the RAS system and an average increase in wet weight over the culture period of 14.8±4.1 g observed in the RAS and 7.9±2.3 g observed in the BFT.

5.3.7 Feed conversion ratio

The results of this study favour the rearing environment and feed application regime of the BFT system above the RAS for low FCR. However, a low FCR with growth compromise may indicate insufficient feeding levels. The observation of no feeding response in the BFT system and a comparatively vigorous feeding response in the RAS system indicates that tilapia in the BFT system were satiated between feeding events due to biofloc consumption. The observation that condition factors between system types for both species did not differ also supports the suggestion that feeding level in the BFT system was not insufficient, as this would have resulted in relatively poorer condition factors. The low feeding response may also be attributed to reduced intake at the lower temperatures recorded in the BFT system (Goolish and Adelman, 1984). The observed response from both species stocked in the RAS is probably a result of the higher feed intake (Brett, Shelbourn and Shoop, 1969; Love, 1980) and requirements due to higher metabolic rates and, consequently, digestion in significantly higher water temperatures (Brett and Higgs, 1970). This increase in metabolic rate is confirmed by higher observed SGRs in RAS. It is also assumed that the lack of filterable feed availability between feeding events contributes to the seemingly higher appetite of fish in RAS. Riche, Haley, *et al.* (2004) found that the appetite of *O. niloticus* returned in approximately 4 hours after satiation at 28˚C with increased periods in cooler water temperatures. The average temperature in the RAS in this study was slightly lower than 28˚C at 26.4±3.3˚C, but feed was delivered at a minimum of 8-hour intervals, so it is expected that a feed response was elicited at feeding events.

O. niloticus displayed a better feed conversion ratio in both system types relative to *O. mossambicus.* This coincides with the results of Day (2015) and is probably a result of the same genetic traits which results in a superior SGR for this species.

At this point it is also necessary to mention that the optimal temperature for a species has been shown to be progressively lowered in circumstances where food was limiting (Brett, Shelbourn and Shoop, 1969; Martinez-Palacios, Chavez-Sanchez and Ross, 1996). This may have played a role in the RAS where the feeding response indicated a higher level of feed limitation than what was present in the BFT. This may have further contributed to the higher SGRs observed in the RAS by making the slightly below optimum temperature profile closer to optimal, thereby enhancing SGR. As mentioned in section 5.2.1, the crude protein level of the combined intake of bioflocs and pelleted feed in the BFT was lower than the diet of pelleted feed only consumed in the RAS. The lower FCR observed for the diet with a lower protein content corresponds to the findings of Hafedh (1999) which demonstrated that the FCR of young (0.51-45 g) *O. niloticus* increased when dietary protein increased from 25-35% to 40-45%.

It is worth noting that, unlike what was done in the RAS system, the mortalities which took place between sampling events in the BFT system could not be visually confirmed due to high turbidity. The feeding rate as a function of tank biomass in the BFT system was therefore an overestimation after a mortality occurred, and this may have resulted in overfeeding and a potential increase in FCR in the associated tank. This phenomenon could be avoided in the RAS system by visually confirming dead fish, recording their WW, and subtracting this from the associated tank biomass to adjust feeding rates for the following days until readjustment to actual tank biomass could take place at sampling. The FCR was also calculated based on

feed delivered, not on feed intake. The observed increase in weight heterogeneity of fish at the conclusion of the trial may, therefore, be a result of differences in individual feed intake, which was not determined.

5.3.6 Linear regression of the growth curve (g)The ‘g’ value is proportional to the daily amount of feed fed. As a result, the accuracy of the growth prediction model to actual daily biomass gain in each tank will determine how closely the delivered feed quantity corresponds to the amount of feed required by the animals housed in each tank for maintenance and growth. Due to its impact on the feed delivered, the ‘g’ values are indirectly reflected in the FCR, which is a function of the actual biomass gain and the feeding level. In the case of an overestimation of growth, the resulting overfeeding will be reflected in a high FCR, and vice versa. Growth prediction based on historic growth performance does not take potential future changes in the environmental conditions which influence growth, such as temperature, into account. Feed delivery was calculated as a proportion of tank biomass, thus was not adjusted when temperature fluctuated. In this study, no significant changes in temperature was observed when considering the averages of 10-day intervals, but day-to-day variation was observed which could have resulted in, for example, overfeeding on days when water temperature was low due to decreased feed intake.

5.4 BIOFLOC CONTRIBUTION TO GROWTH

Differences in average temperature, a parameter which exerts a strong influence on growth performance, was present between the two system types. This difference weakened the system-related growth comparison conclusions which the study aimed to achieve, due to a lack of standardization of external, system-nonspecific factors between the RAS and BFT system. The contribution of biofloc to growth could therefore not reliably be evaluated, quantitatively or qualitatively. However, both tilapia species, and more so *O. niloticus*, cultured in the BFT system demonstrated reasonable growth, albeit at lower rates than that achieved under higher temperature conditions in the RAS, with no significant decrease in survival rate and a substantially lower FCR than that achieved in the RAS. BFT thus demonstrated potential to be a feasible alternative to tilapia aquaculture in RAS. This statement is supported by the results of a similar study performed by Luo *et al.* (2014) in standardized conditions, which demonstrated higher weight gain and SGR coupled with a lower FCR in a BFT system compared to a RAS over an 87-day experiment.

5.5 ECONOMIC ANALYSIS

A financial assessment of the capital and operational costs associated with two candidate systems for intensive, tilapia aquaculture in temperate regions was included in this study since a prominent advantage of utilizing the BFT system is thought to be the potential reduction in associated capital and operational costs (Avnimelech, 2015). As a point of reference, the costs associated with the design, construction and running of the systems and supporting infrastructures utilized in this study were outlined. It is worth noting that these particular systems are not representative of all, or even particularly cost-effective BFT or RAS systems.

In the RAS, water treatment components, more extensive plumbing and a circulation pump were included which increased both the fixed and energy costs of the system. The structural differences between the greenhouses housing the two systems had a big impact on the difference between the total fixed costs of the two systems, with the associated costs of the RAS system greenhouse being substantially higher. This is a very case-specific occurrence, and not an increased cost associated with RAS specifically. However, it was not disregarded in the economic analysis due to its contribution to temperature and therefore its indirect effect on tilapia growth performance and overall productivity of the system. To demonstrate fixed cost differences inherent to the RAS and BFT systems, fixed costs which exclude the cost of the greenhouse were also reported. It was shown that the fixed costs of the BFT system is approximately half that of the RAS, both when the greenhouse costs are excluded and included. A partial cost analysis performed by Luo *et al.* (2014) also revealed lower depreciation costs for water treatment units in BFT systems. Lower water treatment- and pumping-related fixed costs, therefore, seem to be a general cost benefit for BFT systems.

Both culture systems were closed, thus consuming a comparable, low volume of water. Water consumption and the related costs thereof were therefore excluded from the operational cost analysis. Except in cases of considerable water loss, the manager's discretion or the extent of water quality deterioration determines the rate of water consumption, but this consumption is generally not inherent to either system type as a rule. The additional cost of carbon source addition is standard for BFT systems, but was compensated for by the decrease in formulated feed delivered. The related energy costs of the additional circulation pump included in the RAS system, in combination with the increased formulated feed costs, resulted in higher total operational costs for the RAS system. Luo *et al.* (2014) also reported higher costs associated with feed consumption and energy for pumping in the RAS system, so these cost differences can be considered general to the system types. However, Luo *et al.* (2014) reported higher energy costs for aeration in the BFT system. The intensity of aeration varies considerably between BFT-application systems (Gao *et al.*, 2012; Ekasari, 2014; Avnimelech, 2015; Day, 2015), so this cost can be altered in accordance with the scale and stocking densities applied in individual BFT systems.

A cost efficiency (kg/€) for each tilapia species in each systems type was calculated to reflect any impacts the design, construction and ultimately underlying costs might have had on the productivity of the system. Productivity in the BFT system was considerably lower than in the RAS, likely due, at least to some extent, to the temperature difference generated by the difference in housing infrastructures of the two systems. This is a good illustration of how cost-cutting during capital expenditure can result in a decrease of overall cost-effectiveness. Despite lower total fixed and operational costs, cost efficiency in the BFT was lower than in the RAS. Luo *et al.* (2014), on the other hand, performed growth trials in both system types in the same housing structure, therefore in more standardized conditions and obtained a superior growth rate of *O. niloticus* in the BFT system. These results provide evidence that BFT has potential to achieve high productivity despite lower set-up and running costs; thus, that BFT can be a more cost-effective way of culturing tilapia than RAS in standardized conditions.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

Water quality parameters which seemed to be affected by characteristics and processes inherent to the RAS or BFT systems included DO levels, pH, TA, nitrite, UIA and orthophosphate content and turbidity. The system-related properties which are thought to have influenced these parameters include the relative rates of nitrification, heterotrophic proliferation, assimilation and respiration as well as differences in dietary protein content, BOD and mixing intensity. These characteristic properties of the two systems influenced water quality and resulted in comparatively lower DO, TA, nitrite and orthophosphate levels but higher pH, turbidity and UIA levels in the BFT system compared to the RAS.

Both RAS and BFT systems demonstrated technical feasibility for indoor, intensive juvenile tilapia aquaculture. Based on the results of this research, *O. niloticus* was shown to be a superior culture species to *O. mossambicus* for intensive tilapia aquaculture in both RAS and BFT-application based production systems. It consistently outperformed *O. mossambicus* in terms of WW gain, biomass yield, productivity, SGR and FCR. When evaluating suitability of *O. mossambicus* to production system type, it was evident from the results obtained in this study that culture in BFT resulted in a lower FCR, but that a better SGR, biomass yield and survival was achieved in the RAS. The same was true for *O. niloticus*, except that survival was slightly higher in the BFT system. This indicates that both species were better suited to the environmental conditions present in the RAS over the course of this study. Although SGR, biomass yield and productivity were depressed in the BFT system relative to the RAS, this observed depression between systems was lower, as a proportion of each parameter in the RAS, for *O. mossambicus*. This suggests that the growth performance of *O. mossambicus* was less adversely affected by the environmental conditions present in the BFT than *O. niloticus. O. mossambicus* may therefore be considered more suitable for culture in BFT-based production systems, but presumably more because of the circumstantial temperature profile difference between systems and higher cold tolerance of this species than actual system-specific characteristics. On the other hand, contrary to what was observed for O. mossambicus, survival of *O. niloticus* was marginally higher in the BFT system than in the RAS. This suggests that there may be a survival promoting benefit for *O. niloticus* in the BFT system.

The productivity and related profitability of intensive aquaculture was shown to be not only affected by system type, but also by the myriad of choices producers make regarding the nature of housing infrastructure, building materials used as well as supporting appliances such as aerators and pumps, generating substantial variability in the range of initial and working capital required. The economic analysis revealed that the BFT system used in this experiment had lower fixed and operational cost inputs, but that the overall productivity was also substantially lower than that observed in RAS, resulting in a lower overall cost-effectiveness in the BFT system. On laboratory scale and with the materials utilized to construct the systems employed in this study, commercial viability was not attained. However, promising results were obtained for potential reduced feed and energy inputs in BFT systems, below what can be achieved in general RAS.

In future trials of this nature, standardization between systems may be improved by locating the two systems in a single greenhouse and using a single water source for tank filling and top-ups. It would also be of value to introduce a sterilization component to the RAS, to ensure that no level of biofloc development takes place as the experimental period progresses. The complete absence of microbial development in the rearing environment in RAS will allow for stronger conclusions of the effect that the presence of biofloc has on tilapia growth performance and water quality.

REFERENCES

Abdel Magid, A. and Babiker, M. (1975) Oxygen consumption and respiratory behaviour of three Nile fishes, *Hydrobiologia*, 46, pp. 359–367.

Al-Amoudi, M. (1987) Acclimation of commercially cultured *Oreochromis* species to sea water—an experimental study, *Aquaculture*, 65, pp. 333–342.

Albert, A. (1973) *Selective toxicity: the physico-chemical basis of therapy*. 5th edn. London: Chapman Hall.

Alonso-Sáez, L., Gasol, J., Lefort, T., Höfer, J. and Sommaruga, R. (2006) Effect of natural sunlight on bacterial activity and differential sensitivity of natural bacterioplankton groups in northwestern Mediterranean coastal waters, *Applied Environmental Microbiology*, 72, pp. 5806–5813.

Anand, P. S. S., Kohli, M., Roy, S. D., Sundaray, J., Kumar, S., Sinha, A., Pailan, G. H. and Sukham, M. kumar (2013) Effect of dietary supplementation of periphyton on growth performance and digestive enzyme activities in *Penaeus monodon*, *Aquaculture*, 392, pp. 59–68.

AOAC (Association of Analytical Chemists) (2016) *Official methods of analysis*. 20th edn. Washington D.C, USA.: AOAC.

Atwood, H., Tomasso, J., Webb, K. and Gatlin III, D. (2003) Low-temperature tolerance of Nile tilapia, *Oreochromis niloticus*: effects of environmental and dietary factors, *Aquaculture*, 34, pp. 41–251.

Avnimelech, Y. (1999) Carbon/nitrogen ratio as a control element in aquaculture systems, *Aquaculture*, 176, pp. 227–235.

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

Avnimelech, Y. (2015) *Biofloc technology - a practical guide book*. 3rd edn. Baton Rouge, Louisiana, USA: The World Aquaculture Society.

Avnimelech, Y. and Lacher, M. (1979) A tentative nutrient balance for intensive fish ponds, *The Israeli Journal of Aquaculture-Bamidgeh*, 31, pp. 3–8.

Avnimelech, Y. and Ritvo, G. (2003) Shrimp and fish pond soils: processes and management, *Aquaculture*, 220, pp. 549–567.

Avnimelech, Y. and Schroeder, G. L. (1989) Circulated ponds as efficient bioreactors for single cell protein production, *The Israeli Journal of Aquacufture -Bamidgeh*, 41, pp. 58–66.

Avnimelech, Y., Weber, B., Hepher, B., Milstein, A. and Zorn, M. (1986) Studies in circulated fish ponds: organic matter recycling and nitrogen transformation, *Aquaculture and Fisheries Management*, 17, pp. 231–242.

Azaza, M., Dhraïef, M. and Kraïem, M. (2008) Effects of water temperature on growth and sex ratio of juvenile Nile tilapia *Oreochromis niloticus* (Linnaeus) reared in geothermal waters in southern Tunisia, *Journal of Thermal Biology*, 33, pp. 98–105.

Azim, M. E. and Little, D. C. (2008) The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*), *Aquaculture*, 283, pp. 29–35.

Badiola, M., Mendiola, D. and Bostock, J. (2012) Recirculating Aquaculture Systems (RAS) analysis: main issues on management and future challenges, *Aquacultural Engineering*, 51, pp. 26–35.

Balarin, J. and Haller, R. (1982) The intensive culture of tilapia in tanks, raceways and cages, in Muir, J. and Roberts, R. (eds) *Recent Advances in Aquaculture*. London, UK: Croom Helm, pp. 267–355.

Barak, Y., Cytryn, E., Gelfand, I., Krom, M. and van Rijn, J. (2003) Phosphorus removal in a marine prototype, recirculating aquaculture system, *Aquaculture*, 220, pp. 313–326.

Bergheim, A. and Brinker, A. (2003) Effluent treatment for flow through systems and European environmental regulations, *Aquacultural Engineering*, 27, pp. 61–77.

Beveridge, M., Briggs, M., Mowat, A., Northcott, M. and Ross, L. (1988) The function of microbranchiospines in tilapias, in Pullin, R. et al. (eds) *The Second International Symposium on Tilapia in Aquaculture.* Manila, Philippines: Department of Fisheries & International Center for Living Aquatic Resources Management, pp. 311–317.

Bonsdorff, E., Blomqvist, E., Mattila, J. and Norkko, A. (1997) Coastal eutrophication: causes, consequences and perspectives in the archipelago areas of the northern Baltic Sea, *Estuarine, Coastal and Shelf Science*, 44, pp. 63–72.

Bostock, J., Mcandrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward, I. and Corner, R. (2010) Aquaculture: global status and trends, *Philosophical Transactions of the Royal Society*, 365, pp. 2897–2912.

Bowen, S. (1982) Feeding, digestion and growth - qualitative considerations, in Pullin, R. S. V. and Lowe-McConnel, R. . (eds) *The Biology and Culture of Tilapias*. Manila, Philippines: ICLARM, pp. 141–156.

Boyd, C. (2004) *Farm-level issues in aquaculture certification: Tilapia*, *Report commissioned by WWF-US*. Alabama: Auburn University.

Boyd, C. E. (1985) Chemical budgets for channel catfish ponds, *Transactions of the American Fisheries Society*, 114, pp. 291–298.

Boyd, C. and Pillai, V. (1985) Water quality management in aquaculture, *CMFRI special Publication*, 22, pp. 1–44.

Brett, J. and Higgs, D. (1970) Effect of temperature on the rate of gastric digestion in fingerling sockeye salmon, *Oncorhynchus nerka*, *Journal of the Fisheries Board of Canada*, 27, pp. 1767–79.

Brett, J. R., Shelbourn, J. E. and Shoop, C. T. (1969) Growth rate and body composition of fingerling sockeye salmon, *Oncorhynchus nerka*, in relation to temperature and ration size, *Journal of the Fisheries Board of Canada*, 26, pp. 2363–2394.

Brunty, J., Bucklin, R., Davis, J., Baird, C. and Nordstedt, R. (1997) The influence of feed protein intake on tilapia ammonia production, *Aquacultural Engineering*, 16, pp. 161–166.

Bureau, D. P. and Cho, C. Y. (1999) Phosphorus utilization by rainbow trout (*Oncorhynchus mykiss*): estimation of dissolved phosphorus waste output, *Aquaculture*, 179, pp. 127–140.

Bureau, D. P. and Hua, K. (2010) Towards effective nutritional management of waste outputs in aquaculture, with particular reference to salmonid aquaculture operations, *Aquaculture Research*, 41, pp. 777–792.

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

Canagaratnam, P. (1966) Growth of *Tilapia mossambica* Peters in different salinities, *Bulletin of the Fisheries Research Station, Ceylon*, 19, pp. 47–50.

Chakraborty, S. and Chakraborty, S. (1998) Effect of dietary protein level on excretion of ammonia in Indian major carp, *Labeo rohita*, fingerlings, *Aquaculture Nutrition*, 4, pp. 47–52.

Chervinski, J. (1982) Environmental physiology of tilapias., in Pullin, R. and Lowe-McConnell, R. (eds) *The Biology and Culture of Tilapias*. Manila, Philippines: ICLARM, pp. 119–128.

Cho, C. Y. and Bureau, D. P. (2001) A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture, *Aquaculture Research*, 32, pp. 349–360.

Chowdhury, D. K. (2011) *Optimal feeding rate for Nile tilapia (Oreochromis niloticus)*. Norwegian University of Life Sciences.

Cincotta, D. and Stauffer, J. (1984) Temperature preference and avoidance studies of six North American freshwater fish species, *Hydrobiologia*, 109, pp. 173–177.

Cnaani, A., Gall, G. A. E. and Hulata, G. (2000) Cold tolerance of tilapia species and hybrids, *Aquaculture International*, 8, pp. 289–298.

Cnaani, A., Velan, A. and Hulata, G. (2011) Improving salinity tolerance in tilapias: a review, in *Better science, better fish, better life. Proceedings of the Ninth International Symposium on Tilapia in Aquaculture*. Shanghai, China: AquaFish Collaborative Research Support Program.

Crab, R., Avnimelech, Y., Defoirdt, T. and Bossier, P. (2007) Nitrogen removal techniques in aquaculture for a sustainable production, *Aquaculture*, 270, pp. 1–14.

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

Crab, R., Kochva, M., Verstraete, W. and Avnimelech, Y. (2009) Bio-flocs technology application in over-wintering of tilapia, *Aquacultural Engineering*, 40, pp. 105–112.

Crab, R., Lambert, A., Defoirdt, T., Bossier, P. and Verstraete, W. (2010) The application of bioflocs technology to protect brine shrimp (Artemia franciscana) from pathogenic Vibrio harveyi, *Journal of Applied Microbiology*, 109, pp. 1643–1649.

Cruz, E. and Ridha, M. (1995) Survival rates of tilapia, *Oreochromis spilurus* (Günther), fingerlings reared at high densities during winter using warm underground sea water, *Aquaculture Research*, 26, pp. 307–309.

Day, S. (2015) *A growth comparison among three commercial tilapia species in a biofloc technology system in South Africa*. Stellenbosch University.

Dempster, P., Baird, D. J. and Beveridge, M. C. M. (1995) Can fish survive by filter-feeding on microparticles? Energy balance in tilapia grazing on algal suspensions, *Journal of Fish Biology*, 41, pp. 7–17.

Van Dijk, P., Van Den Thillart, G. and Wendelaar Bonga, S. (1993) The influence of gradual water acidification on the oxygen consumption pattern of fish, *Comparative Biochemistry and Physiology. Part C: Comparative Pharmacology*, 105, pp. 421–427.

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

Ekasari, J. (2014) *Biofloc technology as an integral approach to enhance production and ecological performance of aquaculture*. Ghent University.

Ekasari, J., Azhar, M., Surawidjaja, E. and Nuryati, S. (2014) Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources, *Fish & Shellfish Immunology*, 41, pp. 332–339.

Ekasari, J. and Maryam, S. (2012) Evaluation of biofloc technology application on water quality and production performance of red tilapia Oreochromis sp. cultured at different stocking densities, *HAYATI Journal of Biosciences*, 19, pp. 73–80.

El-Sayed, A. (2006a) *Tilapia Culture*. 1st edn. Wallingford UK: CABI Publishing.

El-Sayed, A. (2006b) Tilapia culture in salt water: environmental requirements, nutritional implications and economic potentials, in Cruz, S. et al. (eds) *Advances en Nutricion Acuicola. VIII Simposium Internacional de Nutricion Acuicola.* Nuevo Leon, Mexico, pp. 95–106.

El-Sayed, A. and Kawanna, M. (2008) Optimum water temperature boosts the growth performance of Nile tilapia (*Oreochromis niloticus*) fry reared in a recycling system, *Aquaculture Research*, 39, pp. 670–672.

El-Shafai, S., El-Gohary, F., Nasr, F., van der Steen, N. and Gijzen, H. (2004) Chronic ammonia toxicity to duckweed-fed tilapia (*Oreochromis niloticus*), *Aquaculture*, 232, pp. 117–127.

Emerson, K., Russo, R. C., Lund, R. E. and Thurston, R. V. (1975) Aqueous ammonia equilibrium calculations: effect of pH and temperature, *Journal of the Fisheries Research Board of Canada*, 32, pp. 2379–2383.

Eshchar, M., Lahav, O., Mozes, N., Peduel, A. and Ron, B. (2006) Intensive fish culture at high ammonium and low pH, *Aquaculture*, 255, pp. 301–313.

Eshchar, M., Mozes, N. and Fediuk, M. (2003) Carbon dioxide removal rate by aeration devices in marine fish tanks, *Israeli Journal of Aquaculture - Bamidgeh*, 55, pp. 79–85.

Esselman, P. C., Schmitter-Soto, J. J. and Allan, J. D. (2013) Spatiotemporal dynamics of the spread of African tilapias (Pisces: *Oreochromis spp.*) into rivers of northeastern Mesoamerica, *Biological Invasions*, 15, pp. 1471–1491.

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

FAO (2014) *FAO yearbook. Fishery and Aquaculture Statistics*. Rome, Italy.

FAO (2016) *The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all.* Rome, Italy.

Fitzsimmons, K. (1997) Introduction to tilapia nutrition, in Fitzsimmons, K. (ed.) *Tilapia Aquaculture: Proceedings of the Fourth International Symposium on Tilapia in Aquaculture*. Ithaca, NY: Northern Regional Agricultural Engineering Service (NRAES) Publication No. 106, pp. 9–12.

Funge-Smith, S. and Briggs, M. (1998) Nutrient budgets in intensive shrimp ponds: implications for sustainability, *Aquaculture*, 164, pp. 117–133.

El Gamal, A. (1988) *Reproductive performance, sex ratios, gonadal development, cold tolerance, viability and growth of red and normally pigmented hybrids of Tilapia aurea and T. nilotica*. Auburn University.

Gao, L., Shan, H.-W., Zhang, T.-W., Bao, W.-Y. and Ma, S. (2012) Effects of carbohydrate addition on Litopenaeus vannamei intensive culture in a zero-water exchange system, *Aquaculture*, 342–343, pp. 89–96.

Gaudy, A. F. and Gaudy, E. T. (1981) *Microbiology for environmental scientists and engineers*. New York, USA: McGraw-Hill.

Gelegenis, J., Dalabakis, P. and Ilias, A. (2006) Heating of a fish wintering pond using low-temperature geothermal fluids, Porto Lagos, Greece, *Geothermics*, 35, pp. 87–103.

van Ginneken, V., van Eersel, R., Balm, P., Nieveen, M. and van den Thillart, G. (1997) Tilapia are able to withstand long-term exposure to low environmental pH, judged by their energy status, ionic balance and plasma cortisol, *Journal of Fish Biology*, 51, pp. 795–806.

Goolish, E. and Adelman, I. (1984) Effects of ration size and temperature on the growth of juvenile common carp (*Cyprinus carpio* L.), *Aquaculture*, 36, pp. 27–35.

Gopalakrishnan, V. (1988) Role of tilapia (*Oreochromis andersonii*) in integrated farming systems in Zambia, in Pullin, R. et al. (eds) *The Second International Symposium on Tilapia in Aquaculture, ICLARM Conference Proceedings*. Manila, Philippines: Department of Fisheries & International Center for Living Aquatic Resources Management, pp. 21–28.

Gowen, R. J. (1994) Managing eutrophication associated with aquaculture development, *Journal of Applied Ichthyology*, 10, pp. 242–257.

Guisheng, G., Juan, C., Qiumei, S., Muxiang, G., Yongmei, S., Huang, T., Hongshen, H., Qingchun, S. and Guanglian, R. (2016) Effects of salinity on growth of *Tilapia nilotica* and *Tilapia mossambica*., *Agricultural Science & Technology*, 17, pp. 130–135.

Gupta, M. V and Acosta, B. O. (2004) A review of global tilapia farming practices, in *Aquaculture Asia*, pp. 7–12.

Al Hafedh, Y. (1999) Effects of dietary protein on growth and body composition of Nile tilapia, *Oreochromis niloticus* L., *Aquaculture research*, 30, pp. 385–393.

Hannelly, E. C. (2009) *The effects of introduced trout on native macroinvertebrates from lakes in the Trinity Alps Wildnerness in Northern California*. Humboldt State University.

Hargreaves, J. A. (1997) A simulation model of ammonia dynamics in commerical catfish ponds in the southeastern United States, *Aquacultural Engineering*, 16, pp. 27–43.

Hargreaves, J. A. (1998) Nitrogen biogeochemistry of aquaculture ponds, *Aquaculture*, 166, pp. 181–212.

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

Hargreaves, J. A. (2013) *Biofloc production systems for aquaculture*, *Southern Regional Aquaculture Center*.

Heinen, J. M., Hankins, J. a and Adler, P. R. (1996) Water quality and waste production in a recirculating trout-culture system with feeding of a higher-energy or a lower-energy diet, *Aquaculture Research*, 27, pp. 699–710.

Herbeck, L. S. and Unger, D. (2013) Pond aquaculture effluents traced along back-reef waters by standard water quality parameters, δ15N in suspended matter and phytoplankton bioassays, *Marine Ecology Progress Series*, 478, pp. 71–86.

Herzig, A. and Winkler, H. (1986) The influence of temperature on the embryonic development of three cyprinid fishes, *Abramis brama*, *Chalcalburnus chalcoides mento* and *Vimba vimba*, *Journal of Fish Biology*, 28, pp. 171–181.

Higgs, D., Fagerlund, U., Mcbride, J., Dye, H. and Donaldson, E. (1977) Influence of combinations of bovine growth hormone, 17-methyltestosterone, and L-thyroxine on growth of yearling coho salmon (*Oncorhynchus kisutch*), *Canadian Journal of Zoology*, 55, pp. 1048–1056.

Hofer, S. C. and Watts, S. A. (2002) Cold tolerance in genetically male tilapia (GMT®), *Oreochromis niloticus, World Aquaculture*, 33, pp. 19–21.

Holmer, M., Black, K., Duarte, C. M., Marbà, N. and Karakassis, I. (eds) (2008) *Aquaculture in the ecosystem*. New York, NY, USA: Springer.

Houlihan, D., Boujard, T. and Jobling, M. (eds) (2008) *Food Intake in Fish*, *Aquaculture*. Oxford, UK: Blackwell Science Ltd.

Hu, Z., Lee, J., Chandran, K., Kim, S., Sharma, K. and Khanal, S. (2014) Influence of carbohydrate addition on nitrogen transformations and greenhouse gas emissions of intensive aquaculture system, *Science of the Total Environment*, 470, pp. 193–200.

Hulata, G., Wohlfarth, G. and Halevy, A. (1988) Comparative growth tests of *Oreochromis niloticus* × *O. aureus* hybrids derived from different farms in Israel, in polyculture, in Pullin, R. et al. (eds) *The Second International Symposium on Tilapia in Aquaculture.* Manila, Philippines: ICLARM, pp. 191–196.

Hung, L. T., Luong, V. C., Hoa, N. P. and Diana, J. (2011) Impacts of the introduction of alien tilapias (*Oreochromis spp.*) on the fisheries and biodiversity of indigenous species in Tri An reservoir, Vietnam, in Liping, L. and Fitzsimmons, K. (eds) *Better science, better fish, better life. Proceedings of the Ninth International Symposium on Tilapia in Aquaculture*. Shanghai, China: AquaFish Collaborative Research Support Program, pp. 75–85.

Iwama, G. K. (1991) Interactions between aquaculture and the environment, *Critical Reviews in Environmental Science and Technology*, 21, pp. 177–216.

Jauncey, K. and Ross, B. (1982) *A guide to tilapia feeds and feeding*. Institute of Aquaculture, University of Stirling, Scotland.

Job, S. (1969) The respiratory metabolism of *Tilapia mossambica* (Teleostei), *Marine Biology*, 2, pp. 121–126.

Kaufman, L. (1992) Catastrophic change in species-rich freshwater ecosystems, *BioScience*, 42, pp. 846–858.

Kautsky, N., Rönnbäck, P., Tedengren, M. and Troell, M. (2000) Ecosystem perspectives on management of disease in shrimp pond farming, *Aquaculture*, 191, pp. 145–161.

Kefi, A. S., Kang'ombe, J., Kassam, D. and Katongko, C. (2012) Growth, reproduction and sex ratios in *Oreochromis andersonii* (Castelnau, 1861) fed with varying Levels of 17α-methyl testosterone, *Journal of Aquaculture Research & Development*, 3, pp. 130–137.

Kirchman, D. (1994) The uptake of inorganic nutrients by heterotrophic bacteria, *Microbial Ecology*, 28, pp. 255–271.

Kirchman, D. and Wheeler, P. (1998) Uptake of ammonium and nitrate by heterotrophic bacteria and phytoplankton in the sub-Arctic Pacific, *Deep Sea Research Part I: Oceanographic Research Papers*, 45, pp. 347–365.

Kirk, R. (1972) A review of recent developments in Tilapia culture, with special reference to fish farming in the heated effluents of power stations, *Aquaculture*, 1, pp. 45–60.

Kocher, T. D., Lee, W. J., Sobolewska, H., Penman, D. and McAndrew, B. (1998) A genetic linkage map of a cichlid fish, the tilapia (*Oreochromis niloticus*), *Genetics*, 148, pp. 1225–1232.

Küçük, S., Karul, A., Yildirim, Ş. and Gamsiz, K. (2013) Effects of salinity on growth and metabolism in blue tilapia (*Oreochromis aureus*), *African Journal of Biotechnology*, 12, pp. 2715–2721.

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

Lall, S. P. (2002) The Minerals, in Halver, J. and Hardy, R. (eds) *Fish Nutrition*. 3rd edn. New York, USA: Academic Press, pp. 259–308.

Little, D. C., Murray, F. J., Azim, E., Leschen, W., Boyd, K., Watterson, A. and Young, J. A. (2008) Options for producing a warm-water fish in the UK: limits to 'Green Growth'?, *Trends in Food Science and Technology*, 19, pp. 255–264.

Liu, J., Li, Z., Li, X. and Wang, Y. (2013) *On-farm feed management practices for Nile tilapia in southern China*, *FAO Fisheries and Aquaculture Technical Paper No. 583*.

Liu, L., Hu, Z., Dai, X. and Avnimelech, Y. (2014) Effects of addition of maize starch on the yield, water quality and formation of bioflocs in an integrated shrimp culture system, *Aquaculture*, 418, pp. 79–86.

Losordo, T. M. and Westerman, P. W. (1994) An analysis of biological, economic, and engineering factors affecting the cost of fish production in recirculating aquaculture systems, *Journal of the World Aquaculture Society*, 25, pp. 193–203.

Love, R. (1980) *The chemical biology of fishes: vol. 2*. 2nd edn. London, UK: Academic Press.

Luo, G., Gao, Q., Wang, C., Liu, W., Sun, D., Li, L. and Tan, H. (2014) Growth, digestive activity, welfare, and partial cost-effectiveness of genetically improved farmed tilapia (*Oreochromis niloticus*) cultured in a recirculating aquaculture system and an indoor biofloc system, *Aquaculture*, 422, pp. 1–7.

Luque-Almagro, V., Gates, A. and Moreno-Vivián, C. (2011) Bacterial nitrate assimilation: gene distribution and regulation, *Biochemical Society Transactions*, 39, pp. 1838–1843.

Maicá, P. F., de Borba, M. R. and Wasielesky, W. (2012) Effect of low salinity on microbial floc composition and performance of *Litopenaeus vannamei* (Boone) juveniles reared in a zero-water-exchange super-intensive system, *Aquaculture Research*, 43, pp. 361–370.

Martinez-Palacios, C., Chavez-Sanchez, M. and Ross, L. (1996) The effects of water temperature on food intake, growth and body composition of *Cichlasoma urophthalmus* (Günther) juveniles, *Aquaculture Research*, 27, pp. 455–461.

Masser, M., Rakocy, J. and Losordo, T. (1999) Recirculating aquaculture tank production systems, *Southern Regional Aquaculture Center*, p. 12.

Matty, A. J. and Lone, K. P. (1979) The effect of androgenic steroids as dietary additives on the growth of carp (*Cyprinus carpio*), *Journal of the World Aquaculture Society*, 10, pp. 735–745.

McCambridge, J. and McMeekin, T. (1981) Effect of solar radiation and predacious microorganisms on survival of fecal and other bacteria., *Applied and Environmental Microbiology*, 41, pp. 1083–1087.

Mikkelsen, L., Gotfredsen, A. and Agerbxk, M. (1996) Effects of colloidal stability on clarification and dewatering of activated sludge, *Water Science and Technology*, 34, pp. 449–457.

Morgan, P. (1972) Causes of mortality in the endemic tilapia of Lake Chilwa (Malawi), *Hydrobiologia*, 40, pp. 101–119.

Musuka, C. G. and Musonda, F. F. (2012) Current and future prospects of commercial fish farming in Zambia, *International Journal of the Bioflux Society*, 5, pp. 79–87.

Muthuwani, V. and Lin, C. K. (1996) Water quality and nutrient budget in intensive shrimp culture ponds, in *Book of Abstracts, The 1996 Annual Meeting of the World Aquaculture Society*. Bangkok, Thailand, p. 270.

Northcott, M. and Beveridge, M. (1988) The development and structure of pharyngeal apparatus associated with filter feeding in tilapias (*Oreochromis niloticus*), *Journal of Zoology*, 215, pp. 133–149.

Piedrahita, R. H. (1991) Simulation of short-term management actions to prevent oxygen depletion in ponds, *Journal of the World Aquaculture Society*, 22, pp. 157–166.

Popma, T. and Masser, M. (1999) *Tilapia: life history and biology*, *Southern Regional Aquaculture Center*.

Prein, M., Hulata, G. and Pauly, D. (1993) Multivariate methods in aquaculture research: case studies of tilapias in experimental and commercial systems, *ICLARM Studies and Reviews*. Manila, Philippines: ICLARM, 20, p. 221.

Ray, A. J., Dillon, K. S. and Lotz, J. M. (2011) Water quality dynamics and shrimp (*Litopenaeus vannamei*) production in intensive, mesohaline culture systems with two levels of biofloc management, *Aquacultural Engineering*, 45, pp. 127–136.

Ray, A. J., Lewis, B. L., Browdy, C. L. and Leffler, J. W. (2010) Suspended solids removal to improve shrimp (*Litopenaeus vannamei*) production and an evaluation of a plant-based feed in minimal-exchange, superintensive culture systems, *Aquaculture*, 299, pp. 89–98.

Read, P. and Fernandes, T. (2003) Management of environmental impacts of marine aquaculture in Europe, *Aquaculture*, 226, pp. 139–163.

Riche, M., Haley, D., Oetker, M., Garbrecht, S. and Garling, D. (2004) Effect of feeding frequency on gastric evacuation and the return of appetite in tilapia *Oreochromis niloticus* (L.), *Aquaculture*, 234, pp. 657–673.

Riche, M., Oetker, M., Haley, D. l., Smith, T. and Garling, D. L. (2004) Effect of feeding frequency on consumption, growth, and efficiency in juvenile tilapia (*Oreochromis niloticus*), *Israeli Journal of Aquaculture-Bamidgeh*, 56, pp. 247–255.

Roy, P. K. and Lall, S. P. (2004) Urinary phosphorus excretion in haddock, *Melanogrammus aeglefinus* (L.) and Atlantic salmon, Salmo salar (L.), *Aquaculture*, 233, pp. 369–382.

Samocha, T. M., Patnaik, S., Speed, M., Ali, A.-M., Burger, J. M., Almeida, R. V., Ayub, Z., Harisanto, M., Horowitz, A. and Brock, D. L. (2007) Use of molasses as carbon source in limited discharge nursery and grow-out systems for *Litopenaeus vannamei, Aquacultural Engineering*, 36, pp. 184–191.

Schneider, O., Blancheton, J. P., Varadi, L., Eding, E. H. and Verreth, J. A. J. (2006) Cost price and production strategies in European recirculation systems, in *Linking Tradition & Technology Highest Quality for the Consumer*. Florence, Italy: World Aquaculture Society.

Schneider, O., Sereti, V., Machiels, M. A. M., Eding, E. H. and Verreth, J. A. (2006) The potential of producing heterotrophic bacteria biomass on aquaculture waste, *Water Research*, 40, pp. 2684–2694.

Schramm, A., de Beer, D., Gieseke, A. and Amann, R. (2000) Microenvironments and distribution of nitrifying bacteria in a membrane-bound biofilm, *Environmental Microbiology*, 2, pp. 680–686.

De Schryver, P., Crab, R., Defoirdt, T., Boon, N. and Verstraete, W. (2008) The basics of bio-flocs technology: the added value for aquaculture, *Aquaculture*, 277, pp. 125–137.

Shafland, P. and Pestrak, J. (1982) Lower lethal temperatures for fourteen non-native fishes in Florida, *Environmental Biology of Fishes*, 7, pp. 149–156.

Shirokova, Y., Forkutsa, I. and Sharafutdinova, N. (2000) Use of electrical conductivity instead of soluble salts for soil salinity monitoring in Central Asia, *Irrigation and Drainage Systems*, 14, pp. 199–206.

Siddiqui, A., Howlader, M. and Adam, A. (1988) Effects of dietary protein levels on growth, feed conversion and protein utilization in fry and young Nile tilapia, *Oreochromis niloticus*, *Aquaculture*, 70, pp. 63–73.

Sifa, L., Chenhong, L., Dey, M., Gagalac, F. and Dunham, R. (2002) Cold tolerance of three strains of Nile tilapia, *Oreochromis niloticus*, in China, *Aquaculture*, 213, pp. 123–129.

Sinha, A., Baruah, K. and Bossier, P. (2008) Horizon scanning: the potential use of biofloc as an anti-infective strategy in aquaculture – an overview, *Aquaculture Health International*, 13, pp. 8–10.

Siraj, S. S., Kamaruddin, Z., Satar, M. K. A. and Kamarudin, M. S. (1988) Effects of feeding frequency on growth, food conversion and survival of red tilapia *(Oreochromis mossambicus/O. niloticus*) hybrid fry, in Pullin, R. et al. (eds) *The Second International Symposium on Tilapia in Aquaculture*. Manila, Philippines: Department of Fisheries & International Center for Living Aquatic Resources Management, pp. 383–386.

Skelton, P. H. (2001) *A complete guide to the freshwater fishes of Southern Africa*. Cape Town, South Africa: Struik.

Smith, V. H., Tilman, G. D. and Nekola, J. C. (1999) Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems, *Environmental Pollution*, 100, pp. 179–196.

Steicke, C. R., Jegatheesan, V. and Zeng, C. (2009) Recirculating aquaculture systems - a review, in Vigneswaran, S. (ed.) *Water and Wastewater Treatment Technologies. Encyclopaedia of Life Support Systems*. Oxford, UK: EOLSS, pp. 1–25.

Tacon, A. G. J. and Forster, I. P. (2003) Aquafeeds and the environment: policy implications, *Aquaculture*, 226, pp. 181–189.

Tal, Y., Schreier, H., Sowers, K. and Stubblefield, J. (2009) Environmentally sustainable land-based marine aquaculture, *Aquaculture*, 286, pp. 28–35.

Teichert-Coddington, D. and Green, B. (1993) Tilapia yield improvement through maintenance of minimal oxygen concentrations in experimental grow-out ponds in Honduras, *Aquaculture*, 118, pp. 63–71.

Timmons, M. and Ebeling, J. (2007) *Recirculating aquaculture*. 3rd edn. Ithaca, NY: Cayuga Aqua Ventures.

Toguyeni, A., Fauconneau, B., Boujard, T., Fostier, A., Kuhn, E. R., Mol, K. A. and Baroiller, J. F. (1997) Feeding behaviour and food utilisation in tilapia, *Oreochromis niloticus*: effect of sex ratio and relationship with the endocrine status, *Physiology and Behavior*, 62, pp. 273–279.

Tsadik, G. G. and Kutty, M. N. (1987) *Influence of ambient oxygen on feeding and growth of the tilapia, Oreochromis niloticus (Linnaeus)*, *ARAC/87WP/10*. Port Harcourt, Nigeria.

Ufodike, E. and Madu, C. (1986) Effects of methyltestosterone on food utilization and growth of *Sarotherodon niloticus* fry., 日本水産学会誌, 52, pp. 1919–1922.

Wang, N., Hayward, R. S. and Noltie, D. B. (1998) Effect of feeding frequency on food consumption, growth, size variation, and feeding pattern of age-0 hybrid sunfish, *Aquaculture*, 165, pp. 261–267.

Wangead, C., Geater, A. and Tansakul, R. (1988) Effects of acid water on survival and growth rate of Nile tilapia (*Oreochromis niloticus*), in Pullin, R. et al. (eds) *The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings*. Manila, Philippines: Department of Fisheries & International Center for Living Aquatic Resources Management, pp. 433–438.

Watanabe, W. O., Losordo, T. M., Fitzsimmons, K. and Hanley, F. (2002) Tilapia production systems in the Americas: technological advances, trends, and challenges, *Reviews in Fisheries Science*, 10, pp. 465–498.

Weatherley, A. and Gill, H. (1983) Protein, lipid, water and caloric contents of immature rainbow trout, *Salmo gairdneri* Richardson, growing at different rates, *Journal of Fish Biology*, 23, pp. 653–673.

Wendelaar Bonga, S., Flik, G. and Balm, P. (1987) Physiological adaptation to acid stress in fish, *Annales de la Société royale zoologique de Belgique*. Brussels, 117, pp. 243–254.

Whitfield, A. and Blaber, S. (1979) The distribution of the freshwater cichlid *Sarotherodon mossambicus* in estuarine systems, *Environmental Biology of Fishes*, 4, pp. 77–81.

Wilson, R. P. (2002) Amino acids and proteins, in Halver, J. and Hardy, R. (eds) *Fish nutrition*. 3rd edn. Academic Press, pp. 143–179.

Xu, W. J. and Pan, L. Q. (2012) Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N raio in feed., *Aquaculture*, 356, pp. 147–152.

Xu, W. J., Pan, L. Q., Zhao, D. H. and Huang, J. (2012) Preliminary investigation into the contribution of bioflocs on protein nutrition of *Litopenaeus vannamei* fed with different dietary protein levels in zero-water exchange culture tanks, *Aquaculture*, 350, pp. 147–153.

Xu, W. and Pan, L. (2014) Evaluation of dietary protein level on selected parameters of immune and antioxidant systems, and growth performance of juvenile *Litopenaeus vannamei* reared in zero-water exchange biofloc-based culture tanks, *Aquaculture*, 426, pp. 181–188.

Yada, T. and Ito, F. (1997) Differences in tolerance to acidic environments between two species of tilapia, *Oreochromis niloticus* and *O. mossambicus*, *Bulletin of the National Research Institute of Fisheries Science (Japan)*, 9, pp. 11–18.

Zhao, P., Huang, J., Wang, X. H., Song, X. L., Yang, C. H., Zhang, X. G. and Wang, G. C. (2012) The application of bioflocs technology in high-intensive, zero exchange farming systems of *Marsupenaeus japonicus*, *Aquaculture*, 354, pp. 97–106.