FACTORS AFFECTING GROWTH PERFORMANCE OF *OREOCHROMIS MOSSAMBICUS* IN A LOW TECHNOLOGY AQUAPONIC SYSTEM

MASTER OF SCIENCE (AQUACULTURE)

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FACTORS AFFECTING GROWTH PERFORMANCE OF *OREOCHROMIS MOSSAMBICUS* IN A LOW TECHNOLOGY AQUAPONIC SYSTEM

by

MAHUMA RIBANE

DISSERTATION

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SUPERVISOR: Prof. N.A.G. Moyo

CO-SUPERVISOR: Prof. M.M. Rapatsa-Malatsi

2024

DEDICATION

I am dedicating this to my son Mogau Rabogale, who was conceived and born during my Masters journey. Who has become my source of pride and happiness. My reason for keeping walking the tough journey. This dedication is also extended to my husband Matome Jack, who sticked with me the entire journey, making life as easy as possible for me, comforting and encouraging me to keep working.

DECLARATION

I declare that **FACTORS AFFECTING THE GROWTH PERFORMANCE OF *OREOCHROMIS MOSSAMBICUS* IN LOW TECHNOLOGY AQUAPONICS SYSTEMS** (dissertation) hereby submitted to the University of Limpopo, for the degree of **MASTER OF SCIENCE IN AQUACULTURE** has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

Ribane, M (Ms.)

Date

PLAGIARISM DECLARATION

The thesis has been submitted to the Turnitin module and I confirm that my supervisors have seen my report and any concerns revealed by such have been resolved with them.

Ribane, M (Ms.)	Date

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ABSTRACT

The aim of this study was to enhance fish production in low technology aquaponic systems. Growth parameters and somatic indices were assessed in order to determine the effect of feeding frequency, stocking density and recirculation rate on the growth performance of *Oreochromis mossambicus* in low technology aquaponic systems. Weight gain (%), specific growth rate (SGR), food conversion ratio (FCR), thermal growth coefficient (TGC), condition factor (CF), hepatosomatic index (HSI), viscerosomatic index (VSI) and survival rate (SR) were assessed for each factor. Haematological parameters were also checked to assess the stress levels of fish under different treatments of feeding frequency, stocking density and recirculation rate. Red blood cell (RBC) count, white blood cell (WBC) count, haematocrit, haemoglobin and serum glucose levels were also assessed for each factor. Water quality parameters (pH, dissolved oxygen (DO) concentration, total dissolved solids (TDS), ammonia (NH_3), nitrate (NO_3^-), nitrite (NO_2^-) and phosphorus (P)) were monitored over the duration of the experiment. *Spinacia oleracea* growth parameters (shoot length and the number of leaves), were analysed in order to determine its growth performance when integrated with *O mossambicus*.

The effect of feeding frequency on the growth performance of *O. mossambicus* in low technology aquaponic systems was evaluated over a duration of 28 days. The fish were stocked in 500 L fibreglass tanks. The fish feeding frequency treatments were T1 (once daily), T2 (twice daily) and T3 (thrice daily). The highest %WG, SGR, TGC and SR were recorded in T3, which was significantly different from other treatments ($p < 0.05$, ANOVA). The lowest FCR was recorded in T3. The best growth performance that was observed in the T3 treatment may be due to better feed intake and feed utilization that comes with frequent feeding. The fish feed was also available for prolonged periods. No significant differences ($p > 0.05$, ANOVA) in SR were observed between treatments. Mortalities that occurred in each treatment were not related to feeding frequency, they may have been due to handling. No significant differences ($p > 0.05$, ANOVA) in organosomatic indices (CF, HSI and VSI) were observed with respect to feeding frequency. The health status of the fish was not affected by the different feeding frequencies. Additionally, feeding frequency did not significantly affect ($p > 0.05$, ANOVA) all the haematological parameters. Different feeding frequencies did not cause any significant changes in RBC count, WBC count

or serum glucose. The highest blood performance was recorded at the highest feeding frequency (3 times daily). This suggests better health status of *O. mossambicus* at the highest feeding frequency, where the fish may have been able to feed efficiently. The pH and DO concentration range were 6.99-7.20 and 5.70-8.60 mg/L respectively. No significant ($p > 0.05$, ANOVA) differences in pH and DO were observed between different treatments. Water quality parameters remained within acceptable limits for growth of tilapia. NH_3, NO_3^- and NO_2^- concentrations increased with feeding frequency and the duration of the experiment. This could be due to increasing accumulation of fish waste as feeding frequency increased. Feeding frequency resulted in an increase in P concentrations. This may be a result of increasing accumulation of fish waste in the system. The highest plant growth was also observed in T3. The maximum plant growth in T3 was due to the availability of nutrients in the system for a prolonged period during the day. These results show that feeding frequency had an effect on the growth performance of *O. mossambicus* in low technology aquaponic systems. The feeding frequency of 3 times daily may be the optimum feeding frequency for the growth of *O. mossambicus* and *S. oleracea* in aquaponic systems.

The effect of stocking density on the growth performance of *O. mossambicus* in low technology aquaponic systems was evaluated over a duration of 28 days. The fish were stocked in 500 L fibreglass tanks. The fish stocking density treatments were low stocking density (1.87 kg/m^3), intermediate stocking density (2.50 kg/m^3) and high stocking density (3.13 kg/m^3). The highest %WG, SGR, TGC and SR were recorded in fish that were stocked in ISD. The lowest FCR was also recorded in fish that were stocked in ISD. A significant difference ($p < 0.05$, ANOVA) in SGR and FCR was observed between ISD and other treatments. The best growth performance that was observed in the 2.50 kg/m^3 treatment was due to efficient feeding. At intermediate stocking densities, the was no competition for food and space and as a result, fish stress did not occur. The fish may have been able to channel their energy to feeding instead of stress. SR was not significantly affected ($p > 0.05$, ANOVA) by stocking density. Mortalities that occurred in each treatment were not related to stocking density; they may have been due to handling. The highest organosomatic indices (CF, HSI and VSI) were observed in the ISD treatment. This could suggest that the fish had the best condition and best health

status. Additionally, the highest RBC count, WBC count, haematocrit, haemoglobin, and serum glucose were observed in fish that were stocked at the HSD treatment. This could mean that the fish stocked at the highest density experienced stress which was reflected in high RBC count, WBC count and serum glucose. The highest blood performance was recorded at the lowest stocking density. This could suggest better health status of *O. mossambicus* at the lowest stocking density, where the fish were able to focus their energy on feeding instead of stress resulting from crowding and competition for food. No significant differences in pH were observed between different treatments. The lowest DO concentration was observed in HSD. Water quality parameters remained within acceptable limits for growth of *O. mossambicus*. NH_3, NO_3^-, and NO_2^- concentrations increased with stocking density and the duration of the experiment. This could have resulted from the increasing accumulation of fish waste as stocking density increased. The concentration of P also increased with the stocking density and the experimental duration. This may be owing to the increasing accumulation of fish waste. The highest plant growth was also observed at HSD. This may be due to the increasing accumulation of fish waste in the system that is directly related to the increasing fish biomass with increasing stocking density. These results show that stocking density had an effect on the growth performance of *O. mossambicus* in low technology aquaponic systems. The intermediate stocking density of 2.50 kg/m^3 may be optimal for the growth of both *O. mossambicus* and *S. oleracea* in aquaponic systems. The best fish growth (SGR and FCR) was observed in the intermediate stocking density compared to high stocking density and low stocking density.

The effect of recirculation rate on the growth performance of *O. mossambicus* in low technology aquaponic systems was evaluated over a duration of 28 days. The fish were stocked in 500 L fibreglass tanks. The recirculation rate treatments were LRR (0.5 L/min), IRR (1.5 L/min) and HRR (2.5 L/min). The highest %WG, SGR, TGC and SR were recorded in fish that were stocked in HRR treatments. The lowest FCR was also recorded in HRR. A significant difference ($p < 0.05$, ANOVA) in SGR and FCR was observed between HRR and LRR. The best growth performance of *O. mossambicus* that was observed in the HRR treatment may be due to good water quality (high DO conc. and low TDS). When the water quality is good, fish are able to feed efficiently in the stress-free environment. SR was not significantly affected

($p > 0.05$, ANOVA) by recirculation rate. Mortalities that occurred in each recirculation rate treatment were not related to recirculation rate; they may have been due to handling. The highest ($p < 0.05$, ANOVA) organosomatic indices (CF, HSI and VSI) were observed at HRR. Poor fish health in terms of CF, HIS and VSI was observed in LRR. The highest ($p < 0.05$, ANOVA) haematological parameters were observed in LRR. This could be due to fish stress resulting from the low DO concentrations in the water. The highest blood performance of 3.22 was observed at the high recirculation rate treatment. This could suggest that the high recirculation rate resulted in the best health of *O. mossambicus*. No significant differences ($p > 0.05$, ANOVA) in pH were observed between different treatments. The pH remained within acceptable limits for growth of *O. mossambicus*. The lowest DO concentration was recorded in the LRR treatments. In LRR, the lowest DO concentration was observed. The dissolved oxygen levels in LRR treatment were below acceptable limits for growth of *O. mossambicus*. Recirculation rate led to a decrease in NH_3, NO_3^- and NO_2^- concentrations, while experimental duration led to their increase. This could be attributed to the decreasing accumulation of fish waste as recirculation rate increased. The P concentration also decreased with increasing recirculation rate and increased with the duration of the experiment. This could be as a result of the decreasing accumulation of fish waste as recirculation rates increased. The highest plant growth was observed in IRR. The maximum plant growth in IRR may be due to stable DO concentrations and nutrients in the system from the accumulating fish waste. These results show that recirculation rate had an effect on the growth performance of *O. mossambicus* low technology in aquaponic systems. The intermediate recirculation rate (1.5 L/min) may be optimum for the growth of both *S. oleracea* and *O. mossambicus* in aquaponic systems.

A low technology coupled aquaponic systems was used in this study. The incorporation of a sedimenter into the system presents a solution to the build up of solid waste in the tanks and the subsequent water quality deterioration. This can also minimise the constant water exchanges. The optimisation of the coupled aquaponic system can allow higher fish and plant densities without compromising the water quality.The balance of fish and plant ratios can allow for optimal growth of both entities without water quality deteriorations.The choice of the fish and and plant species can also affect the nutrient build up in the water. Fish polyculture and plant intercropping are also functional in the optimisation of they system’s overall production. Optimal feeding frequencies can also be used without worrying about the nutrient build up.

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ANOVA	Analysis of Variance
C. carpio	*Cyprinus carpio*
C. gariepinus	*Clarias gariepinus*
CF	Condition Factor
CO_2	Carbon Dioxide
DO	Dissolved Oxygen
FCR	Food Conversion Ratio
FW	Final Weight
Hb	Haemoglobin
HCT	Haematocrit
HIS	Hepatosomatic Index
HRR	High Recirculation Rate
HSD	High Stocking Density
IRR	Intermediate Recirculation Rate
ISD	Intermediate Stocking Density
LRR	Low Recirculation Rate
LSD	Low Stocking Density
NFT	Nutrient Film Technique
NH_3	Ammonia
NO_2^-	Nitrite
NO_3^-	Nitrate
O. mossambicus	*Oreochromis mossambicus*
O. niloticus	*Oreochromis niloticus*

P	Phosphorus
PVC	Polyvinyl Chloride
RAS	Recirculating Aquaculture System
RBC	Red Blood Cell
S. oleracea	*Spinacia oleracea*
SGR	Specific Growth Rate
SPSS	Statistical Package for the Social Sciences
SR	Survival Rate
TDS	Total Dissolved Solids
TGC	Thermal Growth Coefficient
VSI	Viscerosomatic Index
WBC	White Blood Cell

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CHAPTER 1: INTRODUCTION

1.1 GENERAL INTRODUCTION

Aquaponics is a bio-integrated food production system that links recirculating aquaculture with hydroponics for simultaneous production of terrestrial plants and aquatic life (Carlson, 2013; Salam *et al*. 2013). In an aquaponic system, aquatic animals excrete waste, bacteria convert the waste into nutrients, and plants take up the nutrients and improve water quality for the aquatic animals (Love *et al*. 2014). An aquaponic system combines hydroponics and aquaculture, eliminating the limitations experienced when they are conducted individually (Somerville *et al*. 2014). Hydroponics requires expensive nutrients to feed the plants and requires periodic flushing of the systems which can lead to waste disposal issues (Blidariu and Grozea, 2011). Recirculating aquaculture systems (RAS) require a constant removal of excess nutrients from the system by removing a percentage of the water on a daily basis and replacing it with clean, fresh water. Factors including high capital and operational costs as well as the need for diligent management and difficulties in treating the diseases are the main limitations of the RAS systems.

The basic principle of aquaponics is the oxidation of ammonia into nitrite and subsequently into nitrate by *Nitrosomonas* and *Nitrobacter* respectively (Pinho *et al*. 2018; Yamane *et al*. 2021). The source of ammonia in aquaponics is fish excrement and the decomposition of uneaten feed (Effendi *et al*. 2017). Ammonia production rate depends on food quantity and feeding frequency (Stathopoulou *et al*. 2021). In addition, ammonia oxidation rate is dependent on the quantity of nitrifying bacteria which is influenced by pH, water temperature and dissolved oxygen concentrations (Stathopoulou *et al*. 2021). The nitrate produced by oxidation is assimilated by plants as the water passes through the hydroponics sub-system (Maucieri *et al*. 2018; Stathopoulou *et al*. 2021).

The accumulation of nutrients in an aquaponic system is good for plant growth but when the nutrients content becomes too high for the plants to assimilate, it becomes a problem for the fish. The appropriate concentration of nutrients in the aquaponic system ensures optimal production of plants which in turn ensures good water quality. When the quality of the water is excellent, optimal growth performance of fish is expected. High concentrations of ammonia and nitrite in the aquaponic system become toxic to the fish. The nitrification process helps by eliminating ammonia and nitrite from the system (Edaroyati *et al*. 2017).

The conditions of an aquaponic system must be balanced in order meet the requirements of the coexisting fish, plants, and bacteria. It is therefore imperative that the system type, fish and plant species as well as the biomass be carefully chosen (Stathopoulou *et al*. 2021). A successful production can be achieved in aquaponic systems when the balance in biomass between fish and plant species has been determined (Wiyoto *et al*. 2023). pH levels and dissolved oxygen concentrations form part of the factors that need to be balanced between the three entities of the aquaponics design. The plants, fish and bacteria thrive at different pH ranges. The pH ranges of 6.5–8.5, 5.5 and 6.5 7.0–8.0 are recommended for fish, plants, and bacteria respectively (Yildiz *et al*. 2017; Stathopoulou *et al*. 2021). So, an optimum pH range for an aquaponic system appears to be 6.5–7.0. Reduced solubility of phosphorus and other micronutrients can result from pH > 7.0 (Stathopoulou *et al*. 2021). Dissolved oxygen levels > 5 mg/L are recommended for the nitrification process (nitrifying bacteria) and for the nutrient uptake by plants as well as strengthening of the plant's root system.

Aquaponic systems use high fish densities in order to supply the required concentration of nutrients for plant growth while at the same time allowing for high production. The fish species selected for culturing in an aquaponic system should be tolerant to these high stocking densities (Sonia and Roopavathy 2021). The survival and growth of the fish in the aquaponic system depends on the quality of the water, which is determined by the plants in the hydroponics sub-system. In this study, *Oreochromis mossambicus* was the chosen fish species because it possesses the ability to grow in high density environments and it can easily adapt to different environments (Akter *et al*. 2018).

The plant species selected for aquaponics production should have the capacity to efficiently take up and utilize the nutrients that accumulate in the system (Pinho *et al*. 2018). The plants should be fast growing with short harvest time and low to medium nutritional requirements (Effendi *et al*. 2017). The current study chose *Spinacia oleracea* as the plant species due to its high ability to absorb nutrients. The ability of *S. oleracea* plants to withstand hydroponics and local climatic conditions makes them good candidates for aquaponics production (Gichana *et al*. 2019). The density of plants is important in aquaponic system production as it directly determines the number of plants required to utilize the waste and nutrients produced by fish (Edaroyati *et al*. 2017). Low plant density leads to low nutrient removal efficiency while the opposite applies for high plant density. Therefore, the density of plants needs to be balanced with that of fish.

The performance production of fish and plants in aquaponics directly depends on the fish management practices. The balance of nutrients in the water (Roy *et al*. 2021), the species of plants and fish, stocking density of fish and the system's recirculation rate form part of the factors that affect the optimal production of an aquaponic system. This study will investigate the effect of feeding frequency, stocking density and recirculation rate on the growth performance of *O. mossambicus* in aquaponic systems.

Feeding frequency is one of the various feed management practices proven to maximize the benefit of feeding in aquaponic systems. It plays an important role in regulating the feed intake, feed utilization efficiency, minimizing feed wastage and optimizing fish growth (Stathopoulou *et al*. 2021). Optimum feeding frequency is fundamental in obtaining the best production of fish and plants in an aquaponics setting (Edaroyati *et al*. 2017). When feeding frequencies become too high, they result in low feed intake. Low feed intake means food gets wasted and the excess dissolved food in the water compromises the quality of the water. Feeding frequencies in aquaculture production systems are affected by stocking densities, since they are directly related to nutrient accumulation. The optimum feeding frequency for *O. mossambicus* in an aquaponic system has been investigated before. In this experiment, feed wastage will be minimized with the appropriate feeding frequency being adapted.

Stocking density has a direct effect on the amount of fish waste and metabolites in the water and as a result the quality of the water. Poor water quality that comes with high stocking densities affects the health, feed intake and the growth performance of fish. Stocking density is considered as one of the important factors in aquaponics, besides feeding rate and frequency since it varies according to fish type (Edaroyati *et al*. 2017). In aquaponic systems, the appropriate fish stocking density can provide suitable concentration of ammonia and nitrate nitrogen that are adequate for the successive plant growth (Roy *et al*. 2021). Stocking density of fish should be optimum to maintain the water quality suitable for fish and plant growth (Rahmatullah *et al*. 2010; Al-Tawaha *et al*. 2021). Through optimum stocking density, one can obtain maximum production without compromising the water quality and fish health (Edaroyati *et al*. 2017). Rahmatullah *et al*. (2010) suggested low stocking densities over high stocking densities for the optimal growth of *Oreochromis niloticus* in aquaponic systems. *O. mossambicus* is the commonly used fish species in aquaponic systems, yet there are no studies on the most appropriate stocking density in aquaponic systems.

Determining the appropriate stocking density of an aquaponic system also relies on the recirculation rate of the system. Regulation of recirculation rates is based on achieving optimum dissolved oxygen (DO) levels in the aquaponic systems and limiting the accumulation of fish metabolites and other wastes (Schram *et al*. 2009). The recirculation rate of the water also affects the accumulation of feed and fish waste in the system, thereby affecting the quality of the water as well. The fish metabolites have a direct effect on the water quality and the accumulation of carbon dioxide and total ammonia (Sun *et al*. 2016). Low recirculation rates lead to increasing accumulation of nutrients and toxic compounds, slow solids removal and slow plant growth (Edaroyati *et al*. 2017). Higher than optimum recirculation rates in aquaponic systems promote greater water agitation that leads to fish stress and lower feed consumption (León-Ramírez *et al*. 2022). Evidently recirculation rates play an important role in aquaponic systems, However, no studies have investigated the effects of recirculation rates of aquaponic systems stocked with *O. mossambicus.*

1.2 PROBLEM STATEMENT

Aquaponic system production has attracted a great deal of attention worldwide as a cost effective, nutrient recycling and water saving alternative to conventional aquaculture (Palm *et al.* 2019). South Africa is a water scarce country (Viljoen and Van der Walt, 2018) and water availability is the key requirement for fish farming, making the development of aquaculture in such a country quite difficult to achieve

(Moyo and Rapatsa, 2021). An aquaponic system allows for the optimal utilization of water in the simultaneous production fish (RAS) and plants (hydroponics) (Pinho *et al.* 2018; Lennard and Goddek, 2019). Aquaponic system production allows for a reduction in feed costs since the system provides a natural food source for the fish. (Blidariu and Grozea, 2011). Furthermore, aquaponic systems are considered the most efficient and sustainable production systems (Palm *et al.* 2019). They ensure good quality of water and there is less monitoring required (Rakocy *et al*. 2006). In order to optimise *Oreochromis mossambicus* production in aquaponic systems, the effect of feeding frequency, stocking density and recirculation rate have to be investigated.

1.3 RESEARCH JUSTIFICATION

South African freshwater aquaculture production is failing due water scarcity. Aquaponics presents a suitable replacement for conventional aquaculture since it allows for the optimal use of the recirculated water between the hydroponics and the aquaculture sub-systems during the simultaneous production of fish and plants. A low technology aquaponic system was chosen for this study because of its simple design, easy management and inexpensiveness. However, aquaponic system production using *Oreochromis mossambicus* has not been adequately investigated. Amongst the aquaponic systems being operated in South Africa, most of them are located in the Eastern Cape and Gauteng Province. No aquaponic systems production were reported in Limpopo Province, the location of the current investigation. *O. mossambicus* is the most commonly cultured species of *O. mossambicus* in South Africa. It is therefore important to evaluate the important factors that affect its production in aquaponic systems, and these include stocking density, feeding frequency and recirculation rate. Evidently there is paucity of information on the factors affecting *O. mossambicus* growth in aquaponic systems.

1.4 MAIN OBJECTIVE / AIM

To enhance fish production in low technology aquaponic systems

1.5 SPECIFIC OBJECTIVES

The objectives of the study are to determine:

I. The effect of feeding frequency on the growth performance of *O. mossambicus* in a low technology aquaponic system

II. The effect of stocking density on the growth performance of *O. mossambicus* in a low technology aquaponic system.

III. The effect of recirculation rate on the growth performance of *O. mossambicus* in a low technology aquaponic system.

1.6 NULL HYPOTHESIS

I. Feeding frequency does not affect the growth performance of *O. mossambicus* in low technology aquaponic systems.

II. Stocking density does not affect the growth performance of *O. mossambicus* in low technology aquaponic systems.

III. Recirculation rate does not affect the growth performance of *O. mossambicus* in low technology aquaponic systems.

1.7 DISSERTATION LAYOUT

Factors affecting the growth performance of *Oreochromis mossambicus* in a low technology aquaponic system. The dissertation has been divided into 6 chapters.

Chapter 1

The chapter introduced the research problem and highlighted the aim of the study. Specific objectives and the research hypothesis have also been outlined.

Chapter 2

The literature on the factors affecting the growth performance of *O. mossambicus* in low technology aquaponic systems was reviewed in this chapter.

Chapter 3

Growth parameters and somatic indices were used to determine the effect of feeding frequency on the growth performance of *O. mossambicus* in low technology aquaponic systems. Blood parameters were used to determine whether the different feeding frequencies caused any stress in the fish. The water quality parameters of the aquaponic systems were analyzed in table 3.1 and figures 3.4, 3.5, 3.6 and 3.7. The growth of *Spinacia oleracea* plants was analyzed in figures 3.8 and 3.9.

Chapter 4

Growth parameters and somatic indices were used to determine the effect of stocking density on the growth performance of *O. mossambicus* in low technology aquaponic systems. Blood parameters were used to determine whether the different stocking densities caused any stress in the fish. The water quality parameters of the aquaponic systems were analyzed in table 4.1 and figures 4.1, 4.2, 4.3 and 4.4. The growth of *S. oleracea* plants was analyzed in figures 4.5 and 4.6.

Chapter 5

Growth parameters and somatic indices were used to determine the effect of recirculation rate on the growth performance of *O. mossambicus* in low technology aquaponic systems. Blood parameters were used to determine whether the different recirculation rates caused any stress in the fish. The water quality parameters of the aquaponic systems were analyzed in table 5.1 and figures 5.1, 5.2, 5.3 and 5.4. The growth of *S. oleracea* plants was analyzed in figures 5.5 and 5.6.

Chapter 6

This chapter evaluated the factors affecting the growth performance of *O. mossambicus* in low technology aquaponic systems. Recommendations were suggested and the conclusion of the study was highlighted.

1.8 SIGNIFICANCE OF THE STUDY

This research will contribute to the advancement of knowledge required for the optimisation of aquaponics system production of *Oreochromis mossambicus* since the factors affecting the growth performance of this species will be elucidated. This study will play a fundamental role in advancing productivity and environmental sustainability of aquaponics for future practices for research purposes or in commercial and subsistence farming.

1.9 LIMITATIONS OF THE STUDY

Ideally, this experiment should have been a 3*3*3 factorial design. Feeding frequency, stocking density and recirculation rate should have been evaluated concurrently. However, due to limitations of resources, it was practically impossible to do the 3*3*3 factorial design.

CHAPTER 2: LITERATURE REVIEW

2.1 LITERATURE REVIEW

2.1.1 Aquaponic system production worldwide and in South Africa

Aquaponics is being practiced in at least 43 countries around the world and on every continent. According to Love *et al*. (2014), the majority of the aquaponics practitioners worldwide were found in the United States of America and were practicing aquaponics as a hobby. Love *et al*. (2014) indicated that the main reason cited by most of the aquaponics practitioners for engaging in aquaponics was subsistence farming. The most common fish cultured in aquaponic systems were *O. mossambicus* and catfish. The plant species of choice by most of the aquaponics participants was lettuce (Love *et al*. 2014).

In Europe, about 68 practitioners from 21 countries were engaging in aquaponics production (Villarroel *et al*. 2016). The aquaponic systems featured *O. mossambicus* and catfish as the fish species of choice. For plant species, herbs and lettuce were commonly used (Villarroel *et al*. 2016). According to Villarroel *et al*. (2016), around 67% of the practitioners were able to produce less than 100 kg of fish per annum and only 30.3% could produce over 1000 kg of fish per annum. In terms of plant production, 55% participants were able to produce less than 100 kg/annum and just 2.3% of participants were able to produce over 10 000 kg/annum (Villarroel *et al*. 2016).

A total of 82 publications on aquaponics were found from 15 African countries. Egypt, South Africa and Kenya (23, 20, and 14 publications, respectively) are the countries that appear to have widely adopted the technology in Africa (Obirikorang *et al*. 2021). According to Obirikorang *et al*. (2021), the rates of adoption of aquaponics practices in the different countries appear to correlate with the scale and development of aquaculture. Countries including Egypt, Nigeria, and Kenya are the major contributors to the continent’s aquaculture production.

In the Northern Africa, aquaponics production is quite established in Egypt, with over 22 publications (Obirikorang *et al*. 2021). The production mostly focused on integrating Nile tilapia (*Oreochromis niloticus*) and olives. In the Western Africa, Ghana and Nigeria are on the forefront when it comes to aquaponics. In Ghana, the implementation of aquaponic systems is slow and reports on aquaponics are very scarce. Fish production was integrated with maize as a plant species of choice (Obirikorang *et al*. 2021). In Nigeria, only 9 publications were made on aquaponics. Aquaponics is a recent concept in East Africa. Most of the East African aquaponics practices are concentrated in Kenya. The hydroponics components of most Kenyan aquaponic systems are sweet wormwood, pigweed, and pumpkin, while the RAS component is Nile tilapia.

In the Southern Africa, aquaponics is practiced in South Africa, Zimbabwe and Namibia (Obirikorang *et al*. 2021). Aquaponics production in Namibia and Zimbabwe is still under development. In Namibia, most aquaponic systems featured tilapia and koi as the fish species.

In South Africa (the focus of the current investigation), aquaponics is an emerging but rapidly evolving practice. According to Mchunu *et al*. (2018), aquaponics production in South Africa has been practiced as a hobby or for subsistence farming, rarely for commercial farming. This explains the little knowledge and development of aquaponics in the country. The plant component of most aquaponic systems in South Africa comprises mostly salad greens, lettuce and spinach (Mchunu *et al*. 2018). Species of tilapia and trout are the commonly raised fish in typical South African aquaponic systems (Mchunu *et al*. 2018).

2.1.2 Tilapia (*Oreochromis mossambicus*) as a fish species of choice for aquaponics

Oreochromis mossambicus (Figure 2.1) is the most commonly cultured tilapia species is South Africa. According to (Alameen *et al*. 2023), *O. mossambicus* is an important aquaculture commodity. The Mozambique tilapia (*O. mossambicus*) industry is the second largest tilapia industry based on its production and exportation rates (Arumugam *et al.* 2023). Masabni *et al*. (2020), listed *O. mossambicus* as one of the excellent fish species for aquaponics. It is exceptionally hardy and can tolerate a wide range of environmental conditions and high stocking densities (Masabni *et al*. 2020), making it highly adaptable for aquaponics production (DAFF, 2018). It is an omnivorous fish that is extensively used in aquaponics (Somerville *et al*. 2014). Additionally, it can survive at low dissolved oxygen concentrations and shows relatively fast growth and efficient food conversion (Hamdy *et al*. 2022). Tilapia species are the most used fish in aquaponic systems for their high availability, fast-growth, stress and disease resistance (Sonia and Roopavathy, 2021). It is easy to breed, has fast growth and eats a cheap vegetarian diet (Abizaka *et al*. 2021; Sonia and Roopavathy, 2021). Although Nile tilapia (*Oreochromis niloticus*) is a more popular fish species of choice for producers, it is somewhat challenging to culture it due to required tedious permits and regulations. that are required (DAFF, 2018). This is due to its classification as an alien invasive species. Mozambique tilapia is native to South Africa, which makes permit applications easier for producers to attain (DAFF, 2018) and the fish species of choice for this study.

Figure 2.1: *Oreochromis mossambicus*.

2.1.3 The factors affecting fish production in aquaponic systems

2.1.3.1. Feeding frequency

Feeding frequency is an important feeding strategy in fish culture. Appropriate feeding frequency reduces feed waste and resulting water quality deterioration while ensuring improved growth of fish. Feeding frequency varies among fish species. The optimum feeding frequency of *Oreochromis niloticus* has been reported to be 3 times daily by Ahsan *et al*. (2009). Jegede and Olorunfemi (2013) also observed the best growth performance of *Oreochromis niloticus* at a feeding frequency of 3 times daily. However, Nasrin *et al.* (2021) stated that the optimum feeding frequency of the same species was 2 times daily. In a similar study, Dediu *et al*. (2011) reported that that the optimum feeding frequency of rainbow trout (*Oncorhynchus mykiss*) was 2 times daily. The optimum feeding frequency of *Clarias gariepinus* was found to be 3 times daily in a study conducted by Aderolu *et al*. (2010). *O. mossambicus* are herbivorous fish which have smaller stomachs with limited storage capacity for food, allowing them to consume small chunks of food at a time (Fava *et al*. 2022). They require more frequent meals are required to meet their nutritional needs due to the nature of their digestive systems and the fact that plant materials often contain lower energy concentrations. African catfish (*Clarias gariepinus*) are considered omnivores, meaning they can consume both plant and animal matter (Fava *et al*. 2022). Their feeding frequency can vary depending on the availability and digestibility of the food sources they encounter. *Oncorhynchus mykiss* are carnivorous fish. They have large stomachs that allow storage of large chunks of food at a time, making them require less frequent feeding (Davis and Hardy, 2022).

Fish size can have a consequential impact when determining the appropriate feeding frequency of fish in fish production systems. According to (Alal, 2018), the optimum feeding frequency for *Oreochromis niloticus* weighing 4 g was 3 times daily. Similarly, Jegede and Olorunfemi (2013) recorded an optimum feeding frequency of 3 times daily in fish of a similar size (3 g). Ahsan *et al*. (2009) also observed an optimum feeding frequency of 3 times daily, however, it was in *O. niloticus* weighing 35 g. However, Chen *et al*. (2014) observed an optimum feeding frequency for *O. niloticus* weighing 3.7 g to be 2 times daily. In another study, Ferdous *et al*. (2014) observed an optimum feeding frequency of 4-5 times daily in three-day old *O. niloticus* fry. Young, juvenile fish generally have higher metabolic rates and growth rates compared to mature fish. They require more frequent feedings to support their rapid growth and energy demands. As fish grow and reach intermediate sizes, their metabolic rates and feeding requirements might stabilize somewhat. Feeding frequency can often be reduced compared to juvenile stages. Mature fish generally have lower metabolic rates and slower growth rates (Esmail and Serluca 2015).

Another factor that can have a direct impact of the feeding frequency of fish is the aquaculture system design. Nasrin *et al*. (2021) recorded an optimum feeding frequency of 2 times daily in pond hapa. Similarly, Chen *et al*. (2014) also stated that the optimum feeding frequency of *O. niloticus* in pond hapa was 2 times daily. However, in a study conducted by Ahsan *et al*. (2009), the optimum feeding frequency of *O. niloticus* in pond hapa was found to be 3 times daily. Thongprajukaew *et al*. (2017) observed the best performance of *O. niloticus* at a feeding frequency of 2 times daily in a recirculating aquaculture system. Falaye and Omoike (2013) recorded a feeding frequency of 2 times daily for *O. niloticus* in glass aquaria. In earthen ponds, feeding frequency can be lower compared to more controlled systems like RAS and aquaponics. Fish in ponds often have access to natural food sources such as algae and insects, potentially reducing the need for supplementary feeding (Stankovic *et al*. 2010). RAS are more controlled environments, and fish in RAS often rely more on the feed provided by the farmer. Feeding frequency in RAS is typically higher compared to ponds due to the need to maintain optimal growth rates and nutrient levels. Fish in RAS might be fed multiple times a day, usually 2 to 4 times. Aquaponic systems rely on nutrient cycling between fish and plants. In aquaponics, feeding frequency can vary based on the fish and plant types, system design, and nutrient cycling efficiency (Stathopoulou *et al*. 2021).

The optimum feeding frequency of juvenile *Oreochromis mossambicus* in low technology aquaponic systems has not been adequately investigated. Therefore, this study will investigate the most effective feeding frequency of juvenile *O. mossambicus* in low technology aquaponic systems.

2.1.3.2. Stocking density

Stocking density is an important culture practice in aquaculture. Suitable stocking densities prevent deterioration of water quality, ensuring suitable growth of fish. Stocking densities vary among fish sizes. Wu *et al*. (2018) stated that the best stocking density for optimal growth performance of *Oreochromis niloticus* was 10 fish per tank in 12 g fish. Opiyo *et al.* (2014) recorded an optimum stocking density of 0.32 g *Oreochromis niloticus* to be 2 fish/L. However, Rahmatullah *et al*. (2010) indicated that optimum stocking density of *Oreochromis niloticus* of similar size (0.76 g) was 106 fish/m^3. Smaller fish require less oxygen and produce less waste compared to larger fish. As fish grow, their oxygen demand and waste production increase. Smaller fish can generally be stocked at higher densities compared to larger fish to maintain water quality and oxygen levels within acceptable ranges.

Another factor that can impact stocking density of fish is the fish species. According to Sabwa *et al*. (2022), the best stocking density for growth of *O. niloticus* is 150 fish/m^3. However, in a study by (Kunda *et al*. 2021), the optimum stocking density of *Oreochromis niloticus* was observed to be 40 fish/m^3. Shubha and Reddy (2011) recorded the best stocking density of 10 fish/aquarium in *Oreochromis mossambicus*. Nuwansi *et al.* (2021) indicated that the best stocking density for *Cyprinus carpio* is 2.1 kg/m^3. However, the optimum stocking density of *Cyprinus carpio* according to Maucieri *et al.* (2019) was 2.5 kg/m^3. Daudpota *et al.* (2014) reported that best stocking density for red tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*) is 200 fry/hapa. The choice of fish species has a profound effect on the appropriate stocking density in aquaculture systems. Different fish species have varying growth rates, behaviors, environmental requirements, and sizes at maturity. Fast-growing species, such as *O. mossambicus* or *C. gariepinus*, reach market size relatively quickly. They might be stocked at higher densities due to their shorter time to harvest. Slow-growing species, like some types of trout or certain ornamental fish, require more time to reach a desirable size. Lower stocking densities might be necessary to allow for proper growth. Fish species with aggressive behaviors or territorial tendencies might require lower stocking densities to reduce stress and minimize competition for resources. Species that school or exhibit more peaceful behaviors might tolerate higher stocking densities.

The appropriate stocking density of fish can be impacted by the aquaculture system design. Sabwa *et al*. (2022) indicated that the best stocking density for growth of *O. niloticus* in aquaponic systems is 150 fish/m^3. However, Rahmatullah *et al*. (2010) found that 106 fish/m^3 is the optimum stocking density of *Oreochromis niloticus* in aquaponic systems. Al-Tawaha *et al.* (2021) stated that 8 kg/m^3 is the best stocking density for *O. niloticus* in aquaponic systems. In another study, Kapinga *et al.* (2014) found that a stocking density of 3fish/m^2 is for *O. niloticus* in ponds. However, Kapinga *et al.* (2014) found that that the preferred stocking density of *O. niloticus* in ponds is 3fish/m^2. Abaho *et al*. (2020) observed better performance of *O. niloticus* in cage culture at a stocking density of 200 fish/m^3. Contrasting observation that the optimum stocking density of *Oreochromis niloticus* is 40 fish/m^3 was made by Kunda *et al*. (2021). Earthen ponds are larger in size and rely on natural processes for nutrient cycling and waste dilution. Stocking densities in ponds are generally lower compared to other systems due to the larger water volume available for fish movement and waste dispersal. RAS are designed for intensive fish production in a controlled environment with advanced filtration and water treatment. Stocking densities in RAS can be higher compared to ponds due to the ability to manage water quality more precisely. Aquaponic systems combine aquaculture and hydroponics, where fish waste provides nutrients for plant growth. Stocking densities in aquaponics are often lower compared to standalone aquaculture systems due to the shared nutrient load between fish and plants. Despite its growing popularity, the stocking density of *Oreochromis mossambicus* in aquaponic systems has not been investigated before. This study will determine the optimum stocking density of *O. mossambicus* in a low technology aquaponic system.

2.1.3.3. Recirculation rate

The recirculation rate can have an impact on the growth and overall health of fish in aquaculture settings. The selection of appropriate recirculation rates for fish production systems can depend on the size of the fish. Obirikorang *et al*. (2019) indicated that for optimal growth of *Oreochromis niloticus* weighing 27 g, the best recirculation rate was 1.5 L/min. In a different study, Hussain *et al.* (2014) stated that the best recirculation rate for the optimal growth of *C. carpio* weighing 6 g was 1.5 L/min. Larger-sized fish generally have higher metabolic rates and oxygen demands compared to smaller fish. Larger fish might require higher recirculation rates to ensure adequate oxygenation of the water. Larger fish produce more waste, primarily in the form of ammonia. Higher waste production might necessitate higher recirculation rates to prevent waste buildup and maintain water quality.

Another factor that can affect the appropriate recirculation rates can is the fish species. According to (Endut *et al*. 2009), the best recirculation rates for growth of *Clarias gariepinus* was 1.6 L/min. Hussain *et al.* (2014) reported that the optimum recirculation rate for *C. carpio* was 1.5 L/min. However, Nuwansi *et al*. (2015) observed the best performance of *C. carpio* at a recirculation rate of 0.8 L/min. In another study, Obirikorang *et al*. (2019) stated that the best flow rate for *Oreochromis niloticus* was 1.5 L/min. Diem *et al*. (2017) stated that the best recirculation rates for growth of *Oreochromis niloticus* was 4 tank volumes/h. Different fish species produce varying amounts of waste. Species that produce more waste might require higher recirculation rates to prevent waste buildup. Fish species with more active feeding behaviors might produce more waste and influence the nutrient load in the system. More active feeders might necessitate higher recirculation rates to manage waste and nutrient levels.

The appropriate recirculation rates can be determined by the aquaculture system design. The best recirculation rates for growth of *Oreochromis niloticus* in aquaponic systems was found to be 4 tank volumes/h in a study by Diem *et al*. (2017). However, Hussain *et al.* (2014) reported a recirculation rate of 1.5 L/min to be the optimal for growth of *Cyprinus carpio* in aquaponic systems. Obirikorang *et al*. (2019) reported that the best recirculation rate for optimal growth of *Cyprinus carpio* in recirculating aquaculture systems was 1.5 L/min. In a different experiment, Endut *et al*. (2009) indicated that the optimum recirculation rates for *Clarias gariepinus* in aquaponic systems was 1.6 L/min. RAS technology enables controlled water quality, potentially allowing for lower recirculation rates due to efficient waste removal. Recirculation rates in pond systems are generally lower due to the dilution effect of larger water volumes. Floating cage systems are located in natural water bodies and rely on water exchange while submersible cage systems might require higher recirculation rates due to reduced water flow and nutrient removal. Tank systems might require moderate recirculation rates to ensure consistent water quality and waste removal. Recirculation rates in aquaponic systems are influenced by nutrient cycling between fish waste and plant uptake.

Appropriate recirculation rates are important in an aquaponic system to prevent the deterioration of the water quality. The appropriate recirculation rates for *Oreochromis mossambicus* cultured in an aquaponic system has not been investigated before.

2.1.4 Low technology aquaponic systems

Low technology aquaponic systems can be characterised by the simplest hydroponics section with the capacity to act as biofilter (Maucieri *et al*. 2019). Low technology aquaponic systems are based on a simple design, easy management, and low capital costs and they are suitable for implementation in various environments or settings (Birolo *et al*. 2020). The absence of power for a specialized heating system, lack of probes for the continuous evaluation of water quality or for remote management or the absence of devices (UV, ozone) for water sanitation make it less complex and inexpensive (Maucieri *et al*. 2019). The lack of a specialized biofilter may result in poor establishment of microbes in the system. The lack of water sanitation devices may present a problem in a case whereby the nutrient uptake by plants isn't enough to improve the quality of the water. Low technology aquaponic systems vary based on the type of hydroponics sub-system used. Floating raft or deep-water culture involves growing plants on pieces of Styrofoam, which glide on the exterior of water-filled grow beds (Sharma *et al*. 2022). The problem with a floating raft system, the roots of the plants are directly floating in the water, exposing them to diseases. In a flood and drain aquaponic system, plants are grown in an intermediate-filled grow bed (Sharma *et al*. 2022). The substrate medium acts as a biological as well as mechanical filter, which helps with better maintenance of plants cultivated. In nutrient film technique (NFT) systems, plants are grown in pipes through which a small amount of water is continually flowing between the hydroponics and the aquaculture sub-systems (Sharma *et al*. 2022).

As it has been established in this literature review, there is a shortage of information on the most effective management strategies for *Oreochromis mossambicus* in low technology aquaponic systems. This includes feeding frequency, stocking density and recirculation rates, all of which will be the focus of the present study.

CHAPTER 3: EFFECT OF FEEDING FREQUENCY ON THE GROWTH PERFORMANCE OF *OREOCHROMIS MOSSAMBICUS* IN A LOW TECHNOLOGY AQUAPONIC SYSTEM

3.1 INTRODUCTION

The success of any fish production system depends on market demand and cost of production (Zafar *et al*.2017). The largest portion of the operational costs in fish production lies in feed (Chepkirui *et al*. 2022). The feeding frequency and amount of food have the highest impact on the water quality (Abdel-Aziz *et al*. 2021) and as a result the stocking density of aquaponic systems (Yildiz *et al*. 2013). It forms part of the different feed management practices that play an important role in regulating the feed intake, minimizing feed wastage, and proven to maximize the benefit of feeding and fish growth (Stathopoulou *et al*. 2021). The determination of an optimum feeding frequency for fish is very important to ensure optimal fish growth, survival, improved immunity and stress resistance (Stathopoulou *et al*. 2021). In aquaponic systems, a properly selected diet must be managed in a way that allows it to meet the nutritional requirements of the fish and plant species integrated in the system. The rate of ammonia and phosphorus production in aquaponic systems depends on feeding frequency, the amount of food and the protein composition of the food. The concentration of ammonia and phosphorus are derived from excess feed and metabolic waste (Stathopoulou *et al*. 2021). Selecting the appropriate feeding frequency and daily feeding rate, can help enhance the growth of fish and reduce feed loss, ultimately improving the water quality of in an aquaponic system (Stathopoulou *et al*. 2021; Liang and Chien, 2013).

The removal of fish metabolic products (nutrients) from the water by plants is directly related to the quantity and quality of the diet, as well as the feeding frequency. Knowledge about the optimum feeding frequency for specific species and size of fish is also important for preventing water quality deterioration as a result of overfeeding. In aquaculture, both overfeeding and underfeeding can have a detrimental effect on the fish health and may cause a marked deterioration in water quality, reduced weight, poor food utilization, and increased susceptibility to infection (Jegede and Olorunfemi, 2013). This can lead to decreased fish and plant production in aquaponic systems. Among the studies that were conducted on feeding frequency in aquaponics, Stathopoulou *et al*. (2021) and Liang and Chien, 2013) focused on *Dicentrarchus labrax* and *Oreochromis niloticus* respectively as their fish species of choice. There is a scarcity of information on the feeding frequency of *O. mossambicus* in low technology aquaponic systems. In literature there is a lot of contradictory information on the optimum feeding frequency of *O. mossambicus*. Jegede and Olurunfemi (2013) suggested a feeding frequency of thrice daily for the best performance of *O. niloticus,* while Nasrin *et al*. (2021) and Ferdous *et al*. (2014) suggested 2 times daily and 4-5 times daily respectively.

3.2 OBJECTIVE: To determine the effect of feeding frequency (once, twice and thrice daily) on the growth performance of *O. mossambicus* in low technology aquaponic systems.

3.3 NULL HYPOTHESIS: Feeding frequency does not affect the growth performance of *O. mossambicus* in low technology aquaponic systems.

3.4 METHODOLOGY AND ANALYTICAL PROCEDURES

3.4.1 Study site

This study was carried out at Aquaculture Research Unit (ARU), University of Limpopo, South Africa. *Oreochromis mossambicus* juveniles were bred at the Aquaculture Research Unit and kept in the hatchery tanks. Before the commencement of the experiment, fish were collected from the hatchery tanks and placed in a 100 L container with water that was mixed with 2 phenoxyethanol (1 mL/L) as an anaesthetic. Fifteen fish were randomly stocked into 500 L fiberglass experimental tanks in a greenhouse (Filled to 400 L mark). The tanks were individually heated with a submersible aquarium heater (ViaAqua Glass heater, 200 W) and aerated with airstones. Fish were left to acclimatise in experimental tanks for 14 days while feeding on commercial pellets (Aqua-plus, Avi Products (Pty) Ltd). After the acclimatisation period, fish were anesthetised, and the initial body weight was recorded for each tank. The *Spinacia oleracea* seedlings were purchased from Greener Tidings Garden Centre in Polokwane.

3.4.2 The integrated aquaponics design

The aquaponic system (Figure 3.1 and Figure 3.2) consisted of two sub-systems, the hydroponics sub-system (plants) and the aquaculture sub-system (fish). The aquaculture sub-system consisted of 500 L fibre glass tanks attached to a WATER PUMPS, 550 W, 450 V mechanical pump (Model no. 140616409) with recirculation rates of 35 L/min. The mechanical pump allowed for the recirculation of water between the aquaculture sub-system and the hydroponics sub-system through PVC pipes of 2.93 cm diameter that connected the two sub-systems. The area where the aquaculture tank attached to the mechanical pump was covered by a mash wire to prevent fish from getting sucked by the pump and killed in the process. The fibre glass tanks were filled with aged tap water and aerated using air stones connected to a Greenco air blower, Model No. BN 130328 21/0093, 3470/min. The water temperature of the system was maintained at 28°C using ViaAqua 300 W glass immersion heaters (Model No.VA 300G) in the fibre glass tanks. The hydroponics sub-system consisted of PVC pipes of 110 mm diameter, each consisting of 18 round holes (50 mm diameter) where the spinach (*Spinacia oleracea*) seedlings were planted at a planting density of 18 plants/hydroponics sub-system. Each aquaponic system consisted of 3 hydroponics sub-systems connected to one aquaculture sub-system.

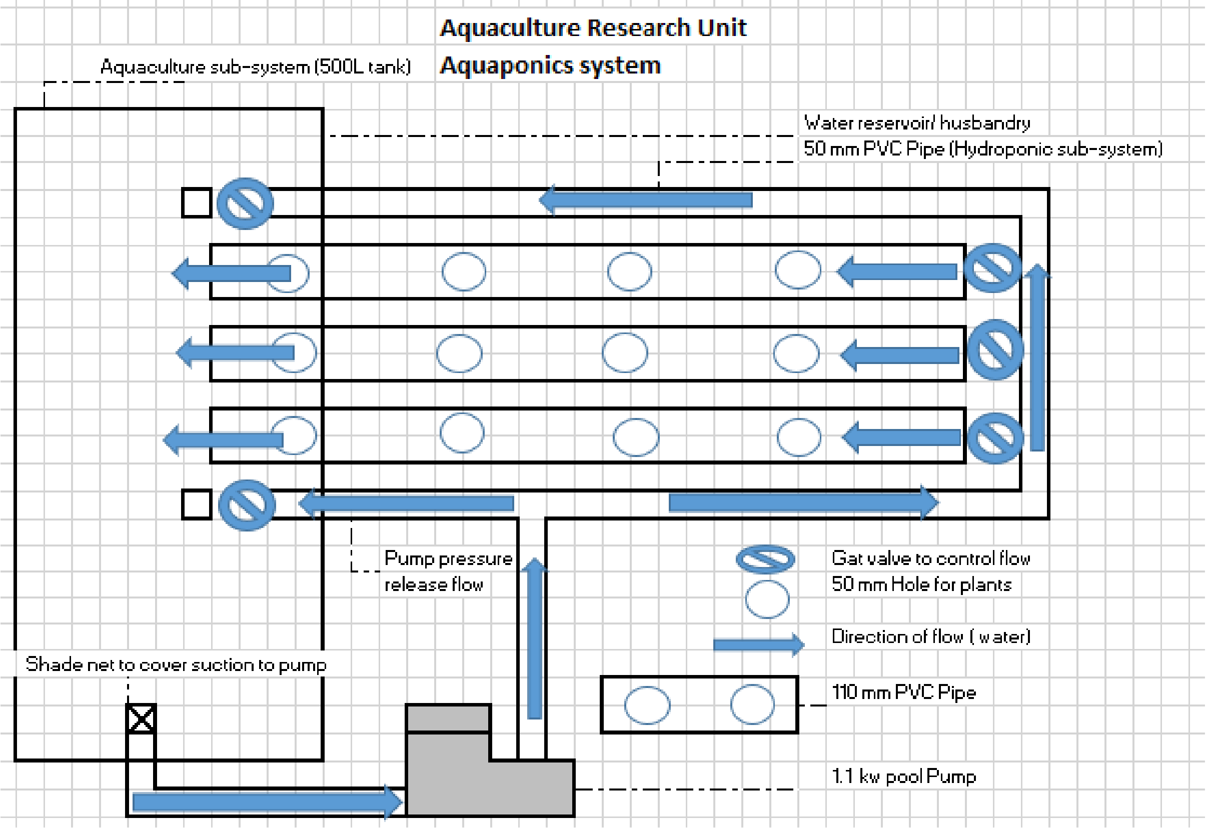

Figure 3.1: The schematic diagram of the integrated aquaponic system.

Figure 3.2: The experimental setup in a greenhouse.

3.4.3 The experimental design

The experiments were carried out in a low technology aquaponic system in a greenhouse. A total of 270 *Oreochromis mossambicus* juveniles with mean weight 37.68±4.14 g were stocked at a density of 3 kg/m^3 in 9 fibreglass tanks (500 L) in a completely randomised design. The experiment was replicated 3 times. The fish were fed 4% of their body weight with pelleted commercial feed over a duration of 28 days. The pellet size was 3 mm. The experiment consisted of three treatments of feeding frequency; T1, T2 and T3, which were assigned once a day at 13h00, twice a day at 08h00 and 18h00, and thrice a day at 08h00, 13h30 and 18h00 respectively. The proximate composition of the fish feed was determined in (Table 3.1). On the 7th, 14th and 28th day of the experiment, fish were weighed using an IGC 201305890 digital balance (Model: HK-HW-3A), maximum weight: 3 kg. The feed allowance was adjusted with the changing weight of fish. The *Spinacia oleracea* plants were planted in hydroponics sub-systems made up of PVC pipes at a planting density of 18 plants/system. All ethical protocols were observed and approved by the University of Limpopo Animal Research Ethical Committee (AREC/23/2023: PG).

Table 3.1: Proximate composition of the commercial *O. mossambicus* diet used in this study.

Ingredients	**g/kg**
Protein	450
Lysine	28
Fat	140
Moisture	100
Crude Fibre	30
Calcium	30
Phosphorus	7

3.4.4 Water quality parameters

Water quality parameters including water temperature, pH, dissolved oxygen (DO) and total dissolved solids (TDS) were monitored daily using a Horiba U-50 multi-parameter water quality meter. Nitrate, nitrite, ammonia and phosphorus were analysed in the laboratoty at Aquaculture Research Unit. The analysis of nitrate, nitrite and ammonia was according to the Cadmium reduction method, the USEPA Diazotization method and the USEPA Nessler method respectively (HACH, 2012). Phosphorus analysis was according to the USEPA PhosVer 3 (Ascorbic acid method) (HACH, 2012). The water quality parameters were recorded on the 7th, 14th and 28th day of the experiment.

3.4.5 Growth performance of *Oreochromis mossambicus*

3.4.5.1 Growth parameters

At the end of the experiment, fish were starved for 24 h before growth performance indices were calculated. Three fish from each replica (9 fish per treatment) were randomly selected for calculation of growth parameter indices. In order to evaluate the growth performance of the fish at the end of the experiment, % weight gain (%WG), specific growth rate (SGR), feed conversion ratio (FCR), thermal growth coefficient (TGC), and survival rate (SR) were calculated.

a) The %weight gain (%WG) of the experimental fish was determined using the following equation:

$$\%\mathrm{WG} = \frac{\mathrm{W_f} - \mathrm{W_i}}{\mathrm{W_f}} \times 100$$

Where W_i is the initial weight of the fish (g) and W_f is the final weight of the fish (g).

b) The specific growth rate (SGR) of the experimental fish was determined using the following equation:

$$\mathrm{SGR} = \frac{\ln \mathrm{W_f} - \ln \mathrm{W_i}}{\mathrm{T}} \times 100$$

Where W_i is the initial weight of the fish (g), W_f is the final weight of the fish (g), In is the natural logarithm and T is the feeding period of the experiment (in days).

c) The thermal growth coefficient (TGC) of the experimental fish was determined using the following equation:

$$\mathrm{TGC} = \frac{(W_f)^{-0.333} - (W_i)^{-0.333}}{\mathrm{T} \times \mathrm{t}} \times 1000$$

Where W_i is the initial weight of the fish (g), W_f is the final weight of the fish (g), T is temperature in (°C) and t is the feeding period of the experiment (in days).

d) The feed conversion ratio of the experimental fish was determined using the following equation:

$$\mathrm{FCR} = \frac{\text{feed consumed (g)}}{\text{weight gained (g)}}$$

Where FCR is the fish conversion ratio.

e) The survival rate (SR) of the experimental fish was determined using the following equation:

$$\mathrm{SR} = \frac{\text{fish stocked} - \text{mortality}}{\text{fish stocked}} \times 100$$

3.4.5.2 Organosomatic indices

Fish were collected from experimental tanks and sedated with 2-phenoxyethanol (1 ml/L). Three fish from each dietary replica (9 fish/diet) were used for organosomatic indices. Organosomatic indices including condition factor (CF), hepatosomatic index (HSI) and viscerosomatic index (VSI) were also calculated to determine the physiological condition of the fish. The organosomatic indices were measured at the end of the experiment. HSI and VSI were measured on the last day of the experiment.

a) The condition factor (CF) of the experimental fish was determined using the following equation:

$$\mathrm{CF} = \frac{\mathrm{W}}{\mathrm{L}^3} \times 100$$

Where W is the weight of the fish (g), and L is the length of the fish (cm).

b) The hepatosomatic index (HSI) of the experimental fish was determined using the following equation:

$$\mathrm{HSI} = \frac{\text{liver weight (g)}}{\text{total weight (g)}} \times 100$$

c) The viscerosomatic index (VSI) of the experimental fish was determined using the following equation:

$$\mathrm{VSI} = \frac{\text{viscera weight (g)}}{\text{total weight (g)}} \times 100$$

3.4.5.3 Haematological parameters

At the end of the trial, fish were starved for 24 h. Three fish samples from each replica were randomly selected and sedated with 2-phenoxyethanol (1 ml/L). The blood samples were drawn from the caudal vasculature of the fish using sterile 1.0 mL syringes (Figure 3.3). The needle size was 21G×11/2 inch. The blood was then stored in 4.0 mL heparinised and non-heparinised tubes. The blood stored in the heparinised tubes (Figure 3.3) was for the full blood count while the one stored in the non-heparinised tubes was for the serum chemistry. The blood samples were then taken for analysis at Lancet laboratory, Polokwane. The blood was analysed for Red Blood Cell count, White Blood Cell count, Haematocrit, Haemoglobin and serum glucose. Using the mean values for RBC count, WBC count, Haematocrit and Haemoglobin, the Blood performance of the fish was calculated.

a) The Blood performance (BP) of the experimental fish was determined using the following equation:

$$\mathrm{BP} = \ln \mathrm{Hct} + \ln \mathrm{Hb} + \ln \mathrm{RBC\ count} + \ln \mathrm{WBC\ count}$$

Where Ht is the haematocrit (%), Hb is the haemoglobin (g/dL), RBC is the white blood cell count ($x10^5/mm^3$) and WBC is the white blood cell count ($x10^3/mm^3$).

Figure 3.3: Blood collection for haematological analysis.

3.4.6 Growth performance of *Spinacia oleracea*

The shoot length of the *Spinacia oleracea* plants was measured from day 7 and weekly thereafter. The length of the main plant from the bedding media of the container to the top of the main plant stem was measured using a ruler. The number of leaves on each *S. oleracea* plant including the new leaves just beginning to emerge were counted from day 7 and weekly thereafter.

3.4.7 Statistical analysis

Analysis of variance (ANOVA) and Tukey’s post hoc analysis were carried out for the analysis of the effect of feeding frequency on the growth performance of *O. mossambicus*. For analysis of fish growth data one way ANOVA was used. Two-way ANOVA was used for the analysis of water quality parameters (ammonia, nitrite, nitrate, phosphorus pH, temperature, DO concentration and TDS levels) and plant growth data (number of leaves and shoot length). The data recorded in this study met the assumptions of ANOVA based on Levine’s test. The statistical analysis was carried out using the Statistical Package for the Social Sciences (IBM SPSS, version 22.0: 2020). All statistical analysis was performed at the 5% significance level.

3.5 RESULTS

3.5.1 Water quality parameters

Feeding frequency did not have a statistically significant effect ($p > 0.05$, ANOVA) on the pH and dissolved oxygen (DO) concentrations (Table 3.2). The pH range was from 6.99 to 7.20 while the DO concentrations ranged from 5.70±0.55 mg/L to 8.60±0.48 mg/L. The DO concentrations decreased with the feeding frequency, with the lowest concentration observed in T1.

The increasing feeding frequency resulted in an increase in the total dissolved solids (TDS) levels (Table 3.2). The TDS levels ranged from 57.55±7.33 mg/L to 213.93±27.37 mg/L. TDS levels recorded between all feeding frequency treatments were significantly different ($p < 0.05$, ANOVA), with the highest TDS recorded in T3.

Table 3.2: pH, DO conc. and TDS levels (mean±SD) recorded on day 7, day 14 and day 28 of the experiment in aquaponic systems stocked with *O. mossambicus* fed at different feeding frequencies for a duration of 28 days.

WQ	**T1**			**T2**			**T3**		
	Day 7	Day 14	Day28	Day 7	Day 14	Day 28	Day 7	Day 14	Day28
pH	7.03^{a}	7.02^{a}	7.16^{a}	6.99^{a}	7.01^{a}	7.16^{a}	7.06^{a}	7.05^{a}	7.20
DO (mg/L)	8.60 ± 0.48^{a}	8.50 ± 0.47^{a}	6.60 ± 0.60^{a}	7.90 ± 0.18^{a}	7.60 ± 0.20^{a}	6.60 ± 0.20^{a}	7.40 ± 0.10^{a}	7.30 ± 0.12^{a}	5.70 ± 0.55^{a}
TDS (mg/L)	57.55 ± 7.33^{a}	86.02 ± 37.21^{a}	99.45 ± 40.56^{a}	63.75 ± 11.87^{b}	132.38 ± 13.48^{b}	156.72 ± 19.93^{b}	72.91 ± 4.22^{c}	168.57 ± 28.55^{c}	213.93 ± 27.37^{c}

Figures on the same row having the same superscript are not significantly different ($p>0.05$, ANOVA).

Ammonia (NH_3) concentrations ranged from 0.021 mg/L to 0.028 mg/L (Figure 3.4). The highest NH_3 concentration was recorded in T3. T1 and T2 recorded equal NH_3 concentrations on day 7 and day 28. There was no significant difference ($P > 0.05$, ANOVA) in NH_3 concentrations between the different feeding frequency treatments. In all feeding frequency treatments, NH_3 concentrations were increasing with the duration of the experiment.

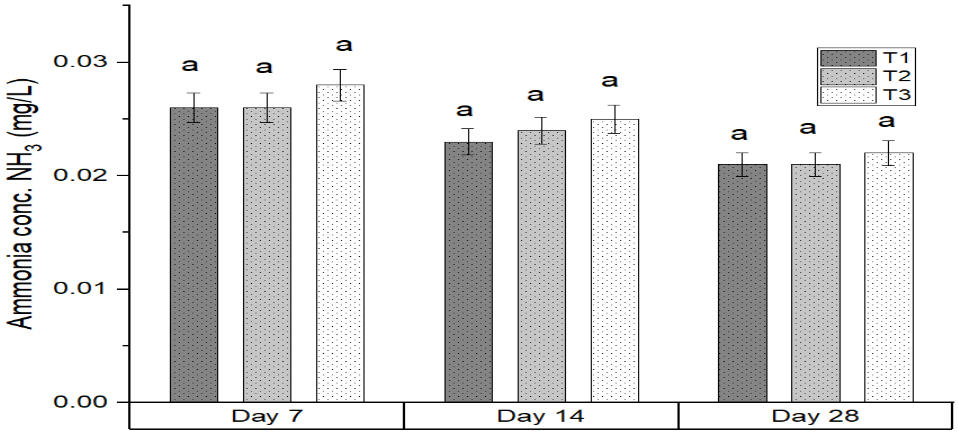

Figure 3.4: The ammonia (NH_3) concentrations recorded on day 7, day 14, and day 28 in aquaponic systems stocked with *O. mossambicus* fed at various feeding frequencies. Bars on the same day having the same letter are not significantly different ($p>0.05$, ANOVA).

The nitrite (NO_2^-) concentrations ranged from 0.019 mg/L to 0.072 mg/L (Figure 3.5). An elevation in NO_2^-concentrations were observed as feeding frequency increases. The highest NO_2^- concentration was recorded in T3, but the difference was not significant ($p > 0.05$, ANOVA) from T1 and T2. NO_2^- concentrations in all feeding frequency treatments decreased with the duration of the experiment.

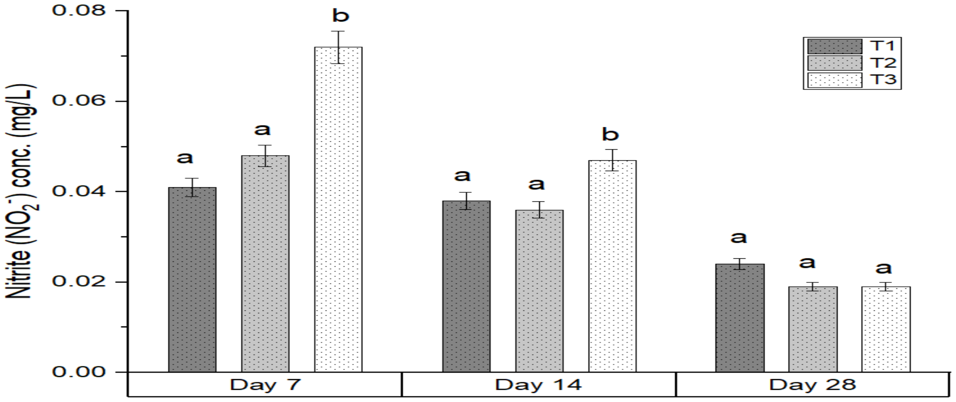

Figure 3.5: The nitrite (NO_2^-) concentrations recorded on day 7, day 14, and day 28 in aquaponic systems stocked with *O. mossambicus* fed at various feeding frequencies. Bars on the same day having the same letter are not significantly different ($p>0.05$, ANOVA).

The nitrate (NO_3^-) concentrations ranged from 1.7± mg/L o 3.30 mg/L (Figure 3.6). T3 recorded the highest NO_3^- concentration on day 7 and day 14, which was significantly different ($P < 0.05$, ANOVA) from T1 and T2. No significant difference ($P > 0.05$, ANOVA) in NO_3^- concentrations was observed between treatments on day 28.

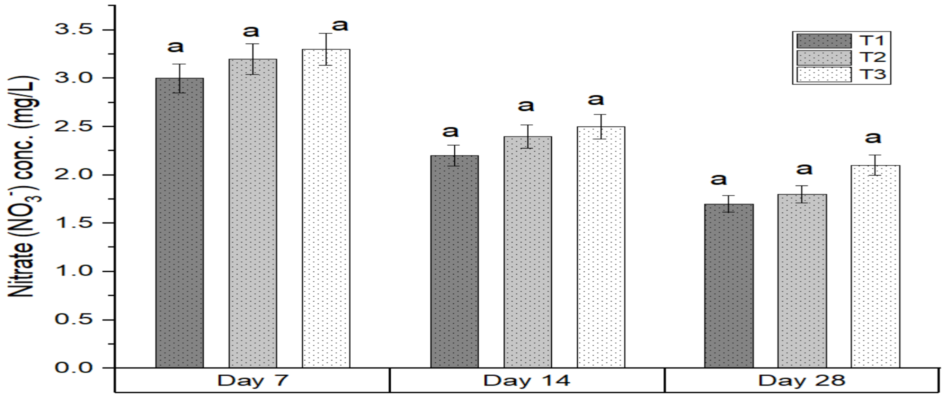

Figure 3.6: The nitrate (NO_3^-) concentrations recorded on day 7, day 14, and day 28 in aquaponic systems stocked with *O. mossambicus* fed at various feeding frequencies. Bars on the same day having the same letter are not significantly different (p>0.05, ANOVA).

The phosphorus (P) concentrations ranged from 0.06 mg/L to 0.16 mg/mL (Figure 3.6). T3 recorded the highest P concentration on followed by T2. A significant difference ($P < 0.05$, ANOVA) in P concentrations was observed between all treatments on day 7 and day 14. On day 28, no significant difference in P concentrations was observed between all feeding frequency treatments. In all feeding frequency treatments, P concentrations decreased with the duration of the experiment. However, P concentrations were not significantly affected ($p > 0.05$, ANOVA) by the duration of the experiment.

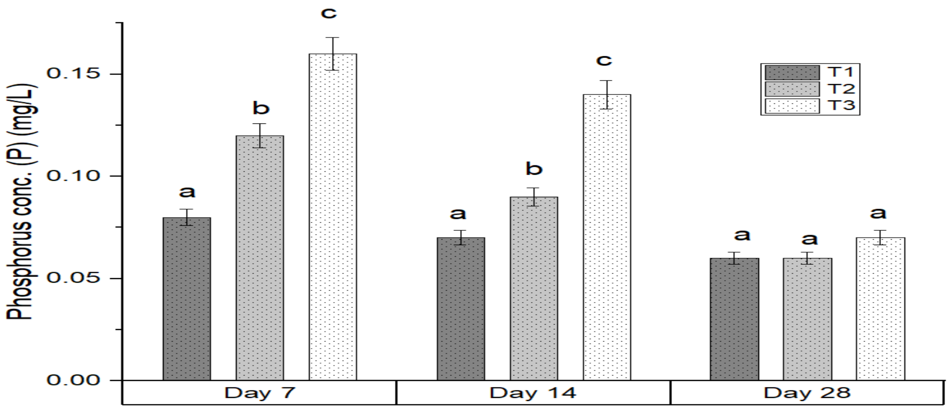

Figure 3.7: The phosphorus (P) concentrations recorded on day 7, day 14, and day 28 in aquaponic systems stocked with *O. mossambicus* fed at various feeding frequencies. Bars on the same day having the same letter are not significantly different (p>0.05, ANOVA).

3.5.2 Growth performance of *Oreochromis mossambicus*

Increasing feeding frequency was followed by an increase in final weights of *O. mossambicus* (Table 3.3). The highest final weight was recorded in T3 (68.70±4.51 g), which was significantly different ($p < 0.05$, ANOVA) from T1 and T2. The highest feeding frequency (T3) also recorded the highest ($p < 0.05$, ANOVA) % weight gain (57.59±6.4%) and specific growth rate (2.53±0.15). However, no significant difference ($p > 0.05$, ANOVA) in %WG and SGR was observed between T1 and T2 (Table 3.3). A similar trend was observed in thermal growth coefficient. The lowest food conversion ratio was observed in T3 (1.27±0.04) which was significantly different ($p < 0.05$, ANOVA) from T1 and T2 (Table 3.3). No significant difference ($p > 0.05$, ANOVA) in FCR was observed between T1 and T2. The increasing feeding frequency was followed by an increase in survival rates of *O. mossambicus* (Table 3.3). The highest SR was observed in T3 (98.89±1.92%) and the lowest in T1 (95.56±7.70%). There was no significant difference ($p > 0.05$, ANOVA) in SR between any of the feeding frequency treatments.

Table 3.3: Final weight, %weight gain, TGC, survival rate (mean ± SD) of *Oreochromis mossambicus* fed at varying feeding frequencies for the duration of 28 days.

Growth parameters	**T1**	**T2**	**T3**
Final weight (g)	47.79±4.60^a	48.15±3.67^a	68.70±4.51^b
% Weight gain	24.10±3.15^a	30.06±2.04^a	57.59±6.46^b
SGR	1.39±0.20^a	1.60±0.08^a	2.53±0.15^b
FCR	2.16±0.15^a	2.04±0.15^a	1.27±0.04^b
TGC	1.02±0.01^a	1.26±0.00^a	1.94±0.02^b
Survival Rate (%)	95.56±7.70^a	97.78±1.92^a	98.89±1.92^a

Figures on the same row having the same superscript are not significantly different (p>0.05, ANOVA).

3.5.3 Organosomatic indices

An increase in feeding frequency led to an increase in condition factor. However, there was no significant difference ($p > 0.05$, ANOVA) in CF between the different feeding frequency treatments. The increase in feeding frequency of *O. mossambicus* juveniles also resulted in an increase in hepatosomatic index (Table 3.4). The highest HSI was observed in T3 (0.87±0.05) and the lowest in T1 (1.37±0.34). No significant difference ($p > 0.05$, ANOVA) in HSI was observed between the different feeding frequency treatments. A similar trend was observed with viscerosomatic index.

Table 3.4: The HSI and VSI (mean ± SD) of *Oreochromis mossambicus* fed at different frequencies for a period of 28 days.

Organosomatic indices	**T1**	**T2**	**T3**
Condition factor	1.20±0.09^a	1.19±0.16^a	1.17±0.15^a
Hepatosomatic index (%)	0.87±0.05^a	1.40±0.24^a	1.37±0.34^a
Viscerosomatic index (%)	2.90±0.32^a	3.68±1.03^a	3.89±1.03^a

Figures on the same row having the same superscript are not significantly different (p>0.05, ANOVA).

3.5.4 Haematological analysis

No statistically significant differences ($p > 0.05$, ANOVA) between feeding frequency treatments were observed in all haematological parameters except for blood performance (Table 3.5).

The increasing feeding frequency led to an increase in the red blood cell count (Table 3.5). T1 (2.00±0.49 x 10^{12}/L), where the fish were fed once daily, recorded the highest RBC count followed by T2.

An increase in feeding frequency caused a decrease in white blood cell count (Table 3.5). The highest WBC count was observed in T3 (1.88±0.64 x 10^9/L) and the lowest in T1 (1.53±0.30 x 10^9/L). A similar trend was observed in serum glucose levels.

An upward trend in haemoglobin and haematocrit levels was observed in relation to feeding frequency (Table 3.5). T3 recorded the highest haemoglobin and haematocrit levels were observed in of 8.63±1.29 g/dL and 0.39±0.07 L/L respectively.

An increase in the feeding frequency resulted in an increase in the fish blood performance. No significant difference ($p > 0.05$, ANOVA) in the blood performance was observed between T2 and T3.

Table 3.5: The haematological parameters (mean ± SD) obtained from *Oreochromis mossambicus* fed at different frequencies for a period of 28 days.

Blood parameters	**T1**	**T2**	**T3**
RBC count (x 10^{12}/L)	1.23±0.57^a	1.91±0.25^a	2.00±0.49^a
WBC count (x 10^9/L)	1.88±0.64^a	1.54±1.14^a	1.53±0.30^a
Haemoglobin (g/dL)	6.37±2.11^a	8.40±1.70^a	8.63±1.29^a
Haematocrit (L/L)	0.13±0.02^a	0.29±0.06^a	0.39±0.07^a
Serum glucose(mmol/L)	5.69±0.82^a	5.42±0.36^a	5.17±0.12^a
Blood performance	0.65^a	1.97^b	2.33^b

Figures on the same row having the same superscript are not significantly different ($p>0.05$, ANOVA).

3.5.5 Growth performance of *Spinacia oleracea*

Increasing feeding frequency was followed by an increase in the number of leaves of *Spinacia oleracea* plants (Figure 3.8). T3 (13.00±3.51) recorded the highest number of leaves, which was significantly different ($p < 0.05$, ANOVA) from T1 and T2 on day 28. However, there was no statistically significant difference ($p > 0.05$, ANOVA) in the number of leaves between T1 and T2. The number of leaves in T1 and T3 increased with the duration of the experiment. In T2, the number of leaves remained the same between day 7 and day 14, then increased between day 14 and day 28. A significant difference (($p < 0.05$, ANOVA) in the number of leaves was observed between day 28 and the other experimental durations. No significant difference ($p > 0.05$, ANOVA) in shoot lengths was observed between day 7 and day 14.

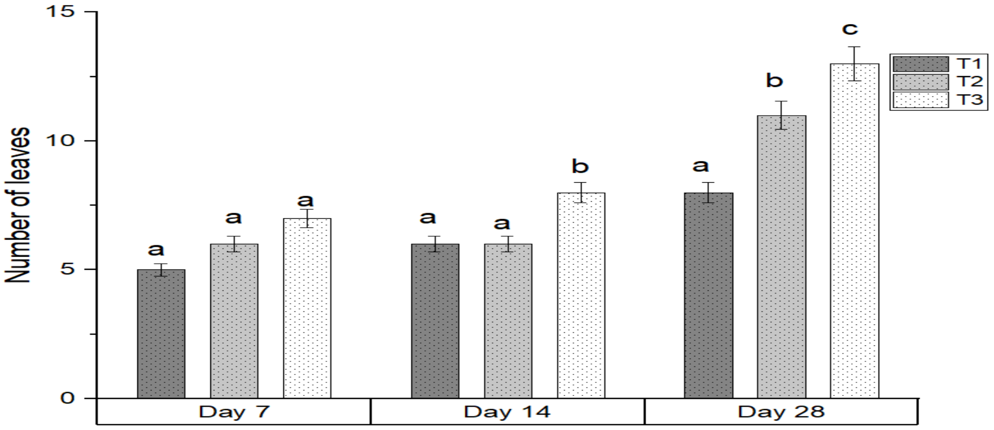

Figure 3.8: The number of *Spinacia oleracea* leaves recorded on day 7, day 14 and day 28 in aquaponic systems stocked with *O. mossambicus* fed at various feeding frequencies. Bars on the same day having the same letter are not significantly different ($p>0.05$, ANOVA).

As the feeding frequency increased, the shoot lengths of *Spinacia oleracea* plants also increased (Figure 3.9). The highest mean shoot length was recorded in T3 (29.20±0.77) while the lowest was recorded in T1 (8.80± 023). A significant difference ($p < 0.05$, ANOVA) in the shoot lengths was observed between all the feeding frequency treatments on day 28. However, there was no statistically significant difference ($p > 0.05$, ANOVA) in the shoot lengths between T1 and T2. The shoot lengths in all treatments increased with the duration of the experiment. A significant difference (($p < 0.05$, ANOVA) in the shoot lengths was observed between day 28 and the other experimental durations. No significant difference ($p > 0.05$, ANOVA) in shoot lengths was observed between day 7 and day 14.

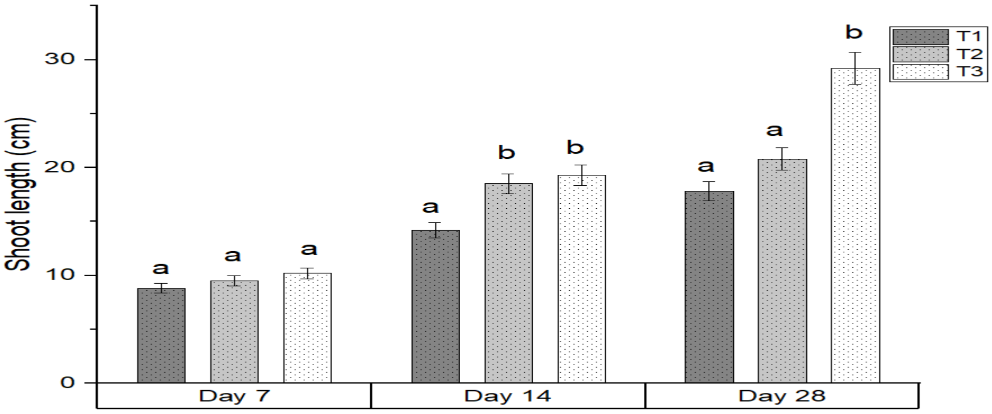

Figure 3.9: The shoot length of *Spinacia oleracea* leaves recorded on day 7, day 14 and day 28 in aquaponic systems stocked with *O. mossambicus* fed at various feeding frequencies. Bars on the same day having the same letter are not significantly different ($p>0.05$, ANOVA).

3.6 DISCUSSION

Water quality parameters are the primary environmental consideration for optimizing the production in aquaponics because they directly affect fish welfare, health, and the nutrient requirement of plants (Yildiz *et al*. 2017). The water temperature setting is dependent on the fish and plant species.

The water temperature and pH remained within the acceptable limits for the growth of *Oreochromis mossambicus* in aquaponic systems. The water temperature was kept at 28°C. The recommended temperature range for optimal growth of *O. mossambicus* is between 27°C and 30°C (El-Sayed, 2006), and 28°C for optimal growth of *Spinacia oleracea (*Nxawe *et al*. 2009*)*. The pH ranged from 6.99 to 7.20. For enhanced nutrient uptake, plants require a pH value between 5.5 and 6.5, and the optimum pH range for nitrifying bacteria is 7.0–8.0, while the recommended pH for fish production is 6.5–8.5 (Yildiz *et al*. 2017; Stathopoulou *et al*. 2021).

Dissolved oxygen levels were observed to increase with increasing feeding frequency throughout the experiment. The highest DO concentration was observed where fish were fed once daily. This could be due to the low accumulation of excess feed and fish excrement seen at low feeding frequencies compared to high feeding frequencies. However, the difference among treatments was not statistically significant. The DO concentrations remained within recommended levels for aquaponics production of *O. mossambicus* as indicated by El-Hack *et al*. (2022), who recommended keeping DO levels above 5 ppm to ensure good growth in *O. mossambicus* production. Stathopoulou *et al*. (2021) also recommended DO concentrations >5 mg/L. This is consistent with the results reported by (Abdelrahman and Abdelrahman, 2018), who recorded a DO concentration range of 5.9-7 3mg/L. Dissolved oxygen concentration can affect the physiological function of *O. mossambicus* (Li *et al*. 2018; Al-Tawaha *et al*. 2021). It is a limiting factor for the life of a fish.

Total dissolved solids (TDS) increased with increasing feeding frequency. The highest TDS level was observed in the thrice daily treatment, where the fish were fed thrice daily. This could be due to the accumulation of fish feed and excrement with increasing feeding frequency. In agreement with this, Abdelrahman and Abdelrahman, 2018) recorded the highest TDS level in the feeding frequency treatment of 3 times daily. Abdelrahman and Abdelrahman (2018) recorded a TDS range of 370.0-837.1 ppm, which was much higher than the range recorded in this study (57.55-213.93 mg/L). The TDS levels remained within acceptable limits for growth to *O. mossambicus*.

Ammonia (NH_3), nitrate (NO_3^-) and nitrite (NO_2^-) concentrations increased with feeding frequency. This could be due to nutrient deposition into the water though excess feed, fish's metabolic waste or microbial oxidation. This observation is in agreement with Abdelrahman and Abdelrahman (2018) and Stathopoulou *et al*. (2021). NH_3, NO_3^- and NO_2^- also decreased gradually with the increasing duration of the experiment, this could be a result of nutrient uptake by the plants or microbial oxidation of NH_3 and NO_2^-. Stathopoulou *et al*. (2021) also observed a decrease in nutrient concentrations as the duration of the experiment increased. The concentrations of NH_3, NO_3^- and NO_2^- were within acceptable limits for growth of *O. mossambicus* in aquaponics. High NH_3 concentrations are known to slow down fish growth and decrease survival (Yildiz *et al*. 2017; Sabwa *et al*. 2022). High concentrations of NO_3^- enhance the productivity of plants (Rakocy *et al*. 2006). However, concentrations of NO_3^- in water stocked with fish should not exceed 50 mg/L since high NO_3^- concentrations usually result in algae blooms (Poxton, 2003; Sabwa *et al*. 2022). High NO_3^- levels affects the fish's ability to transport oxygen via blood (Yildiz *et al*. 2017; Sabwa *et al*. 2022). An increase in feeding frequency led to increasing phosphorus (P) concentrations. The excess feed and fish waste as feeding frequency increased, contributed to the increasing concentrations of P in the water. The P concentrations were also decreasing with the duration of the experiment. This may be due to its uptake by plants. The phosphorus concentrations remained within acceptable limits for growth of *O. mossambicus* in aquaponics.

The growth performance of *Oreochromis mossambicus* in terms of %weight gain, specific growth rate, and thermal growth coefficient increased with increasing feeding frequency. The best WG, SGR and TGC were observed in the fish fed at the highest feeding frequency of 3 times daily. This could be due to increased feed consumption and utilization at high feeding frequencies. *Oreochromis mossambicus* is an herbivorous fish and plant-based feed often contain lower energy concentrations compared to animal-based feed. They also have smaller stomachs with limited storage capacity for food, allowing them to consume small chunks of food at a time

(Fava *et al*. 2022). As a result, *O. mossambicus* require more frequent meals to meet their nutritional needs. These results are in agreement with previous research in several fish species, (Başçınar *et al*. (2007) (*Salmo trutta labrax*), Ahsan *et al*. (2009) (*O. niloticus*), Aderolu *et al*. (2010) (*O. niloticus*), Jegede and Olorunfemi (2013) (*O. niloticus*) and Hassan *et al*. (2021) (*Lates calcarifer*)). However, several other studies presented contrasting findings to those of the present study. Nasrin *et al*. (2021) observed the highest SGR in *O. niloticus* fed twice daily compared to once and thrice; Daudpota *et al*. (2016) recommended feeding *O. niloticus* four to five times daily for optimal growth. Luthanda and Jerling (2013) and Kaya and Bilgüven (2015) observed the best performance of *O. niloticus* at a feeding frequency of 4 times daily and 6 times daily respectively.

A downward trend in food conversion ratios was observed with increasing feeding frequency. The lowest food conversion ratio of *O. mossambicus* was observed in fish that were fed thrice daily. When fish are able to efficiently consume feed and convert a larger portion of the nutrients from the feed into biomass, lower FCR can be expected. The higher FCR that was observed at the lowest feeding frequency might have been due to the fish not getting sufficient feed to meet their nutritional needs. Similar observations were made by Başçınar *et al*. (2007) in *Salmo trutta labrax*, Aderolu *et al*. (2010) in *C. gariepinus* and Jegede and Olorunfemi (2013) in *O*. *niloticus*. According to Ferdous *et al*. (2014), fish that are fed more frequently can utilize the formulated diet more efficiently compared to fish that are fed less frequently. Contrary to the observations made in this study, Zafar *et al*. (2017) and Falaye and Omoike (2013) recorded the best FCR at feeding frequencies of four and twice daily respectively.

The survival rate was fairly high, ranging from 95.56±7.70% to 98.89±1.92%. However, survival was not significantly affected by feeding frequency. A few unexplained mortalities were observed in each treatment, but they were not linked to feeding frequency. The reason for the observed mortalities could have been fish handling.

The highest condition factor was recorded in fish fed 3 times daily. However, the CF was not significantly affected by feeding frequency in all treatments. Higher feeding frequency may have led to elevated feed intake and utilization and a subsequent improvement of the nutritional status of fish. This can result in a higher condition factor. Başçınar *et al*. (2007) in *Salmo trutta labrax* and Thongprajukaew *et al*. (2017) in *O. niloticus* also indicated that the condition factor was not significantly affected by feeding frequency. The condition factor of fish is the manifestation of their nutritional status and physiological condition. It is also related to growth and feeding (Başçınar *et al*. 2007).

The highest hepatosomatic index and viscerosomatic index were observed in fish that were fed 3 times daily. However, in the present study HSI and VSI were not significantly impacted by feeding frequency. Frequent feeding can lead to increased energy intake, which may result in a larger liver size. This can be seen as an increase in the HSI. Increased feeding frequency can result in a higher energy allocation to visceral organs, which can be seen as an increase in the VSI. These findings were consistent with the observations of Hassan *et al*. (2021) in *Lates calcarifer* and Thongprajukaew *et al*. (2017) in *O. niloticus*. HSI and VSI are essential to the evaluation of nutritional status of fish (Rahim *et al*. 2017; Hassan *et al*. 2021).

No significant differences in the haematological parameters (red blood cell count, white blood cell count, Haemoglobin, haematocrit and serum glucose) was observed with respect to the different feeding frequencies. This could mean that feeding frequency did not cause stress in fish that could've caused a spike in blood parameters and serum glucose. No studies that investigated the haematological parameters of fish under different feeding frequencies were found. Inadequate feeding can lead to anaemia (lowered RBC count). RBC count of *O. mossambicus* was not significantly affected by feeding frequency. In addition to this, the WBC was also not significantly affected by feeding frequency Insufficient feeding can result in immune system suppression in fish, to which the body responds by elevating the white blood cell count. Stress can cause an increase in blood glucose levels in fish, similar to the "fight or flight". The release of stress hormones, such as cortisol, can stimulate the liver to release glucose into the bloodstream to provide extra energy for the fish to cope with stress. Feeding frequency did not significantly affect the serum glucose levels of *O. mossambicus*. When assessing the health status of fish, the examination of haematological parameters might prove useful (Tawwab *et al*. 2006; Wu *et al*. 2018). The highest blood performance was recorded at the highest feeding frequency (3 times daily). This suggests better health status of *O. mossambicus* at the highest feeding frequency, where the fish may have been able to feed efficiently.

The growth performance of *Spinacia oleracea* in the present experiment increased with increasing feeding frequency. The best performance of *S. oleracea* was observed in the feeding frequency treatment of thrice daily. Increased feeding frequency could have contributed to more efficient plant nutrition as nitrates were available for a prolonged periods during the day. This is in agreement with the observation made by (Stathopoulou *et al*. 2021) on *Lactuca sativa* in an aquaponic system. In accordance with this finding, Stathopoulou *et al*. (2021) observed a 13.7% increase in lettuce production (kg/m^2) when sea bass was fed at the highest feeding frequencies in comparison with the lowest feeding frequency. Plant growth and production are indirectly related to feeding frequency and microbial activity. Feeding frequency affects nutrient availability in solution inside the system. Plant growth also increased with increasing duration of the experiment. This could be due to the increasing nutrient uptake as plants grow.

Conclusion

The feeding frequency of 3 times daily resulted in the best growth of *O. mossambicus* in terms of SGR and FCR. Feeding frequency did not have a significant effect on CF, HSI, and VSI. Haematological parameters (RBC count, WBC count, haemoglobin, haematocrit and serum glucose were also not significantly affected by feeding frequency. Feeding frequency did not affect pH and DO concentration significantly. The highest NH_3, NO_3^-, NO_2^- and P concentrations, were observed in the 3 times daily treatment, but the concentrations were still within acceptable limits for the growth of fish. Maximum performance of *S. oleracea* was also observed in 3 times daily treatments. Increasing feeding frequency led to better food accessibility which led to better fish growth performance. Therefore, 3 times a day is recommended for optimal growth of *O. mossambicus* and *S. oleracea* in low technology aquaponic systems. In conclusion, feeding frequency had an effect on the growth performance of *O. mossambicus* in low technology aquaponic systems.

CHAPTER 4: EFFECT OF STOCKING DENSITY ON THE GROWTH PERFORMANCE OF *OREOCHROMIS MOSSAMBICUS* IN A LOW TECHNOLOGY AQUAPONIC SYSTEM

4.1 INTRODUCTION

The success of any fish production system depends on market demand and cost of production (Zafar *et al*. 2017). The stocking density of fish is directly linked to the productivity and profitability of any commercial aquaculture unit (Debnath *et al.* 2022). Optimum stocking densities are associated with optimum water qualities, which are a basic requirement for fish growth. In order to achieve better growth of fish and plants in aquaponic systems, optimum fish stocking density is a key requirement. Aquaponic systems stocked at low fish densities may not supply an adequate amount of nutrients to the plants. Stocking density directly impacts water quality (Debnath *et al*. 2022) and the social behaviour of fish such as competition (Yildiz *et al*. 2017). High stocking densities will interfere with the growth of fish even though food needs are fulfilled (Harahap *et al*. 2023). This is due to competition for space and food. High stocking densities may lead to deteriorating water quality resulting in poor growth and physiological performance of cultured species. Stocking fish at low densities results in inefficient utilization of space and low yields whereas stocking at high densities results in impairment of growth performance of fish due to accumulation of metabolic wastes, impairment of fish social interaction and deterioration of water quality. The rate of ammonia (NH_3) and phosphorus (P) production in aquaponic systems depends on feeding frequency, the amount of food and the protein composition of the food since these compounds are derived from fish feed (Stathopoulou *et al*. 2021). This therefore makes it important to determine the optimum stocking density for *Oreochromis mossambicus* in aquaponic systems. Among the studies that were conducted on recirculation rates in aquaponics, Rahmatullah *et al*. (2020, Al-Tawaha *et al*. (2021) and Sabwa *et al*. (2022) had *Oreochromis niloticus* as their fish species of choice. No studies focusing on stocking densities of *O. mossambicus* in low technology aquaponic systems were found. In literature there is a lot of contradicting information on the stocking density of *O. mossambicus*. Sabwa *et al*. (2020) observed the for the best growth of *O. niloticus*, at an intermediate stocking density, while Abaho *et al*. (2020) and Al-Tawaha *et al*. (2021) observed best growth at low and high stocking densities respectively. More comparative studies for aquaponics production are needed in the future to address the fact that there is far too little information about the stocking density required for optimal production of *O. mossambicus* in aquaponic systems worldwide.

4.2 OBJECTIVE: To determine the effect of stocking density (low, intermediate and high) on the growth performance of *O. mossambicus* in low technology aquaponic systems.

4.3 NULL HYPOTHESIS: Stocking density does not affect the growth performance of *O. mossambicus* in low technology aquaponic systems.

4.4 METHODOLOGY AND ANALYTICAL PROCEDURES

4.4.1 Study site

The study site was similar to section 3.4.1.

4.4.2 The integrated aquaponics design

The integrated aquaponics design was similar to section 3.4.2.

4.4.3 The experimental design

A total of 450 *Oreochromis mossambicus* fingerlings with mean weight 10.0±3.0 g were stocked in 9 fibreglass tanks (500 L) in a completely randomised design. The experiment was replicated 3 times. The fish were fed 4% of their body weight with pelleted commercial feed. The pellet diameter was 3 mm. Although the best feeding frequency was established to be 3 times daily in Chapter 3, feeding 3 times daily may invertedly result in higher feed costs. In order to avoid high feed costs during the investigation of the effect of stocking density on fish growth, a feeding frequency of once daily was used. The experiment occurred over a duration of 28 days. The fish were stocked at three different densities: Low stocking density (LSD): 1.87 kg/m^3, Intermediate stocking density: 2.50 kg/m^3 and High stocking density (HSD): 3.13 kg/m^3. The *Spinacia oleracea* plants were planted in hydroponics sub-systems made up of PVC pipes at a planting density of 18 plants/system.

4.4.4 Water quality parameters

The procedure for measurements and analysis of water quality parameters was done in accordance with section 3.4.4.

4.4.5 Growth performance of *O. mossambicus*

The assessment of fish growth was done in accordance with section 3.4.5.

4.4.6 Growth performance of *S. oleracea*

The assessment of plant growth was done in accordance with section 3.4.6.

4.4.7 Statistical analysis

Analysis of variance (ANOVA) and Tukey's post hoc analysis were carried out for the analysis of the effect of stocking density on the growth performance of *O. mossambicus*. For analysis of fish growth data one way ANOVA was used. Two-way ANOVA was used for the analysis of water quality parameters (ammonia, nitrite, nitrate, phosphorus pH, temperature, DO concentration and TDS levels) and plant growth (number of leaves and shoot length data. The data recorded in this study met the assumptions of ANOVA based on Levine's test. The statistical analysis was carried out using the Statistical Package for the Social Sciences (IBM SPSS, 2020) version 22.0 (IBM). All statistical analysis was performed at the 5% significance level.

4.5 RESULTS

4.5.1 Water quality parameters

The pH range was 7.00 to 7.14 (Table 4.1). The was no significant difference ($p > 0.05$, ANOVA) in pH values of the water between the different stocking density treatments.

The dissolved oxygen concentration ranged from 5.13±0.83 mg/L to 8.63±0.40 mg/L (Table 4.1). The increase in stocking density was followed by a decrease in DO concentrations throughout the experiment. HSD recorded the lowest DO concentration compared to LSD and ISD. However, no significant difference ($p > 0.05$, ANOVA) in DO concentrations was observed between LSD and ISD.

The TDS levels ranged from 64.13±2.13 mg/L to 209.58±21.38 mg/L (Table 4.1). As the stocking density increased, TDS levels also increased. A significant variation ($P < 0.05$, ANOVA) in TDS levels between all stocking density groups was observed on day 14 and day 28 of the experiment.

Table 4.1: pH, DO conc. and TDS levels (mean ± SD) recorded on day 7, day 14 and day 28 the experiment in aquaponic systems stocked with *O. mossambicus* under different densities for a duration of 28 days.

WQ	**LSD**			**ISD**			**HSD**		
	Day 7	Day 14	Day28	Day 7	Day 14	Day 28	Day 7	Day 14	Day28
pH	7.06^{a}	7.00^{a}	7.01^{a}	7.03^{a}	7.06^{a}	7.04^{a}	7.03^{a}	7.06^{a}	7.04^{a}
DO (mg/L)	8.63±0.40^{a}	8.47±0.82^{a}	8.23±0.59^{a}	8.37±0.06^{a}	7.80±0.10^{a}	7.37±0.21^{a}	6.20±0.10^{b}	5.80±0.40^{b}	5.13±0.83^{b}
TDS (mg/L)	64.13±2.13^{a}	89.35±11.32^{a}	100.75±17.50^{a}	90.05±12.38^{b}	147.08±13.12^{b}	158.45±12.66^{b}	110.67±5.32^{c}	191.40±17.03^{c}	209.58±21.38^{c}

Figures on the same column having the same superscript are not significantly different ($p>0.05$).

The ammonia (NH_3) concentrations ranged from 0.021 mg/L to 0.028 mg/L (Figure 4.1). HSD recorded the highest NH_3 concentrations the entire duration of the experiment, which was not significantly different ($P > 0.05$, ANOVA) from LSD and HSD. On day 14, LSD and ISD recorded equal NH_3 concentrations. In LSD, NH_3 levels remained constant between day 7 and day 14 then decreased slightly from day 14 until day 28. In ISD and HSD, NH_3 concentrations decreased with the duration of the experiment.

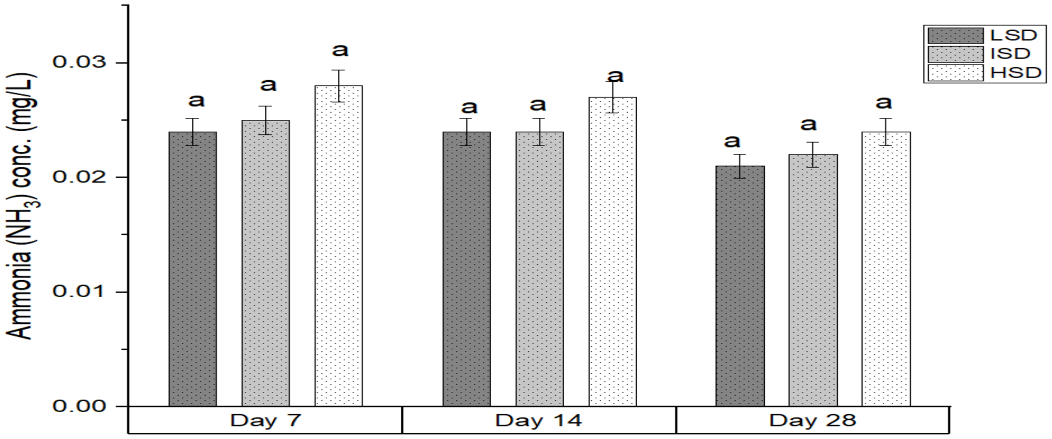

Figure 4.1: The ammonia (NH_3) concentrations recorded on day 7, day 14, and day 28 in aquaponic systems stocked with *O. mossambicus* at various densities. Bars on the same day having the same letter are not significantly different ($p>0.05$, ANOVA).

The nitrite (NO_2^-) concentrations ranged from 0.02 mg/L to 0.11 mg/L (Figure 4.2). HSD recorded the highest NO_2^- concentration throughout the experiment, which was significantly different from LSD and ISD. A significant difference ($p < 0.05$, ANOVA) in NO_2^- concentrations was observed between all the stocking density treatments on day 7 and day 14. In LSD, NO_2^- concentrations decreased slightly between day 7 and day 14, then remained constant between day 14 and day 28. In ISD, NO_2^- concentrations remained constant between day 7 and day 14 and decreased slightly between day 14 and day 28. In HSD, NO_2^- concentrations decreased with the duration of the experiment.

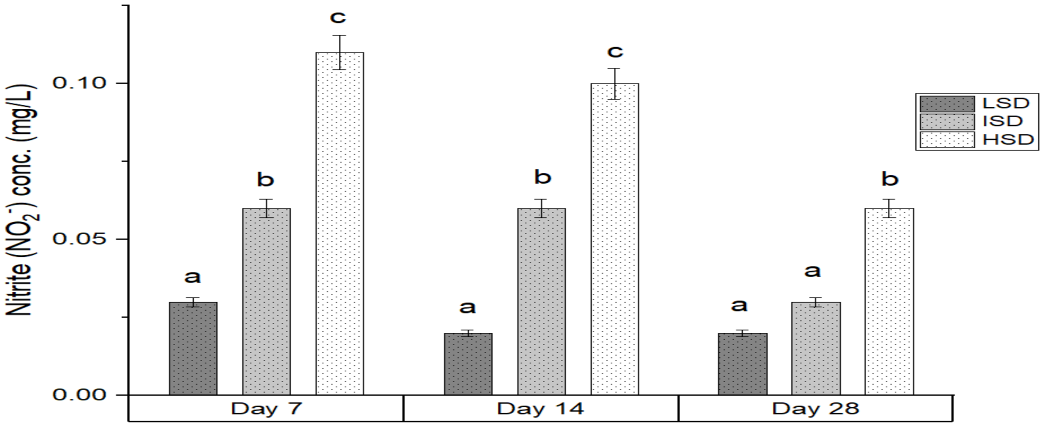

Figure 4.2: The nitrite (NO_2^-) concentrations recorded on day 7, day 14, and day 28 in aquaponic systems stocked with *O. mossambicus* at various densities. Bars on the same day having the same letter are not significantly different ($p>0.05$, ANOVA).

The nitrate (NO_3^-) concentrations ranged from 2.10 mg/L to 2.50 mg/L (Figure 4.3). An increased stocking density led to an increase in NO_3^- concentrations. HSD recorded the highest NO_3^- concentration throughout the experiment. On day 7, NO_3^- concentration in HSD was significantly different ($P < 0.05$, ANOVA) from LSD and ISD. No significant difference ($P > 0.05$, ANOVA) in NO_3^- concentrations was observed between the different treatments on day 14 and day 28. In all stocking density treatments, NO_3^- concentrations decreased with the duration of the experiment.

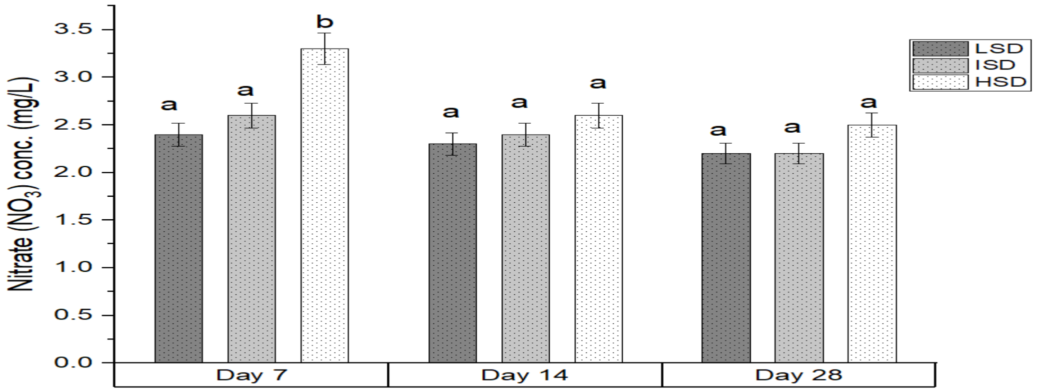

Figure 4.3: The nitrate (NO_3^-) concentrations recorded on day 7, day 14, and day 28 in aquaponic systems stocked with *O. mossambicus* at various densities. Bars on the same day having the same letter are not significantly different ($p>0.05$, ANOVA).

The phosphorus (P) concentrations ranged from 0.04 mg/L to 0.18 mg/L (Figure 4.4). HSD recorded the highest P concentration throughout the experiment. There were significant differences (P < 0.05, ANOVA) in P concentrations observed between the different treatments of stocking density. In all treatments, P concentrations decreased with the duration of the experiment.

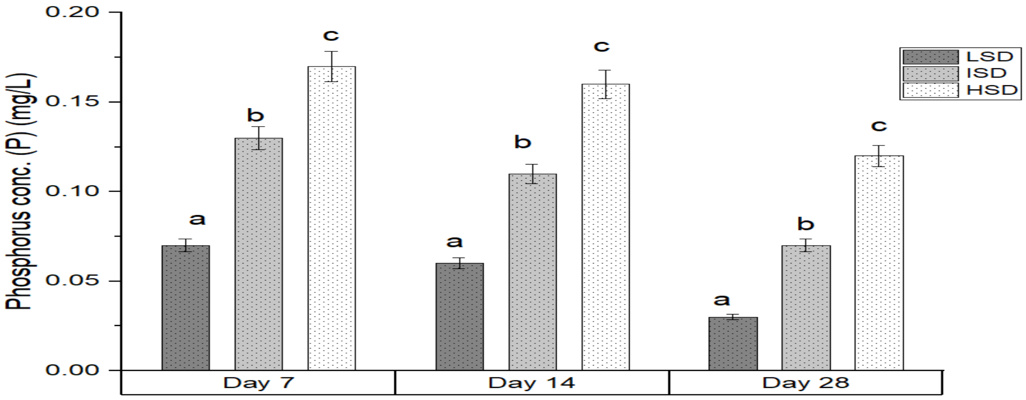

Figure 4.4: The phosphorus (P) concentrations recorded on day 7, day 14, and day 28 in aquaponic systems stocked with *O. mossambicus* at various densities. Bars on the same day having the same letter are not significantly different (p>0.05, ANOVA).

4.5.2 Growth performance of *Oreochromis mossambicus*

The highest final weight of *O. mossambicus* was observed in ISD (17.54±1.17 g) which was significantly different (p < 0.05, ANOVA) from LSD and HSD (Table 4.2). No significant difference (p > 0.05, ANOVA) in FW was observed between treatments LSD and HSD. The intermediate stocking density (ISD) also recorded the highest % weight gain (52.59±3.58%) and specific growth rate (2.26±0.08). However, no significant difference (p > 0.05, ANOVA) in %WG and SGR was observed between treatments LSD and HSD. A similar observation was observed in thermal growth coefficient. ISD recorded the lowest food conversion ratio (1.35±0.16) which was significantly different (p < 0.05, ANOVA) from LSD and HSD (Table 4.2). The highest survival rate was observed in ISD (98.67±2.30%), which was not significantly different (p > 0.05, ANOVA) from LSD and HSD (Table 4.2).

Table 4.2: Final weight, %weight gain, TGC, survival rate (mean ± SD) of *Oreochromis mossambicus* stocked at varying densities in aquaponic systems for a period of 28 days.

Growth parameters	**LSD**	**ISD**	**HSD**
Final weight (g)	$13.08±0.82^a$	$17.54±1.17^b$	$13.11±0.79^a$
% Weight gain	$13.55±5.05^a$	$52.59±3.58^b$	$7.24±1.68^a$
SGR	$1.22±0.16^a$	$2.26±0.08^b$	$1.25±0.05^a$
FCR	$2.41±0.09^a$	$1.35±0.16^b$	$2.27±0.11^a$
TGC	$1.14±0.00^a$	$2.05±0.01^b$	$1.18±0.00^a$
Survival Rate (%)	$96.44±3.36^a$	$98.67±2.30^a$	$96.67±3.06^a$

Figures on the same row having the same superscript are not significantly different (p>0.05, ANOVA).

4.5.3 Organosomatic indices

ISD recorded the highest CF (1.64±0.07) which was significantly different ($p < 0.05$, ANOVA) from LSD and HSD (Table 4.3). No significant difference in CF was recorded between LSD and HSD. The same trend was observed in HSI and VSI.

Table 4.3: The HSI and VSI (mean ± SD) of *Oreochromis mossambicus* stocked at varying densities in aquaponic systems for a period of 28 days.

Somatic indices	**LSD**	**ISD**	**HSD**
Condition factor	$1.17±0.05^a$	$1.64±0.07^b$	$1.11±0.06^a$
Hepatosomatic index (%)	$0.52±0.10^a$	$1.74±0.07^b$	$0.31±0.10^a$
Viscerosomatic index (%)	$1.57±0.31^a$	$2.31±0.16^b$	$1.47±0.19^a$

Figures on the same row having the same superscript are not significantly different (p>0.05, ANOVA).

4.5.4 Haematological analysis

An increase in stocking density caused an increase in red blood cell count (Table 4.4). The highest RBC count was observed in HSD ($2.30±0.16 \times 10^{12}$/L) which was significantly different ($p < 0.05$, ANOVA) from LSD and ISD. No significant difference ($p > 0.05$, ANOVA) in RBC count was observed between LSD and ISD. White blood cell count and serum glucose followed the same trend. There was no visible trend in haemoglobin and haematocrit levels among the different stocking density treatments

(Table 4.4). ISD recorded the highest haemoglobin (8.00±0.52 g/dL) and haematocrit (0.34±0.01 L/L). A significant difference ($p < 0.05$, ANOVA) in haemoglobin and haematocrit levels was observed between all the stocking density treatments.

No discernible trend in the fish blood performance was observed regarding stocking density. A significant difference ($p < 0.05$, ANOVA) in the blood performance was observed between LSD and HSD.

Table 4.4: The haematological parameters (mean ± SD) obtained from *Oreochromis mossambicus* stocked at varying densities in aquaponic systems for a period of 28 days.

Blood parameters	**LSD**	**ISD**	**HSD**
RBC count (x 10^{12}/L)	1.06 ± 0.06^{a}	1.20 ± 0.16^{a}	2.30 ± 0.16^{b}
WBC count (x 10^{9}/L)	14.73 ± 31.48^{a}	16.43 ± 1.08^{a}	36.39 ± 0.60^{b}
Haemoglobin (g/dL)	8.43 ± 0.86^{a}	8.00 ± 0.52^{b}	7.27 ± 0.90^{c}
Haematocrit (L/L)	1.17 ± 0.03^{a}	$0.34\pm.0.01^{b}$	0.22 ± 0.03^{c}
Serum glucose	5.61 ± 0.09^{a}	5.81 ± 1.16^{a}	8.34 ± 0.35^{a}
Blood performance	5.04^{a}	3.98^{ab}	4.29^{b}

Figures on the same row having the same superscript are not significantly different ($p>0.05$, ANOVA).

4.5.5 Growth performance of *Spinacia oleracea*

On day 7, LSD and HSD recorded the same number of leaves of *Spinacia oleracea* plants (Figure 4.5). The highest mean number of leaves was recorded in HSD on day 14 and day 28 followed by ISD. A significant difference ($p < 0.05$, ANOVA) in the number of leaves was observed between HSD and other stocking density treatments on day 14 and day 28. The number of leaves in LSD remained the same between day 7 and day 14, the increased slightly on day 28. In ISD, the number of leaves increased slightly between day 7 and day 14, then remained constant between day 14 and day 28. HSD recorded an increase in the number of leaves with the duration of the experiment.

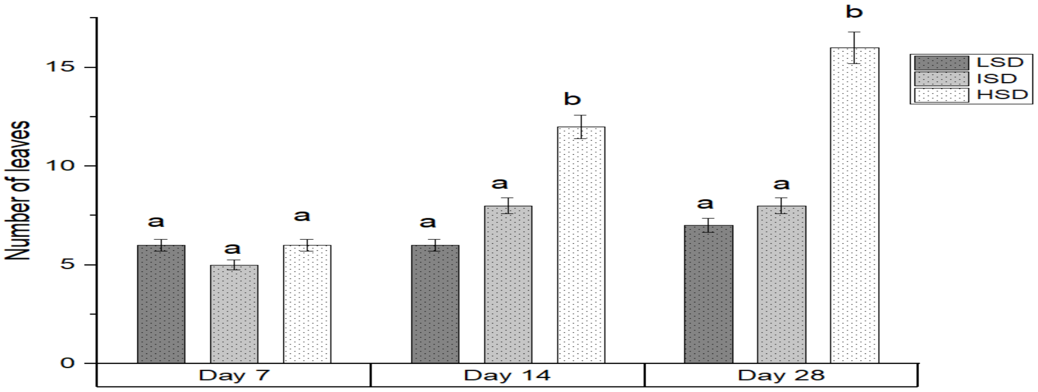

Figure 4.5: The number of *Spinacia oleracea* leaves recorded on day 7, day 14 and day 28 in aquaponic systems stocked with *O. mossambicus* at various densities. Bars on the same day having the same letter are not significantly different (p>0.05, ANOVA).

As the stocking density increased, the shoot lengths of *Spinacia oleracea* plants in all stocking density treatments also increased (Figure 4.6). HSD recorded highest mean shoot length throughout the experiment, followed by ISD. A significant difference ($p < 0.05$, ANOVA) in shoot length was observed between HSD and other stocking density treatments on day 14 and day 28. The shoot length in all treatments increased with the duration of the experiment.

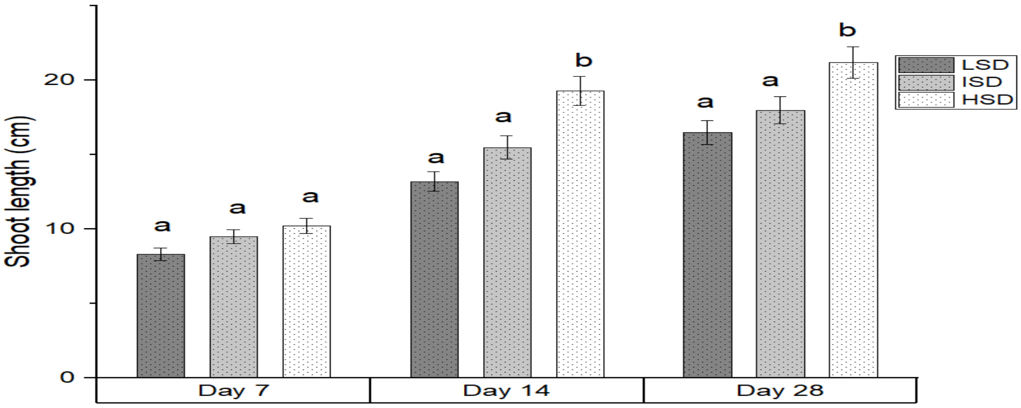

Figure 4.6: The shoot length of *Spinacia oleracea* leaves recorded on day 7, day 14 and day 28 in aquaponic systems stocked with *O. mossambicus* at various densities. Bars on the same day having the same letter are not significantly different (p>0.05, ANOVA).

4.6 DISCUSSION

The water temperature and pH remained within the acceptable limits for the growth of *O. mossambicus* in aquaponic systems. The water temperature was kept at 28°C. The pH range was from 7.01 to 7.14.

A decrease in dissolved oxygen concentrations resulted from the increasing stocking density throughout the experiment. High stocking density led to lower DO concentrations. This could be due to increased oxygen demand and an increased accumulation of fish feed and excrement in the tanks that is directly affected by the higher number of fish in the tank. This observation is consistent with the findings of several studies; Maucieri *et al*. (2019) on *C. carpio* in aquaponics, Abaho *et al*. (2020) on *O. niloticus* in cage culture and Al-Tawaha *et al*. (2021) on *O. niloticus* in aquaponics. The mean DO concentrations recorded in this study were within the recommended range for aquaponics production of *O. mossambicus* as indicated by El-Hack *et al*. (2022) and Stathopoulou *et al*. (2021).

Total dissolved solids (TDS) increased with increasing stocking density. This could be attributed to the increase in the amount of fish feed and excrement as stocking density increases and their accumulation over time. However, TDS levels were not significantly affected by stocking density. Nuwansi *et al*. (2021) observed that the total dissolved solids (TDS) varied within the range of 488.00 to 513, which was much higher than the TDS range of 64.13 to 209.58 recorded in this study. The TDS levels remained within acceptable limits for growth to *O. mossambicus*.

An increase in stocking density resulted in an increase in ammonia (NH_3), nitrate (NO_3^-) and nitrite (NO_2^-) concentrations. This could be due to an increased amount of excess feed and fish excrement being deposited into the water with the increasing number of fish per stocking density. The excess feed and fish excrement release ammonia. Ammonia gets oxidized into nitrite and subsequently nitrate. Conforming to this observation, Sabwa *et al*. (2020) also recorded an increase in NO_3^- and NO_2^- as stocking density increased. Al-Tawaha *et al*. (2021) also reported that an increase in stocking density led to an increase in NH_3, NO_3^- and NO_2^-. With the increasing duration of the experiment, NH_3, NO_3^- and NO_2^- were observed to decrease. This may be due to the plants that were taking up the nutrients. A similar observation was made by Stathopoulou *et al*. (2021) with *Lactuca sativa* plants. The phosphorus (P) concentrations were increasing with increasing stocking density. The phosphorus concentrations are contributed to by the excess feed and fish waste that increases with stocking density. The P concentrations were decreasing with the increasing duration of the experiment. This may be due to P uptake by plants. The phosphorus concentrations were within acceptable limits for the aquaponic production of *O. mossambicus*.

The best WG, SGR and TGC were observed in the fish stocked at an intermediate stocking density of 2.50 kg/m^3. At intermediate stocking density, we expect little to no stress that could result from poor water quality, inadequate space or high competition for food. As a result, the fish could be able to channel their energy to growth instead of dealing with stress. This observation is in agreement with Sabwa *et al*. (2022) who recorded the highest growth of *O. niloticus* at an intermediate stocking density in aquaponics. In contrast to this observation, several studies by Rahmatullah *et al*. (2010) (*O. niloticus*), Wu *et al*. (2018) (*O. niloticus*), Maucieri *et al*. (2019) (*C. carpio*), Abaho *et al*. (2020) (*O. niloticus*), Nuwansi *et al*. (2021) (*C. carpio*) and Al-Zahrani *et al*. (2023) (*O. niloticus*) observed the best growth performance at the lowest stocking density. Surprisingly, Al-Tawaha *et al*. (2021) recorded the highest growth of *O. niloticus* at the highest stocking density. The differences in the results may be attributed to the varying fish species. *O. mossambicus* might not have the same tolerance to higher stocking densities compared to *O. niloticus*, therefore exhibiting the best growth at intermediate stocking densities. The maximum performance of *O. mossambicus* in terms of FCR was also observed at a stocking density of 2.50 kg/m^3. This could be attributed to the higher feed intake and feed utilization taking place at intermediate stocking densities, where there is little crowding stress. An observation made by Sabwa *et al*. (2022) was in agreement with this statement. In contrast to this study, Wu *et al*. (2018) and Nuwansi *et al*. (2021) recorded the lowest (best) FCR at the lowest stocking density. Lower growth performance (poor SGR and FCR) of *O. mossambicus* was observed in the low stocking density and high stocking density treatments compared to the intermediate stocking density. At low stocking densities, fish experience little competition for food and resources, which can lead to reduced motivation to feed actively. This could explain the poor growth performance of *O. mossambicus* observed at the lowest stocking density. Stress resulting from increased competition for food and space may be the reason for the low growth performance that was observed at the highest stocking density.

Stress has a negative effect on the feeding and growth patterns of fish. Stress in fish can cause reduced feeding activity and subsequent growth impairment. Aquaponic systems with high stocking densities have a greater accumulation of waste products and a greater demand for oxygen. Poor water quality can negatively affect fish growth and overall health. Although, the water quality in this study was acceptable for growth of *O. mossambicus* as discussed previously. The low DO concentration in high stocking density was still enough to meet the requirements of the fish. As a result, poor water quality (low DO and high NH_3 and NO_2^-) cannot be the reason for the poor growth of fish observed in high stocking density. According to Wu *et al*. (2018), stocking density can affect fish growth performance even though the water quality is kept the same. The SGR obtained at the best stocking density was 2.26 %/day. A lower SGR was observed here in comparison to Chapter 3 where the best SGR was observed at a feeding frequency of 3 times daily (2.53 %/day). The FCR recorded at the best stocking density was 1.35, which is higher than the one observed in Chapter 3 (1.27). This could be that 3 times daily feeding was required in this experiment in order to obtain better growth performance of the fish.

Survival rate of *O. mossambicus* was fairly high. Intermediate stocking density recorded the highest SR while high stocking density recorded the lowest. However, survival was not significantly affected by stocking density. A few unexplained mortalities were observed in each treatment. The mortalities that occurred were not related to stocking density and could be attributed to fish handling. A similar observation was made by several studies by Rahmatullah *et al*. (2010), Wu *et al*. (2018), Kunda *et al*. (2021) and Sabwa *et al*. (2022). On the other hand, Maucieri *et al*. (2019) and Nuwansi *et al*. (2021) recorded 100% survival in all stocking densities. Wu *et al*. (2018) indicated that that high stocking densities have a negative effect on fish welfare.

The lowest CF was observed at the highest stocking density. Stress can negatively impact the condition factor as stressed fish may allocate more energy to dealing with stressors rather than growth and maintenance. As discussed before, higher stocking densities fish experience stress that results in a lower feeding efficiency. This could lead to reductions in the condition factor. To support this, Wu *et al*. (2018) indicated that there were significant reductions in CF resulting from increasing stocking density. No significant difference in CF was observed between low stocking density and high stocking density. Little motivation to feed at low stocking densities could have resulted the low condition factor.

The highest HSI and VSI were observed at the intermediate stocking density. At intermediate stocking densities, fish may have been able to efficiently consume and utilise their food and channel their energy to growth instead of dealing with stress. The efficient feeding can result in an increase in HIS and VSI. On the other hand, Wu *et al*. (2018), did not observe any significant differences in HSI and VSI in all stocking density treatments. The reduced feed intake and utilization (high FCR) as at low and high densities could have led to the lower HSI and VSI in comparison to fish that were stocked at intermediate stocking densities. HSI and VSI and play an essential role in the assessment of nutritional status of fish (Rahim *et al*. 2017; Hassan *et al*. 2021).

High stocking density recorded the highest red blood cell count, which could have been in response to stress. The production of red blood cells can be a physiological response to help fish maintain adequate oxygen supply when facing stressful conditions such as low oxygen levels. There was no significant difference in the RBC count between the low stocking density and intermediate stocking density treatments. In low stocking density and intermediate stocking density there could have been better oxygenation of the water and improved oxygen delivery to fish tissues, due to lower concentrations of excess feed and fish excrement compared to high stocking density.

The highest white blood cell count was recorded in *O. mossambicus* stocked at the highest density. In stressful conditions elevated WBC counts may occur as an immunological response. The WBC count at stocking density treatments low stocking density and intermediate stocking density did not differ significantly. The highest serum glucose was observed in fish stocked in the high stocking density treatment.

Elevated glucose levels seen in stressful conditions are a result of the liver being stimulated to release glucose into the bloodstream to provide extra energy for the fish to cope with stress. In parallel to this observation, Nuwansi *et al*. (2021) and Onxayvieng *et al*. (2021) observed the highest serum glucose levels in the high stocking density group. This might have been as a result to crowding stress. In contrast to this, Wu *et al*. (2018) indicated that stocking density had no significant effects on serum glucose levels in *O. niloticus*. There was no significant difference in serum glucose between low stocking density and intermediate stocking density. At higher stocking densities, competition for food and limited swimming space could have led to the elevated blood parameters. The lower blood parameters of fish in intermediate stocking density could mean that there was no stress in fish that could've led to a spike in blood parameters. The examination of haematological and blood biochemistry parameters has proven to be effective in monitoring fish health. (Tawwab *et al*. 2006; Wu *et al*. 2018, Esmaeili *et al*. 2021). The highest blood performance was recorded at the lowest stocking density. This could suggest better health status of *O. mossambicus* at the lowest stocking density, where the fish were able to focus their energy on feeding instead of stress resulting from crowding and competition for food.

The growth performance of *Spinacia oleracea* in the present experiment increased with increasing stocking density. These results revealed that the high stocking density of fish supplied more nutrients to plants which resulted in higher plant growth compared to the low and medium stocking density groups. This could be due to more food and fish metabolites entering the system, providing the plants with efficient nutrients for growth. In accordance with this finding, Nuwansi *et al*. (2021) and Sabwa *et al*. (2022). indicated that the highest fish stocking density exhibited the maximum plant growth. Al-Zahrani *et al*. (2023) noticed that the yield of *Spinacia oleracea* increased significantly with the increasing stocking density of *O. mossambicus*, and the highest value was observed in the higher stocking density treatment. Plant growth increased with the increasing duration of the experiment. This could be due to the plants' increasing ability to assimilate nutrients as they grow.

Conclusion

The best growth of *O. mossambicus* in terms of SGR and FCR was found at an intermediate stocking density of 2.50 kg/m^3 and the worst growth was observed at a high stocking density. The highest CF, HSI and VSI were recorded at the intermediate stocking density of 2.50 kg/m^3. The best blood performance was observed at a low stocking density. Stocking density did not significantly impact pH and DO concentration. The highest stocking density (3.13 kg/m^3) recorded the highest NH_3, NO_3^-, NO_2^- and P concentrations. The nutrient concentrations still remained within acceptable limits for the growth of *O. mossambicus* in aquaponic systems. Intermediate stocking density resulted in reduced fish stress resulting from competition for food and space, therefore allowing better food consumption and utilization, leading to a better growth performance. The best plant growth was observed at the highest stocking density while the worst was observed at the lowest stocking density. Therefore, for the best combined performance of *O. mossambicus* and *S. oleracea* in low technology aquaponic systems the stocking density of 2.50 kg/m^3 is recommended. In conclusion, stocking density did affect the growth performance of *O. mossambicus,* high stocking density resulted in reduced fish growth.

CHAPTER 5: EFFECT OF RECIRCULATION RATE ON THE GROWTH PERFORMANCE OF *OREOCHROMIS MOSSAMBICUS* IN A LOW TECHNOLOGY AQUAPONIC SYSTEM

5.1 INTRODUCTION

The design of an aquaponic system is based on the water quality needs of the cultured fish and plant species and the intensity of the production. To maintain good water quality, both dissolved and suspended compounds in the water need to be removed. The recirculation rates have a significant factor in the recirculating aquaponics system. They are responsible for regulating the amount of substances within the system and hence may have a direct effect on the performance of cultured organisms (Mugo-Bundi *et al.* 2024). Recirculation rate directly affects the removal of compounds in aquaponic systems (Schram *et al*. 2009; León-Ramírez *et al*. 2022). The regulation of recirculation rates is based on limiting the accumulation of fish metabolites and achieving optimum dissolved oxygen concentrations in the aquaponic systems. Inadequate recirculation rates allow the accumulation of fish metabolites and wastes that contribute to the concentrations of ammonia (NH_3) and carbon dioxide (CO_2) in the water. Excessive accumulation of NH_3 and CO_2 can result in fish mortality (Hussain *et al*. 2014). Low exchange rates allow for excess accumulation of compounds in the water which can compromise the health of the fish (Davidson *et al*. 2011, Obirikorang *et al*. 2019). Along with balancing the fish to plant ratio, increasing recirculation rates can reduce the rate of nutrient build up in the water. High recirculation rates have the potential to secure good water quality (Diem *et al*. 2017). An optimum plant and fish production can be maximised when the ratio between the number of plants and the number of fish is achieved, and the nutrient build-up is low. There is no single optimum ratio because the rate of nutrient build up can be decreased by increasing the recirculation rate. The importance of recirculation rates has been emphasised by Endut *et al*. (2009) on *C. gariepinus* in aquaponics*,* Hussain *et al*. (2014) on *C. carpio* in aquaponics, Nuwansi *et al*. (2015) on *C. carpio* in aquaponics, Diem *et al*. (2017) on *O. niloticus* in aquaponics, Whangchai *et al*. (2018) on *O. niloticus* in cage culture and Obirikorang *et al*. (2019) on *O. niloticus* in RAS.

This therefore makes it important to determine the optimum recirculation rate for the growth of *Oreochromis mossambicus* in aquaponic systems. Among the studies that focused on recirculation rate, most of them featured other fish species besides *O. mossambicus*. Among the studies that were conducted on recirculation rates in aquaponics, Endut *et al*. (2009) focused on *Clarias gariepinus*, Hussain *et al*. (2014) focused on *Cyprinus carpio* and Diem *et al*. (2017) focused on *Oreochromis niloticus*. No studies focusing on recirculation rates of *O. mossambicus* in low technology aquaponic systems were found. More comparative studies for aquaponics production are needed in the future to address the fact that there is far too little information about the recirculation rates required for the optimal production of *O. mossambicus* in aquaponic systems worldwide.

5.2 OBJECTIVE: To determine the effect of recirculation rate on the growth performance of *O. mossambicus* in low technology aquaponic systems.

5.3 NULL HYPOTHESIS: Recirculation rate does not affect the growth performance of *O. mossambicus* in low technology aquaponic systems.

5.4 METHODOLOGY AND ANALYTICAL PROCEDURES

5.4.1 Study site

The study site was similar to section 3.4.1.

5.4.2 The integrated aquaponics design

The integrated aquaponics design was similar to section 3.4.2.

5.4.3 The experimental design

A total of 450 *O. mossambicus* juveniles of mean weight 15.0±5.0 g were stocked in 9 fibreglass tanks (500 L) in a completely randomised design. The experiment was replicated 3 times. The stocking density for the fish was 2.6 kg/m^3, which allowed the best growth of fish and plants in Chapter 4. The fish were fed 4% of their bodyweight with pelleted commercial feed. The pellet diameter was 3 mm. Although the best feeding frequency was established to be 3 times daily in Chapter 3, feeding 3 times daily may invertedly result in higher feed costs. To avoid high feed costs during the investigation of the effect of recirculation rate on fish growth, a feeding frequency of once daily was used. The experiment occurred over a duration of 28 days. At the Aquaculture Research Unit, fish production is normally done in static systems where numerous fish mortalities occur. As a result, for the purpose of this experiment, we avoided using static systems as a control to avoid fish mortalities, we started our recirculation rate treatments at 0.5 L/min. The experiment was conducted at 3 different recirculation rates: low recirculation rate (LRR) (0.5 L/min), intermediate recirculation rate (IRR) (1.5 L/min) and high recirculation rate (2.5 L/min). The *Spinacia oleracea* plants were planted in hydroponics sub-systems made up of PVC pipes at a planting density of 18 plants/system.

5.4.4 Water quality parameters

The procedure for measurements and analysis of water quality parameters was done in accordance with section 3.4.4.

5.4.5 Growth performance of *O. mossambicus*

The assessment of fish growth was done in accordance with section 3.4.5.

5.4.6 Growth performance of *S. oleracea*

The assessment of plant growth was done in accordance with section 3.4.6.

5.4.7 Statistical analysis

Analysis of variance (ANOVA) and Tukey’s post hoc analysis were carried out for the analysis of the effect of recirculation rate on the growth performance of *O. mossambicus*. For analysis of fish growth data one-way ANOVA was used. Two-way ANOVA was used for the analysis of water quality parameters (ammonia, nitrite, nitrate, phosphorus pH, temperature, DO concentration and TDS levels) and plant growth (number of leaves and shoot length data. The data recorded in this study met the assumptions of ANOVA based on Levine’s test. The statistical analysis was carried out using the Statistical Package for the Social Sciences (IBM SPSS, 2020) version 22.0 (IBM). All statistical analysis was performed at the 5% significance level.

5.5 RESULTS

5.5.1 Water quality parameters

The pH was not significantly affected ($p > 0.05$, ANOVA) by recirculation rate (Table 5.1). The pH range was from 6.78 to 7.45.

An increase in recirculation rate caused an increase in dissolved oxygen concentrations (Table 5.1). The dissolved oxygen concentration ranged from 4.20±0.10 mg/L to 8.17±0.21 mg/L. Low recirculation rate (LRR) recorded the lowest DO concentration, which was significantly different ($p < 0.05$, ANOVA) from other treatments. However, there was no significant difference ($p > 0.05$, ANOVA) in DO concentration between IRR and HRR.

The TDS levels ranged from 65.55±1.17 mg/L to 313.68±23.74 mg/L (Table 5.1). TDS levels decreased with increasing recirculation rates. TDS levels were significantly different ($p < 0.05$, ANOVA) between LRR and other treatments. No significant difference ($p > 0.05$, ANOVA) in TDS levels was observed between IRR and HRR.

Table 5.1: pH, DO concentration and TDS levels (mean ± SD) recorded on day 7, day 14 and day 28 of the experiment in aquaponic systems with varying recirculation rates stocked with *O. mossambicus* for a duration of 28 days.

WQ	**LRR**			**IRR**			**HRR**		
	Day 7	Day 14	Day28	Day 7	Day 14	Day 28	Day 7	Day 14	Day28
pH	6.78^{a}	7.13^{a}	6.80^{a}	7.07^{a}	7.10^{a}	7.45^{a}	7.13^{a}	7.08^{a}	7.07^{a}
DO (mg/L)	4.37 ± 0.21^{a}	4.43 ± 0.35^{a}	4.20 ± 0.10^{a}	7.87 ± 0.25^{a}	7.73 ± 0.31^{a}	6.33 ± 0.12^{b}	7.67 ± 0.10^{a}	8.17 ± 0.21^{a}	7.13 ± 0.15^{b}
TDS (mg/L)	108.62 ± 4.45^{a}	286.87 ± 22.02^{a}	313.68 ± 23.74^{a}	78.88 ± 5.05^{b}	129.49 ± 33.58^{b}	147.06 ± 34.65^{b}	65.55 ± 1.17^{b}	117.49 ± 10.63^{b}	133.70 ± 7.96^{b}

Figures on the same column having the same superscript are not significantly different ($p>0.05$, ANOVA).

The ammonia (NH_3) concentrations ranged from 0.022 mg/L to 0.026 mg/L (Figure 5.1). LRR recorded the highest NH_3 concentration from day 7 to day 28, which was not significantly different ($P > 0.05$, ANOVA) from IRR and HRR. In all recirculation rate treatments, NH_3 concentrations were observed to decrease with increasing recirculation rate.

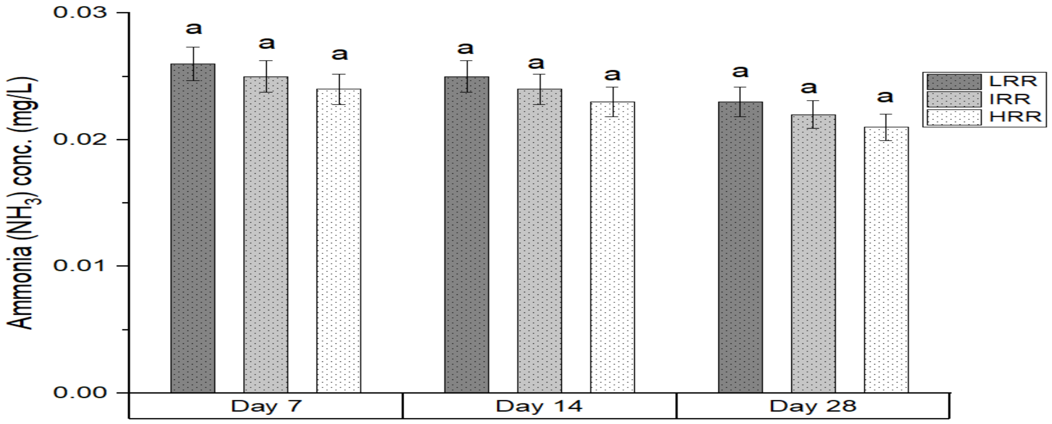

Figure 5.1: The ammonia (NH_3) concentrations recorded on day 7, day 14, and day 28 in aquaponic systems with varying recirculation rates stocked with *O. mossambicus*. Bars on the same day having the same letter are not significantly different ($p>0.05$, ANOVA).

The nitrite (NO_2^-) concentrations ranged from 0.021 mg/L to 0.093 mg/L (Figure 5.2). An increase in recirculation rate caused a decrease in the NO_2^- concentrations. LRR recorded the highest NO_2^- concentration from day 7 until day 28, which was significantly different ($p < 0.05$, ANOVA) from IRR and HRR. No significant difference ($p > 0.05$, ANOVA) in NO_2^- concentrations was observed between IRR and HRR on day 7 and day 28. In all treatments, NO_2^- concentrations were decreasing with the duration of the experiment.

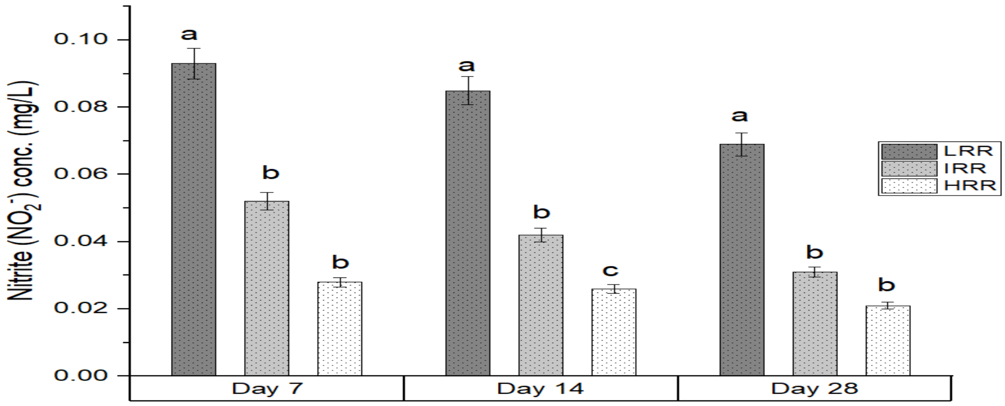

Figure 5.2: The nitrite (NO_2^-) concentrations recorded on day 7, day 14, and day 28 in aquaponic systems with varying recirculation rates stocked with *O. mossambicus*. Bars on the same day having the same letter are not significantly different ($p>0.05$, ANOVA).

The nitrate (NO_3^-) concentrations ranged from 2.07 mg/L to 2.43 mg/L (Figure 5.3). LRR recorded the highest NO_3^- concentration on day 14 and day 28. which was significantly different ($P < 0.05$, ANOVA) from IRR and HRR. NO_3^- concentrations did not differ significantly ($P > 0.05$, ANOVA) between treatments. In LRR and IRR, NO_3^- concentrations remained the same between day 7 and day 14, then decreased slightly on day 28. In HRR, NO_3^- concentrations decreased slightly between day 7 and day 14, then remained the same between day 14 and day 28. However, was no significant interaction ($P > 0.05$, ANOVA) between NO_3^- concentrations and the duration of the experiment.

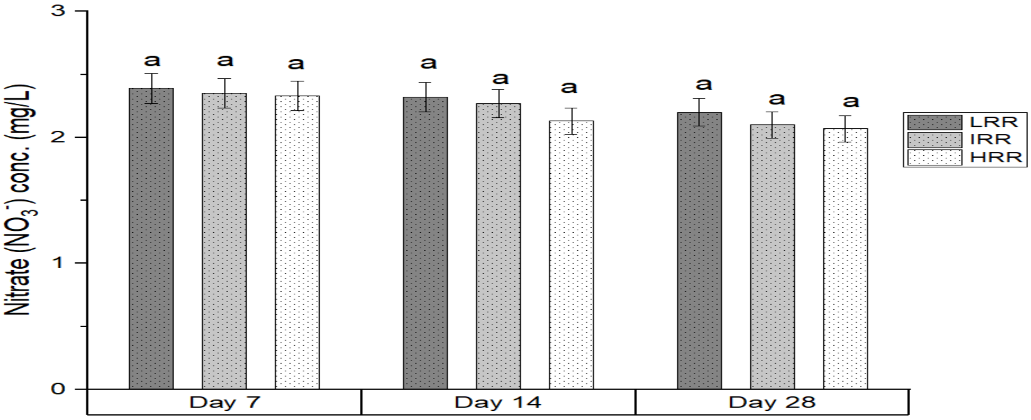

Figure 5.3: The nitrate (NO_3^-) concentrations recorded on day 7, day 14, and day 28 in aquaponic systems with varying recirculation rates stocked with *O. mossambicus*. Bars on the same day having the same letter are not significantly different (p>0.05, ANOVA).

The phosphorus (P) concentrations ranged from 0.03 mg/L to 0.17 mg/L (Figure 5.4). LRR recorded the highest P concentration from day 7 until day 28, which was significantly different ($P < 0.05$, ANOVA) from IRR and HRR. In all treatments, P concentrations were decreasing with the duration of the experiment.

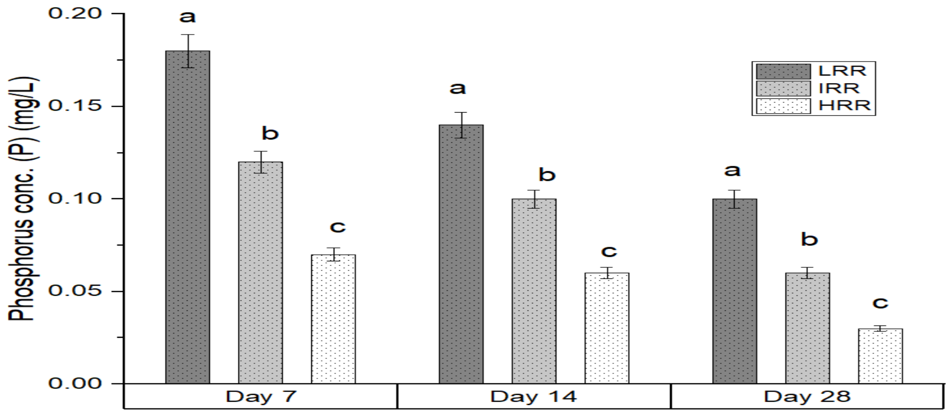

Figure 5.4: The phosphorus (P) concentrations recorded on day 7, day 14 and day 28 in aquaponic systems with varying recirculation rates stocked with *O. mossambicus*. Bars on the same day having the same letter are not significantly different (p>0.05, ANOVA).

5.5.2 Growth performance of *Oreochromis mossambicus*

The increasing recirculation rate was followed by an increase in the final weights of *O. mossambicus* (Table 5.2). The highest FW was recorded in IRR (24.66±0.35 g), which was significantly different ($p < 0.05$, ANOVA) from LRR but not HRR. IRR also recorded the highest ($p < 0.05$, ANOVA) %WG (54.34±4.03%) and SGR (2.35±0.10 %/day). However, no significant difference ($p > 0.05$, ANOVA) in %WG and SGR was observed between IRR and HRR (Table 5.2). A similar trend was observed with TGC. The lowest FCR was observed in HRR (1.32±0.11) which was significantly different ($p < 0.05$, ANOVA) from LRR (Table 5.2). No significant difference ($p > 0.05$, ANOVA) in FCR was observed between IRR and HRR. The increasing recirculation rate was followed by an increase in survival rates of *O. mossambicus* (Table 3.3). The highest SR was observed in IRR and HRR (94.22±1.54%) and the lowest in LRR (94.22±1.54%). There was no significant difference ($p > 0.05$, ANOVA) in SR between any of the recirculation rate treatments.

Table 5.2: Final weight, %weight gain, TGC, survival rate (mean ± SD) of *Oreochromis mossambicus* stocked in aquaponic systems with different recirculation rates for a period of 28 days.

Growth parameters	LRR	IRR	HRR
Final weight (g)	20.59 ± 0.44^a	24.66 ± 0.35^b	23.87 ± 0.56^b
% Weight gain	32.26 ± 5.46^a	52.21 ± 8.01^b	54.34 ± 4.03^b
SGR	2.09 ± 0.15^a	2.26 ± 0.19^b	2.35 ± 0.10^b
FCR	2.35 ± 0.12^a	1.60 ± 0.12^b	1.32 ± 0.11^b
TGC	1.42 ± 0.00^a	2.25 ± 0.00^b	2.34 ± 0.00^b
Survival Rate (%)	94.22 ± 1.54^a	98.67 ± 2.31^a	98.67 ± 2.31^a

Figures on the same row having the same superscript are not significantly different (p>0.05, ANOVA).

5.5.3 Organosomatic indices

An increase in recirculation rate was followed by an increase in CF. No significant difference ($p > 0.05$, ANOVA) in CF was observed between IRR and HRR. However, IRR and HRR were significantly different ($p < 0.05$, ANOVA) from LRR in terms of CF. The increase in recirculation rate also resulted in an increase in the HSI of *O. mossambicus* juveniles (Table 5.3). The highest HSI was observed in HRR (1.53±0.10) and the lowest in LRR (0.06±0.01). No significant difference ($p > 0.05$, ANOVA) in HSI was observed between IRR and HRR. A similar trend was observed with VSI (Table 5.3).

Table 5.3: The HSI and VSI (mean ± SD) of *Oreochromis mossambicus* stocked in aquaponic systems with different recirculation rates for a period of 28 days.

Somatic indices	LRR	IRR	HRR
Condition factor	1.16 ± 0.13^a	1.71 ± 0.10^b	1.63 ± 0.15^b
Hepatosomatic index (%)	0.06 ± 0.01^a	1.43 ± 0.10^b	1.53 ± 0.10^b
Viscerosomatic index (%)	1.55 ± 0.12^a	2.48 ± 0.19^b	2.68 ± 0.18^b

Figures on the same row having the same superscript are not significantly different (p>0.05, ANOVA).

5.5.4 Haematological analysis

With increasing recirculation rates, a decrease in red blood cell count was observed (Table 5.4). LRR recorded the highest RBC count ($2.14 \pm 0.05 \times 10^{12}$/L) which was significantly different ($p < 0.05$, ANOVA) from IRR and HRR. No statistically significant difference ($p > 0.05$, ANOVA) in RBC count was observed between IRR and HRR. White blood cell count, haemoglobin, haematocrit, and serum glucose followed the same trend. However, the lowest WBC count was recorded in IRR.

No discernible trend in the fish blood performance was observed with regard to recirculation rate. A significant difference ($p < 0.05$, ANOVA) in the blood performance was observed between LRR and HRR.

Table 5.4: The haematological parameters (mean ± SD) obtained from *Oreochromis mossambicus* stocked in aquaponic systems with different recirculation rates for a period of 28 days.

Blood parameters	LRR	IRR	HRR
RBC count (x 10^{12}/L)	$2.14±0.05^a$	$1.27±0.04^b$	$1.19±0.01^b$
WBC count (x 10^9/L)	$16.43±1.78^a$	$5.76±0.06^b$	$7.40±1.20^b$
Haematocrit (L/L)	$0.12±0.02^a$	$0.19±0.01^b$	$0.38±0.01^b$
Haemoglobin (g/dL)	$4.40±0.28^a$	$4.75±0.07^b$	$7.45±0.07^b$
Serum glucose (mmol/L)	$8.23±0.56^a$	$5.06±0.82^b$	$4.72±0.74^b$
Blood performance	2.92^a	1.89^{ab}	3.22^b

Figures on the same row having the same superscript are not significantly different ($p>0.05$, ANOVA).

5.5.5 Growth performance of *Spinacia oleracea*

The highest mean number of leaves was recorded in IRR (14.00 ±2.00) while the lowest was recorded in HRR (5.00±1.00) (Figure 5.5). The highest ($p < 0.05$, ANOVA) number of leaves was recorded in IRR throughout the experiment. No significant variation ($p > 0.05$, ANOVA) in the number of leaves was observed between LRR and HRR on day 14 and day 28. In all recirculation rate treatments, the number of leaves increased with the duration of the experiment.

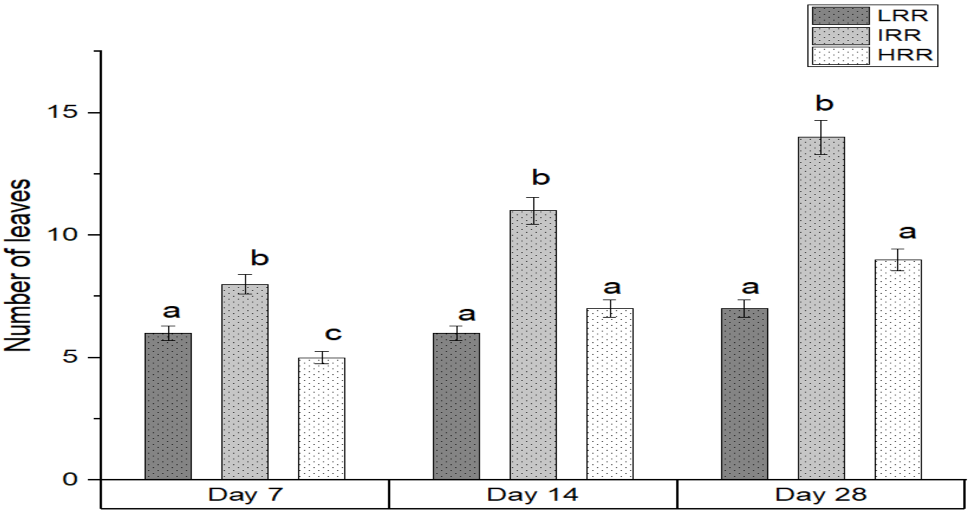

Figure 5.5: The number of *Spinacia oleracea* leaves recorded on day 7, day 14 and day 28 in aquaponic systems with varying recirculation rates stocked with *O. mossambicus*. Bars on the same day having the same letter are not significantly different (p>0.05, ANOVA).

The highest mean shoot length was recorded in IRR (14.30±1.63) while the lowest was recorded in LRR (4.56±0.50) (Figure 5.6). The highest ($p < 0.05$, ANOVA) mean shoot length was recorded in IRR on day 14 and day 28. However, no significant variation ($p > 0.05$, ANOVA) in the shoot length was observed between LRR and HRR on day 14 and day 28. The shoot length in all treatments increased with the duration of the experiment.

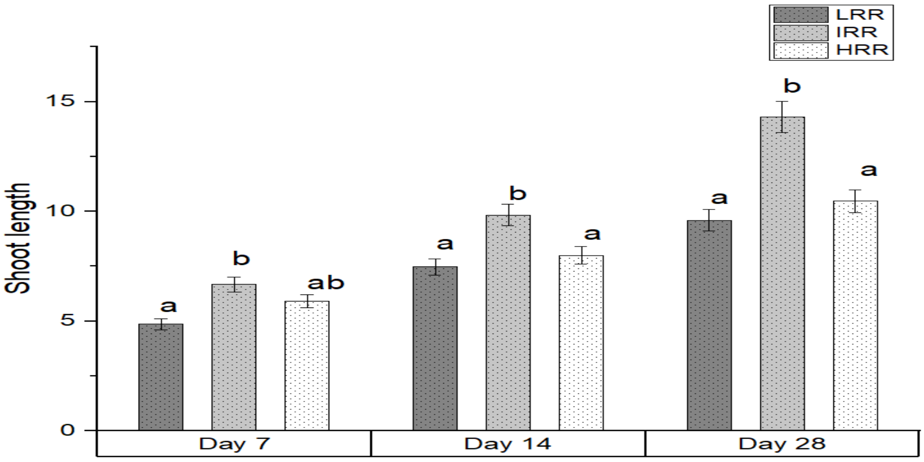

Figure 5.6: The shoot length of *Spinacia oleracea* leaves recorded on day 7, day 14 and day 28 in aquaponic systems with varying recirculation rates stocked with *O. mossambicus*. Bars on the same day having the same letter are not significantly different (p>0.05, ANOVA).

5.4 DISCUSSION

The dissolved oxygen levels were observed to increase with increasing recirculation rate throughout the experiment. The low recirculation rate treatment recorded the lowest DO concentrations which were significantly different from those in the intermediate recirculation rate and high recirculation rate. The reduced DO concentration in low recirculation rate treatment could be a result of microbial oxidation of accumulated organic matter in the water. The dissolved oxygen concentrations in the intermediate recirculation rate and high recirculation rate treatments remained within acceptable limits for the growth of *O. mossambicus* as indicated by (El-hack *et al*. 2022). El-hack *et al*. (2022). However, the DO concentrations at low recirculation rate were below acceptable limits for *O. mossambicus* growth in aquaponics. In agreement with this experiment, Obirikorang *et al*. (2019) observed restricted water flow at low recirculation rates, resulting in reduced DO concentrations. León-Ramírez *et al*. (2022) also observed decreased DO concentrations at low recirculation rates. The DO concentration ranges in Chapter 3 and Chapter 4 were 5.70-8.60 and 8.13-8.63, both which were within acceptable limits for the growth of *O. mossambicus* in aquaponic systems. In this chapter, the lower DO concentration limits were much lower than the ranges in Chapter 3 and Chapter 4, and below acceptable limits for *O. mossambicus* growth in aquaponic systems. However, the upper DO concentration limits were within range.

Total dissolved solids (TDS) decreased with the increasing recirculation rate. This could be attributed to the increased accumulation of fish feed and metabolites as the recirculation rate decreases. A significant difference in TDS levels was observed between all the recirculation rate treatments. Obirikorang *et al*. (2019) also observed decreasing TDS with increasing recirculation (flow) rate at a range of 132.71-143.00 mg/L. This TDS range falls within the TDS range recorded in this study (65.55-313.68 mg/L). The TDS level ranges in Chapter 3 and Chapter 4 were 57.55-215.93 mg/L and 64.13-209.53 mg/L. The upper TDS limit in this chapter was much higher than the ranges in Chapter 3 and Chapter 4, but the lower limit was within range. The TDS levels remained within acceptable limits for growth to *O. mossambicus*.

Ammonia (NH_3), nitrate (NO_3^-) and nitrite (NO_2^-) concentrations decreased with increasing recirculation rate. Increasing recirculation rates could have reduced the build-up of nutrients in the water. At low recirculation rates, the excess feed and fish excrement that enters the system accumulates, leading to poor water quality. Similarly, Sun *et al*. (2016) recorded the highest unionized ammonia (NH_3) at the lowest recirculation rate. Nuwansi *et al*. (2015) also recorded the highest NH_3, NO_3^- and NO_2^- at the lowest flow rate of 0.8 L/min. Li *et al*. (2019) observed no significant differences in NH_3 and NO_2^- with respect to different recirculation rates. NH_3, NO_3^- and NO_2^- also decreased with the duration of the experiment, this could be a result of nutrient uptake by the plants or microbial oxidation of NH_3 and NO_2^-. Similarly, Stathopoulou *et al*. (2021) observed decreasing concentrations of NH_3, NO_3^-, and NO_2^- concentrations with the increasing duration of the experiment. The NH_3, NO_3^- and NO_2^- concentrations remained within acceptable limits for the growth of *O. mossambicus* in aquaponic systems.

The concentration of NH_3 depends on pH and temperature and decreases at high pH and temperature. The temperature was kept constant, and the pH range was kept between 6.4 and 7.4. These parameters did not affect the concentration or the toxicity of NH_3 in the system. The upper limit of NH_3 for the growth of *O. mossambicus* is 2.0 mg/L and the ranges in this study were below that. The NH_3 ranges did not differ between the chapters on feeding frequency, stocking density and recirculation rate.

Decreasing concentrations of phosphorus (P) occurred as result of increasing recirculation rate. The highest P concentration was recorded at the lowest recirculation rate. This could be attributed to the increasing accumulation of excess feed and fish waste with the decreasing recirculation rate. Nuwansi *et al*. (2015) also recorded the highest P concentration at the lowest flow rate of 0.8 L/min. The P concentrations were also decreasing with the duration of the experiment. This may be due to its uptake by plants. Comparably, Stathopoulou *et al*. (2021) observed decreasing concentrations of P concentrations with the increasing duration of the experiment. The phosphorus concentrations remained within acceptable limits for growth of *O. mossambicus* in aquaponics. The P ranges were similar between the chapters on feeding frequency, stocking density and recirculation rate.

The growth performance of *Oreochromis mossambicus* in terms of %weight gain, specific growth rate and thermal growth coefficient increased with increasing recirculation rate. The best WG, SGR and TGC were observed in the fish stocked in aquaponic systems with the highest recirculation rate of 2.5 L/min. This could be due to good water quality (high DO and low TDS) in high recirculation rates, which resulted in the absence of stress in the fish. As a result, the fish were able to feed efficiently and grow better compared to fish with low recirculation rates. At low recirculation rates, there is an accumulation of fish waste and metabolites that can result in poor water quality (low DO and high TDS) which can poorly affect the growth of fish. According to Obirikorang *et al*. (2019), exposing fish to hypoxia (low DO concentrations), can lead to reductions in feed intake and their subsequent growth. Similarly, Schram *et al*. (2009) recorded a higher growth of juvenile *Scophthalmus maximus* at the highest water flow rate. Nuwansi *et al*. (2015) observed the best growth of *C carpio* at the lowest recirculation rate of 0.8 L/min. Obirikorang *et al*. (2019) indicated that the increased growth with increasing flow rate is due to reduced accumulation of fish metabolites and waste in the tank water. In agreement with this, Jorgensen *et al.* (2017) also indicated that the growth of fish was increasingly compromised by decreasing water exchange rate. In relation to this observation, Diem *et al*. (2017) and Obirikorang *et al.* (2019) observed the lowest fish growth in the tanks with reduced water exchange. In contrast to the present study, Li *et al*. (2019) indicated that the highest velocity (0.36m/s) was able to increase fish feed intake but failed to promote growth. Li *et al*. (2019) observed the best feed intake and SGR at the medium recirculation rate of 0.18m/s.

The maximum performance of *O. mossambicus* in terms of food conversion ratio was observed at the highest recirculation rate of 2.5 L/min. This may have been due to better feed intake when fish are not stressed by poor water quality. Endut *et al*. (2009) and Hussain *et al*. (2014) observed no significant difference in the feed conversion ratio at various flow rates. The differences in the results may be attributed to the fish species. Waste production between *O. niloticus*, *C. carpio* and *O. mossambicus* may differ due to the fish's feeding behaviours. More active feeders necessitate higher recirculation rates to manage the waste and nutrient levels. The SGR obtained at the best recirculation rate was 2.35 %/day. A lower SGR was observed here in comparison to Chapter 3 where the best SGR was observed at a feeding frequency of 3 times daily (2.53 %/day). In Chapter 3, the best SGR (2.16 %/day) and FCR (1.27) were reported in the 3 times daily feeding, where the fish was able to feed efficiently. In Chapter 4 the best SGR (2.26 %/day) and FCR (1.35) were observed in the intermediate stocking density, where fish were exposed to minimal stress from crowding and competition for food. They did not vary remarkably from the 2.35 %/day and 1.32 reported in this chapter at the highest recirculation rate, where the fish was exposed to better water quality and as a result, minimal stress. The FCR recorded at the best recirculation rate in this chapter was higher compared to the FCR observed at the best feeding frequency in Chapter 3 (1.27). This could be that 3 times daily feeding instead of once daily was required in this experiment in order to obtain better growth performance of the fish.

The survival rate of *O. mossambicus* was not significantly affected by the recirculation rate. A few mortalities were observed in each recirculation rate treatment, and they were not related to the recirculation rate, they could have been a result of handling. Hussain *et al*. (2014), observed 100 % survival in all recirculation rate treatments. The SR in Chapter 3 and Chapter 4 were both above 95%, indicating low fish mortalities. In this chapter, the lowest SR was 94.22 which is not bad at all in comparison to the 95% in Chapter 3 and Chapter 4.

The best CF was observed at the highest recirculation rate. However, there was no significant difference in CF between the intermediate recirculation rate and the high recirculation rate. Increased water circulation at a high recirculation rate helps maintain optimum water quality parameters, such as dissolved oxygen levels and waste removal, which are essential for fish growth. Improved water quality can reduce stress on fish and support their metabolic processes, resulting in better growth rates. Reduced stress and optimum conditions can contribute to better feed utilization. At low recirculation rates, fish may have been stressed a result of low dissolved oxygen levels caused by the accumulation of organic matter in the water. Stress can negatively impact the condition factor as stressed fish may allocate more energy to dealing with stressors rather than growth and maintenance. Obirikorang *et al*. (2019) observed no significant effect of recirculation rates on the CF of fish. The best condition factor in Chapter 4 was recorded in at intermediate stocking density (1.64) where there was moderate competition for food, no crowding and better fish growth. It did not differ remarkably to one recorded in this chapter at high recirculation rate (1.71), where there was optimal water quality and best fish growth.

The lowest hepatosomatic index and viscerosomatic index were observed in low recirculation rates while the highest were observed at high recirculation rates. This could have resulted from reduced feed intake and utilization caused by stress. Similarly, Obirikorang *et al*. (2019) observed the highest HSI and VSI in fish stocked at high recirculation rates.

The highest red blood cell count was observed at the lowest recirculation rate. This could be in response to fish stress resulting from low DO concentrations. The production of red blood cells can be a physiological response to help fish maintain an adequate oxygen supply when facing stressful conditions such as low oxygen levels. Similarly, Obirikorang *et al*. (2019) observed higher RBC counts at low recirculation rate treatments. Higher recirculation rates result in better oxygenation of the water, which can lead to improved oxygen delivery to fish tissues. As a result, higher recirculation rates may help maintain RBC counts. In Chapter 4, the highest RBC count was observed at the highest stocking density, where there was reduced fish growth due to crowding stress and competition for food. In the current chapter, the highest RBC was observed at the highest recirculation rate, where the best fish performance was observed. Therefore, elevating or reductions in RBC count cannot be a reliable measure of the health status of fish. The highest blood performance (BP) was recorded at the lowest stocking density where there was no crowding stress. At the highest recirculation rate where there was no stress related to DO concentrations, the highest BP was also observed. The BP was consistent with the conditions where the fish were not experiencing any stress. The high BP value can be a signal of better growth and health of fish (Esmaeili *et al*. 2021).

Any change in the physiological status of the fish, from pollution to nutritional stress, can cause changes in the blood parameters (Esmaeili *et al*. 2021). The highest white blood cell count was observed at the lowest recirculation rate, this could have been due to stress resulting from hypoxia. Similarly, Obirikorang *et al*. (2019) observed higher WBC counts in low recirculation rate treatments. In Chapter 4, the highest WBC count was observed at the highest stocking density as a result of crowding stress. The highest BP and the WBC count had opposite trends. The increase in

WBC count was as a result of increasing and the opposite happens with BP. At this stocking density, the lowest haematocrit and haemoglobin were observed. The highest recirculation rate led to the highest haematocrit and haemoglobin. Increasing concentrations of haematocrit and haemoglobin within a normal range can represent a good sign of optimized oxygen transport thus improving fish growth (Esmaeili *et al*. 2021). The haematocrit and haemoglobin levels were in the same trend as the BP, decreasing with increasing stress levels.

The highest serum glucose levels were recorded at the low recirculation rate treatments, where DO concentrations were below acceptable limits for the growth of *O. mossambicus*. The low DO concentrations could have led to stress in the fish. Similarly, León-Ramírez *et al*. (2022) recorded the highest serum glucose level in *O niloticus* juveniles stocked at the lowest recirculation rate. Li *et al*. (2019) observed no significant differences in serum glucose levels among the different recirculation rates. The highest blood performance of 3.22 was observed at the high recirculation rate treatment. This could suggest that the high recirculation rate resulted in the best health of *O. mossambicus.* This could suggest that the DO concentrations were optimal for the functioning of the fish. In Chapter 3, the highest fish blood performance was observed in 3times daily feeding (2.33), this may be due to better feed intake and utilization resulting in better fish health. In Chapter 4, the best blood performance was seen at the low stocking density treatment (5.04). This suggests that better fish health was observed at this stocking density, which could a result of minimal exposure to crowding stress and competition for food. The blood performance seen in this chapter was lower that the one recorded in Chapter 4, but higher than the once seen in Chapter 3.

The growth performance of *Spinacia oleracea* in the present experiment increased with increasing recirculation rate. The best growth performance of *S. oleracea* was observed at an intermediate recirculation rate of 1.5 L/min. This revealed that the intermediate recirculation rate treatment could have had sufficient nutrients which resulted in higher plant growth compared to the low and high recirculation rate groups. In accordance with this finding, Endut *et al*. (2009) observed the best plant growth at a recirculation rate of 1.6 L/min. The lower performance of *S. oleracea* was observed at the recirculation rates 0.5 L/min and 2.5 L/min. At a low recirculation rate, the reduced plant growth could be due to marginal DO concentrations that restrict nutrient uptake through plant roots (Nuwansi *et al*. 2015), leading to lower plant growth. At high recirculation rates, there is reduced contact between plant roots and the water (Wonkiew *et al.* 2017), which could be the reason for the lower plant growth observed. Plant growth increased with increasing duration of the experiment. This could be due to the increasing ability of plants to assimilate more nutrients as they grow. In Chapter 3 and Chapter 4, the best *S. oleracea* growth was observed 3 times daily feeding and high stocking density, where the nutrient accumulation was the highest. However, in this Chapter, the treatment with the highest nutrient accumulation exhibited DO concentrations that were below the plants' requirements. As a result, the best plant growth was observed at an intermediate recirculation rate.

Finding the optimum recirculation rates can allow the adjustment of feeding frequencies and stocking densities without compromising the water quality. Higher fish and plant densities can be used without the concern of deteriorating water quality as a result of nutrient build-up in the water. Optimum feeding frequencies can also be applied without having to worry about the accumulation of fish waste and compromising the water quality.

Conclusion

The recirculation rate of 2.5 L/min resulted in the best growth of *O. mossambicus* in terms of SGR and FCR. The lowest fish growth was observed at a low recirculation rate. The best CF, HSI and VSI were recorded at the highest recirculation rate of 2.5 L/min. The best blood parameters were also observed at the highest recirculation rate. The recirculation rate did not affect pH. The low recirculation rate treatment resulted in DO concentrations that were below acceptable limits for the growth of *O. mossambicus*. The highest NH_3, NO_3^-, NO_2^- and P concentrations, were observed at a low recirculation rate treatment, but the concentrations were still within acceptable limits for the growth of fish. High recirculation rates a resulted in lower concentration of dissolved solids (excess fish feed and excrement) and higher dissolved oxygen concentration and as a result, lower fish stress leading to a better growth performance. The best plant growth was observed at the lowest recirculation rate while the worst was observed at the highest recirculation rate. Therefore, for the best combined performance of *O. mossambicus* and *S. oleracea* in low technology aquaponic systems, the recirculation rate of 1.5 L/min is recommended. In conclusion, recirculation rate did affect the growth performance of *O. mossambicus,* low recirculation rates resulted in reduced fish growth.

CHAPTER 6: GENERAL DISCUSSION, RECOMMENDATIONS AND CONCLUSION

Aquaponics is an emerging fish production technique in aquaculture. There is very limited information on the fish husbandry practices in aquaponics. In this study, three main fish husbandry practices were investigated namely, feeding frequency, stocking density and recirculation rate. The study was carried out in a low technology aquaponic system that is deemed to be inexpensive. The fish species of choice for this study was *Oreochromis mossambicus*, a commonly culture fish species in South Africa. *O. mossambicus* is exceptionally hardy and can tolerate a wide range of environmental conditions and high stocking densities (Akter *et al*. 2018; Masabni *et al*. 2020), making it highly adaptable for aquaponics production (DAFF, 2018).

Furthermore, *O. mossambicus* is native to South Africa, which makes permit applications easier for producers to attain (DAFF, 2018) and the fish species of choice for this study. *Other* studies focused on *O niloticus* (Liang and Chien, 2013; Diem *et al*. 2017; Nasrin *et al*. 2021; Sabwa *et al*. 2022; Alameen *et al*. 2023; Al-Zahrani *et al*. 2023) while others focused on *C. carpio* (Hussain *et al*. 2014; Nuwansi *et al*. 2015; Maucieri *et al*. 2019; Obirikorang *et al*. 2019; Nuwansi *et al*. 2021) as their fish species of choice. For even better combined fish and plant growth, fish polyculture can be considered. The combination of herbivorous and omnivorous fish would result in a better use of food, as herbivorous fish would feed on both pellet and crop residues while the omnivorous fish would also seek wastes accumulating at the bottom of the tank (Somerville *et al*. 2014).

Polyculture in coupled aquaponics has the potential to increase plant yield (Knaus and Palm, 2017). El-Sayed *et al*. (2022) indicated that *O. niloticus* and *C. carpio* performed better (in terms of FCR, SGR and SR) in polyculture compared to monoculture. The plant species of choice was *Spinacia oleracea*, which a commonly cultivated vegetable. Hussain *et al*. (2014), Kabir *et al*. (2021) and Al-Zahrani *et al*. (2023) also focused on *S oleracea* as the plant species for the hydroponic system. Other studies used *Lactuca sativa* (Effendi *et al*. 2017; Maucieri *et al*. 2019; Stathopoulou *et al*. 2021; Yamane *et al*. 2021; Sabwa *et al*. 2022), while others used *Ipomoea aquatica* (Endut *et al*. 2009; Liang and Chien, 2013; Nuwansi *et al*. 2015; Akter *et al*. 2018; Pamula *et al*. 2019) for their hydroponic system. At the highest feeding frequency of three times daily, the highest fish and plant growth was achieved. Similarly, studies by Ahsan *et al*. (2009) on *O niloticus*, Jegede and Olorunfemi (2013) on *O niloticus*, Aderolu *et al*. (2010) on *C. gariepinus* and Alal (2018) on *O niloticus*. Other studies by Falaye and Omoike (2013), Chen *et al*. (2014), Thongprajukaew *et al*. (2017) and Nasrin *et al*. (2021) recommended the feeding frequency of 2 times daily for optimal aquaponic production of *O niloticus*. However, it must be noted that the feeding frequency may be affected by both biological and non-biological factors.

Feeding frequency can be affected by the size of the fish. The results obtained in this study were specific a given size of fish and should not therefore be used as representing across the board for every size range. The type of feed also has an effect on the feeding frequency. Poor quality feed (low nutrient content) may require high feeding frequencies while good quality feed may require lower feeding frequencies. These factors must be taken into account before making recommendations on the most appropriate feeding frequency. Water temperatures can also affect the frequency of feeding. *O. mossambicus* will stop feeding at temperatures below 16°C and above 32°C. In this study, the temperature was kept at 28°C which is the optimal for the growth of *O. mossambicus*. The feeding frequency obtained must always refer to the temperature that is used, because temperature has an important barring on the feeding frequency.

In aquaponics, the majority of these nutrients arise from the fish wastes. However, fish feed and waste do not contain all the nutrients needed for plant growth or they are found in low quantities causing nutrient deficiencies in the plants. As a result, the plant chosen for the aquaponic system needs to be a low nutrient-demanding plant. In general, leafy vegetables have been the preferred crop to grow in aquaponic systems instead of fruiting plants. The plant and fish mature at different times, the fish had not reached marketable size when the plants were ready to be harvested. This presents a management problem because harvesting plants collapses the hydroponic system. To prevent the collapse of the hydroponic system as a result for plant harvesting, two plants with different culture periods can be intercropped so that when the first plant gets harvested, the other remains and continues to act as filter. Intercropping also has the potential to increase the overall productivity of the system (El-Sayed *et al*. 2022). The plant densities during intercropping may need to be adjusted based on the plants' nutrient requirements. The fish stocking density can also be affected by the fish size, the water quality, and the temperature. Thus, any recommendation on stocking density must be made in relation to these factors. However, in this study the best growth performance of both the fish and plant was achieved at the intermediate stocking density.

In tilapia species, it has been shown that at low stocking densities, the growth of tilapia regresses. At very high stocking densities, there is reduced growth of tilapia resulting from crowding stress and competition for food. This can also trigger precautious breeding *O. mossambicus*, resulting in reduced fish growth because the fish channels most of the energy to reproduction instead of growth. Thus, intermediate stocking densities are recommended for the aquaponic production of *O. mossambicus*. Sabwa *et al.* (2022) also recommended intermediate stocking densities for aquaponic production of *O. niloticus.* Other studies by Rahmatullah *et al.* (2010) on *O. niloticus*, Wu *et al.* (2018) on *O. niloticus*, Maucieri *et al.* (2019) on *C. carpio*, Abaho *et al.* (2020) on *O. niloticus*, Nuwansi *et al.* (2021) on *C. carpio* and Al-Zahrani *et al.* (2023) on *O. niloticus* recorded the best fish growth at the lowest stocking density. Intermediate recirculation rates are recommended the best growth of both *O. mossambicus* and *S. oleracea* in low technology aquaponic systems. Hussain *et al.* (2014) also recorded the best growth of *C. carpio* at an intermediate stocking density. Other studies by Diem *et al.* (2017) and Obirikorang *et al.* (2019) on *O. niloticus* recommended high recirculation rates for optimal fish growth.

The stocking density and feeding frequency can affect the recirculation rate, therefore the recommended recirculation rate for aquaponic systems must be based on the feeding frequency and the stocking density. An optimal ratio for plant and fish in aquaponic systems can only be achieved if an appropriate recirculation rate is determined. It is thus recommended that a model that can be used to predict the optimal plant to fish ratio for different recirculation rates. Different experiments should be carried out to determine the potential plant to fish ratio at different stocking densities. Many studies have suggested the use of a sedimenter for the collection of accumulating solid matter, however the aquaponic system design used in this study did not have a sedimenter. The sedimenter was deemed unnecessary since there was very little build up of solid material in the tanks. The design of the system used in this study is recommended for household use. This system can be used in rural households in a case whereby solar power can be secured to run the system. Therefore, the system is recommended for both rural and urban households.

Ideally, this experiment should have been a 3*3*3 factorial design. Feeding frequency, stocking density and recirculation rate should have been evaluated concurrently. However, due to limitations of resources, it was practically impossible to do the 3*3*3 factorial design.

Recommendations:

- It is recommended that *O. mossambicus* of mean weight 38 g be fed 3 times daily to optimize growth. However, fish that are larger than 38 g must be fed at lower feeding frequencies.
- An intermediate stocking density is recommended for *O. mossambicus* of mean weight 10 g. This stocking density enhances growth *O. mossambicus* growth because low stocking density leads to regressed growth due to reduced motivation to feed and high stocking density also leads to regressed growth due to competition for space and food.
- For optimal growth *O. mossambicus* of mean weight 15 g, an intermediate recirculation rate of 1.5 L/min is recommended. At this recirculation rate there is sufficient DO concentrations for optimal fish growth.
- A planting density of 18 plants/system of *Spinacia oleracea* is recommended to optimize nutrient removal in aquaponic systems. A higher plant density may be required when fish stocking densities are higher than 2.50 kg/m^3, and vice-versa when lower fish stocking densities are used.
- It is recommended that a model be developed that can be used to predict the optimal plant to fish ratio for different feeding frequencies, stocking densities and recirculation rates.
- It is recommended that more studies be conducted to optimize fish husbandry practices in aquaponic systems.

CHAPTER 7: REFERENCES

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