RHODES UNIVERSITY

An aquaponics production output comparison between drain-fill versus flooded bed cultures and between solids removal versus no solid removal, using African catfish and Swiss chard

A thesis submitted in fulfilment of the requirements for the degree:

Master of Science in Fisheries Science

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Submitted on: 30 April 2025

DECLARATION

I, **Franck Mateso Lutili**, declare that this work has not been previously submitted in whole, or in part, for the award of any degree. It is my own work. Each significant contribution to, and quotation in, this dissertation from the work, or works, of other people has been attributed, and has been cited and referenced.

Signed

By: **Franck Mateso Lutili**

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At: Grahamstown

ABSTRACT

Aquaponics is one of the solutions for food self-sufficiency which constitutes a challenge for many countries. This technology is based on the integration of aquaculture and hydroponic production, through water recycling. The result is the availability of fresh fish and vegetables for the benefit of communities and the reduction of environmental pressure associated with conventional production systems.

With this technology, the problem of eliminating solid waste from uneaten food and fish excretions arises. These wastes can become the ultimate source of water pollution in aquaponics. These pollutants are rich in nitrogen compounds, which are toxic in certain forms. Nitrogen exists in various forms, and among these forms are ammonium (NH_4^+), ammonia (NH_3), nitrite (NO_2^-), nitrate (NO_3^-), and nitrogen gas (N_2). In its NH_3 form, it is toxic to aquatic life, but also in its NH_4^+ form, but at higher concentrations. However, nitrogen is important in its nitrate form. In this form, it assists in plant growth integrated with soilless growing technology, although plants can also absorb nitrogen in other forms. In addition, most vegetables are terrestrial, and their root system requires access to air. Drain-fill technology is used in hydroponics and has been shown to increase the biological filtration capacity of systems, and increases plant production, but this technology has not been widely studied in aquaponics.

The aim of this study was to evaluate the performance of primary facultative pond (PFP) used to maintain solid waste in the system, and drain-fill technology in aquaponics. This was done by comparing water quality parameters, growth and health of African catfish (*Clarias gariepinus*) and Swiss chard (as a crop) between conventional systems where solids were removed (which acted as the control) and those with a PFP that maintain and treat solid waste as a loop in the system. The same parameters were compared between aquaponics systems with flooded grow beds (which acted as the control), to grow beds using drain-fill technology.

Fish were significantly larger and about 37.6% heavier in the drain fill compared to the flooded aquaponics system after five months, and specific growth rate was significantly higher in the drain-fill system at 1.41±0.03% bw/day. The wet leaf biomass of Swiss chard was significantly different between the flooded (689.3±90.1 g) and drain-fill treatments (17529.6±2731.9 g).

In the no-PFP treatment (where solid waste was removed from the system), fish weight and length were not significantly different to the PFP (where solid waste was maintained in the system). The wet leaf biomass of Swiss chard was also similar between the two treatments (18191.00±4765.39 g, in no-PFP and 21618.67±7805.50 g in PFP).

This study contributed to the knowledge and understanding of the operation of different systems in aquaponics. It showed that the drain-fill technology is a better alternative to the flooded as it produced better plant and fish biomass, probably due to the aeration (and additional oxygen) associated with drain-fill technology. The addition of a PFP however did not appear to have a significant impact on fish and plant production, but this requires further investigation because the benefit of maintaining nutrients in the system (with the addition of the PFP) might have been masked by excessive nutrient availability and/or the inclusion of insufficient vegetable biomass in the system to realise the benefit of the additional nutrients that were made available by the PFP.

Keywords: aquaponics, drain-fill, flooded bed, solids removal, African catfish, Swiss chard.

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LIST OF ABBREVIATIONS

AM	Ante meridiem (before noon)
BW	Body weight
CCI	Chlorophyll concentration index
CF	Condition factor
COD	Chemical oxygen demand
DIFS	Department of Ichthyology and Fisheries Science
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen.
EC	Electrical conductivity
EXP	Experiment
FAO	Food and Agriculture Organization of the United Nations
N	Nitrogen
NH_3	Ammonia
NH_4^+	Ammonium
NO_2	Nitrite
NO_3^-	Nitrate
NO PFP	No primary facultative pond
N_2	Nitrogen gas
mm	Millimeter
$mg \cdot L^{-1}$	Milligram per liter
$\mu S \cdot cm^{-1}$	Microsiemens per centimeter
PO_4	Phosphate
PM	Afternoon
FCR	Food conversion ratio
g	Gram
H^+	Proton
L	Litter
O_2	Oxygen gas
P	Phosphorus

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PVC	Polyvinylchloride
POM	Particular Organic Matter
PFP	Primary facultative pond
pH	Hydrogen potential
RU	Rhodes University
RU-AREC	Rhodes University Animal Research Ethics Committee
RNA	Ribonucleic acid
R-CH-NH_2-COOH	Organic nitrogen including amino acids.
SDGs	Sustainable Development Goals
SGR	Specific growth rate
TAN	Total ammonia nitrogen
TL	Total length
TDS	Total dissolved solids
T	Treatment
%	Percentage
‰	For a thousand
ºC	Degree Celsius

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ACKNOWLEDGEMENTS

May the Rhodes University community in general and the DIFS receive my sincere thanks for all their participation. I am very grateful to my promoters. Professor C.L.W. Jones and Dr Nyiko Mabasa welcomed me in these hands, as well as in the various DIFS laboratories and who trusted me until the end.

Professor Mbalassa Mulongaibalu and the entire COTRA project team, thanks to whom I obtained this scholarship. May they find here all the expressions of my gratitude. As for Professor Philip Okoth Raburu, I remember the first contact I had with him, it was on the phone very early in the morning around 6:00 am and that was the start of the metamorphosis for me. May he also receive my sincere thanks. Our thanks go to my lecturers: Lina Aleke, Muhigwa Bahananga, Cikwanine Kasigwa, Murhabale Cisirika, Nshombo Muderhwa, ChantalNzabandora, Isumbisho Mwapu, Akonkwa Balagizi, Amani yq Igugu for the training of high quality they offered me (in DR Congo).

Professor H. Kaiser never stopped encouraging and supporting me when I needed water in the tank. May he accept my sincere thanks. Our thanks to Mr. Selapa N who entrusted me with his water quality analysis laboratory at the University of Rhodes for the analysis of the water qualityparameters, we let him know that this opened doors to obtaining this master's degree. I would like to thank here, Masudi Wiya, Elizabeth Obado, Abbie Tee, Shamim Naigaga, Thierry Lwanzo, Justin Safari, Dr Urbain Ndagano, Joseph Matungulu, God only knows why our paths crossed, in RU in South Africa but it was the start of a great and eternal friendship.

I thank the staff members of the UOB Faculty of Science: Professor Balezi Zihalirwa, Professor Lina Aleke. Many other people have contributed in one way or another to the achievement of this work, I thank them. This is the case of Mwezani Mukamba, Balalwa

Lwesso, Ntole Birere, Bahati Maheshe, Desire Maheshe, Lukunga Iragi, Claire Sakina, Safi Claudine, Esther Maheshe, my friends, brothers and sisters, uncles and aunts, cousins, nephews, and nieces... And then there is Brigitte Amina whose knowledge is probably very far from Aquaponics, flooding, PFP, no-PFP, but who knew how to manage and educate, all alone, Josias Mutekya while providing me with what I needed.

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DEDICATION

To the Almighty, the God of all graces who governs all and who had planned everything.

To

Brigitte Amina my wife,

Josias Mutekya my son,

My father Ir. Marcel Mutekya and my mother Adelle Wabusala,

All those who are dearest

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Chapter 1. Introduction

Food self-sufficiency is a challenge for many countries and is an integral part of the Sustainable Development Goals (SDGs) (FAO, 2012). The sustainable development program starts from its objective of transforming the world according to its 2030 horizon adopted by all the Member States of the United Nations in 2015 (FAO, 2015). Finding one of the solutions to this challenge lies in intensive aquaculture, as it contributes protein supply to communities (FAO, 2012; Antonio *et al.*, 2013). The production of fish using intensive aquaculture has recorded an increased rate of 6.1% in 10 years (2002-2012) ranging from 36.8 million tons to 66.6 million tons (Nawwar, 2016; Bosire and Sikora, 2017).

However, as the balance between intensive production and environmental preservation can be disrupted, implementing a new mode of production using recyclable resources such as aquaponics technology is necessary. It manifests itself by providing crops and fish and maintaining a circular economy (John *et al.*, 2005; Simon *et al.*, 2015; Viladomat and Jones 2011). Aquaponics technology is based on aquaculture and hydroponic production, allowing the recycling and valorization of nutrients. This is a process that promotes aquaculture farming, and crop production (Laidlaw and Magee, 2016). This technology is most needed for aquaculture industries as it can treat wastewater and reuse this waste as nutrients for agriculture (Graber *et al.*, 2014; Bosire and Sikora, 2017). The development of aquaponics technology in developing countries requires a structured approach to consolidate the achievements of the combined process including aquaculture and substrate-based hydroponics (Schneller, 2015; Maucieri *et al.*, 2018). The result then is the permanent availability of fresh products (fish and vegetables) for the benefit of the communities and the reduction of the environmental pressure caused by the conventional production system (FAO, 2014; Genello *et al.*, 2015).

Fish waste can be converted by bacteria into nutrients that sustain plants, while treated effluent is returned to fishponds (aquaponics) (Diver and Rinehart, 2010). It is the cycle of the symbiotic relationship between organisms (fish, bacteria, and plants) that promotes animal and plant productivity. Thus, bacteria are important as they transform toxic nitrogenous waste from fish farming into essential nutrients for plants, and this results in treated wastewater for reuse (Maucieri *et al.*, 2018).

To increase the productivity of fish farming, the addition of a biofilter containing a substrate acting as additional support for the growth of bacteria is a necessity (Somerville *et al.*, 2014). Like waste stabilization pond technology, primary facultative units are the most widely used

and studied; they can be designed as individual cells or a series of ponds.

Thus, within the framework of this study, the technology for drain filling and solids removal in aquaponics pursues the general objectives of evaluating the performance of this waste stream treatment, whilst also determining the nitrogen as water quality parameters for both systems. To find a solution in the use of drain-fill vs flooding, this work compares the water quality, fish and plant production in a drain-fill aquaponic systems to those in conventional constant flow cultivation (Experiment 1, Chapter 3). Similarly, this work also compared these parameters in an aquaponic system that includes a PFP and where solids are maintained in the system to those not having a PFP and where solids are removed from the system (Experiment 2, Chapter 4).

1.1. Problem statement

Aquaponics is the integration of fish farming and plant culture carried out on a neutral and inert substrate, which is irrigated by a current of a solution to provide the mineral salts and nutrients, provided by the farmed fish (aquaculture) and that are essential for plant growth in water (hydroponics) (APHA, 2005; Scheidegger and Wilhelm, 2006). With this technology, there is the problem of disposal of solid wastes from fish food and excretion (Jensen, 2003). These wastes are of inorganic and organic nature and are introduced into the aquaponics system through fish feed (Hochmuth and Hanlon, 2010; Junge *et al.*, 2017). Solid wastes can become the detrimental source of water pollution in aquaponics (Ghaly *et al.*, 2005). These pollutants are very rich in nitrogen compounds, which are toxic in certain forms (Coche, 2014), but these solids are made up of components that include N and P which could be used by the plants if they were presented in the correct form.

Many plants that are produced in aquaponics are terrestrial that require a well aerated root system and these plants are not as productive if produced in flooded systems (Ghaly, *et al.*, 2005). There is also evidence that the efficiency with which biofilters convert ammonia into nitrite and nitrate increases if the biofilter medium is well aerated (Goddek, 2017). It therefore stands to reason that drain-fill technologies that draw oxygen rich air into the filter medium when the vegetable grow-bed is repeatedly drained of water, might result in better effluent treatment efficiency and improved plant production.

In aquaponics, microorganisms co-cultivated in biofilters, and plants play a very important role in wastewater treatment (Turcios and Papenbrock, 2014; Khokhar, 2017). In this case, the organisms of the aquaponic system should be considered holistically in all aquaponic operations

(Rodier, 2009; Love *et al.*, 2014). Water quality parameters are the primary environmental consideration for optimizing aquaponics production (Jensen, 2003; Hargreaves *et al.*, 2005). These parameters have a direct impact on health problems and the survival of fish and plants in aquaponics (Jensen, 2003). In an aquaponic system, nutrient (Nitrogen) uptake must be maximized for healthy plant biomass production. It is also important not to neglect the best living conditions for fish in terms of water quality (Jensen, 2003; Person-Le Ruyet *et al.*, 2008). Aquaponic production is conditioned by the maintenance of good water quality and the proper functioning of the system (Love *et al.*, 2014). The proper functioning of the aquaponics system depends on the removal or treatment of solid waste (fish excrement and remains of food not consumed by the fish) (Hargreaves *et al.*, 2005).

This research tested the use of PFP in aquaponics at making components of solid fish waste available to the plants by treating this solid waste as part of a loop in the system. It also tested the use of drain-fill technology to aerate the vegetable grow-beds.

1.2. Hypotheses, overall aims and objectives

Hypotheses

The following was hypothesised:

- More fish production would be expected from the drain-fill as opposed to the flooded bed (H1);
- More vegetable production would be expected from the drain-fill as opposed to the flooded bed (H2);
- Higher NO_3 concentrations would be expected from the drain-fill as opposed to the flooded bed (H3); and
- Plant production would be expected to increase in aquaponics if solid wastes were maintained in the system and mineralised using a PFP (H4).

These hypotheses were backed by the expected advantages in the drain-fill versus the flooded aquaponics design. That is, the drain-fill aquaponics design was expected to:

1) reduce toxicity by transforming ammonia to nitrate;

2) improve fish and plant growth due to reduced toxicity and boosted nutrients released by solids treatment; and to

3) improve water quality due to solid waste and fish excreta treatment.

The flooded aquaponic design was expected to result in:

1) still water leading to reduced water treatment and overall poorer water quality;
2) ammonia not transformed, and contributing to increased toxicity;
3) excess nutrients in the system;
4) lower nitrate concentrations; and
5) lower fish and vegetable production due to nutrient shortage and toxicity.

Overall aims and objectives

This study aims to evaluate the performance of PFP and drain-fill in aquaponics at producing fish and vegetables.

The specific objectives of this study are to compare:

- the water quality parameters,
- fish health and production, and
- plant production,

between aquaponics systems that included:

(1) a vegetable grow-bed that used drain-fill technology, versus
(2) a system that included a grow-bed that was permanently flooded;

and between aquaponics systems that included:

(1) a loop in the system that maintained solids within the system using a PFP, versus
(2) a system with no-PFP and from which solids were removed.

1.3. Thesis structure

Chapter 1 of this thesis includes the general introduction of two experiments that were carried out. Experiment 1 addressed the use of drain-fill versus flooded aquaponic grow beds. Keeping solids in the system using primary facultative ponds (PFP) was addressed in Experiment 2. Most of the materials and methods that were used were common to both experiments, so the materials and methods were presented in a single chapter that was dedicated to material and methods only (Chapter 2). Chapters 3 and 4 are used to present and discuss the results of Experiments 1 and 2, respectively. The final Chapter 5 includes a general discussion and a general conclusion is presented in Chapter 6.

1.4. Literature review

1.4.1. Aquaponics history and evolution in the world

Aquaponic production, which consisted of combining fish and vegetable production in an integrated circulation system, is not new (Turcios, 2014). It was first used in Chinampa in Mexico and Asia (southern China, Thailand, and Indonesia) where the fish were used in rice cultivation, to produce fertilizer that supported plant productivity (Jones, 2002; Turcios, 2014). Aquaponics appeared at least 1,500 years ago in China (Xu *et al.*, 2014). This is a practice that was used by an entrepreneur, where co-products from ducks were used to support finfish and catfish production (Tura *et al.*, 2019). Ducks were stacked in cages above the water containing the finfish and catfish (Mateo-Sagasta *et al.*, 2017; Palm *et al.*, 2018). When the ducks were fed, their excrement and uneaten food fell into the water containing the fish (Lennard, 2012). The fish waste sank to the bottom of the pond, providing food for the catfish, which are bottom feeders and natural scavengers (DeLong *et al.*, 2009). In addition, the processed food, and all the waste from the fish was removed in the form of einkorn and transported to the rice fields and used as fertilizer (Joyce *et al.*, 2019). These fields were fertilized once, and this supported four harvests (Mchunu *et al.*, 2018).

The Incas of Peru practiced a different style of aquaponics before the arrival of the Conquistadors. Living in the centre of the island, they dug oval ponds near their mountain dwellings (Li *et al.*, 2019; Yildiz *et al.*, 2019). Once these ponds were filled with water, they stocked them with fish (Lennard, 2012). The geese arrived by flight, and fed form the water in the ponds (Jones, 2002). Waste (excrement) and fish remains quickly transformed the island into a super-rich high-quality garden (Turcios and Papenbrock, 2014). Now, not only did the Incas have the geese to do the work of fertilizer, but they also had fresh fish. Additionally, the system of mini ponds (islands) created a local microclimate that remained somewhat warmer than the surrounding mountains, providing additional harvest days each year (Mateo- sagasta *et al.*, 2017). This is how the production of the Inca's aquaponic systems fed more people than any other type of agriculture to date, in this arid environment (Turcios and Papenbrock, 2014; Khokhar, 2017; Palm *et al.*, 2018).

Currently, Aquaponics is not new, and is a solution to old problems (Lennard, 2012). Commercial aquaponics has begun to develop, both in the United States and around the world (Jones, 2002). In South Africa, aquaponics originated in the aquaculture industry, when fish

farmers tried to reduce dependency on resources when exploring methods of fish farming and crop cultivation (Mchunu *et al.*, 2019) Aaquaponics is able to produce and ecosystem maintenance as well. This technology can strengthen the capacity of humans to adapt to climate change, as well as to extreme weather conditions, and adaptation to climate change, as it recycles water (Yildiz *et al.*, 2019). The system water is mechanically filtered to remove solids and biological filtration is used to remove other dissolved elements (e.g., ammonia and ammonium) (Nelson and Siegel, 2014). It is necessary to control and maintain water quality parameters, such as NO_2^-, NO_3^-, $NH_3^+$and PO_4^{-3}, at the normal operating conditions required by the fish reared in the system. The aquaponics system has developed in recent years, especially regarding the treatment and disposal of solids, including work on mechanical filtration and bio-filtration (Bowers and Brett, 2008). This development of aquaponics is driven by profitability and stricter environmental requirements (Lockhart *et al.*, 1955; El-Bouhy *et al.*, 2006).

Aquaponics is a modern technology for recirculating aquaculture and agriculture systems that require continuous treatment of wastewater (Mongirdas and Kusta, 2006). Therefore, in aquaponics, the drain-fill technique using the water reuse system must be efficient, profitable, and simple to use (Friedman *et al.*, 1991). Aquaponics is a technology that produces a lot, using very little water, but it requires highly skilled personnel to operate (El-Bouhy *et al.*, 2006).

1.4.2. Definition of concepts

Typical aquaponics systems consist of a fish tank and a vegetable grow-bed (Figure 1.1). They usually also include a sump, from which water is moved using a pump (Figure 1.1). The configuration, the way water is moved, and the material used to construct them, may vary between system.

Figure 1.1: The diagram depicting the components of an aquaponics system, used in this study.

Drain-fill systems

The drain-fill systems are culture containers and aquariums that are used in aquaponics, to grow plants and fish (Palm *et al.*, 2018; Zwane, 2019). Drain-filling can be an effective means of disposing of wastewater in the hydroponic bed of an aquaponic system (Palm *et al.*, 2018; Zwane, 2019). The water is circulated to the culture bed, which is drained by the siphon and it is then returned to the aquarium (Xu *et al.*, 2014).

Mechanical and bio filters

Mechanical filtration is used to remove solids from an aquaponic system (Mchunu *et al.*, 2018; Zwane, 2019). In aquaponics, it is advised that the solids are not broken down into smaller particles, which can make them expensive or difficult to process (Li *et al.*, 2019; Zwane, 2019). Mechanical filters are usually located after the fish tanks, just before the biofilter. The PFP is a form of mechanical filtration or are considered an alternative to mechanical filtration. The PFP mineralises solid waste, and converts them into nutrients that can be utilised by plants in the system.

For any aquaponic unit designed, the operation and performance of its biofiltration depend on

the technology used and the characteristics of the filter media used (Thorarinsdottir *et al.*, 2015). There are several types of mechanical and bio-filters such as:

- The trickle filter consisting of a tank having a circular shape and is filled with various specific artificial media (balls or plastic balls, gravel, pumice stone). In this biofilter, the water is sprayed in its upper part (the water trickles through the filter medium). It is a filter grade that can provide passive aeration with the ability to remove carbon dioxide from system water.

- Moving bed bioreactors are filtration units containing filter media that float neutrally in the culture bed and are constantly agitated by the effects of aeration. Here the water is aerated by active diffusion, whilst excess carbon dioxide is driven off. For this biofilter, the filter media to be used are generally made up of plastic beads or another type of plastic product that can float on the surface to create a screen effect.
- The bed filter is a form of filter that generally uses a cylindrical pressurized tank. For this type of filter, the pearls of filtration media located inside it are temporarily agitated and then self-cleaned (backwashing) to avoid being clogged by solid waste.
- The sand filter, with a shape comparable to the bead filter, is a pressurized cylinder using sand as filtration media (Love *et al.*, 2014). It is a filter created specifically at an elevated surface where the sand ensures a well-defined rate of mechanical filtration with additional nitrification (Naddafi *et al*, 2009).

From the above, we can say the aquaponic system needs a large amount of oxygen for its operation (Love *et al*, 2014). This is explained by the process of nitrification consuming oxygen; hence the active aeration created in aquaponics by the biofilter properties. The water in the system must be adequately and constantly aerated to supply sufficient oxygen to the microbial community, and other living beings (fish and plants) of the integrated aquaponic system (Thorarinsdottir *et al.*, 2015).

The biofilter is a component of the system facilitating the process of mineralization of nitrogen or nutrient, for crop production (Mchunu *et al.*, 2018; Zwane, 2019). This mineralization is understood as a step by which the solids formed by organic matter decompose, resulting in treated wastewater (Xu *et al.*, 2014). In aquaponics, the biofilter is located after the mechanical filter, thus with a PFP function (Von Sperling and de Lemos, 2005).

Aeration and nitrification are important as a function of substrate surface required to convert ammonia to nitrite/nitrate. Aerobic biofilters (nitrification) and anaerobic biofilters (mineralization) need to be distinguished. The latter is a slower process requiring more retention

time (Tura *et al.*, 2019). This differentiation is important for sequential filtration positioning and exposure time (a design requirement). Also, both the aqueous nitrified product (NH_3-NO_3) and the mineralized products (from solids) are required for plant growth.

Nitrogen and nitrification are central to optimising plant production in an aquaponics system, since the process makes nitrogen available to the plants. That is, with biofiltration and with a primary facultative pond, inorganic/organic wastewater is converted into bioavailable nutrients for plants. In an aquaponic system, the microorganisms include archaea, protists, bacteria, fungi, and viruses, whose composition intervenes according to an ebb and flow of nutrients in aquaponics (Goddek, 2017; Khandaker and Kotzen, 2018).

Growing bed

A grow bed is part of an aquaponic unit, the most productive consisting of the vegetative system containing selected plants for growth and harvest (Xu *et al.*, 2014; Mchunu *et al.*, 2018). It is a small, potentially nutrient-rich growing unit supporting a viable root system for the final growth of selected plants transferred to it after seed germination (Tura *et al.*, 2019). This is a very important place in an aquaponic unit as it can be successfully used to produce a variety of plants (Mchunu *et al.*, 2018). Thus, the plant system chosen depends on the type of production, the environment, and the improving of the aquaponic plants being cultivated (Xu *et al.*, 2014; Mchunu *et al.*, 2018).

Constant flow bed

The system uses the flow process, with a constant flow rate. It is easy because the water in its system flows permanently, continuously, and constantly in the culture bed (Rakocy *et al.*, 2006). Here the water from the system enters through a single path from the end and thus fills the hydroponic culture bed until it reaches the desired maximum level, and then the water flows out through an overflow (outlet) (Thorarinsdottir *et al.*, 2015). This overflow is usually located at the other end of the system's hydroponic grow bed (Xu *et al.*, 2014). While the reflux process can be achieved by installing an influent pump that turns on and off automatically at well-determined time intervals (Rakocy *et al.*, 2006).

Drain-fill bed and a comparison with flooded bed

A bell-siphon unit is installed at the overflow of the drain-fill bed to facilitate filling up and draining. This is the model mostly used by many researchers (Love *et al*, 2014). Water fills to the maximum level, prior to automated draining (bell-siphoning), after which the siphon is

opened when air is sucked into the drain pipe. The process then restarts with a new cycle (Love *et al*, 2014). During the draining phase, the plant roots and bacteria in the system are exposed to air, facilitating increased oxygenation. This intermittent exposure to air promotes robust root development and prevents problems related to oxygen deprivation. This method is hypothetically superior to constant flow beds that are dependent on limited dissolved oxygen. On the other hand, in the flow (variant with the constant flow) the same substrate of the culture bed and the plant roots are always flooded under water making the latter not well aerated (Love *et al*, 2014). During the aquaponics of plants with a high level of nutritional need, it is recommended to operate a constant flow system (Rakocy *et al*., 2006). Since the reciprocating flow system has the best biofiltration unit, as well as a good quality PFP to remove the solid waste from the system, many workers resort to using this type of aquaponic system (Thorarinsdottir *et al*., 2015).

In aquaponics, hydroponic grow beds can use ebb and flow systems with the support of gravel, rock, sand, or even floating rafts (Thorarinsdottir *et al*., 2015). Thanks to this, aquaponics is classified into three main techniques. These aquaponics techniques are:

- Aquaponics with culture beds on support (gravel, rock, and sand);
- Aquaponics with deep water culture (floating rafts);
- Aquaponics with the nutrient film technique.

The pump

A pump is a device that brings water from the aquarium to the plant culture bed; it will pump enough water into the culture beds so that all the cultural supports are underwater (Zwane, 2019). The exact height of the water level can be set and the water is then drained form the grow bed using a siphon or overflow (Tura *et al*., 2019).

Primary facultative ponds

The primary facultative ponds (PFPs) are those created artificially, anaerobically, or aerobically filled with pre-screened wastewater, which is then treated by natural processes through micro-organisms (Artur, 1983; Zwane, 2019). Their basins can be used individually or combined in series for enhanced treatment. They have a major role in the waste stream environment to receive low organic loads, treated in a short period of time (Artur, 1983; Mara, 1997). Some may be called aerated facultative ponds, if the water in the ponds is conditioned by the

oxygenation of air diffusers, which are installed at the bottom or on the surface of these (Mara, 1997). In the optional basins, the mixing of these waters is partial, thus causing the energy of their mixing to be weak or even absent to eliminate sedimentological deposits (Mara, 1997; Von sperlin de Lemos, 2005; Zwane, 2019). Some types of PFPs include sedimentation tanks. When solid waste is allowed to settle without mixing, an anaerobic layer forms at the bottom of the basin, and the allows for the mineralization of nutrients in the layer of settled solid waste (Hargreaves *et al.*, 2005; Thorarinsdottir *et al.,* 2015).

1.4.3. Abiotic factors

Abiotic factors are the physical parameters, also of the aquaponics environment. The abiotic components of a given aquatic ecosystem are temperature, water, air, reservoirs, etc. (Francis-Floyd *et al.*, 2009). Abiotic factors are universal (terrestrial, climatic, aquatic, etc.) and dictate the sustenance of life and distribution and diversity of organic species in all types of environments (Ghaly *et al.*, 2005).

Water as a renewable resource

Water is one of the essentially renewable resources that ensure the biological maintenance of organisms (Turcios and Papenbrock, 2014). Freshwater demand has increased dramatically due to a rapid population growth rate and accelerated industrialization process in recent decades (Ramanan *et al.*, 2016). As a unique gift from nature (Ghaly *et al.*, 2005; Miller *et al.*, 2001) it is necessary to manage this natural resource that can be diverted, transported, stored, and recycled to maintain the ecosystem balance in terms of life and food production (Ghaly *et al.*, 2005). Globally, water occupies a volume of about 1.4×10^9 km^3 (Wang *et al.*, 2009). However, rivers as sources of freshwater represent an insignificant volume of this earth's water (0.0001%) as illustrated in Table 1.1 (Wang *et al.*, 2010; usbr.gov). Despite their small quantity, the rivers are important for life as almost all rivers are fresh. As many rivers are perennial, while some are seasonal (Coche, 2014), recycling processes are needed to maintain the water resources balance.

Table 1.1: Global distribution and supply of water resources (modified from Wang *et al*., 2010; usbr.gov).

Worldwide water volume distribution (%)	
Oceans	97.2
Glaciers	2.0
Groundwater	0.62
Freshwater lakes	0.009
Seas salt lakes	0.008
Atmosphere	0.001
Rivers	0.0001
Total	99.8381

Water quality parameters in aquaponics

The aquaponics combines fish farming and plant cultivation as a mutualistic interaction, sustained by microbial nutrient cycling. Living organisms depend on the balance of water quality as well as dissolved nutrients (Francis-Floyd *et al*., 2009; Nicolae, 2014). For this purpose, the good production and health performance of fish and plants depend on this balance. Therefore, the parameters below are going to give more details concerning the quality of the water used for this research (Bernstein, 2013; Nicolae, 2014).

pH and temperature variations

The pH values required for the proper functioning of the aquaponics system range between 5.5 and 7.0. Below and above these pH values, nutrient uptake by the plants becomes low or even impossible, creating a decrease in plant growth and development (Francis-Floyd *et al*., 2009; Bernstein, 2013). Thus, plants begin to wilt and curl and yellow their leaves (Bernstein, 2013). This phenomenon leads to a delay or a complete absence of inflorescence (no reproduction). In this case, the plants may no longer produce and may even die (Nicolae, 2014).
On the contrary for catfish, they prefer a pH of 7.0 or neutral, but they can live beyond these pH limits (Bernstein, 2013; Nicolae, 2014). For temperature parameters, it was generally documented that good temperature ranges between 15 and 30°C allow good growth of fish species and selected plants, but this is species specific (Rakocy *et al*., 2006; Bernstein, 2013).

Dissolved oxygen

Dissolved oxygen (DO) is required in terrestrial and aquatic environments (Bernstein, 2013). For an aquaponics system, enough DO (4.5 - 10 $mg \cdot L^{-1}$) in the ponds is very important because it ensures the respiration and subsequent growth rate of fish and plants (Bernstein, 2013; Nicolae, 2014).

Chemical oxygen demand

The chemical oxygen demand (COD) is defined as a parameter used to characterise the organic pollution of water (Moore *et al.*, 1949). This is an oxygen concentration that is equivalent to the quantity of a chemical oxidant consumed by organic matter, expressed in $mg \cdot L^{-1}$ (Francis-Floyd *et al.*, 2009). These organic materials can be dissolved and suspended when treating a water sample with an oxidant under defined conditions. It is an analysis that is done by oxidation of potassium dichromate (Moore *et al.*, 1949). In previous years (1872), this analysis was carried out by the titration method, a method which was based on the reaction of a solution of the water sample with the chemical reagents (permanganate) by observing the colour change (Miller *et al.*, 2001).

Total dissolved solids

Total dissolved solids (TDS) are part of the dissolved organic waste leading to the pollution of a given aquatic ecosystem. For fish farming, TDS is normally derived from solid organic waste which consists of uneaten food and fish excrement (Bouma *et al.*, 2005). Others may be airborne due to dust accumulation or sedimentation and changes in water quality of system supply water (Francis-Floyd *et al.*, 2009).

Electrical conductivity and salinity

Electrical conductivity (EC) determines the ability of water to conduct electricity (Pagand *et al.*, 2000). This capacity is linked to the concentration of dissolved ions water (DeLong *et al.*, 2009; Fowler *et al.*, 2013). However, these ions may originate from inorganic alkaline materials, chlorides, and dissolved salts within the supply water (Fowler *et al.*, 2013).

Salinity is the concentration of dissolved salts in an aquatic ecosystem; that is, dissolved electrolytes form ionic particles. Each particle has one of two opposing charges, positive or negative. Salinity, therefore, plays an essential role in determining the EC (Nelson and Siegel, 2014). A high concentration is observed in marine waters. Research has shown that, most often, the determination of concentration is not directly noticeable. It is often recognized as a derivative of the EC measure (Nelson and Siegel, 2014). These derivatives include the conductive force related to their salinity.

Nitrogen cycle

Nitrogen was ranked among the most important essential elements involved in the growth of living beings (Akan *et al.*, 2010). It occupies 5th place in the classification of the most abundant elements forming the galaxy (Ramanan *et al.*, 2016). These biological molecules were then transformed into amino acids and nucleic acids, proteins, vitamins, as well as other molecules essential to metabolism (Resh, 2013). Nitrogen transformation processes are involved in the biogeochemical nitrogen cycle leading to nitro-fertilization which includes N_2 fixation, ammonification, and nitrification (Van Den, 1974; Ramanan *et al.*, 2016).

Nitrate is the primary source of nitrogen for plants. Hydroponically, the PFP treats solid waste which contains ammonia and nitrite contained in the unused feed and fish excreta. Figure 1.2 illustrates the nitrification dentification cycle (Fowler *et al.*, 2013). These are well-known processes that occur in soil and aquatic environments for good production (Al Bloushi, 2018).

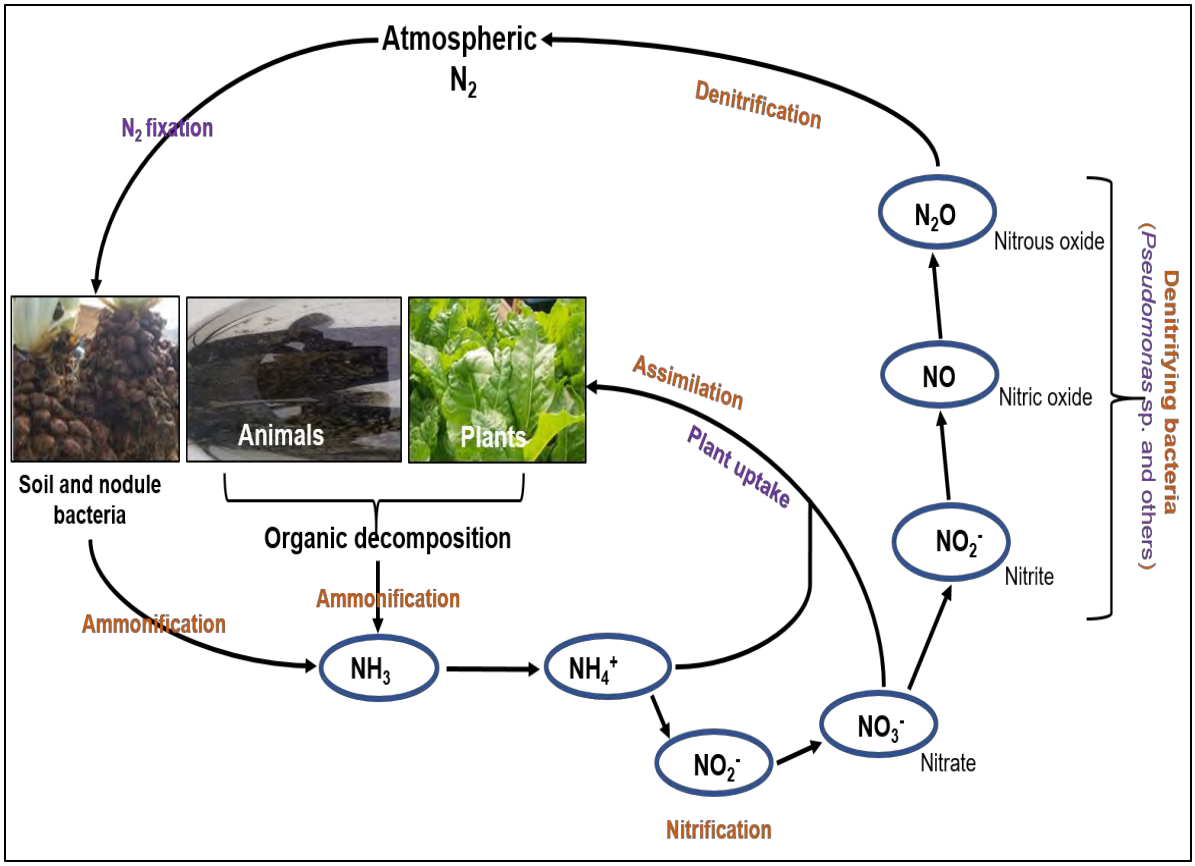

Figure 1.2: Diagram illustrating the nitrogen cycle in nature (modified from Fowler *et al.*, 2013).

Biogeochemical cycle in an aquaponics system

Living organisms are composed of essential elements, of which nitrogen is one of the most important (Atwater, 1900). Occupying 80% of atmospheric air, it is inaccessible to most plants

due to its triple molecular bond (Barua *et al.*, 2000; Bolger and Connolly, 1989; Love *et al.*, 2015). However, certain bacteria and cyanobacteria that can produce nitrogenase can convert N_2 into NH_3 (Kim *et al.*, 2014).

The chlorophyll pigments and proteins are among the biomolecular resources that often limit the primary productivity of many environments (Kiple, 2002). Nitrogen becomes accessible to primary production (phytoplankton and plants) only when it is converted from its atmospheric state (N_2) into NH_3 (Li *et al.*, 2014). Nitrogen can take many different forms (MacIntrye *et al.*, 2008), comprising inorganic nitrogen (NH_3 or NH_4^+ and NO_2^-), and organic nitrogen including amino acids (R-CH-NH_2-COOH), nucleic acids, vitamins, and other molecules essential to metabolism (Kadlec and Wallace, 2008; Goddek *et al.*, 2016). As certain forms of nitrogen can be toxic to aquatic ecosystems, their transformation by nitrifying organisms is necessary for primary producers (Gopalakrishnan, 2007). This transformation into its many states occurs through oxidations, making it a source of food in the biosphere chain productivity (Koch, 2001; Lanl, 2016).

Ammonification process in aquaponics

In aquaculture, ammonia is produced by the excretion of fish and by decaying plants and animal matter (Lennard and Leonard, 2004). It occurs in water in two forms, (1) as the ammonium ion (NH_4^+) and the other in the simple form, un-ionized ammonia (NH_3) (Ramanan *et al.*, 2016). Its third form is taken as total ammoniacal nitrogen, which is also its most toxic form for fish (Li and Li, 2009). All these forms depend on the temperature and pH of the water (Sunarma, 2004; DeLong *et al.*, 2009). In other words, adjusting the pH greatly impacts the ammonia concentration (Syeda *et al.*, 2022). For example, at a pH of 7.0 or less, ammonia is 95% higher in most cases. In this form, it is non-toxic (NH_4^+) (Shi-Weng *et al.*, 2009).

This content of non-toxic ammonia versus toxic ammonia increased dramatically as the pH increased (Rakocy, 1999). The same goes for the water temperature which can also influence the NH_3 and NH_4^+ content (Francis-Floyd *et al.*, 2009). Water temperature is also the element that influences nitrogen in its NH_3 form, which is toxic at a given pH in warmer rather than colder water (De Moor and Bruton, 1988). For example, at 40 °C, the percentage of ammonia in toxic form (NH_3) can reach 2% at pH 7.5, compared to 18% at pH 8.5 (DeLong *et al.*, 2009). The process of decomposition of ammonia (NH_3) and ammonium (NH_4^+) is carried out by synthesizing proteins and other nitrogenous organic substances (Ramanan *et al.*, 2016).

These elements are always present in humus and are broken down by bacteria such as *Bacillus*

sp. (FAO? 20214; Delaide *et al.*,2017). Among the forms of nitrogen that plants can use by absorption, ammonium (NH_4^+) is one. The bio-filter mainly contains ammonifying bacteria which break down NH_4^+ to release NH_3 (Ramanan *et al.*, 2016). At the same time, N_2- fixing microorganisms also transform atmospheric N_2 into NH_3. So, under these two conditions, the NH_3 produced can capture a proton (H^+) in aquatic environments or the soil, thus forming NH_4^+ (DeLong *et al.*, 2009; Fernandes *et al.*, 2017).

Nitrification in an aquaponics system

Nitrite is one of the forms of nitrogen that exists in water in aquaponics (DeLong *et al.*, 2009). It is also toxic to fish, and it can produce nitrate through, it is reduction by denitrifying means (Bernstein, 2013; Nicolae, 2014). The nitrification process works well in an aquaponic system (Britz and Hecht, 1987). It starts with the increased level of dissolved oxygen in the water and with a low concentration of organic matter (Bernstein, 2013). This material is produced by excrement and food not consumed by the fish; others can come from solid waste accumulated by the water in small quantities (Brikké and Bredero, 2003). The phenomenon is explained as follows if the oxygen levels are too low, the nitrification rate was slowed down or stops completely; this was causing a large build-up of ammonia to levels toxic to fish. Nitrite can also be toxic to fish if found at concentration levels over 5 ppm (Fox *et al.*, 2012).

The ammonia to nitrite and nitrite to nitrate conversion that takes place in an aquaponic system is a process of biofiltration (Clunie and John, 2001). Nitrifying bacteria carry out biofiltration, and this is the link between the fish component and the hydroponics component in an aquaponics system, thus forming a cycle (Bernstein, 2013). In an aquaponic system without a healthy and functional biofilter, the waste produced in the fish component is accumulated in large quantities. If the supply of nutrient to the plants is too high or too low, the aquaponic system will malfunction (Christensen and Harremoes, 1978).

In short, nitrification means the transformation of NH_4^+ or NH_3 into nitrite (NO_2^-) and the transformation of nitrite to nitrate (NO_3^-). It is done by the process of oxidation which is carried out in two phases: A priori, it is the passage of NH_4^+ or NH_3 into NO_2 through the *Nitrosomonas,* which are the bacteria oxidizing ammonia. Second is the conversion of NO_2^- into NO_3^- via nitrite-oxidizing bacteria, usually *Nitrobacter* species (Danaher *et al.*, 2013). Lastly, we have nitrate as the third form of nitrogen. It is the form required in aquaponics, thus allowing good

production (Greenfield and Southgate, 2003; Nicolae, 2014). But it can also be harmful if present in too high a concentration, and this concentration varies with fish species (Bernstein, 2013). As such, nitrification is an important consideration in aquaponics.

Denitrification process in the aquaponics system

In an aquaponic system, the nitrogen cycle begins when food is first added to fishponds (Junge *et al.*, 2014; Ramanan *et al.*, 2016). Since fish food is rich in protein, it is the main source of nitrogen in aquaponics systems (Bernstein, 2013). The different steps observed during denitrification are conditioned and accelerated by enzymes produced by microorganisms and mainly bacteria (Hickey *et al.*, 2018). Several heterotrophic organisms (dependent on external carbon sources), such as bacteria, are involved in the denitrification of the aquaponic system (Bernstein, 2013).

The systems can use methyl alcohol and vinegar as carbon sources under artificial conditions. Bacteria of the genera *Alcaligenes*, *Bacillus*, and *Pseudomonas* can perform denitrification (Hanssens, 2009; Nicolae, 2014). The denitrification process that occurs at the end of the nitrogen cycle, is known as a phenomenon that appears mainly under anoxic conditions by heterotrophic bacteria except for the presence of nitrate and nitrite (Li *et al.*, 2005; Bernstein, 2013; Nicolae, 2014). During denitrification, NO_3^- is reduced to NO_2^- and then to N_2O which is finally reduced to N_2 (Ramanan *et al.*, 2016).

The nitrogen cycle is one of the most crucial processes in the terrestrial environment and aquaponic systems (Mancuso, 2013). It is an essential component of many vital molecules such as DNA, RNA, and proteins of all living organisms and fish are part of them (Bernstein, 2013). The structure of the nitrogen cycle in the aquaponics system is summarised in Figure 1.2 (Bernstein, 2013, FAO, 2014). The wastewater from the hydroponic culture of African catfish (*Clarias gariepinus*) was treated with nitrifying bacteria for its reuse to evaluate and grow these fish (Khokhar, 2017; Hughey, 2005). Through this system, the fish was reared considering three development stages (fingerling, juvenile and adult) (Bernstein, 2013). For all these stages, the concentrations of the water in nitrite, nitrate, ammonium, and phosphorus were determined, to know the quantity of nutrient excreted by the fish at each stage of development (Liddell and Scott, 1980).

Phosphates in the aquaponics system

The knowledge of the effect of wastewater phosphorus on the evolutionary and productive stages of the aquaponic system is of paramount importance (Moore *et al.*, 1951). The highest

rate of nutrient concentration (phosphorus and nitrogen) in aquaponics suggests a treatment using bio-filters in the early stages of growth illustrating in Table 1.2 (Bernstein, 2013). As previously reported, the system's bio-filter after a certain period becomes a positive test for solubilization (Papatryphon *et al.*, 2005). On the solubilization of phosphate, several strains of bacteria can be isolated and tested; for example, let us quote 5 strains such as *Bacillus*, *Arthrobacter*, *Enterobacter*, *Pseudomonas*, and *Ancylobacter*. Colonies of these types of bacteria can determine the solubility potential of phosphate in aquaponics (Schober and Vetter, 2018). Several methods have been tested to estimate the amount of soluble phosphate in water, but the most used is colorimetric because of its liquid culture media (Rakocy *et al.*, 2004).

Table 1.2: Average water qualities in aquaponics system(Timmons and Ebeling, 2010).

Parameter	Aquaponics system
Sodium ($mg \cdot L^{-1}$)	10.79
Electrical conductivity ($\mu S \cdot cm^{-1}$)	27.61
Temperature (ºC)	22.69
pH	7.01
Chemical oxygen demand ($mg \cdot L^{-1}$)	53.21
Ammonia ($mg \cdot L^{-1}$)	0.53
Nitrite ($mg \cdot L^{-1}$)	0.07
Nitrate ($mg \cdot L^{-1}$)	1.97
Phosphate ($mg \cdot L^{-1}$)	12.49
Chloride ($mg \cdot L^{-1}$)	482.25

Water pollution

Water pollution is often linked to human activities (Okeke and Igboanua, 2003). Its source is industrial or domestic activities and the excessive use of chemical fertilizers in agriculture (Aguilar *et al.*, 2012). Pollution affects human health (Okeke and Igboanua, 2003) and can also be caused by uncontrolled population increase (demography) (Peralta *et al.*, 2000; Yavuzcan *et al.*, 2017, Wang *et al.*, 2009). The wastewater recycling process is increasingly becoming a requirement to safeguard human health due to pollution and lack of water (Sabwe, 2021). The problem of water pollution was and remained the focus of much research funded by governments and scientists, including in South Africa (Bernstein, 2013). To avoid national or global disasters and preserve aquatic ecosystems, there is an urgent need to minimize water pollution and maximize its renewal (Vymazal, 2014).

Pollution sources

Water pollution can be of inorganic or organic origin (Ram and Irfan, 2021) and can come from two sources: direct and indirect (Okeke and Igboanua, 2003). The direct source of pollution

known as a point source, includes, for example, the pipes attached to a factory, whose discharges (effluents) are not treated (Yang and Kim, 2019). It is formed by wastewater effluents (domestic and industrial) and generally affects environmental storm sewer discharges by the industrial area (Sarker and Oba, 2019). However, in the indirect or diffuse source, the contaminants come from groundwater or surface water (Ricker, 1979; Saxton and Rawls, 2006). They thus arrive in the environment by various unidentified routes (Schneider *et al.*, 2005; Mitchell, 2010). Such is the case with runoff from agricultural fields, and urban and industrial waste (Okeke and Igboanua, 2003). Eventually, these contaminants can sometimes enter the environment hundreds or even thousands of kilometres away from the source and cause water pollution (Wang *et al.*, 2009). This can lead to so-called cross-border pollution, such as radioactive waste that crosses the oceans (Okeke and Igboanua, 2003).

Inorganic and organic pollutants

Inorganic pollutants may be caused by heavy metals released by mining industries that process ores, sand, and silt from runoff and surface water (Vymazal, 2007). In addition, the effects of deforestation, bushfires, and the burial of animal and plant debris (Wang *et al.*, 2009; Sánchez, 2014) are sources of pollution. Inorganic pollutants are also of agricultural origin brought by fertilizers and industrial effluents containing chemical waste (Rupasinghe and Kennedy, 2010; Shava and Gunhidzirai, 2017). Organic pollutants come from agro-industrial companies that use organic matter (hormones) to increase productivity (Wang *et al.*, 2009). Others may come from pathogenic wastewater microorganisms as well as cattle excrement, which can be used as organic fertilizers (Wang *et al.*, 2009).

Ecosystem degradation is often due to these inorganic and organic pollutants from contaminants released by industries and households (Shilton and Mara, 2005). Pollutants can consist of toxic or non-toxic heavy metals dumped into nature without treatment (Wang *et al.*, 2009). These heavy metals are found in sediments and can reach aquatic ecosystems in several ways, including runoff, erosion, and wind (Peralta *et al.*, 2002; Wang *et al.*, 2009). Therefore, the physicochemical water parameters, such as temperature, can also impact its pollution because they can influence the concentration of minerals in an aquatic ecosystem (Post note, 2006; Rakocy, 2012).

Finally, transmitting these metals (pollutants) to humans is possible from the trophic chain (Rakocy and Hargreaves, 1993). Therefore, research in sedimentology, including heavy metals in an aquaponic system, will be of great importance because it will shed light in the knowledge

of the impact of sediments (pollution indicators) on the health of the ecosystem (Junge *et al.*, 2014). In addition, it will enable us to understand how microorganisms transforming heavy metals into plant nutrients can attenuate the effects of heavy metals in the aquaponic system (Peralta *et al.*, 2002; Wang *et al.*, 2009). These heavy metals such as K, Mg, Fe, Mn, and Ca, in balanced concentration are essential in aquaponics because they can promote growth and increase productivity in aquaculture and agriculture over a well-defined period (Hargreaves *et al.*, 2005; Li *et al.*, 2014; Simon *et al.*, 2015).

Biological wastewater treatment

Sanitation of solid or liquid waste in fish farming such as in aquaponic systems is recommended (Simon *et al.*, 2015). Without sanitation, the accumulation of debris is possible, and this can harm the health not only of fish but also of aquaponics (Li *et al.*, 2014). Fish farming practices lead to diversified pollution caused by the feeding and defecation of fish (Li *et al.*, 2014). As this waste is stored in fishponds FAO (2014), it is, therefore, advisable for fish farming in aquaponics to carry out regular analyses of the quality of the water, which is an indicator of water pollution in the aquatic environment. Nitrogen in the form of ammonia is toxic; it cannot be ignored, alike oxygen (Figure 1.3). Ammonia is also known as a by-product of the natural metabolic reaction of fish (Francis-Floyd *et al.*, 2009).

The non-ionized form of nitrogen (NH_3) is more toxic to fish than its ionized form (NH_4^+), and the intervention of nitrifying microorganisms and plants is necessary to reduce this toxicity (Li *et al.*, 2014). Ammonia is not a problem in free-flowing systems because mixing, dilution and turbulent flow are optimal (Xu *et al.*, 2004). However, in standard recirculation systems, in cases of aquaponics, the use of a microbial biofilter and plants for its operation and regular observations of these parameters are necessary (Rakocy, 1989; Xu *et al.*, 2004). In short, microorganisms and plants act as filters in recirculation systems, promoting oxygenation (Xu *et al.*, 2004). More often in the aquaponic system, even if it uses a biofilter, the effluents from these recirculating aquaculture systems have good nutrient concentration levels (Xu *et al.*, 2004). The concentrations may reach 200 $mg \cdot L^{-1}$ of nitrate nitrogen and 20-30 $mg \cdot L^{-1}$ on average of total phosphorus (FAO, 2004), nutrient levels which are sufficient to support the good production of hydroponic plants (Rakocy, 1989).

Recirculation of water in an aquaponics unit

The aquaponics system forms a closed cycle with water circulation, producing both fish and vegetables (Mongirdas and Kusta, 2006). Also recognized as a bio-integrated system that

simultaneously combines fish farming and recirculating plant cultivation (Moore *et al.*, 1949; Moore *et al.*, 1951). This mode of water circulation allows good production of fish and plants while quantitatively saving water (Bowers and Brett, 2008; Turcios and Papenbrock, 2014). This is a method that can be very important for countries with the problem of water scarcity such as South Africa (Mchunu *et al.*, 2019). Beyond food production, aquaponics also contributes to maintaining environmental balance (Friedman, 1991; El-Bouhy *et al.*, 2006). Nutrients in the effluent (manure and food not used by the fish) from the fishponds is pump/gravity transferred to feed the plants in the hydroponic bed (Lockhart *et al.*, 1955; Rakocy, 2006). As soon as it returns to the process, the water already treated by the rhizobacteria and filtered by the root system of the plants, is returned, it is re-used for the growth of the fish in the aquaponics system, added that the water is first aerated in the sump (Lockhart *et al.*, 1955; Nelson and Siegel, 2014). For a recirculating aquaponics unit, the plants in the hydroponic bed act as a biofilter that removes ammonia, ammonium, nitrite, nitrate, phosphorus, and other trace elements that can be lethal to fishes in the ponds (Graber *et al.*, 2014; Thorarinsdottir *et al.*, 2015).

Water sampling and frequency

The aquaponics integrates fish farming and the cultivation of certain types of plants, forming a mutualistic interaction between them through bacteria (Antonio *et al.*, 2013; Junge *et al.*, 2017). Here, living organisms depend on the balance of water quality and dissolved nutrients (Francis-Floyd *et al.*, 2009; Nicolae, 2014). The production rate of fish and plants is governed by this balance (Ram and Irfan, 2021). Therefore, the health performance of fish and plants in an aquaponic system depends on water quality (Bernstein, 2013; Nicolae, 2014).

1.4.4. Fish, bacteria, and plants as biotic components

Apart from the three replicated aquaponics system used as the abiotic components in the current research project, fish, bacteria, and plants in mutualistic interactions have been used as the biotic components.

African catfish (Clarias gariepinus) as a choice for fish

Clarias gariepinus belongs to one of the six genera of fish reared in aquaponics. These genera include *Oreochromis sp*, *Oncorhynchus sp*, *Carassius sp*, *Cyprinus sp*, *Siluriformes sp*,

Clarassius sp, and *Micropterus salmoides*. They are easy to raise in the aquaponics system (Antonio *et al.*, 2013; Sánchez, 2014).

However, the present work will be focused on culturing African catfish only (*Clarias gariepinus*) because this fish grows fast, produces good quality proteins, and has an economical feed conversion rate (Rakocy, 1989; Maucieri, 2018). They are omnivorous predators with an additional air breathing apparatus with a capability to thrive opportunistically on many types of feeds, as long as the protein content is above 35% (Rakocy, 1989). They can be raised at high stocking densities (Viladomat and Jones, 2011; Maucieri, 2018). For these reasons, African catfish at high densities can produce the necessary waste products to be biologically (filter and hydroponics) treated for reuse (Viladomat and Jones, 2011). They can easily reach a weight of about 450 g within 18 months from breeding (Clunie and John, 2001).

As robust fish, they are resistant to environmental stress such as temperature variations and water quality conditions (Maucieri, 2018). Catfish are found in freshwater environments and can live in terrestrial waters of all the continents except Antarctica (Maucieri, 2018). However, growth rates will be limited outside of the ideal temperature range between 25-30°C.

Native to Africa, the African catfish is most common in Africa and has a natural range that extends from South Africa to the Middle East and Eastern Europe through Central, Western, and Northern Africa (Barua *et al.*, 2000). This fish is indigenous thus colonizing many rivers that form the source of the Nile to the Orang River. In the southern part of Africa, the African catfish occurs in the ecology of the Limpopo, Okavango, Cunene, and Orange-Vaal lotic systems. A species of fish is also found in northern and southern KwaZulu Natal at elevations of 1400 m and 500 m (De Moor and Bruton, 1988). Around the 1950s, African fish was introduced to Indochina by the French. It was widely used in South and Southeast Asia for fish farming (Barua *et al.*, 2000). This species is known as the walking or Thai catfish; it was introduced to Florida in the United States in the 1960s and is currently widespread throughout that country (De Moor and Bruton, 1988; Barua *et al.*, 2000).

Today, African catfish are found internationally. They have been found in Asia (from the Philippines to Java), Lebanon, Syria, Israel, Turkey, and America (Rakocy, 1989). They have been produced and commercialized for more than 100 years in the USA (Kansas and Arkansas). So, due to its various qualities (i.e., the hardiness, the omnivorous diet, the vitamin D reach meat, the ability to withstand high water toxicity), the catfish is commercially important (Clunie and John, 2001). However, for these reasons, the African catfish can greatly overstretch the environment and pollute the water that needs to be biologically treated (Viladomat and Jones, 2011). The African catfish is an alien and invasive species in many countries (including some

river systems in South Africa), and currently identified as an agent of native biodiversity loss (Barua, 2000; Rakocy, 2012).

Density investigations and transportation of fish

For the stocking density, Hussain *et al.*, (2018) found that the 120 fish/m^3 (of 10-15 g) density with tomato cultivation was suitable and gave the best yield. The specific growth rate (SGR) was reduced with increasing stocking densities whereas food conversion ratio (FCR) increased with stocking density (Naddafi *et al.*, 2009). Aquaponic systems with respectively the lowest stocking densities (150 fish/m^3) performed better than those of 300 and 450 fish/m^3 (Diver and Rinehart, 2010; Bosire, 2017).

The most important thing to take into consideration in an aquaponics system is first, the abundant aeration, and secondly, a pH value of 7 is ideal for a rapid and efficient water cycle (Rakocy, 1987). It is always required to have a power source with two basic backup systems powered by deep-cycle energy if high stocking densities are used. For a fish farm, the stocking is very important, because of the direct effects on the quality of water, the behaviour, the growth, and the survival of the fish (Antonio *et al.*, 2013; Laidlaw and Magee, 2016; Bosire, 2017).

Feeding African catfish fry

The fry is fed until satiety (≥ 5.5% weight of their body/day, illustrated in Table 1.3) (FAO, 2014). This feed is a unique and important guideline based on water quality parameters (Bosire, 2017).

Table 1.3: Global feeding protocol for African catfish in an aquaponics system (FAO 2012).

Feeding days	Average weight (g)	Feed size/level (%BW/day)	Feed type/Feed size (mm)
1	0.0025	Live feed	Artemia
7	0.044	90% 0.2-0.3 + 10% artemia	Essence + Artemia
14	0.221	0.3-0.5	Advance
21	0.63	0.5-0.8	Advance
27	1.2	0.5-0.8	Advance
34	2.3	0.8-1.2	Advance
41	3.9	1.0	Start Premium
47	5.8	1.0	Start Premium
54	8.6	1.5	Start Premium
3	13	5.55	2.0
28	90	4.04	3.0
77	514	1.70	4.5 / 6.0
126	1144	1.18	4.5 / 6.0
175	1948	0.86	4.5 / 6.0

Bacterial biofilter (Bacteria nitrification)

In an aquaponic unit, aquaculture tanks produce a large amount of waste (remains of food not consumed by fish and these excrements) which can be high in ammonia and ammonium (Love *et al.*, 2014). The solid wastes produced by fish are important for plant production since they contain sources of plant nutrients (Antonio *et al.*, 2013; Love *et al.*, 2014). However, this fish waste cannot be utilized by plants without going through the mineralization process (Somerville *et al.*, 2014). Mineralization is a process by which organic matter (solid waste) is decomposed in a filtration component (Somerville *et al.*, 2014; Khandaker and Kotzen, 2018).

Mineralization includes five main responsible mechanisms including ammonification, nitrification, denitrification, immobilization, and volatilization (Maucieri *et al.*, 2018). These mechanisms are the basis of nutrient formation in aquaponics (Nawwar, 2016). Mineralization can take place quickly in an aquaponic unit, only when the conditions are perfect that the microorganisms reproduce for at least 3 to 7 days (Love *et al.*, 2014; Nawwar, 2016). It is conditioned by optimal parameters such as appropriate temperature and pH, balanced mineral nutrients, and high aeration (Naddafi *et al.*, 2009).

In aquaponics, microbial activity is significantly reduced at low temperatures and at a pH below 5.5 (Nawwar, 2016). This microbial activity also increases with increasing temperature and pH (Lund, 2014). For a given aquaponic unit, maximum nitrogen mineralization can only occur when the water in the system reaches temperatures between 30–36°C (Nawwar, 2016). Nitrogen mineralization in aquaponics can also decrease (Love *et al.*, 2014; Nawwar, 2016). This decrease is indicated by low microbial activity and degradation of the biological properties of the growing bed or hydroponic media (Love *et al.*, 2014). Therefore, when the temperature, the pH, and the humidity are favourable to the metabolism of microorganisms in an aquaponic unit, this results in mineralization while it's opposite produces a process of immobilization (Love *et al.*, 2014; Nawwar, 2016).

The biofiltration component is a key element characterizing aquaponic systems as well as all recirculating aquacultures. During the recirculation of the same water in aquaponics, there are dangerous toxins that accumulate and should be eliminated (Okeke and Igboanua, 2003). This removal is achieved by the microorganisms by biotreating the water in the system, as well as converting dissolved ammonia and ammonium into nutrients (Williams and Long, 1997).

In aquaponics, ammonia and ammonium come from excreta and food waste not eaten by fish, they are toxic to aquatic animal (fish) life. After metabolic reactions by micro-organisms, these are transformed into nitrite and finally into nitrate which is less harmful to fish. Through the

process of biofiltration, derived from beneficial microorganisms, PFPs can prevent the replenishment as well as the rejection of most of the water in the system. Therefore, in aquaponics, enormous water savings can be obtained. For PFP, a biofiltration unit is crucial and must be sound and well-designed to have a stable and functioning aquaponics system.

The biofiltration process is ensured by three genera of bacteria (*Nitrosomonas*, *Nitrobacter*, and *Nitrospira*) which are all nitrifying. These bacteria play an important role in maintaining optimal water quality parameters. In aquaponic water, *Nitrosomonas* convert ammonium and ammonia into nitrite. Then *Nitrobacter* and *Nitrospira* convert nitrite into nitrate which is a nutritive and essential element for the growth of plants (Nawwar, 2016). These three genera of bacteria mentioned above are naturally present in the environment.

The bio-filter can be defined as a tank or a cartridge having a polyhedral or cylindrical shape, containing porous filtration media, bacteria, and water. The biofilter must be well aerated to allow the nitrification process, which is the consumer of oxygen. In an aquaponic system, the design of the biofilter can be of several forms (rudimentary or complex industrial). Since the bacteria are invisible to the naked eye on the filtration media, monitoring the levels of ammonia, ammonium, nitrites, and nitrates in the system water remain the only way.

In aquaponics, there may be other environmental factors related to water such as turbidity, transparency, temperature, pH, dissolved oxygen, and salinity that can influence the proper functioning of this system (Ramanan *et al.*, 2016). During the start-up phase of aquaponics, there is an acclimatization period of up to six weeks, to allow the bacteria in the biofilter to establish. This phase is the most important because the nitrifying bacteria resulting from the process needs time to multiply and finally colonize the filter medium.

In other words, the existing bacteria may cause a high rate of fatal toxicity because they will not be able to properly treat the water in the system. For this reason, the operation the system without fish to condition the filters is recommended before fish are included. To accelerate the process of bacterial development, and another source of ammonia and ammonium must be added to the system. The system can also be inoculated with water from the filtration media or with aquaponic water from another system already established; this is another way to speed up system acclimatization time.

The process of nitrification is carried out by the interrelation of two groups of nitrifying bacteria, namely *Nitrosomonas* and *Nitrospira spp* (Figure 1.3) (Goddek, 2017). They can use different substrates in the aquaponics environment. The basins of the aquaponics system operate under aerobiosis (O_2 needed). The aerobic stabilization pond also contains bacteria and algae in suspension. The only difference, in this case, is that aerobic conditions prevail even

throughout its depths (Maucieri *et al.*, 2018).

During the start-up of a new aquaponics system, the levels of ammonia and ammonium will increase until the first bacteria genus (*Nitrosomonas*) colonizes the entire system to convert the ammonia and ammonium into nitrite (Figure 1.3). When ammonia and ammonium levels begin to decline, nitrite levels rise until the second and last bacteria genus (*Nitrobacter* and *Nitrospira*) subsequently colonize the system, decreasing and converting nitrite into nitrate (Saxton and Rawls, 2006). Nitrate is an element in the aquaponic system that is relatively harmless to fish, and an essential element for plant growth.

Nitrate is the primary source of nitrogen for plants. Hydroponically, the PFP treats solid waste which contains ammonia and nitrite contained in the unused feed and fish excreta.

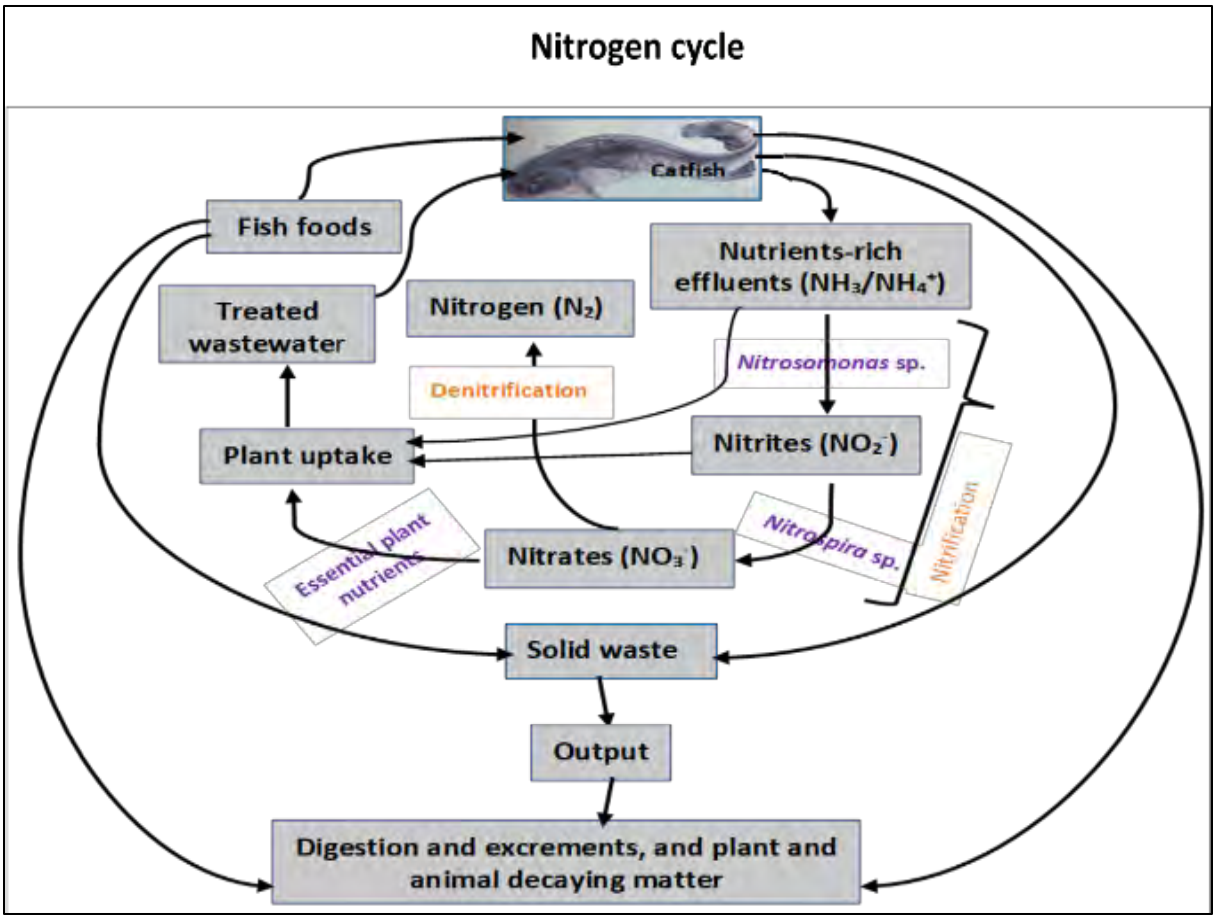

Figure 1.3: Diagram illustrating the nitrogen cycle in aquaponics process, modified from FAO (2014).

Plants choice

Eleven vegetables, namely *Ocimum basilicum*, *Brassica oleracea*, *Lactuca sativa*, *Cucumis sativus*, *Solanum melongena*, *Capsicum*, *Solanum lycopersicum*, *Phaseolus vulgaris*, *Spinacia oleracea*, *Beta vulgaris*, and *Petroselinum crispum*, are known as the most used in aquaponics (Sarker and Oba, 2019). Their growth depends on optimal water quality conditions, cultivation

instructions, and harvesting techniques (Schneller, 2015). These conditions are pH (5-7.5), temperature (8-30°C), exposure to light in full sun (partial shade for temperatures > 26°C), plant spacing, growth time (24-140 days), germination time, plant height and width (3-21 days), recommended methodology in aquaponics and hydroponic beds (Hochmuth and Hanlon, 2010; Genello *et al.*, 2015). All these conditions may vary depending on the plants.

Swiss chard was chosen because of its growth times (60-65 days) and the suitable set environmental factors such as the pH, temperature ranges between 5.5 to 7.0, and 10 to 30°C as required in the cultivation of this plant in the aquaponic system (Sarker and Oba, 2019). Swiss chard is of the order Caryophyllales, it is one of the species of the *Amaranthaceae* family, and the *Chenopodioideae* subfamily is named *Beta vulgaris*; it is one of the most popular vegetables on the market; it is a leafy green vegetable (Schneller, 2015).

Its leaves are a common edible vegetable that are consumed fresh. It may be eaten cooked or raw, and the high oxalate content may be reduced by steaming (Hochmuth and Hanlon, 2010). Swiss chard has the nutritional value of 23 calories, 91% of water, 2.9 g of protein, 3.6g of carbohydrates, 0.4 g of which was sugar, 2.2 g of fibre, and 0.4 g of fat per 100 g. In contains vitamins and minerals, including vitamins A, C, and K1, folic acid, iron, and calcium (Rodier, 2009).

Again, Swiss chard contains several important compounds including lutein, nitrates, zeaxanthin, quercetin, and kaempferol. It has the health benefits of oxidative stress, eye health, blood pressure control and cancer prevention (Love *et al.*, 2014). This species derives its origin from central and western Asia, and it is an annual plant, growing as tall as 30 cm. It is from a well-known family of food plants that produce only consumable leafy greens (Schneller, 2015).

Lighting

In aquaponics as in any form of aquaculture, lighting is a necessary element for productivity. For the quick and appropriate development of any aquatic ecosystem, as well as the aquaponics system, it has to be exposed to light sources (Liddell and Scott, 1980; Miller *et al.*, 2001). There are several options to choose from, ranging from natural light to light bulbs (e.g., fluorescent or metal halide).

Natural light was used in this aquaponics system, and the light intensity was measured using a light meter (Figure 1.4) and it varied between 1985 to 9301 Lux.

The electrical lights can have different wavelengths and they can come from several sources. In an aquaponic system, the lights must cover a considerable area and can be mounted at specific heights above the plant growing bed, depending on the plant species to be grown (Lennard and

Leonard, 2004; Mchunu, 2019).

During the day, the evaluation of the operating time of the lighting can vary according to the plants to be cultivated as well as to the annual period of cultivation.

To choose the solution between the types of lighting that can operate an aquaponic system, an aspect is needed, the ratio between the acquisitions costs divided by the operating cost.

Figure 1.4: The device used to capture light intensity inside the greenhouse during the experiments.

Chapter 2. Materials and methods

2.1. Study site

The experiment was conducted in an existing aquaponics system located at the Department of Ichthyology and Fisheries Science (DIFS), Rhodes University (RU). This aquaponic unit is the main site of this study in Makhanda/Grahamstown (33° 19' 07'' S, 26° 33' 25'' E), Province of Eastern Cape, South Africa (Figure 2.1). For the current study, nine aquaponics systems were investigated for five months.

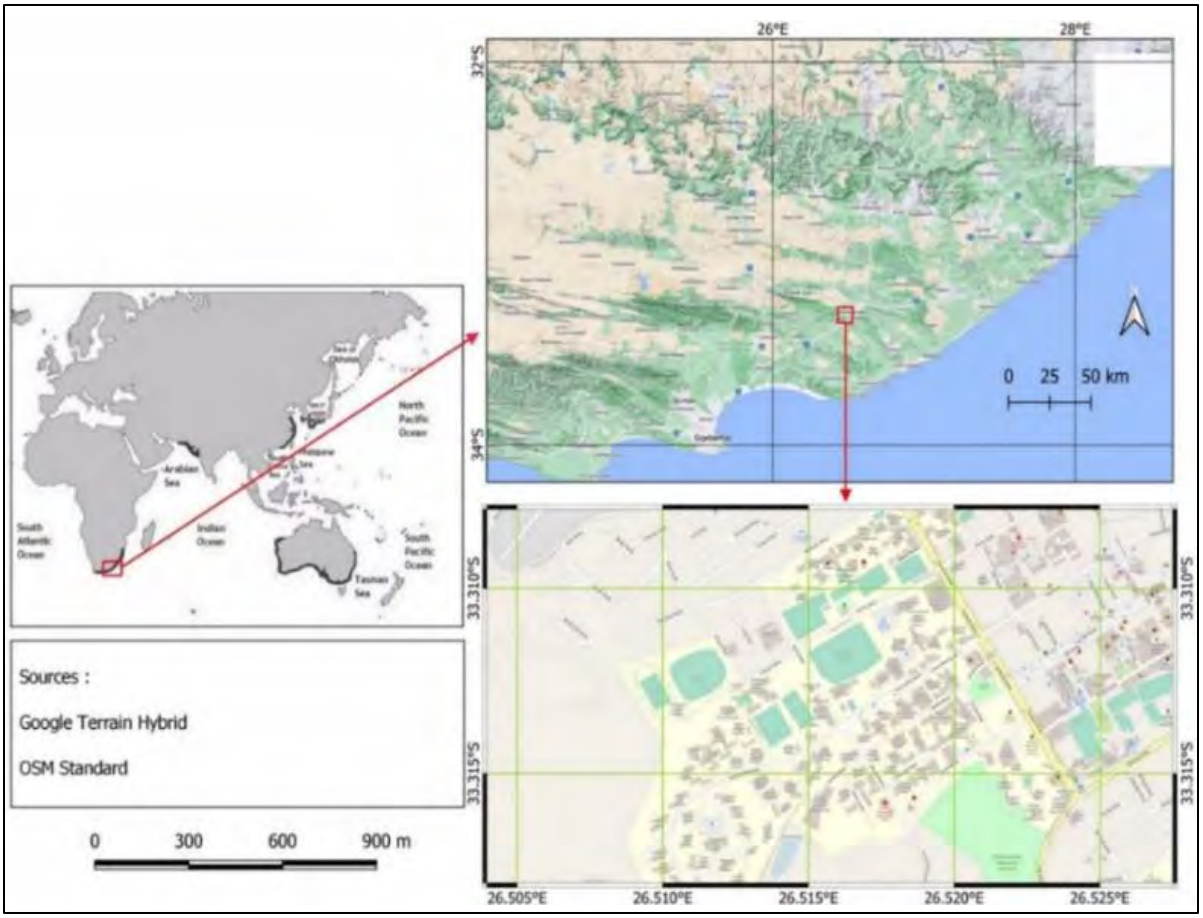

Figure 2.1: Location of Rhodes University on the map of South Africa (Google terrain satellite image and the Open Street Map (OSM).

2.2. Conceptual framework

There were numerous aspects to this work, including a primary facultative pond (PFP) where solids remained in the system, no-PFP where fish solid waste was removed manually, as well as flooded and drain-fill vegetable grow-beds. They formed three treatments (T; Box 1). Treatments T1 and T2 both use drain-fill technology and together formed a single experiment

with an experimental design aimed at comparing the use of PFP vs no-PFP (control; Box 1). Treatments T1 and T3 both include the use of a PFP and together form an experimental design aimed at comparing drain-fill with flooded (control) production systems (Box 1). Note that T2 was a common item to both experiments; which was necessary because there were only nine experimental systems, so this allowed for the work to be carried out with three replicates per treatment (Figure 2.2).

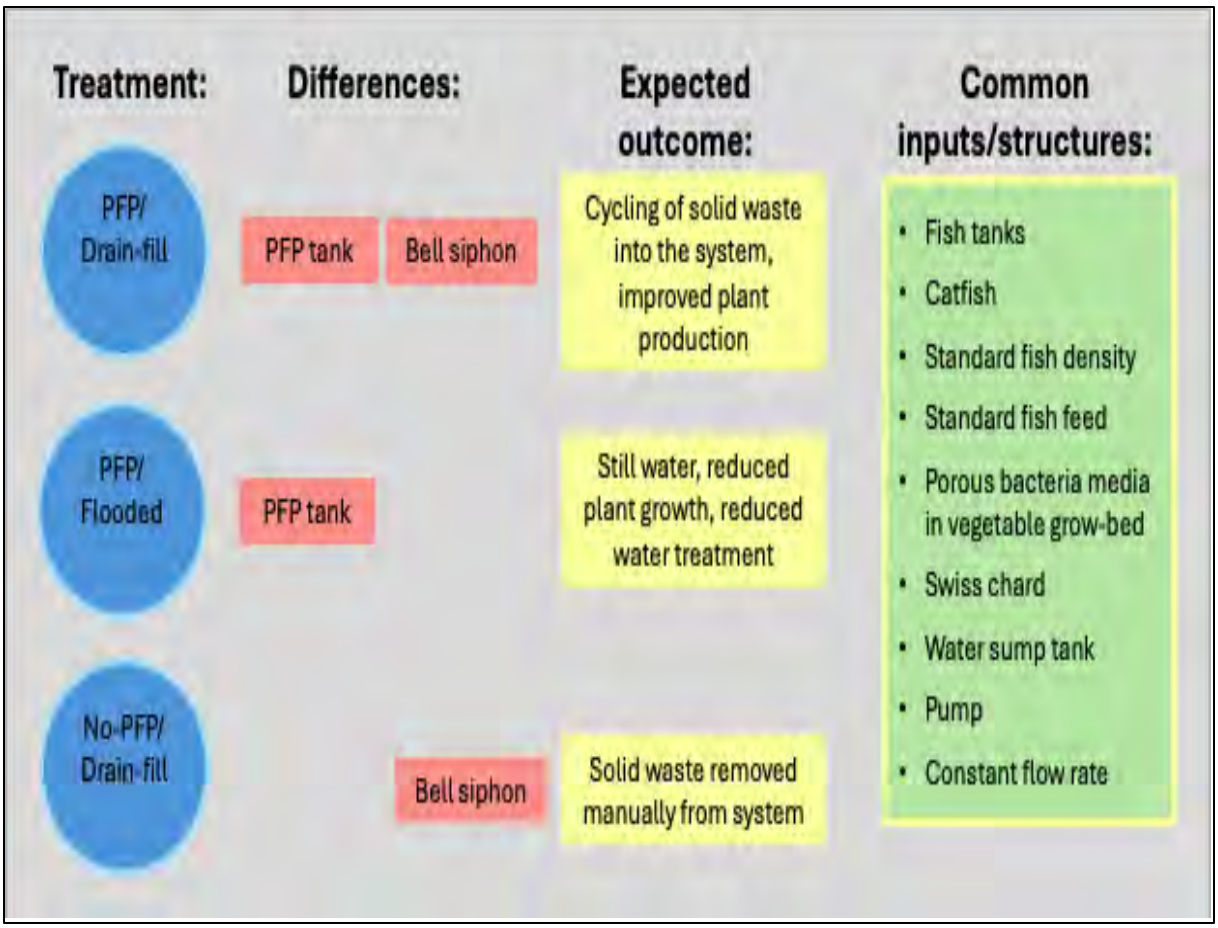

Figure 2.2: Illustration of the three treatments in aquaponics systems (customized concept).

Box 1.2: The combination of variables that were common or different between the three treatments (T) used in this project.

(T1) PFP* (fish solid waste remain in the system) and flooded grow-bed.
(T2) PFP* (fish solid waste remain in the system) and drain-fill grow-bed;
(T3) No-PFP* (fish solid waste removed manually) and drain-fill grow-bed;

Exp 1: Flooded (control) vs drain-fill included T1 and T2 (both treatments used a PFP)
Exp 2: PFP* vs no-PFP* (control) included T2 and T3 (both treatments used drain-fill)

*PFP stands for primary facultative pond.

Also note that it was not possible to compare all three treatments in a single experimental design and statistical analysis, since there would always be more than one variable different between two of the three treatments.
Elements, such as water, media, bacteria, fish and plants were used in all three treatments that were tested in this study.

2.3. Experimental system

2.3.1. Experimental hardware

An experimental unit consisted of a recirculating aquaponics system (Figures 2.3 to 2.5). The system included a sump (1000 L), in which a submersible pump (Dolphin PH-2500 of 240, 50 HZ, 30W and 2.75 m head) was positioned. This pump moved water via a 25 mm pipe at 13.3 $L \cdot min^{-1}$ from the sump into two fish tanks (each tank was 100 L, with an operational water volume of 80 L). To prevent the fish from jumping, all the fish tanks had a net cover that was black in colour (Figure 2.3-B). The fish tanks were circular (diameter: 62 cm) with an inverted conical bottom. Inside each fish tank was placed a polyvinyl chloride (PVC; FLO-TEK 110 mm CLASS 6) pipe that was 0.50 m long, serving as a "cave" that made it possible for the fish to escape direct sunlight (Figure 2.3-A). The water level of the fish tanks was maintained by a 25 mm overflow pipe positioned at the water surface that drained by gravity into one plant grow-bed.

The plant grow-bed (220 L and a surface area of 1.0 m^2; Figures 2.5 and 2.6), was filled with clay pebbles as a growth medium, and the water level in the grow bed was maintained at 220 mm using a 50 mm upstand pipe in the centre of the grow-bed (Figures 2.4 and 2.5), which allowed for a layer of clay pebbles of about 5 to 8 cm above the water level. The grow-bed could be run as a flooded system using the upstand pipe only, or a drain-fill system using a bell siphon (Figure 2.7). Water from the grow-bed flowed by gravity via the 50 mm PVC pipe back into the sump, completing the recirculating system.

In addition, to the overflow drain at the water surface of the fish tanks, there was also an effluent valve positioned at the apex of the inverted cone-shaped tank bottom, which allowed for the periodic removal of settled solid waste from the bottom of the fish tank, but only when the valve was opened. In each tank, a protective screen was placed inside to prevent fish from reaching the sediment at the bottom of the tank Figure 2.3-A). The purpose of this shield was to separate

fish and waste, to allow solid to settle for easier removal from the bottom of the conical fish tank. This solid waste drained either into a primary facultative pond (PFP; 210 L) or to waste via a 25 mm pipe (Figure 2.5). The PFP operated as a stagnant bioreactor (Figure 2.8). When fish tank effluent containing solid was periodically drained (i.e., daily) into the PFP, an equivalent volume of PFP water (approximately 13.3 L) was displaced and this post-PFP water was drained into the plant grow-bed. Black covers were manufactured and placed above each sump tank.

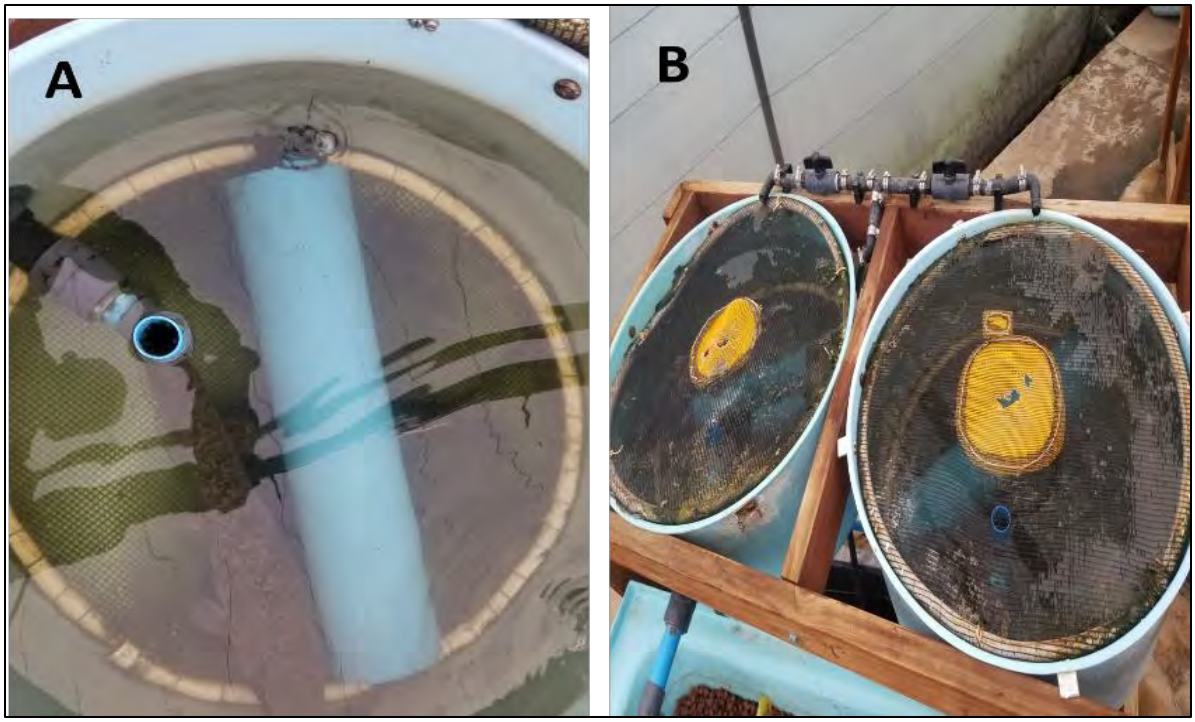

Figure 2.3: The circular screen preventing the African catfish from reaching the bottom of the tank and the PVC "cave" (A), and the circular screen above the tank to prevent the fish from jumping out of the tank (B) (customized concept).

Figure 2.4: The nine individual and isolated recirculating aquaponics systems used in the experiments all positioned in a green-house tunnel. In the foreground are two completed systems showing one green sump at ground level, the plant grow-bed above the sump and the two circular fish tanks above the grow-bed. The other seven systems are shown behind the two in the front (customized concept).

2.3.2. Water sampling points

There were five points at which water samples were collected from the system (Figure 2.5A, B and C). These included: (1) post-sump to fish (Figure 2.5 C-A), (2) post-fish to hydroponic grow-bed (Figure 2.5 C-B), (3) post-fish to primary facultative ponds (Figure 2.5 C-C) or to waste, (4) post-hydroponic grow-bed (plants) to sump (Figure 2.5 C-D), and finally (5) post-PFP to hydroponic grow-bed (Figure 2.5 C-E).

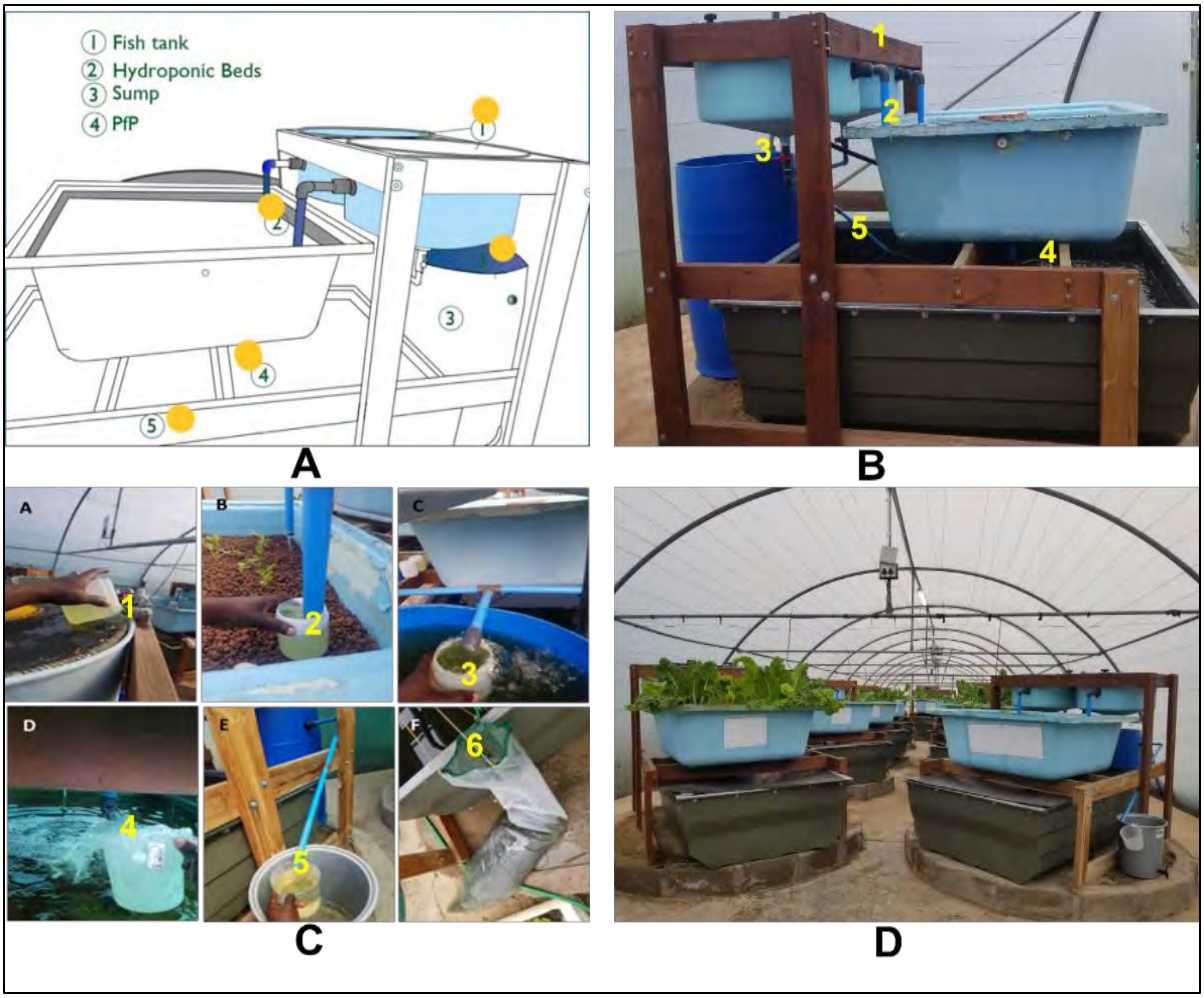

Figure 2.5: Sampling points for each aquaponics unit and the various positions at which water was sampled from the system(customized concept).

2.4. The experimental fish

Clarias gariepinus juveniles weighing approximately 1.5-2.0 g each were obtained from a commercial hatchery (Gariep ATDC, the Department of Forestry, Fisheries, and Environment, Republic of South Africa; Figure 2.6-B). The juvenile African catfish were transported to the Department of Ichthyology and Fisheries Science (DIFS) of Rhodes University in Grahamstown, in a 1000 L plastic container and were supplied with oxygen (Figure 2.6-A). During the 6-hours trip, 650 specimens of the African catfish were transported, and eight mortalities were recorded.

Fish were handled in accordance with the ethical guidelines for the use of animals in research and was approved by RU-AREC references 2022-5738-6852.

Figure 2.6: Equipment and gear used for transporting African catfish fry (A) and the fish on arrival (B).

Clarias gariepinus was acclimatized for four weeks before the start of the trial, in the tanks that were subsequently used for the experiment. During this period, they were fed a commercial tilapia feed (1-2 mm grower pellets, 35% protein and 7.4% fat; Aqua Plus, Avi Products Pty Ltd, Linkhills, South Africa). They were fed to apparent satiation four times a day at 08h00, 10h00, 12h00; and 14h00 for the first two weeks and twice a day at 08h00 and 16h00 for the last two weeks of acclimatisation.

2.5. Experimental vegetables

Sixteen Swiss chard (*Beta vulgaris*) seedlings, purchased from a commercial supplier (Moorland seedlings, Pty, Ltd, Humansdorp), were planted per grow bed (Figure 2.7). Before being introduced into the hydroponic bed, each seedling was weighed (ADAM PGL 2002 electronic scale). The weights varied between 1.8 to 2.3 g. A total of 144 Swiss chard specimens were planted, i.e., a total starting biomass of 288 g and 32 g per grow-bed. Inside each hydroponic grow-bed were stands that provided support for the plants. These plants were maintained in the greenhouse and subject to natural photoperiod and light conditions.

2.6. Experimental design for Exp. 1: The use of drain-fill versus flooding

The experiment included two treatments: (T1) a flooded grow-bed systems (control); and (T2) a drain-fill system (noting that both these systems included primary facultative ponds; Table 2.1 and Figures 2.2 and 2.8). Each of these treatments were carried out in the aquaponics system described in Section 2.2. The treatments were represented by three replicates each, where a

replicate was one aquaponics system, so this experiment included a total of six aquaponics systems.

For the drain-fill treatment, there was a second PVC pipe that formed a siphon but only when the grow-bed was filled with water; once formed, the siphon then drained the grow-bed (Figure 2.7 D, E and F). The siphon-effect was broken when the bed was empty of water, after which the bed then filled up again. Whereas, for the flooded treatment, the siphon-pipe was excluded, and the grow-bed remained permanently flooded (Figure 2.7 A, B and C).

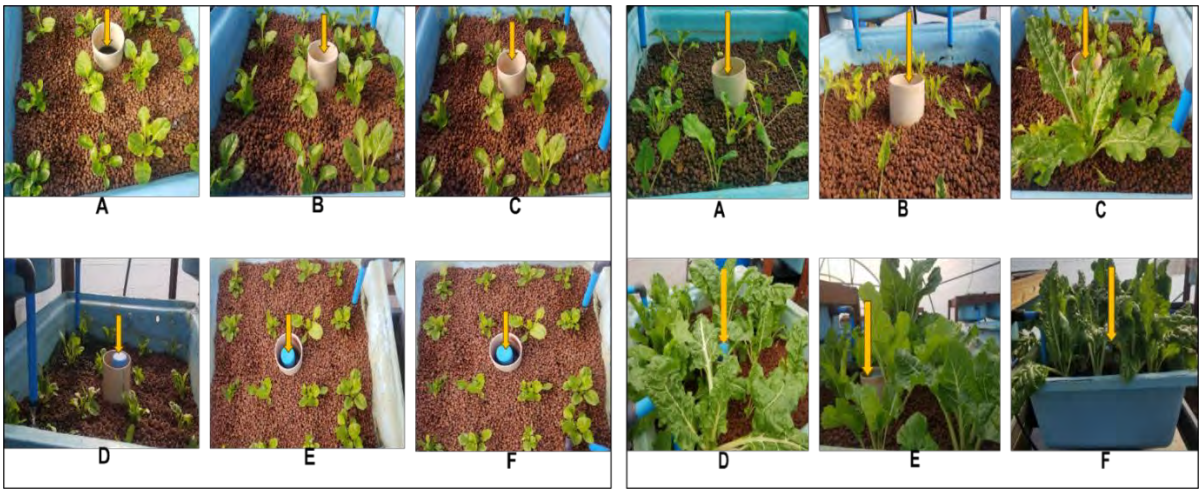

Figure 2.7: Three-and six-week's duration experimental vegetables: flooding (A, B and C), drain-fill (D, E and F) using primary facultative ponds. The growth-bed remained flooded using an upstanding pipe only (arrow in A, B and C). In D, E and F the growth-bed water was drained by a bell-syphon (indicated by the arrow in D, E and F) that allowed for tidal drain-fill (customized concept).

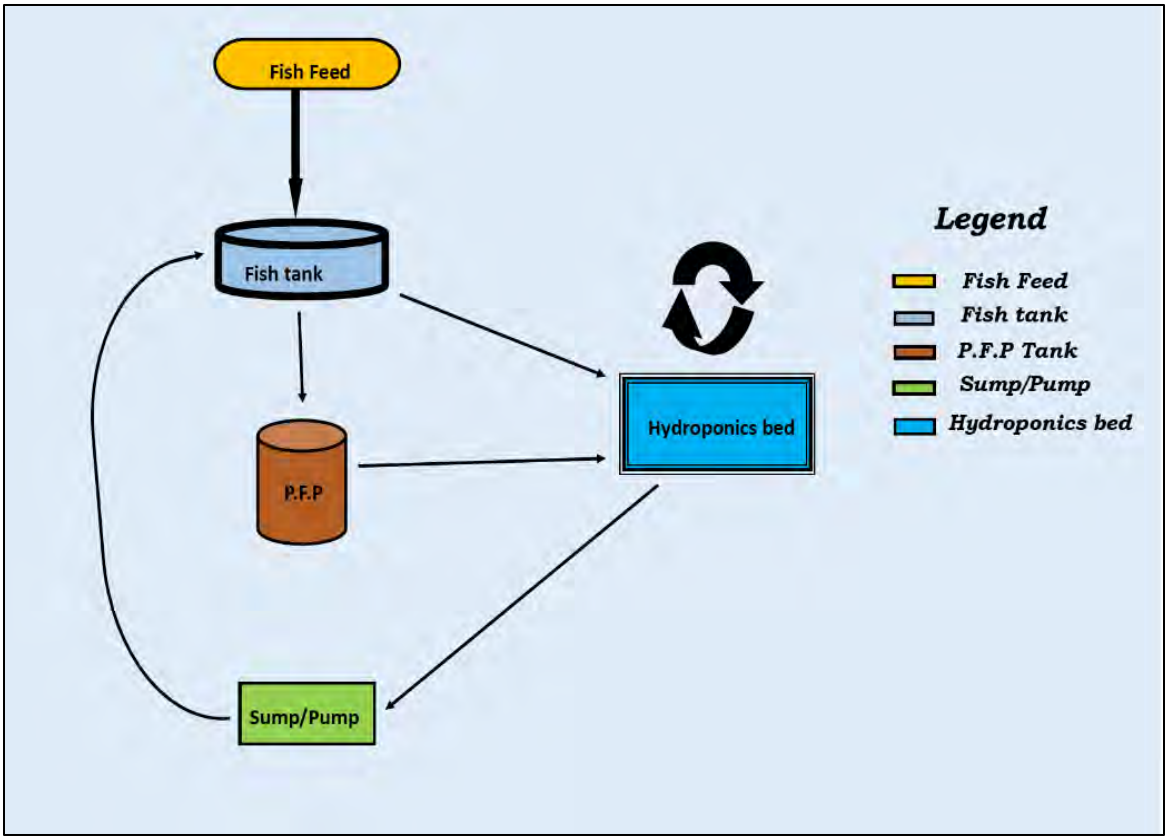

Figure 2.8: System design: drain-fill and flooding using primary facultative ponds (PFP). The hydroponic bed is either flooded or subject to drain-fill. Illustration of the drain-fill and solids removal technology systems in aquaponics (customized concept).

Table 2.1: Experimental design of two experiments that share treatment with and without drain-fill or with and without primary facultative ponds (PFP).

Treatment No.	Grow-bed type	Solids removal	Flow rate ($L \cdot min^{-1}$)[a]	Approximate no. drain-fills per hour in the grow-bed[b]	Approximate water retention cycle in grow-bed (min)	Initial mean fish stocking density (kg $\cdot kL^{-1}$)	No. reps
T1	Flooded (control)[c]	PFP	13.3	N/A	6.6	9	3
T2	Drain-fill	PFP	13.3	9	6.6	9	3

[a] This is the flow rate into the fish tanks, i.e. split between the two fish tanks in each system.
[b] This is the approximate number of exchanges in the grow-bed considering that the grow-bed was 220 L and that it was filled with clay growth media (i.e., approximately 40% water and 60% clay media and plant matter).
[c] This treatment is considered the control since flooded grow beds are historically more the conventional production system and the aim was to establish the effect of drain-fill on the productivity of the system; a flooded grow-bed is thus the control.

2.7. Experimental design for Exp. 2: Dealing with solids using primary facultative ponds

Two treatments were compared in this experiment: (T2) an aquaponics system where the solid fish waste was retained in the system using a PFP; and (T3) an aquaponics system where solid fish waste was removed from the system (Table 2.2; Figure 2.9). Each of these treatments were carried out in the aquaponics system described in Section 2.3, with the exception that one included the PFP and the other did not (Figure 2.9). The treatments were represented by three replicates each, where a replicate was one aquaponics system, so this experiment included a total of six aquaponics systems. Note that treatment T2 was the same treatment described in Section 2.6 as "drain-fill" and since both experiments were run simultaneously, T2 was a shared treatment in both experiments.

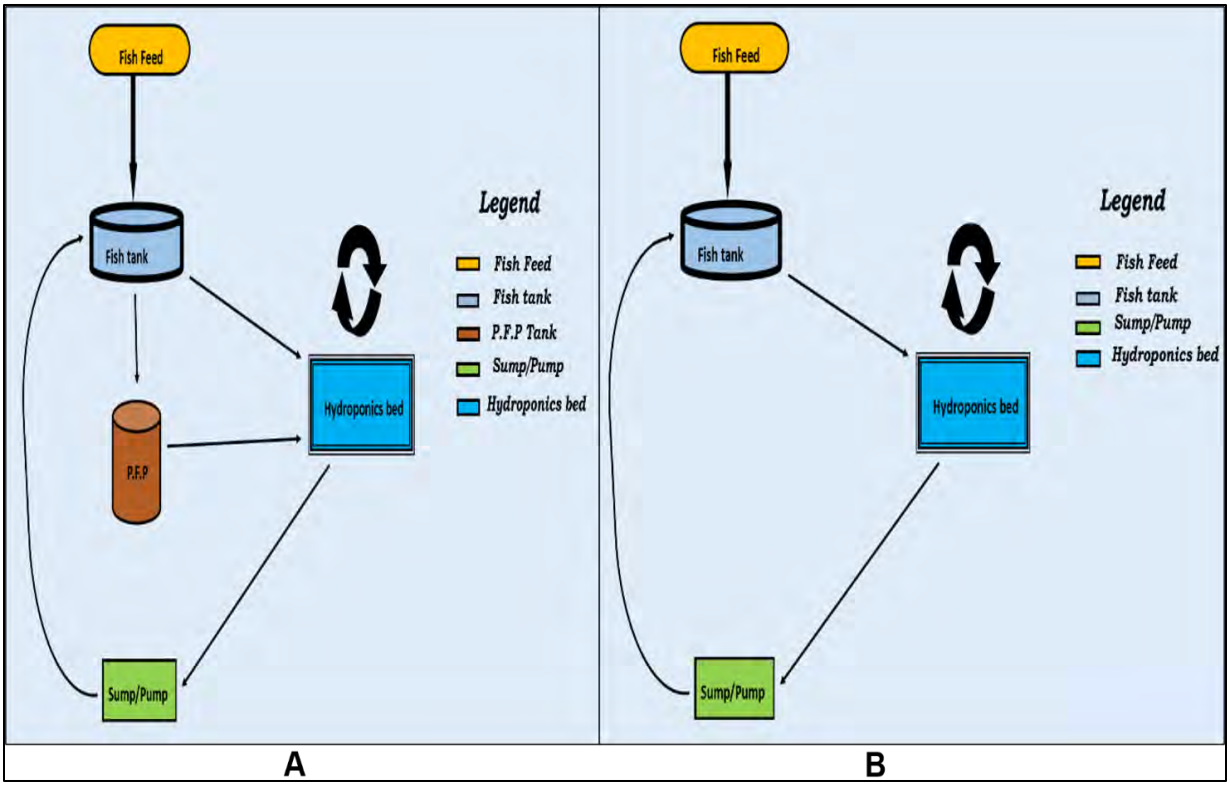

Figure 2.9: System design of primary facultative ponds (A) and no primary facultative ponds (B) (customized concept). Both systems were identical with the exception in A, solid waste was retained in the system using a primary facultative pond (PFP), whereas in B the solid waste was removed from the system.

Table 2.2: Experimental design of two experiments that share treatment with and without drain-fill or with and without primary facultative ponds (PFP).

Treatment No.	Grow-bed type	Solids removal	Flow rate ($L \cdot min^{-1}$)[a]	Approximate no. drain-fills per hour in the grow-bed[b]	Approximate water retention cycle in grow-bed (min)	Initial mean fish stocking density ($kg \cdot kL^{-1}$)	No. reps
T2	Drain-fill	PFP	13.3	9	6.6	9	3
T3	Drain-fill	No-PFP (control)[c]	13.3	9	6.6	9	3

[a] This is the flow rate into the fish tanks, i.e. split between the two fish tanks in each system.
[b] This is the approximate number of exchanges in the grow-bed considering that the grow-bed was 220 L and that it was filled with clay growth media (i.e., approximately 40% water and 60% clay media and plant matter).
[c] This treatment is considered the control since the aim was to establish the effect that maintaining solids in the system using a PFP had on the productivity of the system; no-PFP is thus the control.

2.8. Data collection

In order to obtain good results, characterized by precise and reliable data, allowing informed decisions to be made when comparing these nine aquaponic replications, this research proceeded by a systematic process of collecting and measuring all the information entering or leaving the system. This collection made it possible to test the hypotheses of the present work. It used several experimental methods carried out in the field and in the laboratory.

2.8.1. Water quality

Aquaponics research requires knowledge of the water quality used in the system. It assesses the physical, chemical, biological, and radiological characteristics of water used in an aquatic ecosystem. This water quality helps assess whether water meets the national or international standards established for its use.

Water sampling points

Data were analysed according to the procedures described by Rodier (2009). Water quality parameters such as dissolved oxygen (DO $mg \cdot L^{-1}$), temperature (°C), pH, electrical conductivity (EC $\mu S \cdot s^{-1}$), and salinity (‰ or ppt) were measured once a day during the five months of the experiment. These parameters were taken using a portable electrical probe (Hach HQ30d Multimeter probe, n.d.) (Figure 2.10 H). Total dissolved solids (TDS; $mg \cdot L^{-1}$),

ammonia ($mg \cdot L^{-1}$), nitrate ($mg \cdot L^{-1}$), nitrite ($mg \cdot L^{-1}$), phosphorus (PO_4^{3-}; $mg \cdot L^{-1}$), and chemical oxygen demand (COD; $mg \cdot L^{-1}$) were monitored once a week, over the same period, using a spectrophotometer (Hach, DRB200 Digital Reactor Block for TNT plus; 21X13 mm vial wells, 4x20 mm vial ells, 115 VAC, USA) and commercial colorimetric test kits (DR9000, Sera Aquarium; NH_3, NH_4^+, NO_2^-, NO_3^- test kits, Heinsberg, Germany) (Figure 2.10). The COD was determined using a hot reaction with a volume of 1.50 ml of 0.04 M potassium dichromate and 3.50 mL of concentrated sulfuric acid in a kit. A sample of 2.50 mL of water that was tested, was gradually added to the reaction (Figure 2.10 E-G). The reaction took place at a temperature of 148°C in a mineralizer for two hours (Figure 2.10 F). After cooling, the amount of oxidant remaining in this test kit (Heinsberg, Germany) was measured by photometry (Figure 2.10 G). The reading was made by the appropriate spectrophotometer (Nelson and Siegel, 2014). In aquaponics, values between 25 to 80 $mg \cdot L^{-1}$ are required (Bowers and Brett, 2008).

Figure 2.10: The various kits according to the toning colour: ammonia (A), phosphorus (B), nitrate (C), nitrite (D) and chemical oxygen demand (E), (F) is the mineralizer to cook the water sample for the COD, (G) spectrophotometer for reading the concentration values in mg·L-1 and (H) the indicated HACH multi-parametric probes for the in-situ parameters.

Solid waste (Particular Organic Matter)

Sediments that were removed from the three no-PFP systems were assessed to measure the weight of solids that were removed from the system. In each of these systems, a water sample of a minimum volume of 20 L that contained the solid fish waste that was removed from each system, was collected in a sediment trap. This water sample was left to settle for approximately 24 h. After settling, the water was drained from the fish tanks into the container to keep the

already settled sediments in the bottom of the bucket. The fresh weights of sediments were taken the same day using an electronic balance (RADWAG WTC 2000), and then the samples of particular organic matter (POM) were kept in the oven where it was dried for three days at a temperature of 40°C. Finally, these samples were reheated in the oven (temperature 40°C) until a constant weight was obtained to determine the dry weights of these sediments (Figure 2.11 F**)** using the following equation, where POM is particular organic matter ($mg \cdot L^{-1}$), Ɛ is fresh weight (g) and α is dry weight (g):

$$POM = Ɛ - \alpha \text{ Equation} \quad (1)$$

Figure 2.11: The methods of processing algae and sediments (organic meter), (2.12A-C-D) algae and (B) sediments. (E) Algae dry sample, and (F) dry sediment (organic matter) sample.

2.8.2. Fish production

Weighing and measuring

After the acclimation period, fish were purged for 24 h and anesthetized with clove oil at 0.05ml/500 ml (Fernandes *et al.*, 2017). The fish were scooped out of the tanks with a fish net, one to three fish at a time. These fish were then transferred to the clove oil bath solution and observed until they lost balance as they swam. They were then weighed to the nearest gram

using an electronic scale (ADAM Highland HCB 3001, n.d), and the total length (TL) was measured to the nearest millimetre using a measuring board (Figure 2.12). This was carried out at the start of the trial and was repeated every four weeks for a total of five months.

Figure 2.12: African catfish.

The fish were monitored for signs of illness, disease, and infections throughout the trial, and these were recorded and photographed. Dead fish were removed from the tanks, which were checked daily, and mortalities were recorded to calculate survival rates.

At the end of the experiment (after five-months) clove oil was used to euthanize the fish in a 0.2 mL/500 mL bath solution (Fernandes *et al.*, 2017). Three fish per treatment were frozen and sent to a commercial lab (Agricultural Research Council, Irene, Gauteng) for whole-body proximal analysis (Khokhar, 2017). After each month, the fish that reached about 300 g were removed (i.e., harvested) from the aquaponics system to maintain constant stocking densities (Figure 2.13 F).

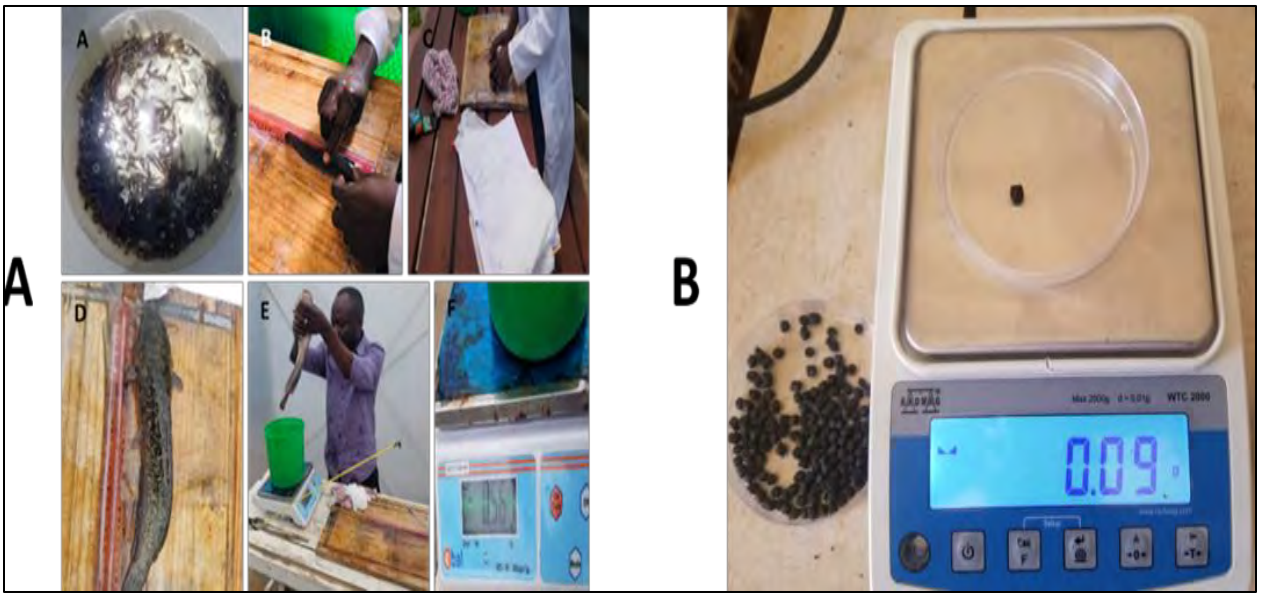

Figure 2.13: Determination of weight using an electronic scale (AE, AF) and measurements (AB, AD) to assess the growth of African catfish in aquaponics and (B) the weight of feed.

Stocking density

The fish tanks used had a capacity of 80 L and were each stocked with 20 African catfish, which conformed to densities used by Hochmuth and Hanlon (2010), FAO (2012 and 2014), Bosire and Sikora (2017) and Khandaker and Kotzen (2018). As such, 360 African catfish were stocked into the tanks at the start of the experiment. The mean density at the start was 9 $kg \cdot kL^{-1}$ for each tank (Love *et al.*, 2014; Hussain *et al.*, 2018). This stocking density increased to means of 15 and 23.0 $kg \cdot kL^{-1}$ after four weeks and eight weeks respectively, and was maintained between a mean of 35 to 36 $kg \cdot kL^{-1}$ form week 12 to 16. Fish were removed (i.e. harvested) when they reached 300 g to maintain the stocking density within this range. The weight of harvested fish was recorded and subtracted from the stocked biomass.

Condition factor, feeding and feed conversion ratio

The same diet used during acclimation was provided twice daily at 9 a.m. and at 4 p.m., except for the day before sampling and if the ammonia level was high (i.e., >3 $mg \cdot L^{-1}$). Feed was provided at 3% of the total body weight per day per tank and this was adjusted after every weighing and measuring. However, feeding was stopped if they reached satiation, which was assumed when foraging at the top of the tank stopped. They were never fed more than 3% of their body weight per day. The fish were fed an average of 60 g of feed per day per tank over the trial. As such, the African catfish were feed 10.8 kg of feed over the period of the trial, which was equivalent to 10.8 $t \cdot m^{-3}$.

Condition factor (CF; Bolger and Connolly 1989) was calculated using Equation 2:

$$CF = \frac{\text{body weight}}{\text{total length}^3} \text{ x}100 \qquad (2)$$

where body weight was wet weight (g) and total length was measured in millimetres. This is a numerical value that expresses the ratio of a fish's weight to its length (Bolger and Connolly 1989). It serves as an indicator of the fish's overall health and well-being, reflecting its fat reserves and weight (Bolger and Connolly 1989).

The feed conversion ratio (FCR) was determined according to Cowey (1992), and obtained by calculating the ratio between the dry weight of feed that was fed and the wet gain in live weight at a certain time interval, using the Equation 3 where Q is the amount of dry feed given during experiment (g), Ga is the initial wet fish biomass (g), Gn is the wet biomass of dead fish or fish

that were harvested from the treatment (g) and Gt is the wet final biomass of the fish (g).

$$FCR = \frac{Q}{(Gt + Gn - Ga)} \quad \text{Equation (3)}$$

Specific growth rate (SGR) was calculated using Equation 4.

$$SGR = \left[Ln(\text{final weight g}) - Ln\frac{\text{initial weight g}}{\text{days number}} \right] x\ 100 \quad \text{Equation (4)}$$

Mortality (M) was calculated using Equation 5.

$$M = \left(\text{initial number of fish} - \text{final number of}\frac{\text{fish}}{\text{initial}}\text{number of fish x } 100\right) \text{ Equation (5)}$$

The length (mm) and weight (g) of African catfish were monitored during this five-month experimental period to determine, and these were measured and recorded every four weeks from the start of the test, according to the methods described previously. Fish growth was recorded as specific growth rate (SGR) using Equation 4.

Cleaning

The cleaning of the aquariums was done once a week. Waste that consisted of fish excrement and uneaten fish food was removed from the fish tanks, by opening the valve at the bottom of the conical-shaped fish tank (Figure 2.11-F); Section 2.7 Sediments (particular organic matter). This waste either drained into the PFP (in the case of treatments that included the PFP) or into a waste bucket for treatments that did not include a PFP. This solid was used to determine POM (Section 2.8.1).

2.8.3. Plant production

Weighting and measuring

Morphological features such as leaf length, leaf width and stem length of the plants in the aquaponics systems were measured in millimetres, using a tape measure and then weighed on an electronic scale (ADAM PGL 2002 and using a 3M AUTO-STOP TAPE METRIC 16mm). There were two factors that were measured: plant growth and biomass harvest (Figures 2.14 and 2.15, AC-AD and 2.15 BA-BB-BC-BE). Plant growth: here, measurements were taken on

three randomly selected plants in each growing bed every four weeks for twenty weeks. The following parameters were measured: the number of leaves, leaf height and width, and the height of the stem of the plants.

Harvesting leaves: After three months of growth, the largest leaves were harvested. A leaf area index (LAI) was considered "large" and of harvestable size when it reached a length of 260 mm. They were weighed directly to record the fresh, wet weight and then they were put in the oven at 40°C for three days and the dry weight was recorded (Figures 2.16-B and 2.16-D). Thereafter, large leaves were harvested once a week. Wet weight and dry weight were recorded using the method presented above. The biomass production in the last quarter of the experiment was too large and it was not possible to dry all the plant material that was harvested. As such, only the fresh weight was recorded and a sample of 10 leaves per harvest was dried to obtain the average dry weight; and the wet-dry-weight-ratio of the sample was used to calculate the total dry weight harvest. The characteristics assessed in these plants included the number of leaves, and their heights and widths were determined. Root, stem and leaf fresh weight was quantified at the end of this experiment.

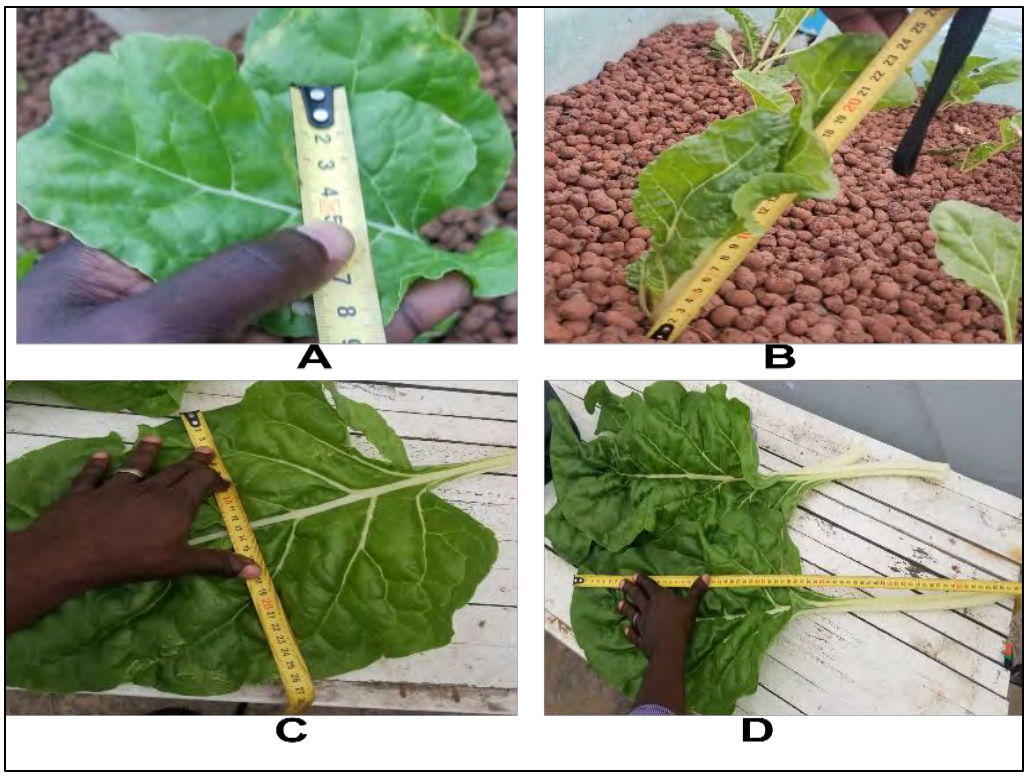

Figure 2.14: Measurement of Swiss chard leaves in width (A) and (C); length (D), as well as the length of the stem B using a slate and a SELF-STOP TAPE to determine the leaf area index (LAI) for plant growth.

Figure 2.15: The chlorophyll-meter (AA) and the determination of chlorophyll-a concentration (AB), and the measurements by means of a slat and a TAPE SELF-STOP for plant growth in length (AC-AD and BA-BB-BC-BC).

Figure 2.16: Vegetable harvest (A), fresh weight gain (B), baked vegetables (C) and dry weight gain of vegetables after three days at 40°C in the oven (D).

Chlorophyll-a concentration

The same three plants, randomly selected to be sampled in each hydroponic bed, were also sampled for their chlorophyll-a concentration during this research. It was determined using a chlorophyll meter (CCM-200 plus, NH 03051, n.d) once a month (i.e., every four weeks) (Figure 2.15-AA).

Plant biomass calculated

Swiss chard seedling developed into a plant of a harvestable size in all three treatments (Figure 2.17). Treatment one (T1) included the PFPs, treatment two (T2) was the flooded grow beds, and treatment three (T3) included no-PFPs (Figure 2.17). The same materials and methods were used for the production of plants in all the treatments.

The leaves, stems and roots were weighed separately at the end of the experiment to obtain biomass (Figure 2.17). Plant biomass was calculated from the entire composition of the plant (i.e., using the leaves, stems and roots) that remained at the end of the trial and leaves that were harvested before the end of the trial were added to the total biomass.

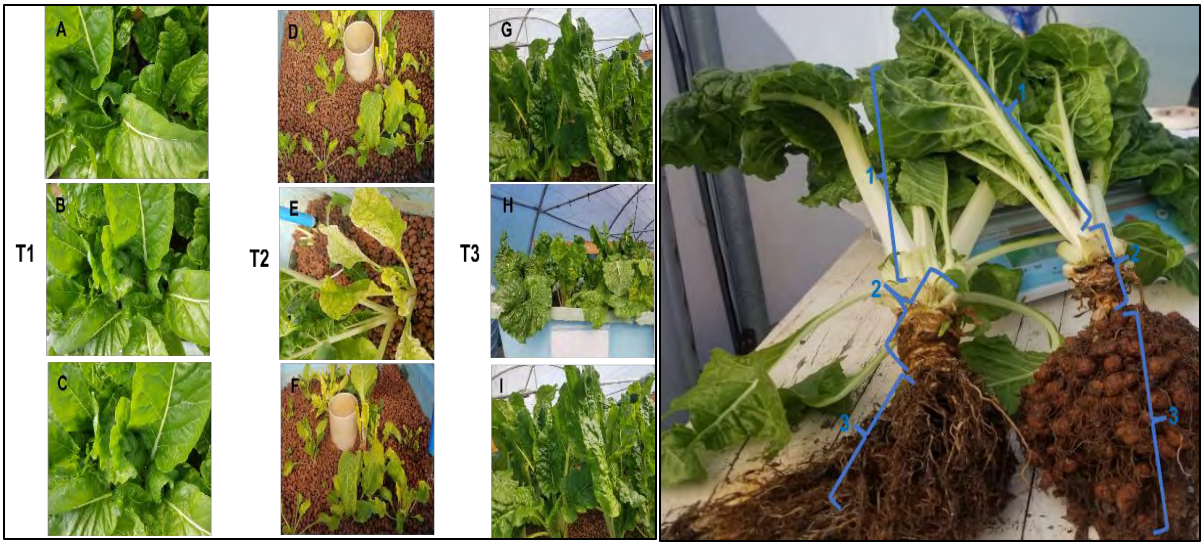

Figure 2.17: The dynamics of plants according to the various treatments (T1 Drain-fill PFP, T2 Flood PFP, T3 Drain-fill no PFP) and the Plant biomass (1 leaves; 2 stems; 3 roots biomass) of the system.

2.9. Statistical analysis

2.9.1. Statistical analysis-Exp. 1: The use of drain-fill vs flooding

The data were archived using Microsoft Excel and analysed using Statistica 10, Stata 13 and Past 4.11 Software's (Cox, 2002; Mitchell, 2010). Treatment means were compared using a

multi-factor, repeated measures analysis of variance (RM ANOVA) for all data that were collected over the course of the experiment (Schober and Vetter, 2018; Hickey *et al.*, 2018). The factors included: system type (drain-fill versus flooded) and different sampling points in the system, for all data collected over the course of the experiment. If these data did not meet the assumptions of equality of variance and normal distribution of residuals, they were adjusted using Mauchley Spherity. For data that were not collected repeatedly, the two-treatment means were compared using either Student's t-test (for parametric data sets) or a Man-Whitney U test (for data sets that there were not parametric). All treatments were considered significantly different at $p < 0.05$ and Mann-Whitney U-test as a non-parametric test when normality was not achieved.

The relevant graphs of means comparison were drawn in Statistica. The fish weight-length regression was linearized by log10 transformation. Daily feeding was obtained by summing up the morning and afternoon feed. The monthly averages of all quantitative variables per experiment were unified in one table to allow the evaluation of the relationships between the fish data, vegetables, and water quality parameters.

2.9.2. Statistical analysis-Exp. 2: Solids dealing using primary facultative pond

The same statical analysis that was described in Section 2.9.1 was used to compare the two treatments in this experiment, only the system type in this experiment included PFP versus no-PFP.

Chapter 3. Experiment 1: The use of drain-fill vs flooding

3.1. Introduction

Drainage, filling and flooding technology in an aquaponic system was designed for improved growth of fish and plants in a closed environment. Fish tank effluent provides most of the nutrients needed by plants (Papatryphon *et al.*, 2005). The system can perform well if the optimal ratio of daily nutrient intake matches the area maintained for plant growth (Rakocy *et al.*, 2004). However, most vegetable crops are terrestrial and their root system requires atmospheric oxygen (Hochmuth and Hanlon, 2010). In the hydroponics bed, plants must be produced under ideal water conditions, and they should not be exposed to excess water (Lennard and Leonard, 2004). As such, vegetable production in an aquaponics system, where the grow bed is permanently flooded, is probably not an ideal production environment and may be anoxic in parts with poor water exchange. The draining and refilling of the vegetable grow bed has been tested and been shown to keep aquaponic systems operating optimally (Rupasinghe and Kennedy, 2010; Li *et al.*, 2019). Unlike a flooded system, this was a system in which water continually filled and drained the vegetable grow bed throughout the research (Van Den Driessche, 1974; Rakocy *et al.*, 2004). The flush and fill action refers to a process of removing all the old water from the system and replacing it with new water in the culture bed (Mongirdas and Kusta, 2006; Lennard 2012). This movement was carried out regularly, to ensure the proper functioning of this system (Shava and Gunhidzirai, 2017; Khandaker and Kotzen, 2018).

It should be noted that, in an aquaponics system, the addition of "splash" water to the fish tank provides the fish with additional oxygen, which is needed for the fish to meet their oxygen demand (Van Den Driessche, 1974). As such, a system with well oxygenated water should result in faster growing fish. Fish production is characterized by three functions: natality, growth and mortality (Rakocy, 1999; Goddek, 2017). In aquaponics grow-out and mortality are most important. The control of these two functions is needed for sustainable management (Rakocy, 1999; Goddek, 2017). Thus, knowledge of mortality rate and growth is essential (Von Sperling and de Lemos, 2005; Xu *et al.*, 2014). In aquaponics, the use of the new practice that uses drain-fill might affect fish growth and mortality.

Hypotheses:

The same hypotheses that were described in Section 1.2 were used to compare the two treatments in this experiment, only the system type in this experiment included drain-fill versus flooded.

A higher fish production would be expected from the drain-fill as opposed to the flooded system (H1),

A higher vegetable production would be expected from the drain-fill as opposed to the flooded bed culture (H2),

Higher NO_3 concentrations would be expected from the drain-fill as opposed to the flooded bed culture (H3).

3.2. Materials and methods

This chapter used the materials and method already described in Chapter 2 (Section 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.8 and 2.9). In this first experiment, two treatments were used: (1) an aquaponics system that included a drain-fill vegetable grow-bed, and (2) an aquaponics system that used a flooded vegetable grow bed (i.e. the control). Both treatments were presented in triplicate (i.e., there were six systems in total). The study duration was five months.

3.3. Results

3.3.1. Results - Water quality

Dissolved oxygen

The dissolved oxygen (DO) concentration was not affected by the interaction between time, treatment and sampling point (RM-ANOVA $F_{(20,700)}=0.13$, $p=1.00$). Similarly, it was also not affected by the interaction between time and treatment (RM-ANOVA $F_{(5,700)}=1.68$, $p=0.14$). However, it was affected by the interaction between treatment and sampling point (RM-ANOVA $F_{(4,140)}=4.20$, $p=0.003$; Figure 3.1). The DO concentration in sampling points 1, 2, 3 and 4 were similar but sampling point 5 was significantly lower than the others, with respect to the flooded treatment. It followed a similar trend in the drain-fill treatment. The DO of flooded systems, however, was significantly lower at all sampling points compared to drain-fill (Figure 3.1).

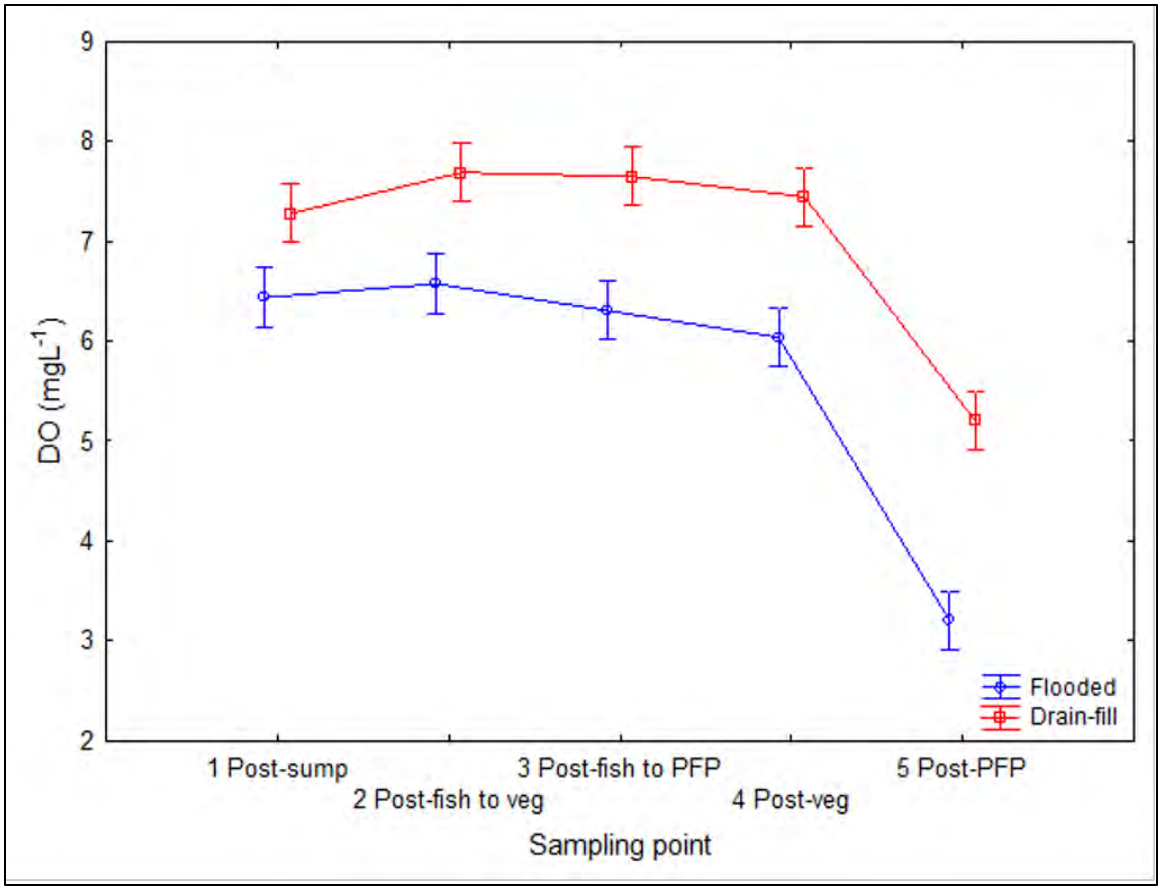

Figure 3.1: Mean (± 95% confidence interval) dissolved oxygen (DO, $mg \cdot L^{-1}$) at the outlet of the sump (1 Post-sump), the outlet of the fish tank that drains to the vegetable grow-bed (2 Post-fish to veg), the outlet of the fish when solid waste was removed (3 Post-fish to PFP) the outlet of the vegetable grow bed (4 Post-veg to sump) and the outlet of the primary facultative pond (5 Post-PFP) in the drain-fill and flooded aquaponic systems, with all sample periods combined (RM ANOVA, $F_{(20,700)}=4.20$, $p=0.003$).

Temperature (°C)

The temperature was not influenced by the interaction between time, treatment and sampling points (RM-ANOVA $F_{(20,700)}=0.001$, $p=1.00$). Similarly, temperature was not influenced by the interaction between time and sampling point during the experimental period (RM-ANOVA $F_{(20,700)}=0.12$, $p=1.00$). It was also not influenced by the interaction between the treatment and the sampling point (RM-ANOVA $F_{(4,140)}=0.11$, $p=0.98$). However, the temperature was influenced significantly by the interaction between time and treatment during this study (RM-ANOVA $F_{(5,700)}=21.42$, $p<0.001$; Figure 3.2). For both flooded and drain fill systems, temperature steadily decreased from the beginning of the experiment until month three, and then remained constant from month three to four. At month five, the temperature in both systems increased, and that of the flooded was significantly higher than that of the drain fill system (Figure 3.2).

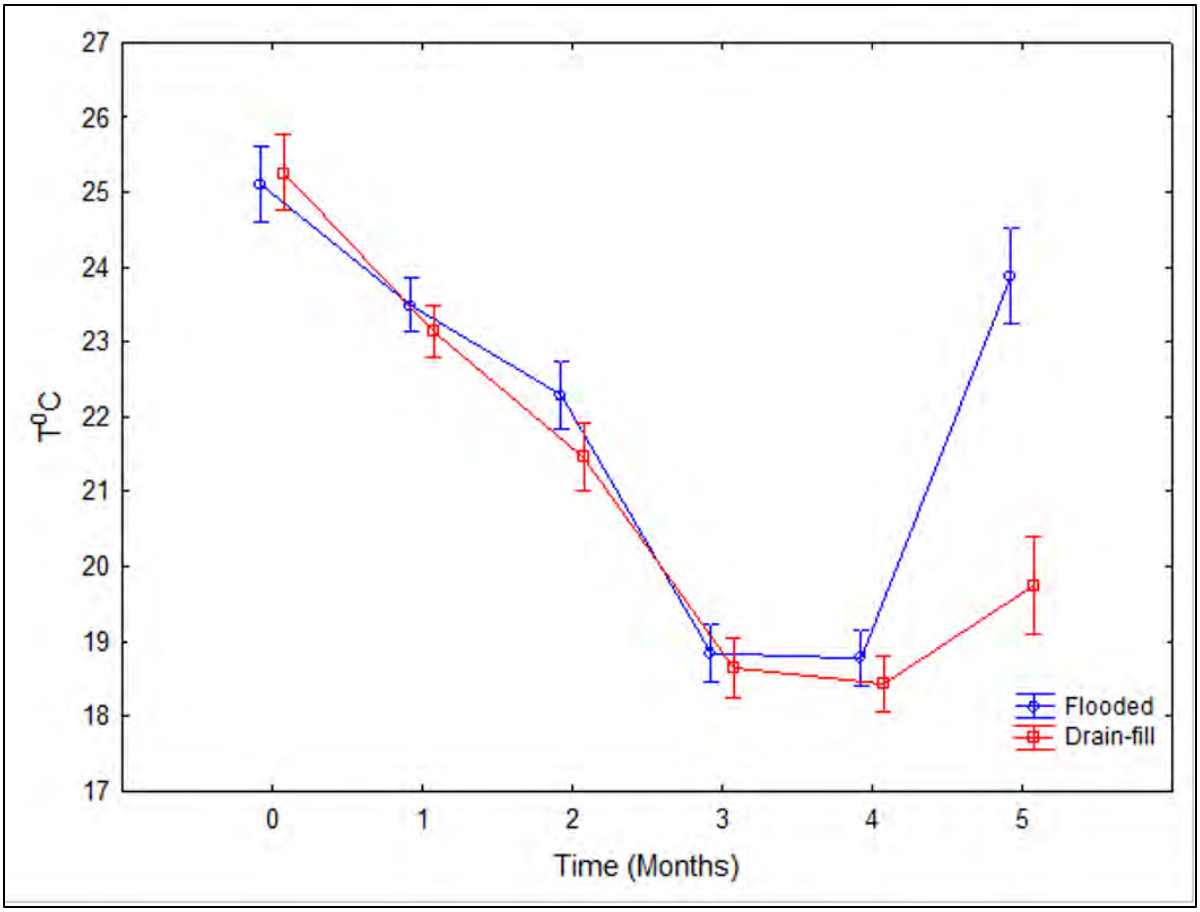

Figure 3.2: Comparison of mean (± 95% confidence interval) temperature (T°C) in the drain- fill and flooded aquaponic systems, with all sample periods combined (ANOVA $F_{(5,700)}$=21.42, p<0.001).

Hydrogen potential

The pH was not influenced by the interaction between time, treatment and sampling points (RM-ANOVA $F_{(20,700)}$=0.10, p=1.00). Similarly, pH was not influenced by the interaction between time and sampling point in period of this experiment (RM-ANOVA $F_{(20,700)}$=0.10, p=1.00). Also, the pH was not influenced by the interaction between treatment and the sampling points (RM-ANOVA $F_{(4,140)}$=0.16, p=0.96). However, pH was influenced by a significant interaction between time and treatment during this study (RM-ANOVA $F_{(5,700)}$ =16.38, p<0.001; Figure 3.3). The pH decreased at the same rate for both treatments in the first month of the experiment. For months two and three, the flooded system had a significantly higher pH than the drain fill. The pH of the two treatments was similar in the fourth month but the water pH of flooding treatments increased significantly in the last month of the study. This pH was the highest (>9.5) recorded in the entire experimental period (Figure 3.3).

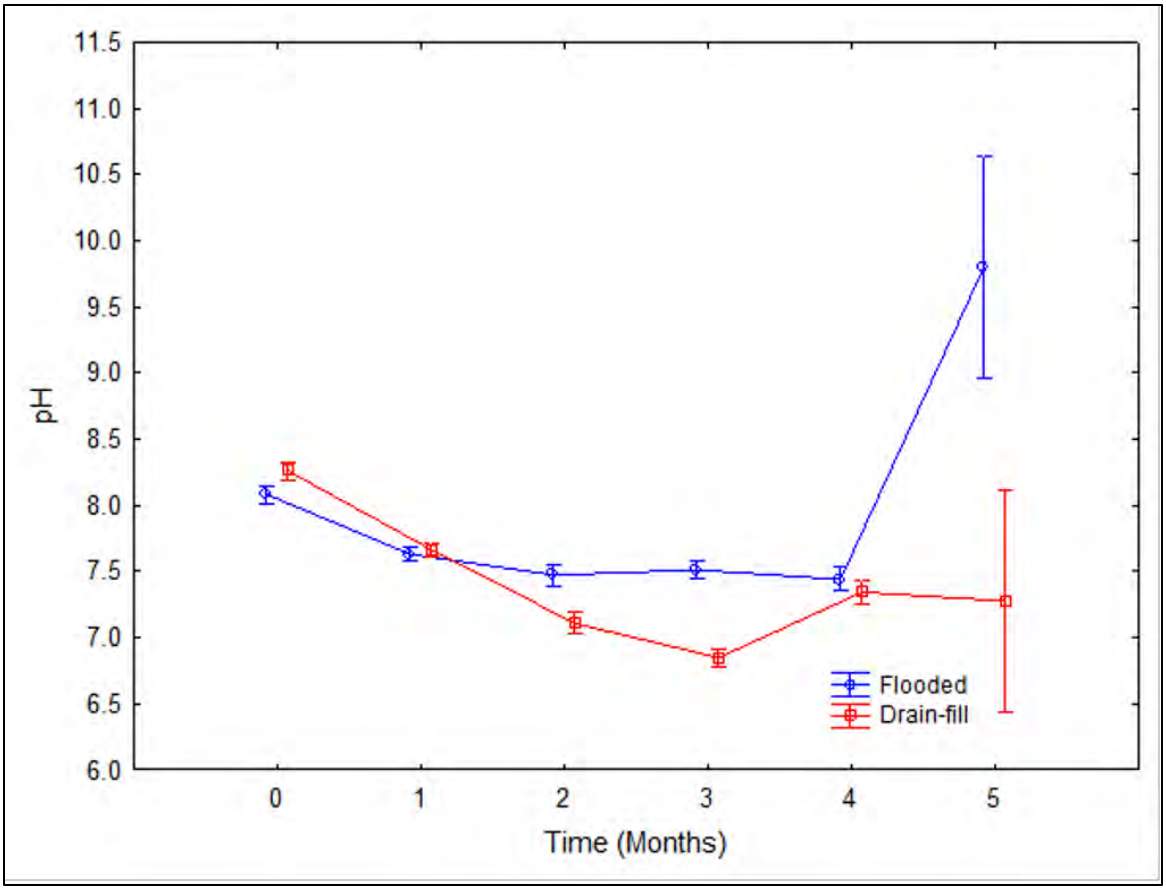

Figure 3.3: Comparison of mean (± 95% confidence interval) Hydrogen potential (pH) in the drain-fill and flooded aquaponic systems, with all sample periods combined (RM-ANOVA $F_{(5,700)} = 16.38$, $p < 0.001$).

Electrical conductivity

Electrical conductivity was not influenced by the interaction between time, treatment and sampling points (RM-ANOVA $F_{(20,700)}=0.08$, $p=1.00$). Similarly, EC was not influenced by the interaction between time and sampling points during this experiment (RM-ANOVA $F_{(20,700)}=0.29$, $p=0.99$). Moreover, EC was not influenced by the interaction between treatment and sampling points (RM-ANOVA $F_{(4,140)}=0.18$, $p=0.95$). However, EC was significantly affected by the interaction between time and treatment in this study (RM-ANOVA $F_{(5,700)}=6.21$, $p<0.001$; Figure 3.4). The EC decreased for both treatments for the first month. It then increased at different rates for the two treatments, with drain fill increasing at a higher rate until month five, where EC was once again equal between the treatments (Figure 3.4).

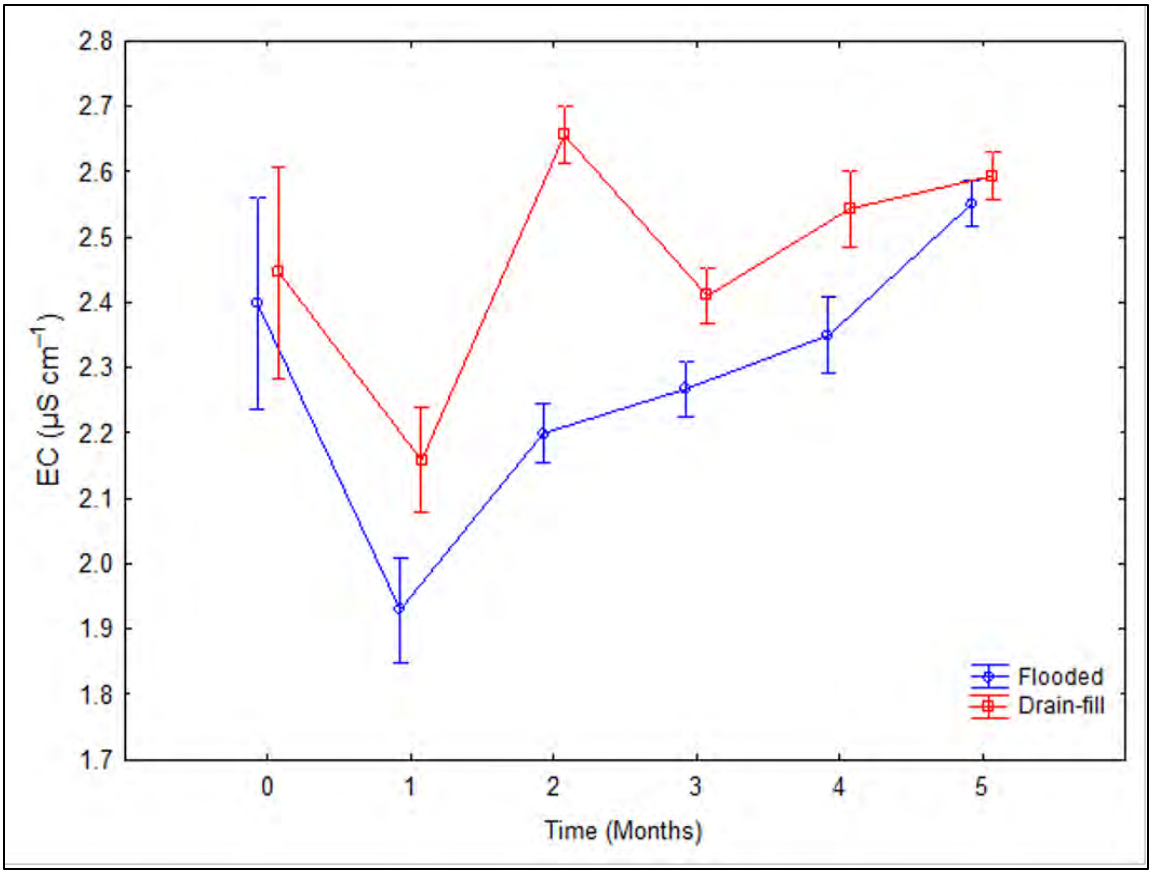

Figure 3.4: Comparison of mean (± 95% confidence interval) electrical conductivity (EC, µS·cm^{-1}) in the drain-fill and flooded aquaponic systems, with all sample periods combined (RM-ANOVA $F_{(500,700)}$=6.21, p<0.001).

Salinity

Salinity was not influenced by the interaction between time, treatment and sampling points (RM-ANOVA $F_{(20,700)}$=0.09, p =1.00). It was also not influenced by the interaction between time and sampling points during this experiment (RM-ANOVA $F_{(20,700)}$=0.18, p=0.99). Moreover, salinity was not influenced by the interaction between treatment and sampling points (RM-ANOVA $F_{(4,140)}$=0.59, p=0.67). However, it was significantly affected by the interaction between time and treatment in this study (RM-ANOVA $F_{(5,700)}$=3.40, p=0.005; Figure 3.5). Salinity was equal between the drain fill and flooded treatments at the start of the experiment. From month zero to month one, salinity in the flooded treatment decreased while that of the drain fill remained constant. From month one to two salinity increased in both treatments. The drain fill treatment had a significantly higher concentration from this time until the end of the experiment (Figure 3.5).

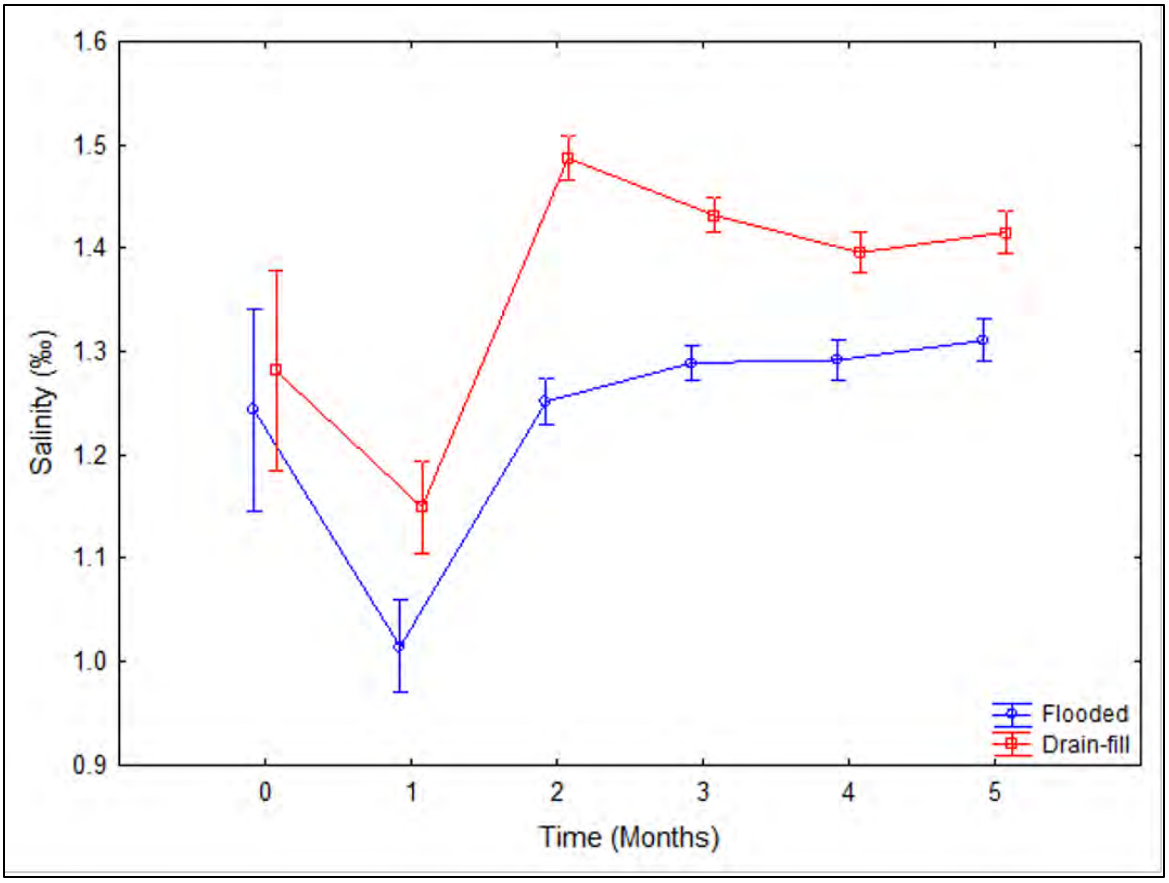

Figure 3.5: Comparison of mean (± 95% confidence interval) salinity (‰) in the drain- fill and flooded aquaponic systems, with all sample periods combined (RM-ANOVA $F_{(5,700)}$=3.40, p=0.005).

Total dissolved solids

The TDS was significantly influenced by the interaction between time, treatment and sampling points (RM-ANOVA $F_{(20,700)}$=1.87, p=0.01; Figure 3.6). The concentration of TDS was similar between treatments and sampling points at the beginning of the experiment, as well as months one, three and four. At month two, TDS was significantly higher in the flooded treatment at sampling points three and five. In the final month of the experiment, the flooded treatment had lower TDS concentration in sampling point two. It was however significantly higher than that of the drain fill treatment in sampling point five (Figure 3.6).

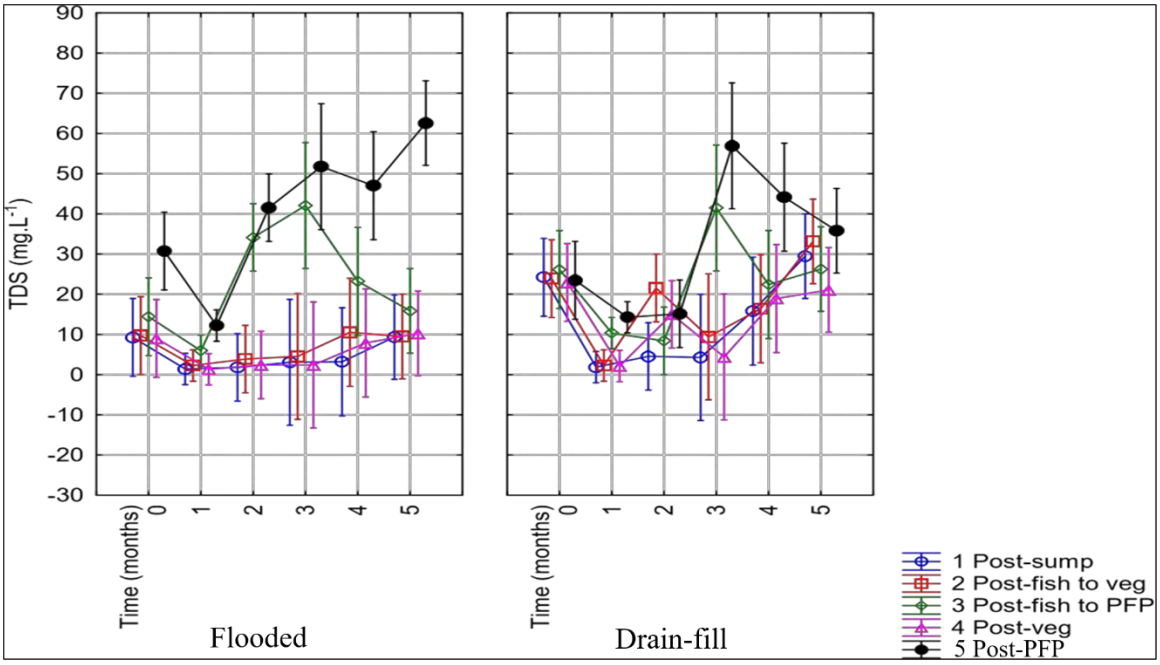

Figure 3.6: Mean (± 95% confidence interval) total dissolved solids (TDS, $mg \cdot L^{-1}$) at the outlet of the sump (1 Post-sump), the outlet of the fish tank that drains to the vegetable grow-bed (2 Post-fish to veg), the outlet of the fish when solid waste was removed (3 Post-fish to PFP) the outlet of the vegetable grow bed (4 Post-veg to sump) and the outlet of the primary facultative pond (5 Post-PFP to veg) in the drain-fill and flooded aquaponic systems (RM-ANOVA $F_{(20,700)}=1.87$, $p=0.01$).

Ammonia

The NH_3^- was significantly influenced by the interaction between time, treatment and sampling points during this study (RM-ANOVA $F_{(20,700)}=2.66$, $p<0.001$; Figure 3.7). The concentration of NH_3 was similar over time between the flooded and drain fill treatments, across the five sampling points, except for month three where NH_3 was significantly higher in the drain fill treatment, at sampling points one to four.

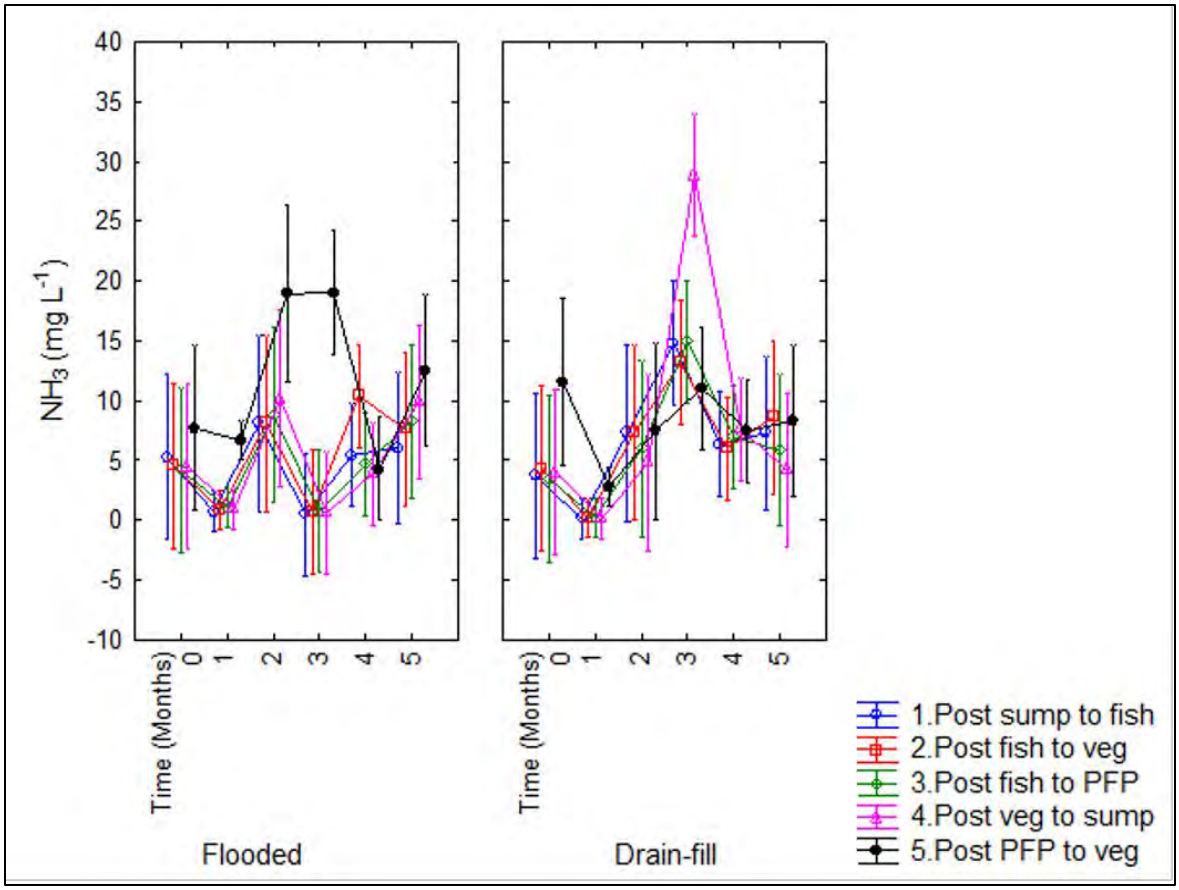

Figure 3.7: Mean (± 95% confidence interval) ammonia (NH_3, mg·L^{-1}) at the outlet of the sump (1 Post-sump), the outlet of the fish tank that drains to the vegetable grow-bed (2 Post-fish to veg), the outlet of the fish when solid waste was removed (3 Post-fish to PFP) the outlet of the vegetable grow bed (4 Post-veg to PFP) and the outlet of the primary facultative pond (5 Post-PFP) in the drain-fill and flooded aquaponic systems (RM-ANOVA $F_{(20,700)}$=2.66, p<0.001).

Nitrite

The concentration of NO_2^- was not influenced by the interaction between time, treatment and sampling points (RM-ANOVA $F_{(20,700)}$=0.19, p=1.00). Similarly, it was not influenced by the interaction between time and sample points during this period of this experiment (RM-ANOVA $F_{(20,700)}$=0.15, p=1.00). Nitrite concentration was also not influenced by the interaction between the treatment and the sampling point (RM-ANOVA $F_{(4,140)}$= 0.14, p=0.97). However, a significant interaction between time and treatment influenced the concentration of NO_3^- during this study (RM-ANOVA $F_{(5,700)}$= 4.97, p<0.001; Figure 3.8). The NO_2^- concentration was similar between treatments at the beginning of the experiment. It then decreased for the drain fill treatment at month one, showed a slight increase again at month two where it was significantly higher than that of the flooded treatment. Concentration in the flooded treatment was constant through the experiment (Figure 3.8).

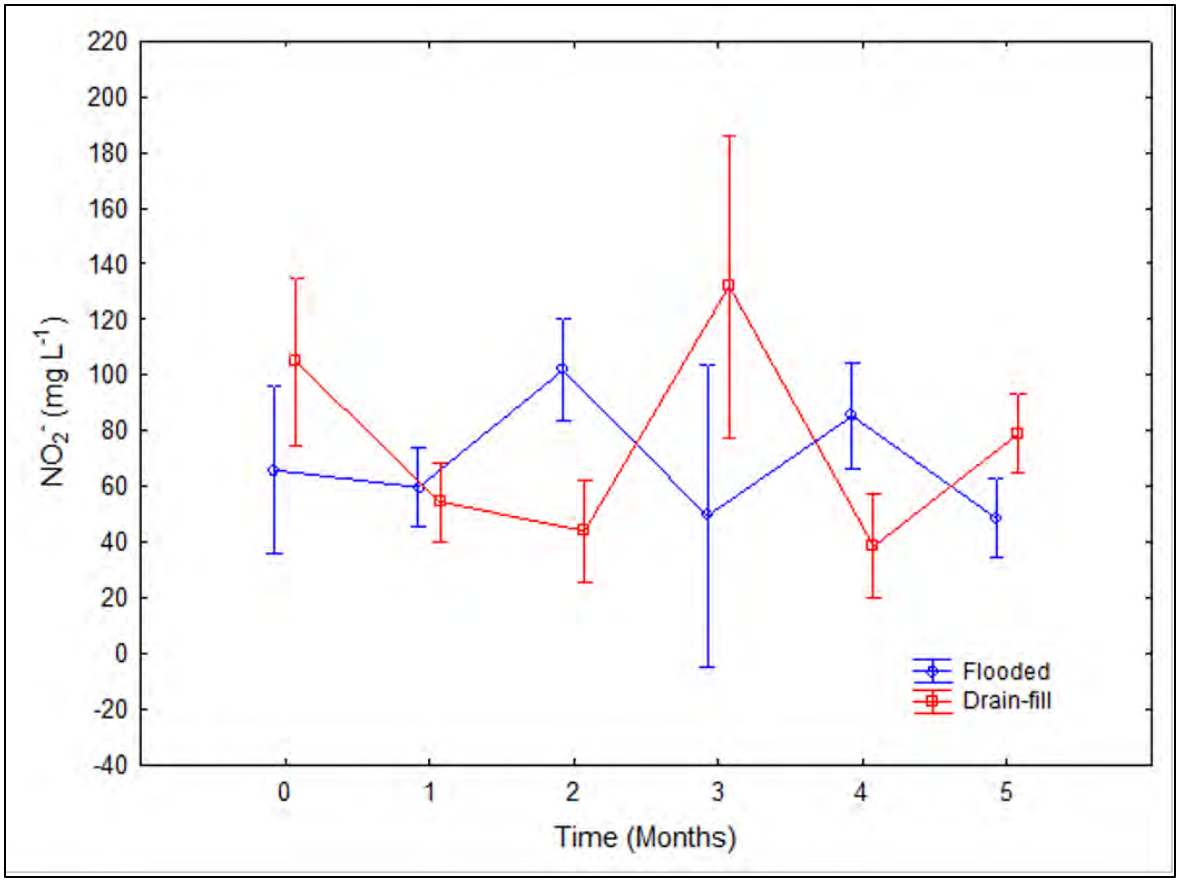

Figure 3.8: Comparison of mean (± 95% confidence interval) nitrite (NO_2^-, mg·L^{-1}) in the drain-fill and flooded aquaponic systems, with all sample periods combined (RM-ANOVA $F_{(5,700)}$=4.97, $p < 0.001$).

Nitrate

The concentration of NO_3^- was not influenced by the interaction between time, treatment and sampling points (RM-ANOVA $F_{(20,700)}$=0.79, p=0.72). Similarly, the NO_3^- was not influenced by the interaction between time and sampling points during the period of this experiment (RM-ANOVA $F_{(20,700)}$=0.78, p=0.74). The NO_3^- concentration was also not influenced by the interaction between the treatment and the sampling points (RM-ANOVA $F_{(4,140)}$=0.58, p=0.68). However, NO_3^- was significantly influenced by the interaction between time and treatment during this study (RM-ANOVA $F_{(5,700)}$=6.99, p<0.001; Figure 3.9). The NO_3^-concentration of the flooded treatment was significantly higher than that of the drain fill at months two and four, and the opposite was true for month five (Figure 3.9).

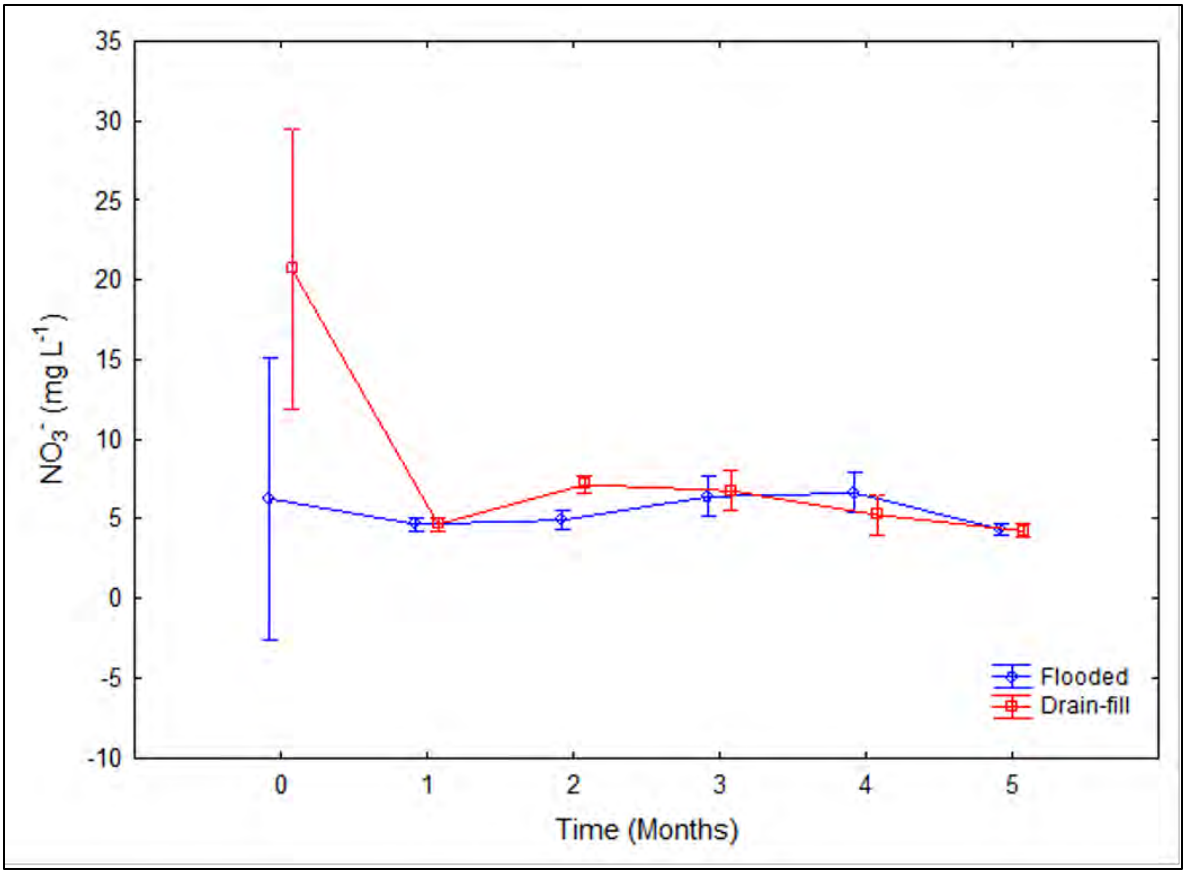

Figure 3.9: Comparison of mean (± 95% confidence interval) nitrate (NO_3^-, mg·L^{-1}) in the drain-fill and flooded aquaponic systems, with all sample periods combined (RM-ANOVA $F_{(5,700)}$=6.99, p<0.001).

Phosphates

The concentration of PO_4^{-3} was not influenced by the interaction between time, treatment and sampling points (RM-ANOVA $F_{(20,700)}$=0.81, p=0.70). Similarly, it was not influenced by the interaction between time and sampling points during this period of this experiment (RM-ANOVA $F_{(20,700)}$=1.43, p=0.10). The PO_4^{-3} was also not influenced by the interaction between the treatment and the sampling points (RM-ANOVA $F_{(4,140)}$=0.24, p=0.91). However, PO_4^{-3} was significantly influenced by the interaction between time and treatment during this study (RM-ANOVA $F_{(5,700)}$=6.61, p<0.001; Figure 3.10). The PO_4^{-3} remained constant from month 0 to month two in the two treatments. A sharp increase of the same rate was then observed in both the drain fill and flooded systems in from month two to three. At month four the phosphate concentrations in the drain-fill treatment were significantly higher than those in the flooded systems. After this, there was a significant decrease in the PO_4^{-3} concentration in the two treatments (Figure 3.10).

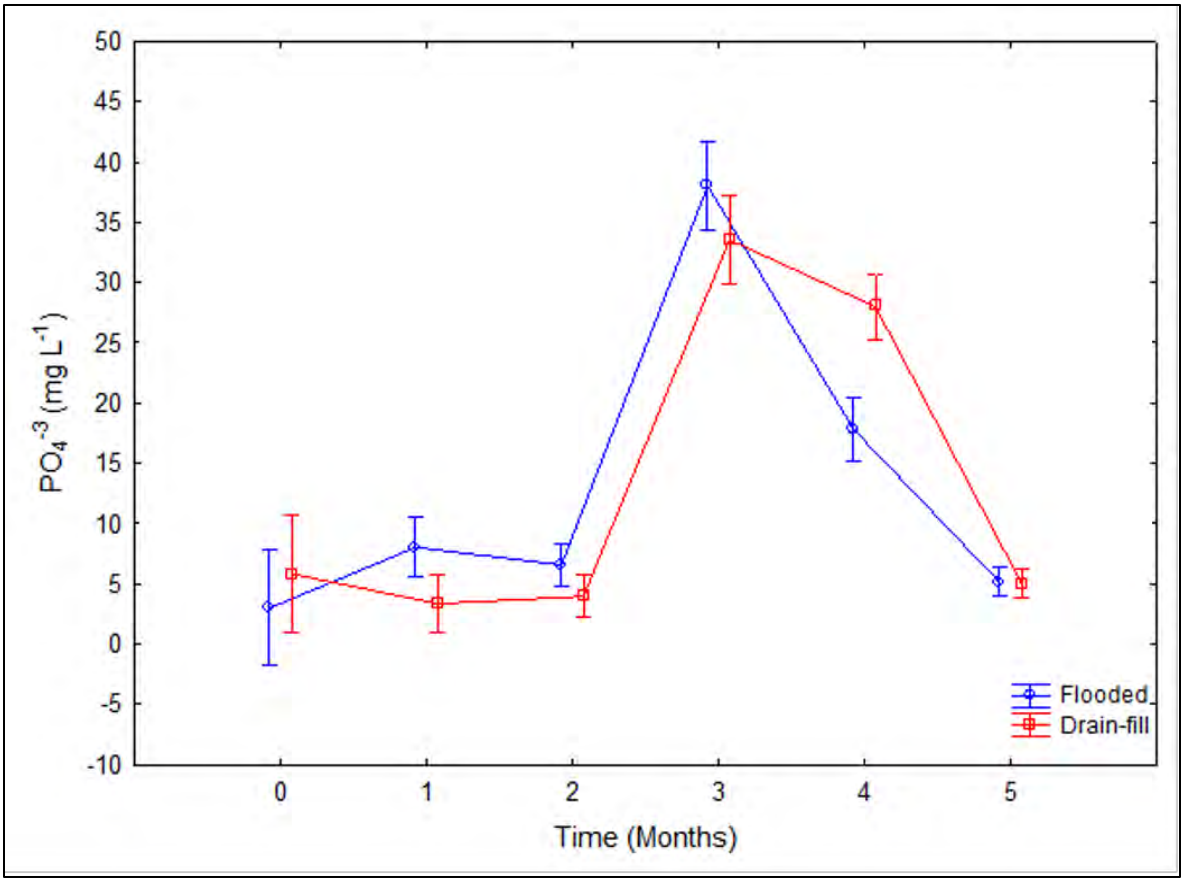

Figure 3.10: Comparison of mean (± 95% confidence interval) Phosphates (PO_4^{-3}, mg·L^{-1}) in the drain-fill and flooded aquaponic systems, with all sample periods combined (RM-ANOVA $F_{(5,700)}$=6.61, p<0.001).

Chemical oxygen demand

The COD was not influenced by the interaction between time, treatment and sampling points (RM-ANOVA $F_{(20,700)}$=1.30, p=0.17). Similarly, it was not influenced by the interaction between time and treatment during this period of this experiment (RM-ANOVA $F_{(5,700)}$=1.05, p=0.39). The COD was also not influenced by the interaction between the treatment and the sampling points (RM ANOVA $F_{(4,140)}$=1.70, p=0.15). However, COD was significantly influenced by the interaction between time and sampling points during this study, which appeared to be related to dissolved and solid waste after the fish tank (RM-ANOVA $F_{(20,700)}$=2.00, p=0.006; Figure 3.11). Sampling point five (post-fish tank effluent) had a significantly higher COD than all other sampling point at the start of the experiment. After the first month of the experiment, sampling point three (post-fish tank to PFP; i.e., effluent that contained solid fish waste) had a significantly higher COD than all other points. At month two, all sampling points had similar COD. From month three to month five, a similar trend was observed where COD in sampling point five (fish tank effluent) was significantly higher than point four (post-vegetable grow bed), but all other sampling point were similar (Figure 3.11).

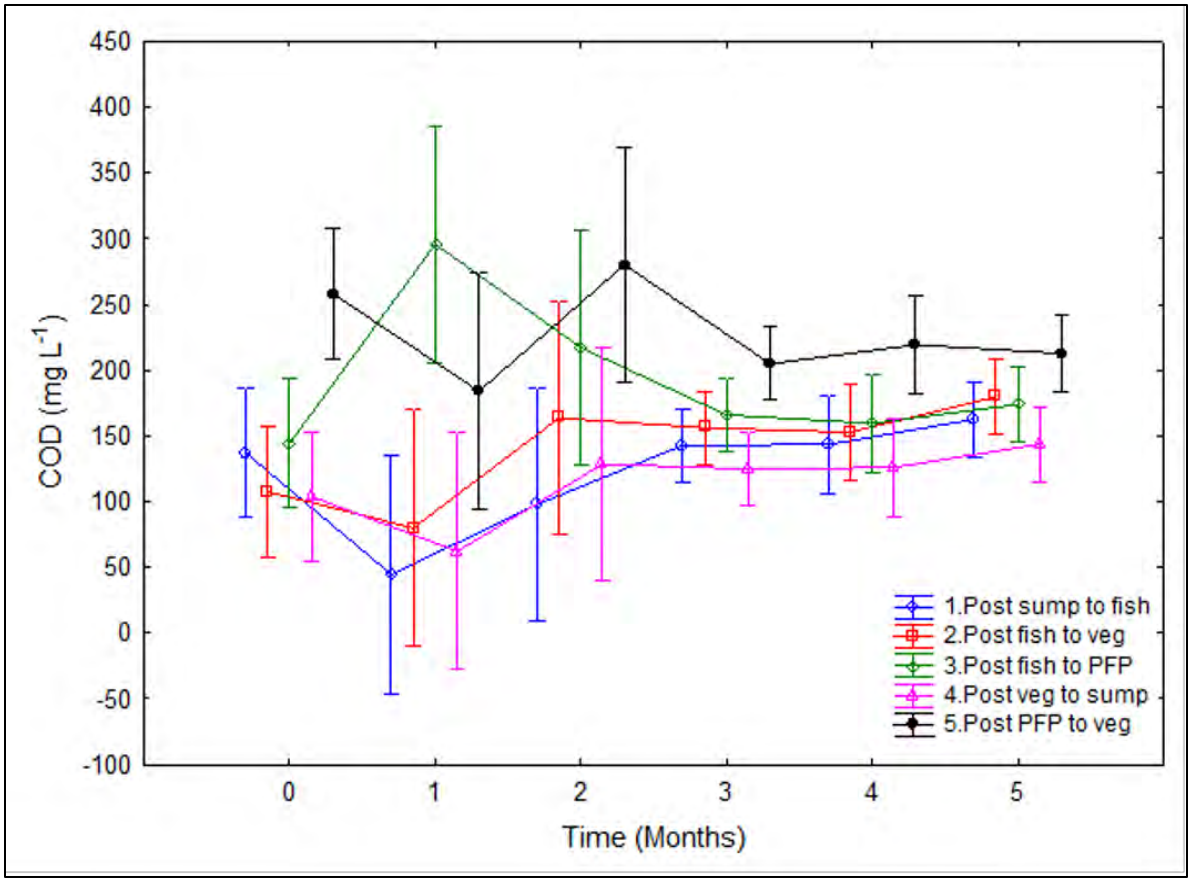

Figure 3.11: Mean (± 95% confidence interval) chemical oxygen demand (COD, mg·L^{-1}) at the outlet of the sump (1 Post-sump), the outlet of the fish tank that drains to the vegetable grow-bed (2 Post-fish to veg), the outlet of the fish when solid waste was removed (3 Post-fish to PFP) the outlet of the vegetable grow bed (4 Post-veg to sump) and the outlet of the primary facultative pond (5 Post-PFP) in the drain-fill and flooded aquaponic systems(RM-ANOVA $F_{(20,700)}$=2.00, p=0.006).

Solid waste

There were no significant differences in the mean solid waste collected in the drain-fill and flooded aquaponics systems (Mann-Whitney U-test=0.87, p=0.56; Table 3.1). The mean weight of the solid waste in the flooded treatment was 328.00±44.07 g and 393.66±76.90 g in the drain-fill (Table 3.1).

Table 3.1: Comparison of mean (± stand error) final dry weight for the solid waste in flooded verses drain-fill (two aquaponic system treatments) for five months (Mann-Whitney U-test, U=1.00, P=0.56).

Flooded vs Drain-fill		Means (g)	N	p-value
Final dry weight of sediments (g)	Flooded	328±44	3	0.56
	Drain-fill	394±77	3	

3.3.2. Results - Fish production

During this research, juvenile African catfish were introduced to see their growth in this aquaponics system. Before the end of the experiment, a harvest was made to assess the scale of fish production in these aquaponics systems. This fish production allowed comparison and designated which of these different types of fish farming was performing best. The fish catch in each breeding tank was determined in terms of weight in kg and was oriented to human consumption and other uses in the department's fish ponds.

Individual fish growth

There was a significant difference between the initial weights of African catfish stocked in drain-fill and flooded treatments (Student's t-test=-2.98, df=4, p=0.04, Table 3.2). The mean starting weight of the fish in the flooded treatment was higher than that in the drain-fill, at 33.90±1.35 g and 38.03±1.91 g in the drain-fill and flooded systems respectively (Table 3.2). The final weights of the fish in the two treatments were also significantly different (Student's t-test=6.00, df=4, p=0.003, Table 3.2). The mean final weight of the fish in the drain-fill (245.36±7.48 g) was higher than the fish in the flooded treatment (177.18±5.90 g). Fish in the drain-fill systems were, on average, about 37.6% heavier at the end of the trial, compared to those produced in the flooded systems (Table 3.2). On the other hand, there was no significant difference between the starting lengths of the fish stocked in the drain-fill and the flooded treatments (Student's t-test=-2.43, df=4, p=0.07, Table 3.2). According to Table 3.2 there was a significant difference between final lengths of the fish raised in drain-fill and flooded treatments (Student's t-test=2.74, df=4, p=0.05, Table 3.2). The fish had an average length of 162±1 mm in the drain-fill and 166±3 mm in flooded systems. The initial and final CF were also similar for the catfish in the both systems (Student's t-test=-1.22, df=4, p=0.28 and t=-1.41, df=4, p=0.22, respectively, Table 3.2).

There was a significant difference between SGR of catfish in drain-fill and flooded treatments (Student's t-test=4.83, df=4, p=0.008, Table 3.2), where the fish in drain-fill had a higher SGR (1.41±0.03 % bw d^{-1}) than the fish in flooded system (1.09±0.01 % bw d^{-1}). The FCR of the fish in the flooded system was significantly higher at 1.67±0.18, than that in the drain fill treatment at 1.06±0.08 (Student's t-test=-3.09, df=4, p=0.04, Table 3.2).

Table 3.2: Mean (± stand error) weight, length, condition factor, specific growth rate (SGR) and feed conversion ratio (FCR) of African catfish grown in drain-fill and flooded (two aquaponic system treatments) for five months (Student's t-test, $p<0.05$).

	Drain-fill	Flooded	t value	df	P value
Initial weight (g)	34±1	38±2	-2.98	4	0.04
Final weight (g)	245±7	177±18	6	4	0.003
Initial length (mm)	162±1	166±3	-2.43	4	0.07
Final length (mm)	308±8	235±45	2.74	4	0.05
Initial condition factor	0.78±0.02	0.80±0.01	-1.22	4	0.28
Final condition factor	0.81±0.01	0.87±0.08	-1.41	4	0.22
Specific growth rate (SGR) (%bw day^{-1})	1.41±0.03	1.09±0.1	4.83	4	0.008
Feed conversion ratio (FCR)	1.06±0.08	1.67±0.18	-3.09	4	0.04

Degrees of freedom (df)

The fish weight was affected by an interaction between time and the treatments drain-fill and flooded (RM-ANOVA $F_{(5, 20)}=7.17$, $p=0.0005$, Figure 3.12)**.** Drain fill system had a mean fish length that was higher than that in the flooded systems. In the flooded treatment, the fish weight was low in December, increased through to March, and then remained constant from March to May (Figure 3.12**)**. In the drain-fill, the fish weight followed the same trend from December to March, and it also levelled off in the last two months, only it levelled off with a significantly higher final weight (Figure 3.12).

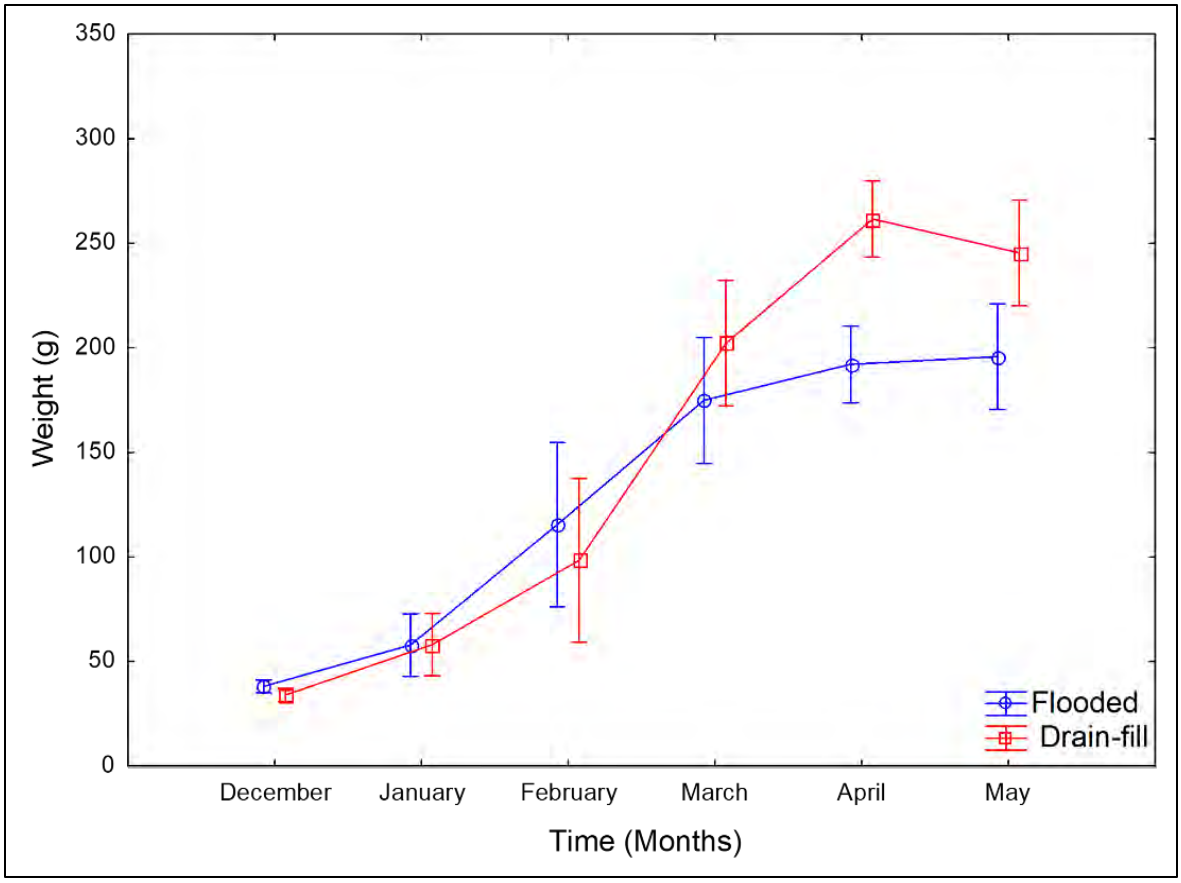

Figure 3.12: Comparison means (± standard error) individual fish weight over five months produced in flooded and drain-fill aquaponics systems (RM-ANOVA $F_{(5,20)}=7.17$, $p=0.0005$).

The fish length was not affected by an interaction between time and treatments drain-fill and flooded (RM-ANOVA $F_{(5,20)}=1.73$, $p=0.17$, Figure 3.13). However, the fish length was significantly influenced by time (RM-ANOVA $F_{(5,20)}=50.40$, $p<0.0001$). Over time, the length of African catfish increased, and this was similar between these two treatments. From the beginning of the experiment (December), it increased until the end of the experiment (May) and in drain fill system mean fish lengths was higher than flooded (Figure 3.13).

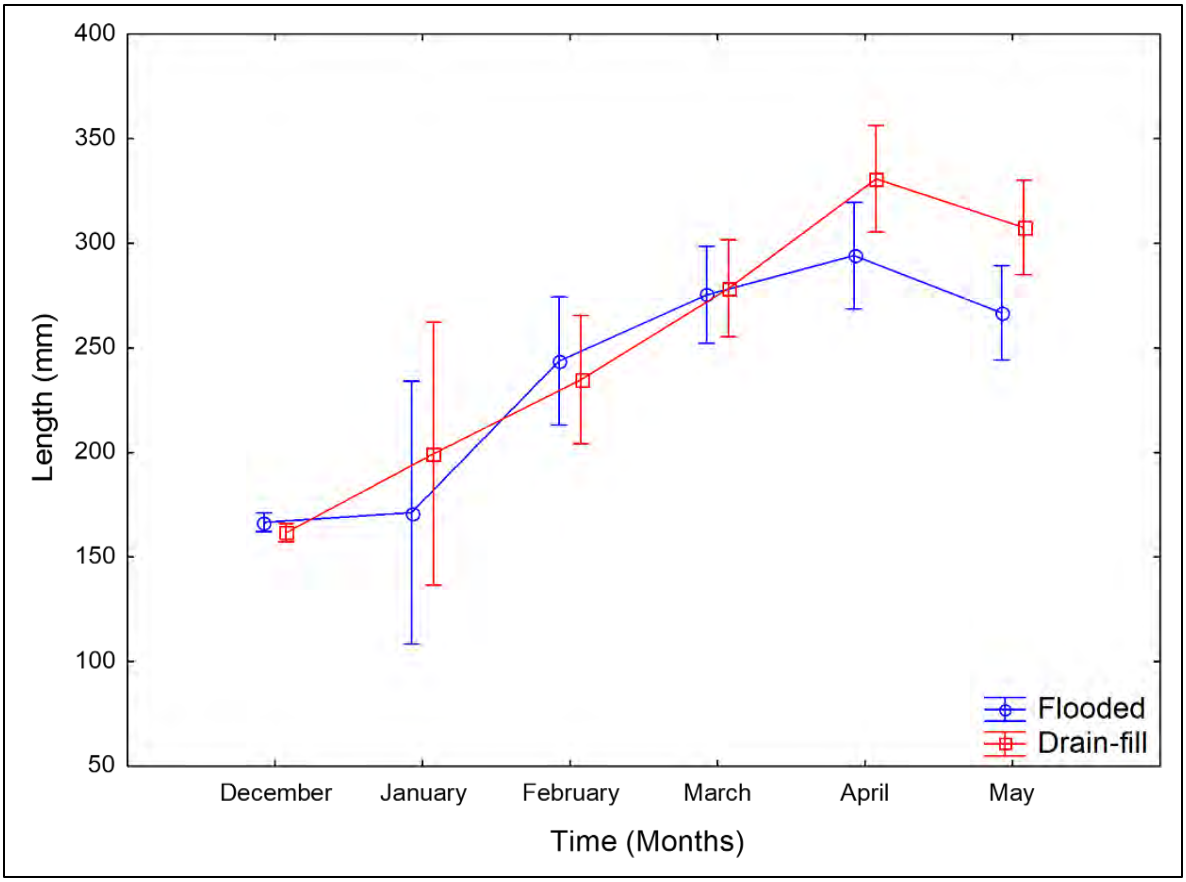

Figure 3.13: Comparison of means (± standard error) individual fish length over five months produced in flooded and drain-fill aquaponics systems (RM-ANOVA $F_{(5,20)}=1.73$, p=0.17).

Fish survival and mortality

The drain-fill treatment had a final fish survival rate of 88.33%, while the African catfish reared in the flooded treatment had a survival rate of 80.83%. A total of 37 fish died in this experiment; 23 in the flooded systems and 14 in the drain-fill systems; however, the distribution of fish that died in the two systems was not significantly different from a distribution that assumed an equal number of deaths in both systems (X^2=2.19, p=0.139, Table 3.3). It should be noted that morality was checked daily. It should also be noted that fish were removed from the systems to balance and maintain stocking density consistency, and these harvests were not included in the morality recorded here.

Table 3.3: Survival and mortality of African catfish reared in flooded and drain-fill treatments.

	Flooded	Drain-fill	Chi-square	p-value
Total number stocked	120	120		
Total number that died	23	14	2.19	0.139
Survival (%)	80.83	88.33		

Fish biomass

Initial total biomass was similar in both treatments at the start of the trial (Student's t-test = -2.35, p = 0.08, Table 3.4). There was a 333% and a 570% increase in biomass over the course of the five-month trial, in the flooded and drain-fill systems, respectively. Final mean biomass and biomass increase was significantly more for African catfish stocked in the drain-fill compared with the flooded treatment (Student's test-t=3.26, df=4, p=0.03, Table 3.4).

Table 3.4: Mean (± standard error) African catfish biomass (grams) in flooded and drain-fill systems at the start of the trial (initial biomass) the total biomass at the end of the trail including all of the fish that were left and all that were harvested during the trial (final biomass), and the biomass increase over the five-month trial (Biomass increase = Final weight less Initial weight).

	Flooded (g)	Drain-fill (g)	t-value	Df	p-value
Initial biomass (g)	1512±47	1387±30	-2.35	4	0.08
Final biomass (g)	6552±583	9302±608	3.26	4	0.03
Biomass increase (g)	5039±626	7915±617	3.26	4	0.03

Degrees of freedom (df)

3.3.3. Results - Plant production

The chlorophyll-a concentration was affected by an interaction between time and the treatments drain-fill and flooded (RM-ANOVA $F_{(5,20)}$=12.35, p=0.00001, Figure 3.14). The chlorophyll-a concentration increased between December and February in the drain-fill. It then remained constant between February to April. This was followed by a slight increase in May (Figure 3.14).

In the flooded system, chlorophyll-a was similar from December to February. In March, it was similar to the concentration in February but higher than in the first two months. It was then constant between April and May. Over the course of the trial both treatments followed a similar trend, whereas in the last two months, the chlorophyll-a concentrations in the drain-fill systems were significantly higher than those in the flooded treatments (Figure 3.14).

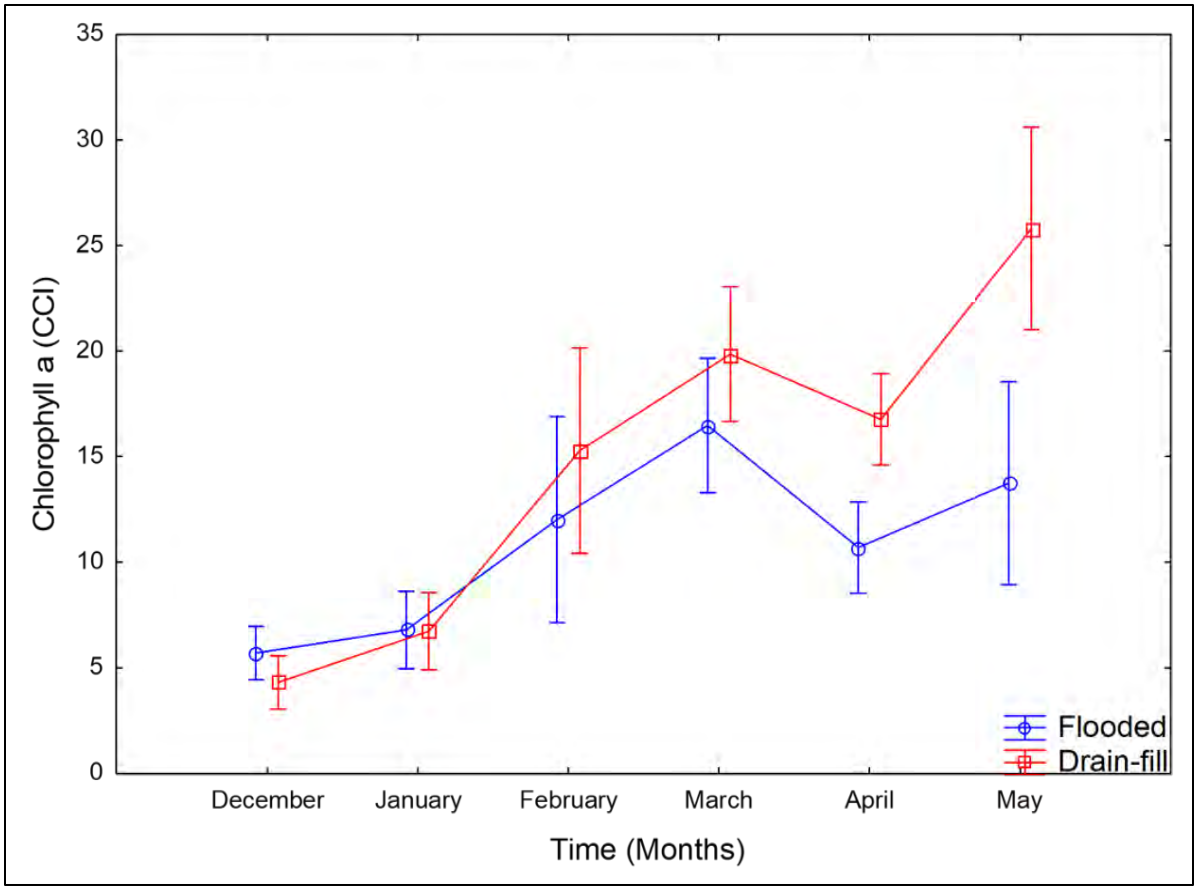

Figure 3.14: Comparison of mean (± standard error) chlorophyll-a concentration index (CCI) by month in the flooded and drain-fill treatments (RM-ANOVA $F_{(5, 20)}$=12.35, p=0.00001).

The leaf length of the plants was affected by an interaction between time and treatments drain-fill and flooded (RM-ANOVA $F_{(5, 20)}$=9.71, p=0.00008, Figure 3.15). It was similar between treatments in the first two months of the study (Figure 3.15). In the drain-fill treatment, leaf length increased from January to February, and then remained constant for the rest of the experiment (Figure 3.15). The flooded treatment maintained consistent growth rates for entire experimental period and was lower than the drain-fill in February, March and May (Figure 3.15).

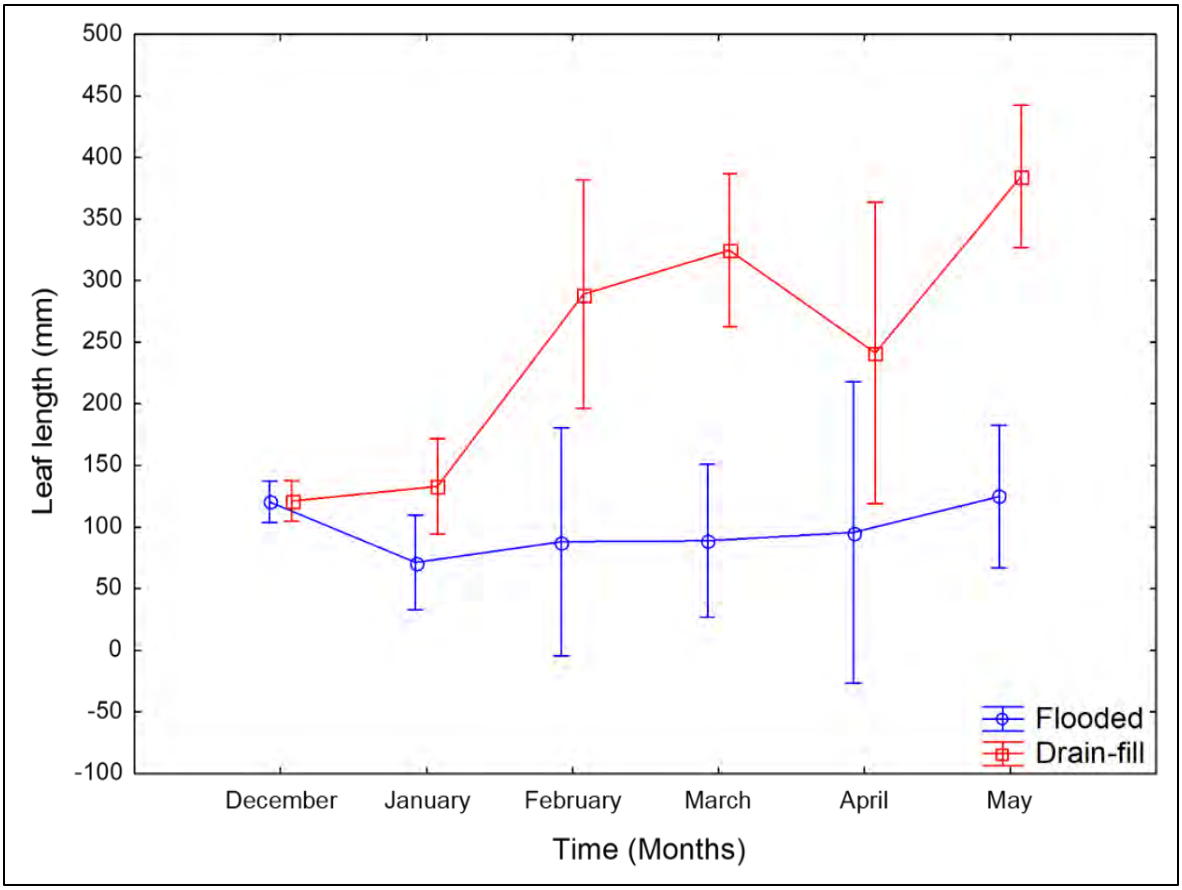

Figure 3.15: Comparison of means (± standard error), of the leaf's length of plant by month in the Flooded vs drain-fill treatments (RM-ANOVA $F_{(5,20)}$=9.71, p=0.00008**).**

Leaf width was affected by an interaction between time and the treatments (RM-ANOVA $F_{(5,20)}$=32.51, p<0.0001, Figure 3.16). It was similar between treatments in the first two months. The drain-fill treatment then increased from February to March, where it was significantly higher than the flooded (Figure 3.16). The treatments were then again similar in April, and once again the drain-fill increased and was significantly higher than the flooded in the final month (Figure 3.16).

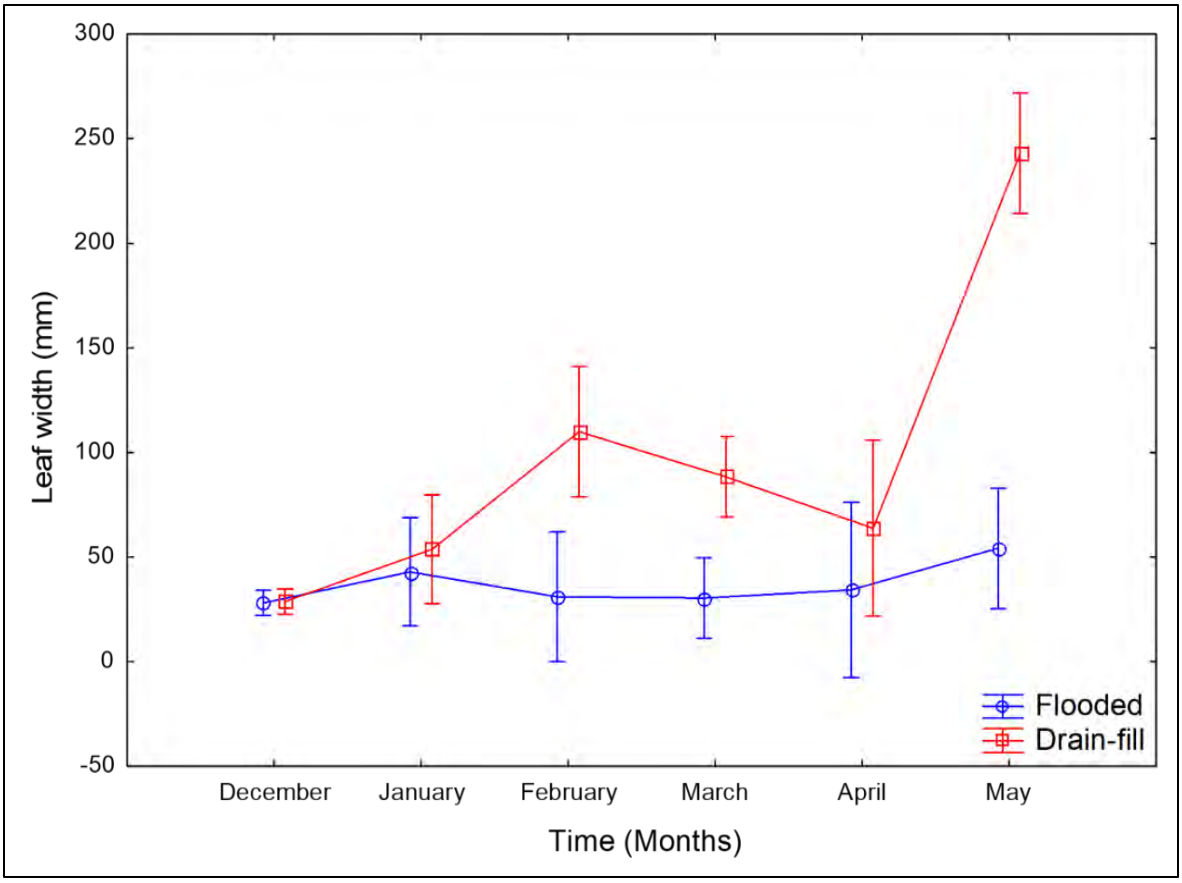

Figure 3.16: Comparison of means (means ± standard error), of the leaf's width of plant by month in the flooded vs drain-fill treatments (RM-ANOVA $F_{(5,20)}=32.51$, $p<0.0001$**).**

Swiss chard stem length was affected by an interaction between time and the treatments drain-fill and flooded (RM-ANOVA $F_{(5,20)}=3.87$, $p=0.01$, Figure 3.17). In the drain-fill, stem length increased progressively from the beginning to the end of the experiments (Figure 3.17). Unlike in the flooded, the length of the stems increased in length from December to February, then remained constant until the end of the experiment (Figure 3.17). In the last two months of the study, the flooded treatment was significantly lower than the drain-fill (Figure 3.17).

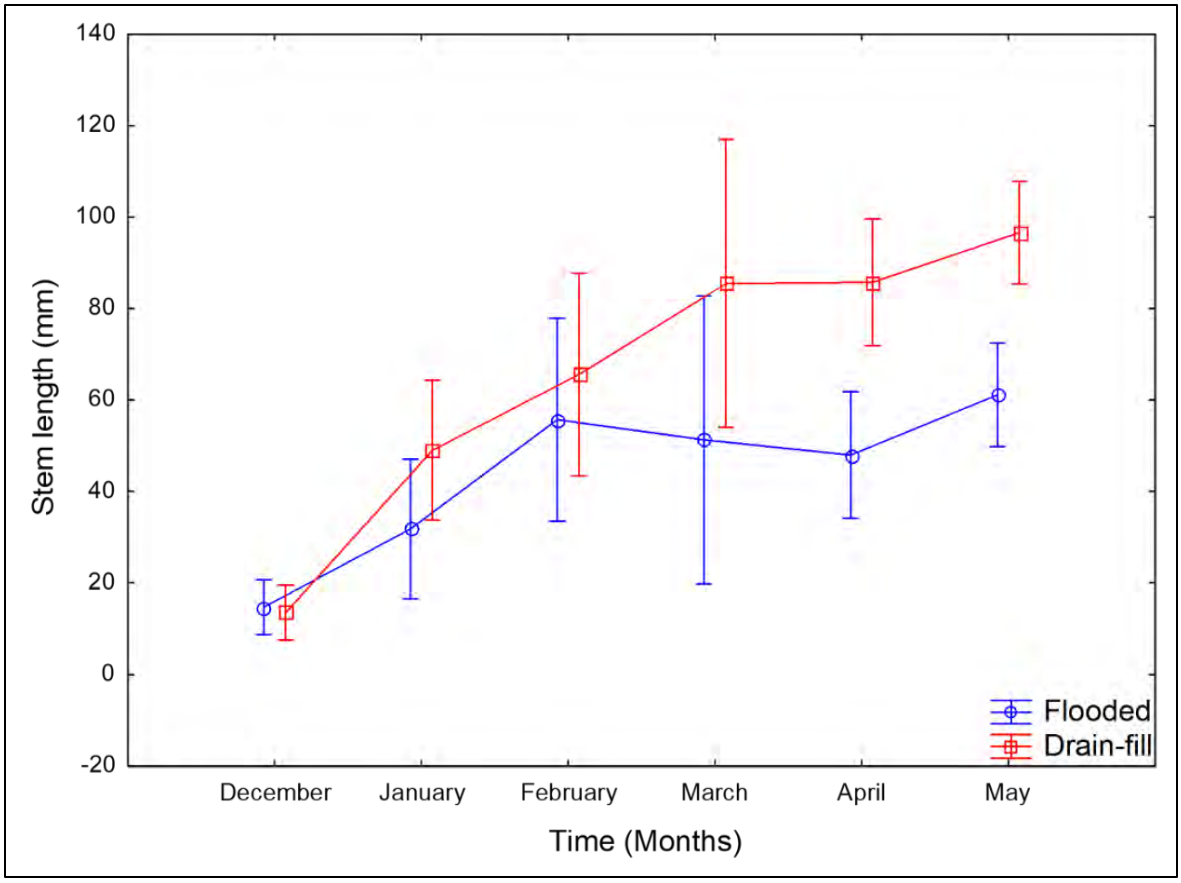

Figure 3.17: Comparison of means (± standard error), plot of the stem length of plant by month in the flooded vs drain-fill treatment (RM-ANOVA $F_{(5,20)}$=3.87, p=0.01).

Plant biomass

The wet plant biomass was significantly different between the flooded and drain-fill treatments (Student's t-test=-5.33, n=4, p=0.005, Table 3.5). The dry biomass of Swiss chard in the flooded treatment were significantly lower (104.06±7.07 g) than that of Swiss chard in the drain-fill treatments (1797±210.60 g; Student's t-test=-6.95, n=4, p=0.002, Table 3.5). There was also a significant difference between the stem biomass of the Swiss chard in drain-fill and flooded treatments (Student's t-test=-361, n=4, p=0.02, Table 3.5). Similarly, there was a significant difference between the root biomass in the drain-fill and the flooded treatments Student's t-test=-3.01, n=4, p=0.03, Table 3.5).

Table 3.5: Mean (± standard error) Swiss chard biomass (grams) in flooded and drain-fill treatments, the total biomass at the end of the trail including all of the plants that were harvested during the trial (wet biomass), and the dry biomass over the five-month trial, Stem and root biomass are both dry weights (Total wet biomass was the sum of wet leaf, wet stem and wet root biomass).

	Flooded (g)	Drain-fill (g)	t value	df*	p value
Dry leaf biomass (g)	104±7	1798±211	-6.95	4	0.002
Wet leaf biomass(g)	689±90	175210±2732	-5.33	4	0.005
Wet stem biomass (g)	57±14	713±156	-3.61	4	0.02
Wet root biomass (g)	59±14	707±371	-3.01	4	0.03
Wet total biomass (g)	**800±118**	**18950±3260**	**-2.11**	**4**	**0.004**

*Degrees of freedom (df)

Chapter 4. Experiment 2: Dealing with solids using primary facultative ponds

4.1. Introduction

Aquaponic production requires good water quality and the proper functioning of the system (Moore *et al.*, 1949; Maucieri *et al.*, 2018). The buildup of solid waste in an aquaponics system, if not removed, will compromise fish and plant production (Palm *et al.*, 2018), so the elimination of this solid waste is an essential process, which must be addressed (El Bouhy *et al.*, 2006; Mongirdas and Kusta, 2006).

This waste not only increases the risk of parasites, disease, and gill problems for fish, but it also increases ammonia and ammonium in the water, and it decreases oxygen concentration due to biological oxygen demand (BOD), and waste products also reduced the efficiency of the functioning of the mechanical filter (Lockhart *et al.*, 1955; Von Sperling and de Lemos, 2005). Waste products can also influence the hydroponic bed by clogging it, and this in turn could compromise heterotrophic bacterial function (Genello *et al.*, 2015; Palm *et al.*, 2018). This clogging can lead to the release of hydrogen sulphide which is a gas and can be toxic to fish and nitrifying bacteria (Thorarinsdottir *et al.*, 2015). Therefore, it is important to eliminate solid waste by mechanical filtration in an aquaponic system (Coche, 2014). This will reduce the solid waste content and retention time of solids in the system; thus, reducing the risk of oxygen consumption by microorganisms (Moore *et al.*, 1951; Antonio *et al.*, 2013). If the solid waste is maintained in the settling tank, an anaerobic layer forms at the bottom and the this allows for the mineralization of nutrients in the layer of settled solid waste (Hargreaves *et al.*, 2005; Thorarinsdottir *et al.*, 2015), and these nutrients may then be used to improve growth of vegetables in the aquaponics system (Khandaker and Kotzen, 2018). This system is called a primary facultative pond (PFP) (Jones, 2002).

The solid wastes, which are removed as sludge from aquaponics system, are rich in nutrients. This sludge has the potential to act as a nutrient source in agriculture and in aquaponic systems (Turcios and Papenbrock, 2014). Thus, fish farm solid waste can be used as natural organic fertilizers by farmers in their fields (Rupasinghe and Kennedy, 2010). Settling tanks, where solid waste is allowed to settle out of suspension, is one way of removing solids in aquaponics and is an alternative (or supplemental) to mechanical filtration (Mancuso, 2013; Shava and Gunhidzirai, 2017).

Hypotheses

- A higher fish production would be expected from the PFP as opposed to the no-PFP (H1),
- More vegetable production would be expected from the PFP as opposed to the no-PFP (H2),
- The addition of more mineralised nitrogen would be available for plant production in the treatments with a PFP (more NO_3^- would be avaible in the PFP compared systems where the solid waste is removed (i.e. he no-PFP) (H3).

The no-PFP aquaponics design is expected:

1) to grant only partial elimination of solid waste by mechanical filter;
2) to be impeded by the shortage of biological treatment of solid waste remaining in the hydroponic bed, subsequently, poor in nutrients, thus less vegetable production and less fish production due to residual toxicity remaining in the hydroponic; and
3) to yield reduced water quality due to residual ammonia from unused feed and excreta.

These hypotheses were backed by the expected advantages in the PFP versus the no-PFP aquaponics design. That is, the PFP aquaponics design was expected to:

1) reduce toxicity by transforming ammonia to nitrate by the bacteria in the PFP;
2) improve fish and plant growth due to reduced toxicity and boosted nutrients released by solids treatment; and to
3) improve water quality due to solid waste and fish excreta treatment in the PFP.

The no-PFP aquaponic design was expected to result in:

1) lower nitrogen (ammonia to nitrite to nitrate) available for plant growth; and
2) lower vegetable production due to less nutrient availability.

4.2. Materials and methods

This chapter used the materials and method already presented in Chapter 2 (Sections 2.1, 2.2, 2.3. 2.4, 2.5, 2.7, 2.8 and 2.9). In this second experiment, two treatments were used: (1) aquaponics systems where solids were maintained in the systems with the inclusion of a PFP, and (2) aquaponics systems where solids were removed from the system (no-PFP; control). Both these treatments were represented in triplicate (i.e. there were six systems in total). Note

that both these treatments included a drain-fill vegetable grow bed, so the PFP treatment here was the same treatment that was referred to as the "drain-fill" treatment in Chapter 3. That is, this treatment was shared between both experiments, which was necessary since there were only nine systems available for this research project. This study duration was five months and it ran concurrently with the experiment presented in Chapter 3.

4.3. Results

4.3.1. Results - Water quality

Water quality

Dissolved oxygen

The DO concentration was not influenced by the interaction between time, treatment and sampling point (RM-ANOVA $F_{(20,700)}=0.99$, $p=0.48$). It was also not influenced by any interaction between time and sampling point (RM-ANOVA $F_{(20,700)}=1.00$, $p=0.46$), time and treatment (RM-ANOVA $F_{(5,700)}=0.93$, $p=0.46$) or treatment and sampling point (RM-ANOVA $F_{(4,140)}=0.97$, $p=0.43$). The DO ranged between a mean of 8.59 $mg \cdot L^{-1}$ in the no-PFP and 5.93 $mg \cdot L^{-1}$ in the PFP throughout the duration of the experiment.

Temperature (ºC)

Temperature was not influenced by the interaction between time, treatment and sampling points (RM-ANOVA $F_{(20,700)}=0.11$, $p=1.00$). Similarly, temperature was not influenced by the interaction between time and sampling point during the entire period of this experiment (RM-ANOVA $F_{(20,700)}=0.05$, $p=1.00$). It was also not influenced by the interaction between the treatment and the sampling points (RM-ANOVA $F_{(4,140)}=0.36$, $p=0.84$). However, temperature was significantly influenced by the interaction between time and treatment during this study (RM-ANOVA $F_{(5,700)}=16.29$, $p<0.001$; Figure 4.1). In the first month of the experiment, the PFP treatment had a significantly higher temperature than the no-PFP. The temperature in both systems decreased in the first and second months. From month three to five, the no-PFP treatment had a significantly higher temperature than the PFP (Figure 4.1).

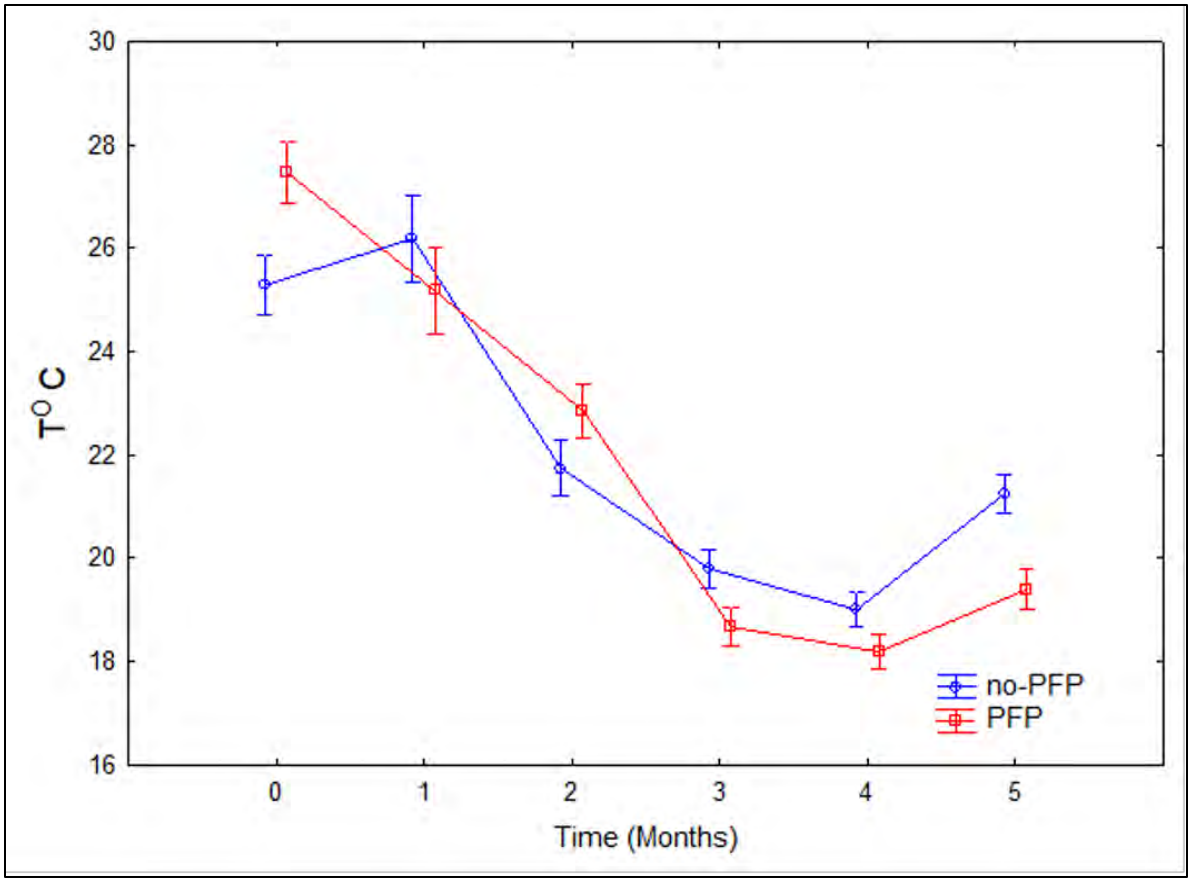

Figure 4. 1: Comparison of mean (± 95% confidence interval) temperature (TºC) in the no primary facultative ponds and primary facultative ponds (PFP) aquaponic systems, with all sample periods combined (RM-ANOVA $F_{(5,700)}$=16.29, p<0.001).

Hydrogen potential

The pH was not influenced by the interaction between time, treatment and sampling point (RM-ANOVA $F_{(20,700)}$=0.75, p=0.76). It was also not influenced by interactions between time and sampling point (RM-ANOVA $F_{(20,700)}$=0.75 p=0.78), time and treatment (RM-ANOVA $F_{(5,700)}$=2.01, p=0.74) or treatment and sampling point (RM-ANOVA $F_{(4,140)}$=0.74, p=0.57). The mean pH ranged between 11.11 in the no-PFP and 7.47 in the PFP throughout the duration of the experiment.

Electrical conductivity

The EC was not influenced by the interaction between time, treatment and sampling points (RM-ANOVA $F_{(20,700)}$=0.05, p=1.00). Similarly, the EC was not influenced by the interaction between time and sampling points during the period of this experiment (RM-ANOVA $F_{(20,700)}$=0.05, p=1.00). The EC was also not influenced by the interaction between treatment and sampling point (RM-ANOVA $F_{(4,140)}$=0.36, p=0.84). However, EC was influenced by a

significant interaction between time and treatment during this study (RM-ANOVA $F_{(5,700)}=15.29$, $p<0.001$; Figure 4.2). The EC of both treatments was similar at the start and after the first month of the experiment. An increase in EC was observed after month two, with the PFP treatment having a higher increase than the no-PFP. In month-three, the EC in both treatments decreased, however the PFP treatment still had a significantly higher EC. For the last two months of the experiment, the EC in the PFP treatment had a further decrease while that in the no-PFP increased and was significantly higher (Figure 4.2).

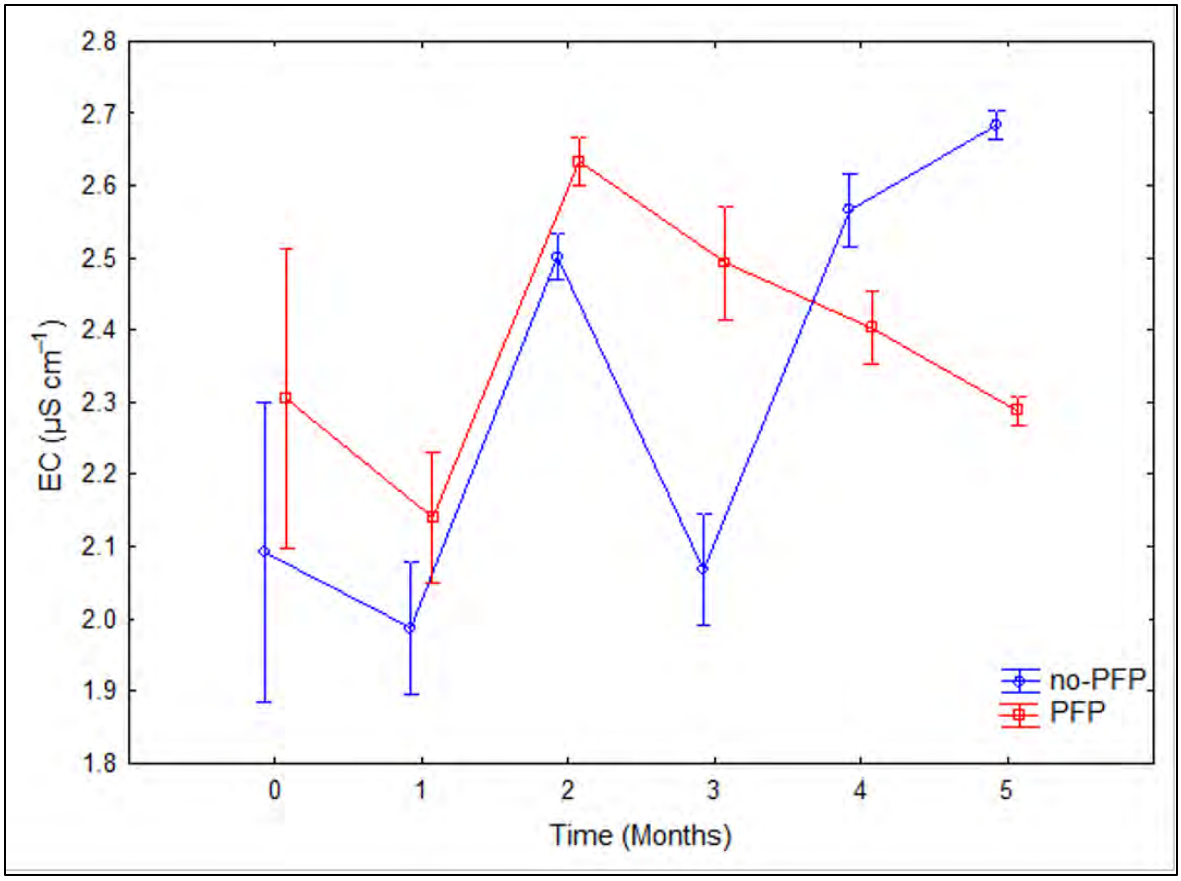

Figure 4. 2: Comparison of mean (± 95% confidence interval) electric conductivity (EC, $\mu S \cdot cm^{-1}$) in the no primary facultative ponds and primary facultative ponds (PFP) aquaponic systems, with all sample periods combined (RM-ANOVA $F_{(5,700)}=15.29$, $p<0.001$).

Salinity

The salinity was not influenced by the interaction between time, treatment and sampling points (RM-ANOVA $F_{(20,700)}=0.00$, $p=1.00$). Similarly, the salinity was not influenced by the interaction between time and sampling points during the period of this experiment (RM-ANOVA $F_{(20,700)}=0.11$, $p=1.00$). The salinity was also not influenced by the interaction between the treatment and the sampling points (RM-ANOVA $F_{(4,140)}= 0.12$, $p=0.97$). However, salinity

was significantly influenced by the interaction between time and treatment during this study (RM-ANOVA $F_{(5,700)}=6.18$, $p<0.001$; Figure 4.3). The salinity in both the no-PFP and PFP treatments remained constant from the beginning of the experiment to month one. It then increased from month one to month two, after which PFP had a significantly higher salinity until the end of the experiment (Figure 4.3).

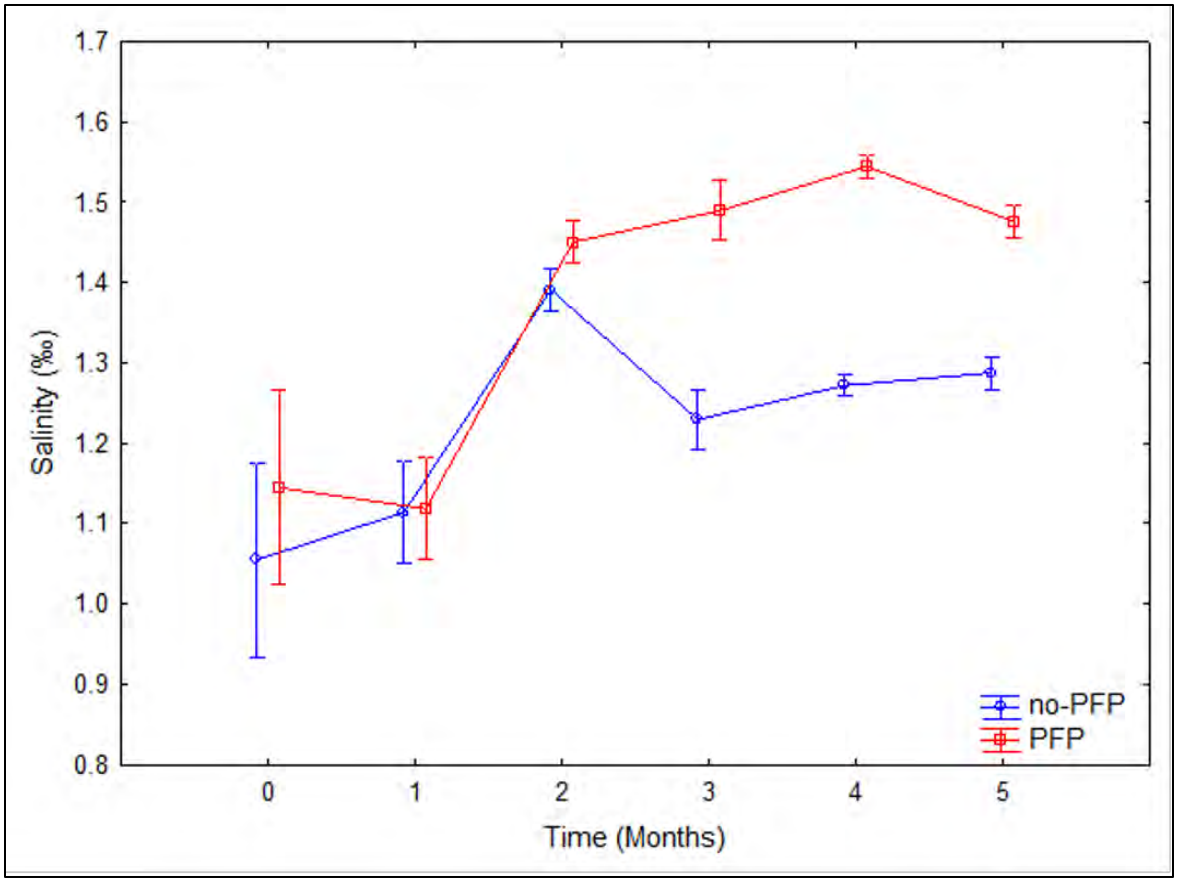

Figure 4. 3: Comparison of mean (± 95% confidence interval) salinity (‰) in the no primary facultative ponds and primary facultative ponds (PFP) aquaponic systems, with all sample periods combined (RM-ANOVA $F_{(5,700)}=6.18$, $p<0.001$).

Total dissolved solids

The TDS was significantly influenced by the interaction between time, treatment and sampling points during this study (RM-ANOVA $F_{(20,700)}=2.00$, $p=0.006$; Figure 4.4). The no-PFP treatment had higher TDS at sampling point five in month one, sampling point three; month two at sampling point three. It once more had a higher concentration of TDS than the PFP in month three, sampling point four. This concentration was however significantly lower than that of the PFP treatment at sampling point five (Figure 4.4).

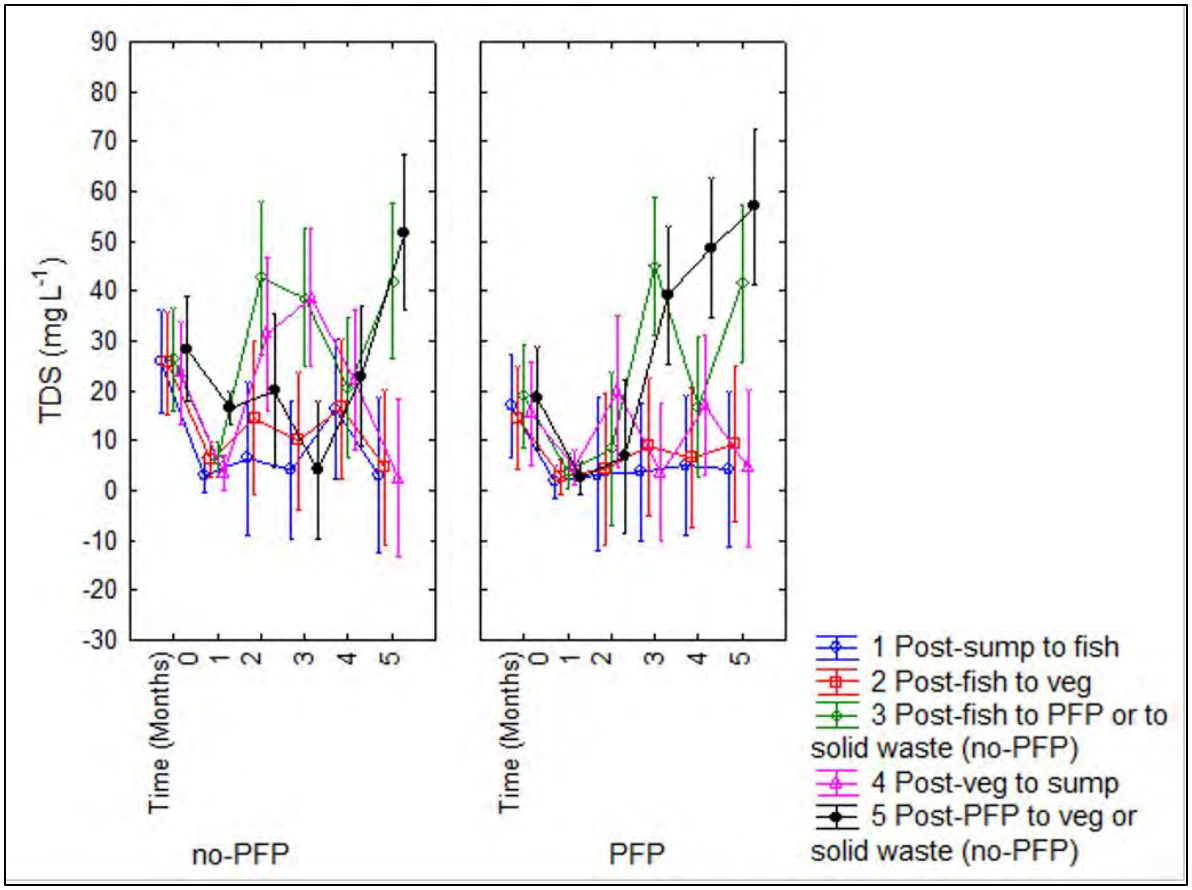

Figure 4. 4: Mean (± 95% confidence interval) total dissolved solids (TDS, mg·L^{-1}) at the outlet of the sump (1 Post-sump to fish), the outlet of the fish tank that drains to the vegetable grow-bed (2 Post-fish to veg), the outlet of the fish when solid waste was removed (3 Post-fish to primary facultative ponds or to solid waste) the outlet of the vegetable grow bed (4 Post-veg sump) and the outlet of the primary facultative pond (PFP) (5 Post- primary facultative ponds to veg) in the no primary facultative ponds and primary facultative ponds aquaponic systems (RM-ANOVA $F_{(20,700)}$=2.00, p=0.006).

Ammonia

The NH_3 was significantly influenced by the interaction between time, treatment and sampling points during this study (RM-ANOVA $F_{(20,700)}$=2.98, p<0.001; Figure 4.5). Ammonia concentration was equal between the no-PFP and PFP treatments, across sampling points at the start of the experiment. After the first month, the no-PFP treatment had a significantly higher concentration of NH_3 at sampling point five. The concentration of NH_3 was once again similar between the two treatments, across the five sampling points for months two and three. At month four, the PFP treatment had a higher NH_3 at sampling point three. It also had higher concentrations of NH_3 at sampling points one to four, in the final month of the experiment (Figure 4.5).

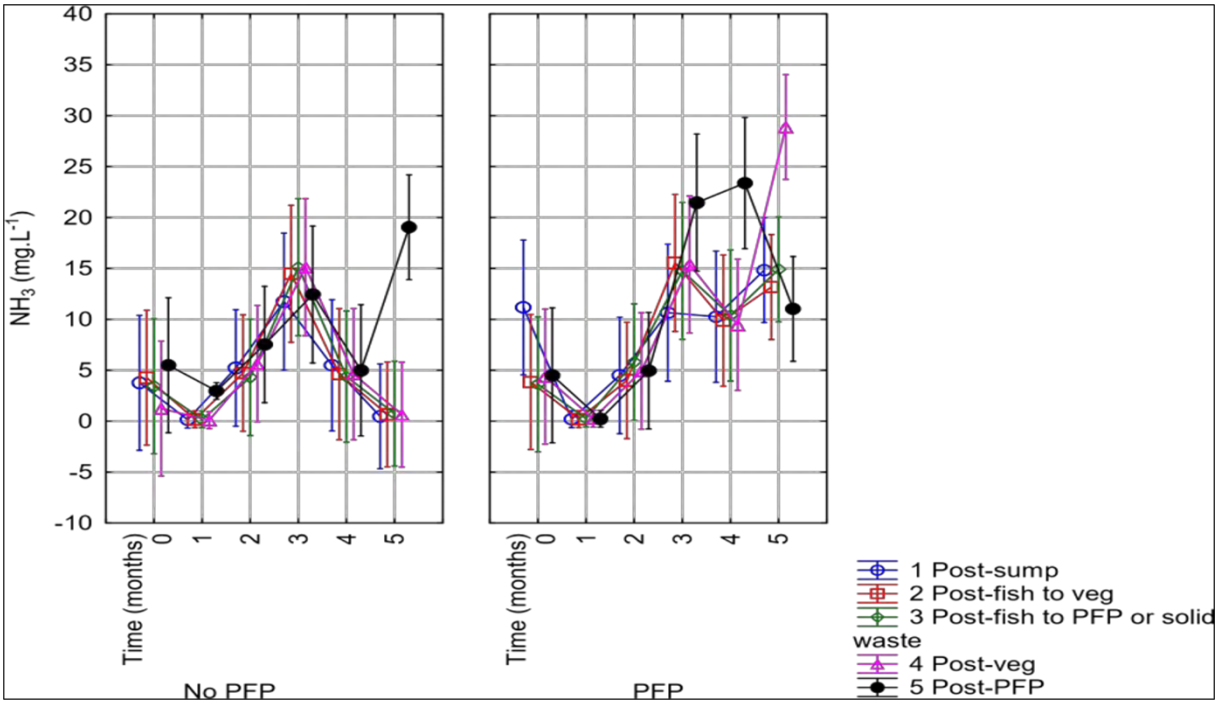

Figure 4. 5: Mean (± 95% confidence interval) ammonia (NH_3, $mg \cdot L^{-1}$) at the outlet of the sump (1 Post-sump to fish), the outlet of the fish tank that drains to the vegetable grow-bed (2 Post-fish to veg), the outlet of the fish when solid waste was removed (3 Post-fish to primary facultative ponds or to solid waste) the outlet of the vegetable grow bed (4 Post-veg to sump) and the outlet of the primary facultative pond (PFP) (5 Post- primary facultative ponds to veg) in the no primary facultative ponds and primary facultative ponds aquaponic system (RM-ANOVA $F_{(20,700)}=2.98$, $p<0.001$).

Nitrite

The concentration of NO_2^- was not influenced by the interaction between time, treatment and sampling points (RM-ANOVA $F_{(20,700)}=0.10$, $p=1.00$). Similarly, the NO_2^- was not influenced by the interaction between time and sampling points during the period of this experiment (RM-ANOVA $F_{(20,700)}=0.10$, $p=1.00$). It was also not influenced by the interaction between the treatment and the sampling points (RM-ANOVA $F_{(4,140)}=0.07$, $p=0.99$). However, NO_2^- concentration was significantly influenced by the interaction between time and treatment during this study (RM-ANOVA $F_{(5,700)}=3.27$, $p=0.006$; Figure 4.6). The NO_2^- decreased over time in the no-PFP treatment from month zero to one, then had an increase in month two, after which it remained constant throughout the experiment. The NO_2^- concentration of the PFP treatment did not vary from the start concentration throughout the study (Figure 4.6).

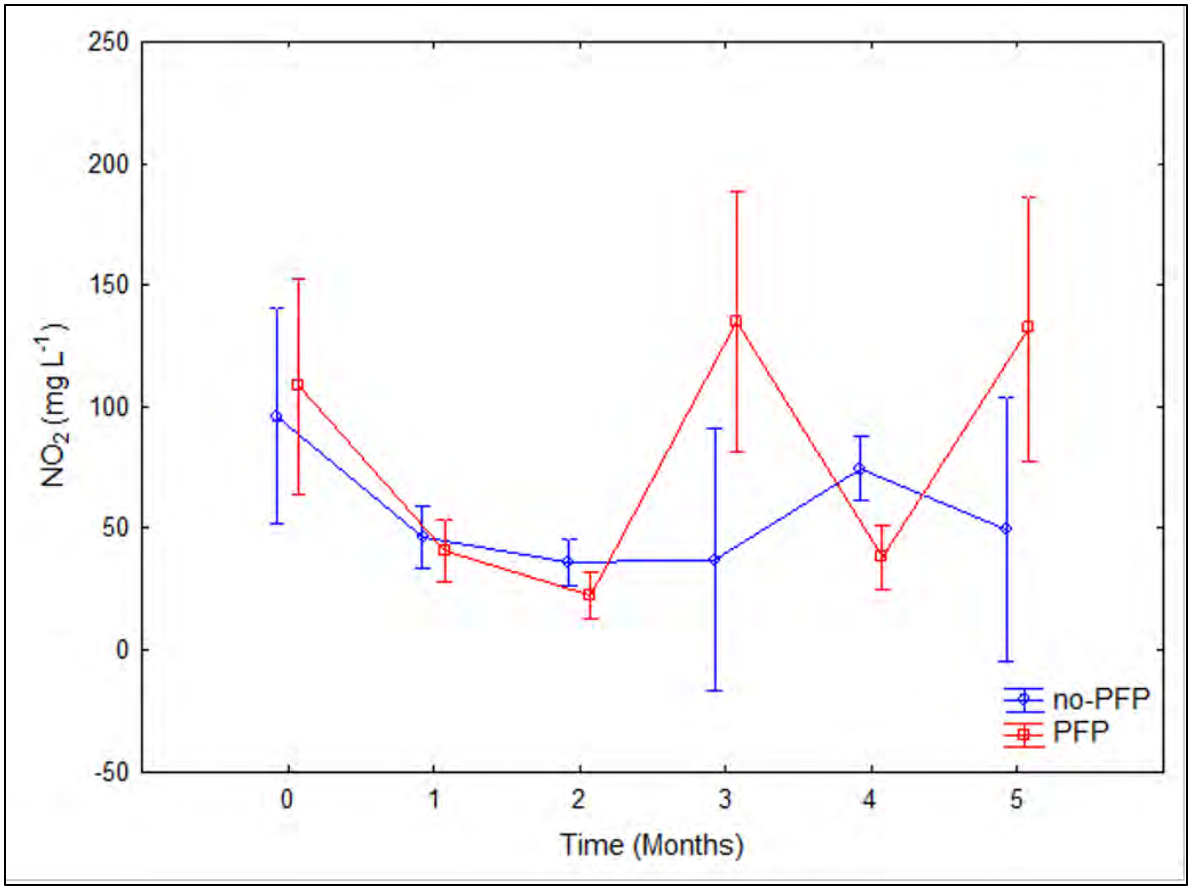

Figure 4. 6: Comparison of mean (± 95% confidence interval) nitrite (NO_2^-, mg·L^{-1}) in the no primary facultative ponds and primary facultative ponds (PFP) aquaponic systems, with all sample periods combined (RM-ANOVA $F_{(5,700)}$=3.27, p=0.006).

Nitrate

The NO_3^- concentration was not influenced by the interaction between time, treatment and sampling points (RM-ANOVA $F_{(20,700)}$=0.54, p=0.95). Similarly, it was not influenced by the interaction between time and sampling points during the period of this experiment (RM-ANOVA $F_{(20,700)}$=0.58, p=0.93). The NO_3^- concentration was also not influenced by the interaction between the treatment and the sampling points (RM-ANOVA $F_{(4,140)}$=1.17, p=0.32). It was significantly influenced by the interaction between time and treatment during this study (RM-ANOVA $F_{(5,700)}$=4.18, p=0.001; Figure 4.7). The NO_3^- concentration was similar between the two treatments at the start of the experiment. It the PFP treatment, it showed a decrease after the first month of the experiment while the no-PFP showed no significant change. This continued until the second month of the experiment. In month three, the NO_3^- concentration increased from the previous month. It continued to oscillate until the end of the experimental period and was only significantly lower than that of the PFP at month four (Figure 4.7).

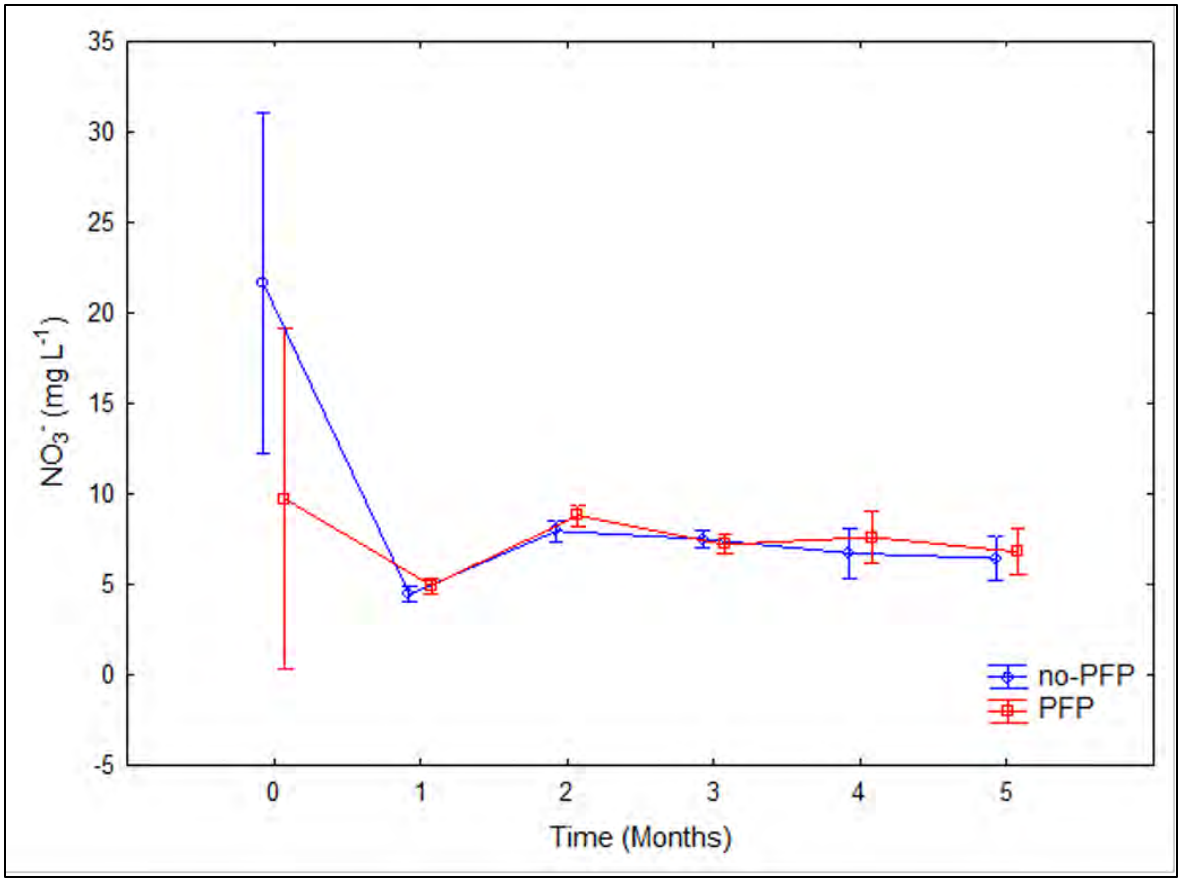

Figure 4.7: Comparison of mean (± 95% confidence interval) nitrate (NO_3^-, mg·L^{-1}) in the no primary facultative ponds and primary facultative ponds (PFP) aquaponic systems, with all sample periods combined (RM-ANOVA $F_{(5,700)}$=3.27, p=0.006).

Phosphates

The PO_4^{-3} was not influenced by the interaction between time, treatment and sampling point (RM-ANOVA $F_{(20,700)}$=0.81, p=0.70). It was also not influenced by any interaction between time and sampling point (RM-ANOVA $F_{(20,700)}$=0.77 p=0.75), time and treatment (RM-ANOVA $F_{(5,700)}$=2.02, p=0.07) or treatment and sampling point (RM-ANOVA $F_{(4,140)}$=0.22, p=0.93). The PO_4^{-3} concentration ranged between 15.50 in the no-PFP mg·L^{-1} and 14.58 in the PFP mg·L^{-1} for the duration of the experiment.

Chemical oxygen demand

The COD was significantly influenced by the interaction between time, treatment and sampling points during this study (RM-ANOVA $F_{(20,700)}$=2.04, p=0.005; Figure 4.8). For the first two months of the experiment, there were no significant differences in the COD between the no-PFP and PFP treatments, in the different sampling points. However, on month three, the no-PFP treatment had a significantly higher COD than the PFP, at sampling points three and four.

On the final month of the experiment, the no-PFP treatment once again had higher COD than the PFP at sampling point five.

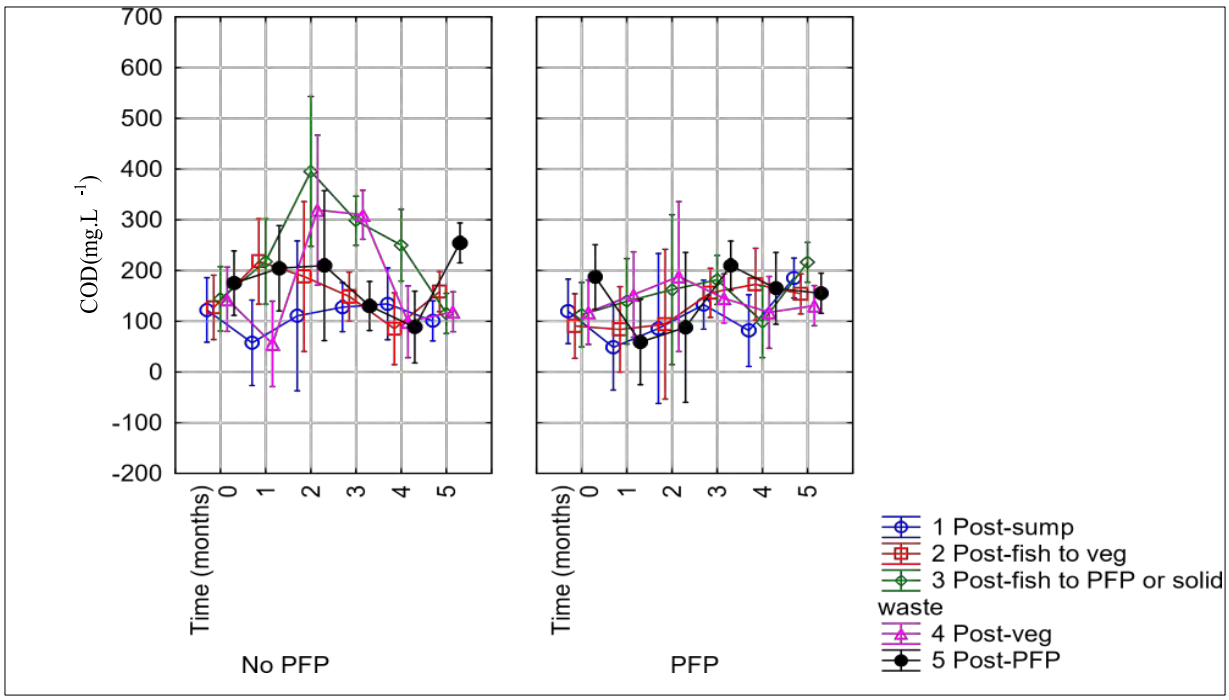

Figure 4.8: Mean (± 95% confidence interval) dissolved oxygen demand (COD, mg·L^{-1}) at the outlet of the sump (1 Post-sump to fish), the outlet of the fish tank that drains to the vegetable grow-bed (2 Post-fish to veg), the outlet of the fish when solid waste was removed (3 Post-fish to primary facultative ponds (PFP) or to solid waste) the outlet of the vegetable grow bed (4 Post-veg to sump) and the outlet of the primary facultative pond (5 Post-primary facultative ponds to veg) in the no primary facultative ponds and primary facultative ponds aquaponic systems (RM-ANOVA $F_{(20,700)}$=2.04, p=0.005).

Solid waste

There were no significant differences in the mean solid waste collected in the PFP and no-PFP aquaponics systems (Mann-Whitney U-test=1.00, p=0.26; Table 4.1). The mean weight of the solid waste in the PFP treatment was 360.00±120.90 g and 415.00±33.18 g in the no-PFP (Table 4.1).

Table 4. 1: Comparison of mean (± stand error) final fresh and dry weight for the solid waste in aquaponic systems with a primary facultative pond (PFP) and with no primary facultative ponds (no-PFP) for five months (Mann-Whitney U-test, U=1.00, P=0.26).

PFP vs No-PFP		Means (g)	n	*df	p-value
Final dry weight of sediments	PFP	361±121	3	3	0.26
	No-PFP	415±33	3	3	

*Degrees of freedom (df).

4.3.2. Results - Fish production

There was no significant difference between the initial weights of African catfish stocked into the PFP and no-PFP treatments (Student's t-test=-1.92, n=4, p=0.13, Table 4.2). The mean starting weight of the fish in the no-PFP was 37±1 g and 34±1 g in the PFP systems (Table 4.2). The final weights of the fish in the two treatments were also not significantly different (Student's t test= -1.40, n=4, p=0.23, Table 4.2). The mean weight of the fish in the no-PFP was 255±4 g and that in the PFP treatment was 245±6 g.

There was also no significant difference between the starting lengths of the fish stocked in the PFP and the no-PFP treatments (Student's t-test=2.05, n=4, p=0.11, Table 4.2). Similarly, there was no significant difference between final lengths of the fish raised in PFP and no-PFP treatments (Mann-Whitney U-test, U = 1.00, n_1=n_2=3, p=0.19, Table 4.2). The fish had an average length of 268±1 and 238±25 mm in the no-PFP and PFP systems.

The initial CF was not significantly different between the African catfish in the no-PFP and the PFP (Student's t-test=-2.72, n=4, p=0.05, Table 4.2). Similarly, CF was not significant difference between fish in the no-PFP and the PFP treatments (Student's t-test=1.03, n=4, p=0.36, respectively, Table 4.2).

There was also no significant different between SGR of the fish in the no-PFP and PFP treatments (Student's t-test=1.94, n=4, p=12, Table 4.2), where the fish in PFP had a SGR of 1.40±0.01% bw d^{-1} and those in the no-PFP system had a SGR of 1.38±0.01% bw d^{-1}.

The FCR between cultured catfish was not significantly different between no-PFP (1.16±0.04) and PFP (1.15±0.09) (Student's t-test=-0.07, n=4, p=0.96, Table 4.2).

Table 4.2: Mean (± stand error) weight, length, condition factor, specific growth rate and feed conversion ratio of African catfish grown in primary facultative ponds and no primary facultative ponds systems (two aquaponic systemtreatments) for five months (Student's t-test, p<0.05, Mann-Whitney, p<0.05).

	PFP	no-PFP	t/*U* value	Df */n	P value
Initial weight (g)	34±1	37±1	t = -1.92	df = 4	0.13
Final weight (g)	245±6	255±4	t = -1.40	df = 4	0.23
Initial length (mm)	172±12	147±4	t = 2.05	df = 4	0.11
Final length (mm)	238±25	268±1	U = 1	n_1=n_2=3	0.19
Initial condition factor	0.73±0.04	0.93±0.06	t = -2.72	df = 4	0.05
Final condition factor	0.88±0.11	0.75±0.06	t = 1.03	df = 4	0.36
Specific growth rate (SGR) (% bw day^{-1})	1.40±0.01	1.38±0.01	t = 1.94	df = 4	0.12
Feed conversion ratio (FCR)	1.15±0.09	1.16±0.04	t = -0.07	df = 4	0.95

*Degrees of freedom (df); sample size (n).

Fish weight was not affected by an interaction between time and the treatments (no-PFP and PFP) (RM-ANOVA $F_{(5,20)}$=1.00, p=0.44, Figure 4.9). However, it was affected by the interaction with time (RM-ANOVA $F_{(5,20)}$=439.57, p<0.0001), where from the beginning of the experiment in December, mean weight increased until April, and this increase was the same for both treatments (Figure 4.9).

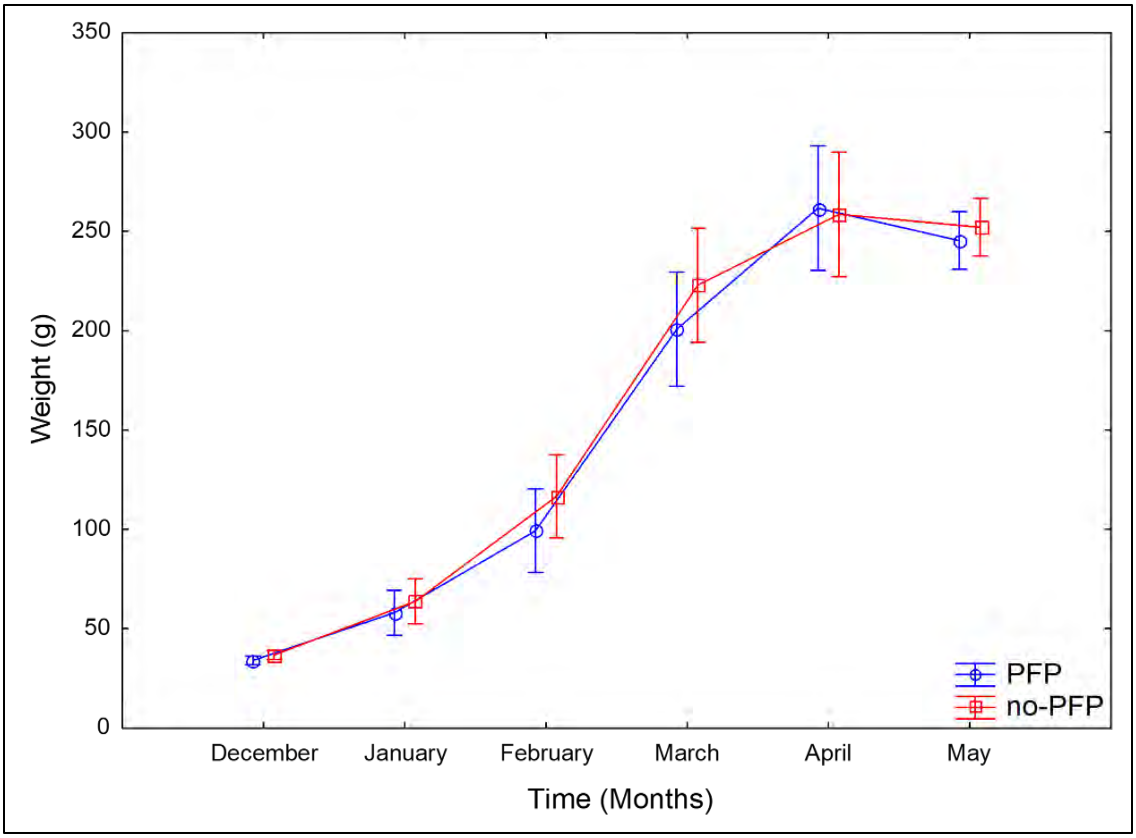

Figure 4.9: Comparison of means (± standard error), the fish weight growth, plots by time (months) in the no primary facultative ponds vs primary facultative ponds (RM-ANOVA $F_{(5,20)}$=1.00, p=0.44).

Fish length was not affected by the interaction between time and the treatments PFP and no-PFP (RM-ANOVA $F_{(5,20)}$=1.93, p=0.13, Figure 4.10). However, fish length was influenced by a significant interaction with time in this study (RM-ANOVA $F_{(5,20)}$=384.58, p<0.0001). From the start of the experiment (December), mean length increased until the end of the experiment (May), and this increase was similar for both treatments (Figure 4.10).

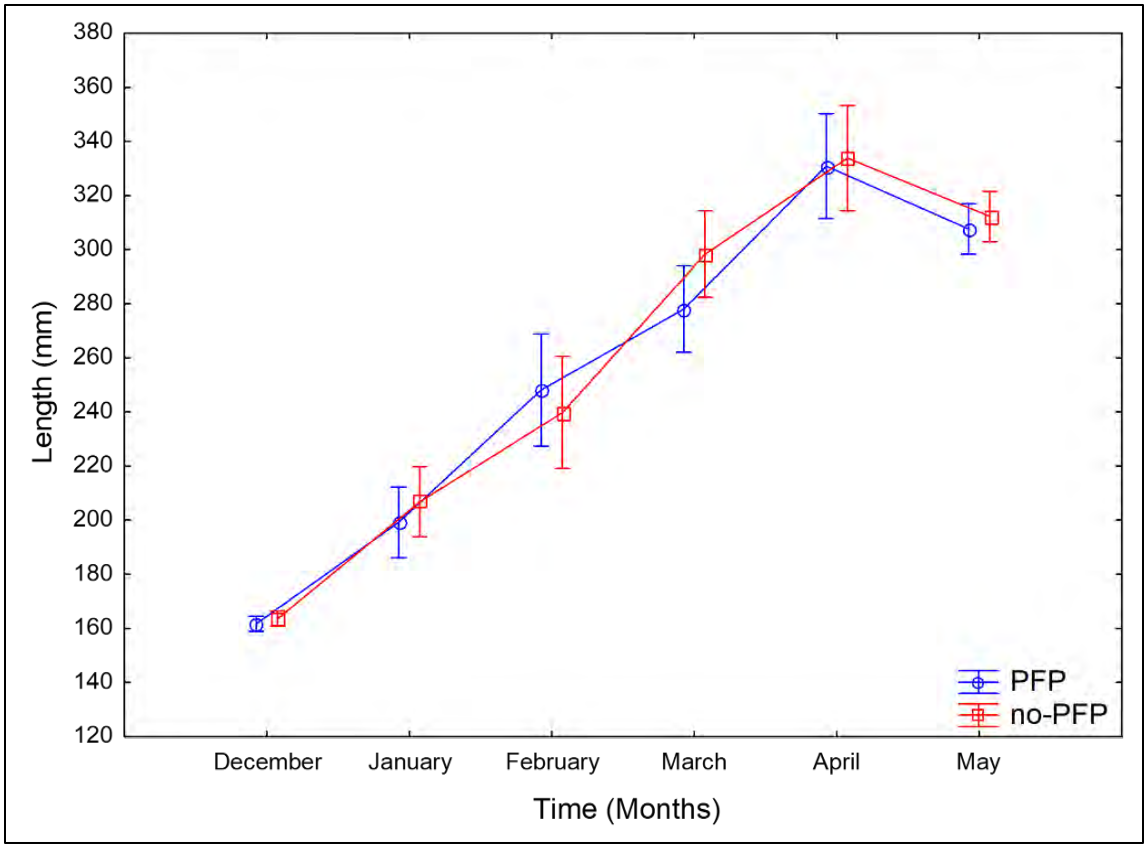

Figure 4.10: Comparison of mean (± standard error) fish length by month in the primary facultative pond vs no primary facultative ponds treatments (RM-ANOVA $F_{(5,20)}$=1.93, p=0.13).

Fish Survival and mortality

The systems that included solids removal (no-PFP) and that, where solids were maintained in the system using a PFP, had survival rates of 89.16% and 88.33% respectively. The distribution of fish that died was not significantly different in the two aquaponics systems (X^2=0.04, p=0.847, Table 4.3).

Table 4.3: Survival and mortality of African catfish reared in aquaponics systems with no primary facultative ponds (no-PFP) and primary facultative ponds (PFP).

	no-PFP	PFP	Chi-square	p-value
Total number stocked	120	120		
Total number that died	13	14	0.04	0.84
Survival (%)	89.16	88.33		

Fish biomass

Initial biomass of African catfish was not significantly different between the no-PFP and PFP treatments (Student's t-test=2.29, n=4, p= 0.08, Table 4.4). Similarly, there was no significant difference between the final biomass in the no-PFP and the PFP (Student's t-test=0.31, n=4, p=

0.76, Table 4.4). Similarly, the increase was not significantly different between the fish in no-PFP and in PFP (Student's t-test=0.21, n=4, p=0.84, Table 4.4).

Table 4.4: Mean (± standard error) African catfish biomass (grams) in no primary facultative ponds and primary facultative ponds (PFP) treatments at the start of the trial (initial biomass) the total biomass at the end of the trail including all of the fish that were left and all of the fish that were harvested during the trial (final biomass), and the biomass increase over the five-month trial (Biomass increase=Final weight less Initial weight).

	No-PFP (g)	PFP (g)	t-value	*df	P value
Initial biomass (g)	1466±41	1387±43	2.29	4	0.08
Final biomass(g)	9539±743	9302±1053	0.31	4	0.76
Biomass increase (g)	8073±701	7915±1069	0.21	4	0.84

*Degrees of freedom (df).

4.3.3. Results - Plant production

Chlorophyll-a concentrations in Swiss chard leaves were not affected by the interaction between time and treatments PFP and no PFP (RM-ANOVA $F_{(5,20)}$=1.98, p=0.12, Figure 4.11). However, chlorophyll-a was significantly affected by an interaction with time (RM-ANOVA $F_{(5,20)}$=8.78, p=0.00015), Chlorophyll-a overtime was similar between these two treatments. It increased from the start of the experiment (December) until February, then levelled off for the remainder of the experiment (Figure 4.11).

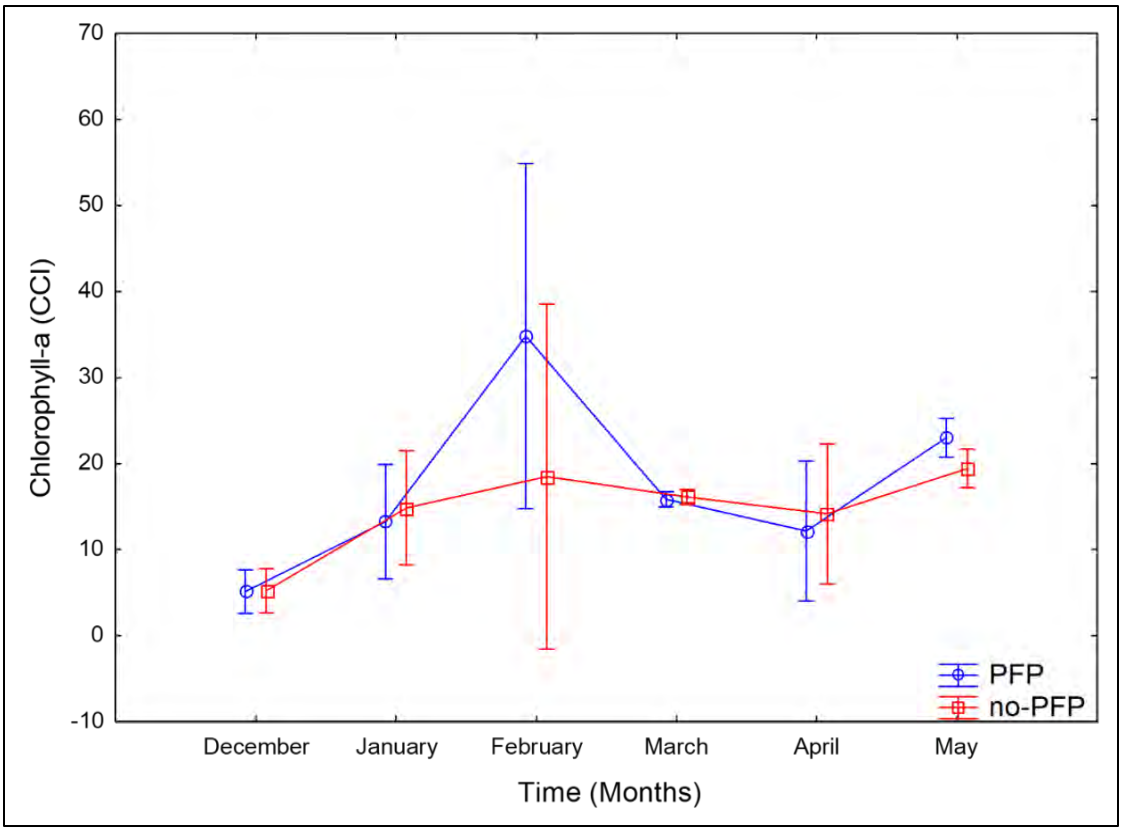

Figure 4.11: Comparison of mean (± standard error) chlorophyll-a by month in the no primary facultative ponds vs primary facultative ponds (PFP) (RM-ANOVA $F_{(5,20)}$=1.98, p=0.12).

Mean leaf lengths were not affected by an interaction between time and treatments PFP and no-PFP (RM-ANOVA $F_{(5,20)}$=1.46, p=0.24, Figure 4.12). However, leaf length was significantly affected by an interaction with time (RM-ANOVA $F_{(5,20)}$=35.79, p<0.0001). The leaf length over time was similar between these two treatments. It increased from January until March, then decreased to April, then increased to the end of the experiment (May) (Figure 4.12).

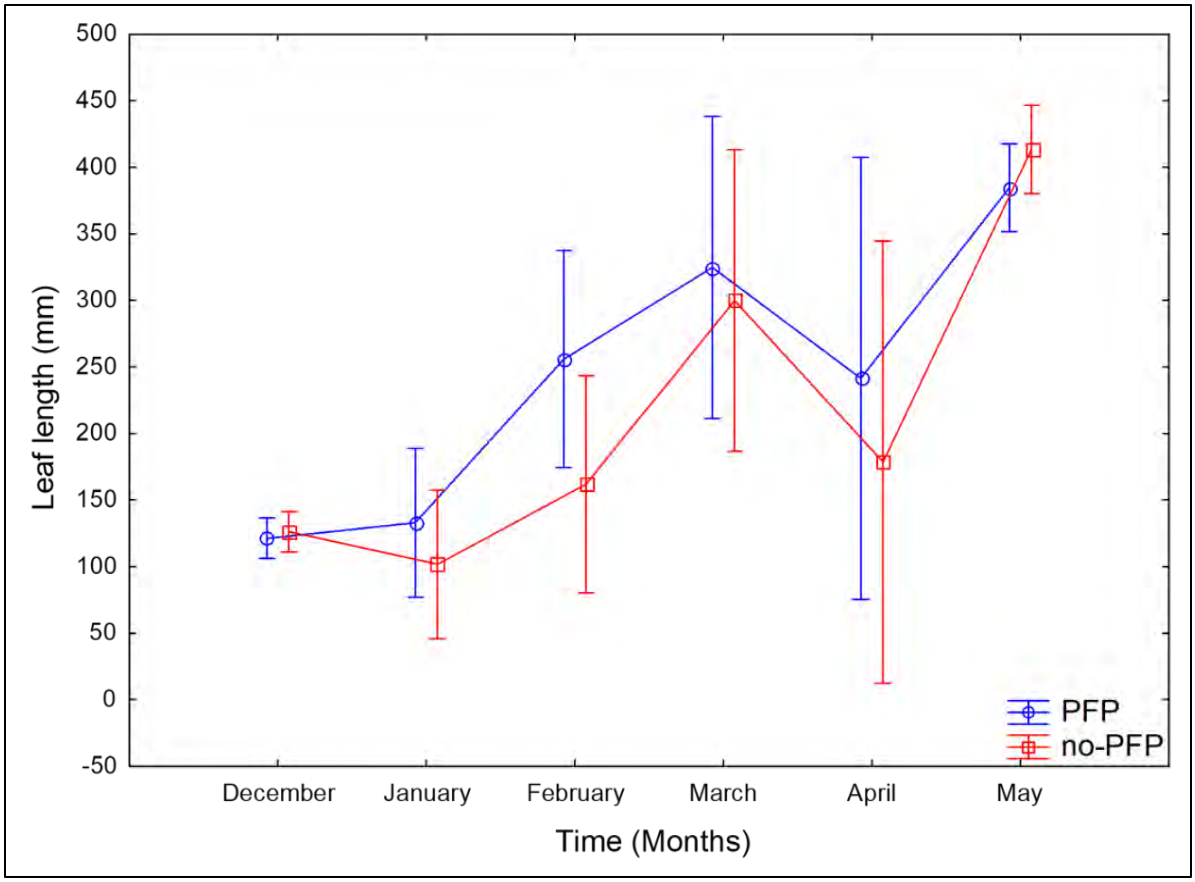

Figure 4.12: Comparison of mean (± standard error) leaf length of plant by month in the no primary facultative ponds vs primary facultative ponds (PFP) treatments (RM-ANOVA $F_{(5,20)}$=1.46, p=0.24**).**

Mean Swiss chard leaf width was not affected by an interaction between time and the treatments PFP and no-PFP (RM-ANOVA $F_{(5,20)}$=2.49, p=0.06, Figure 4.13). However, leaf width was significantly influenced by an interaction with time during this study (RM-ANOVA $F_{(5,20)}$=102.31, p<0.0001). The leaf width over time was similar between these two treatments, and both increased from December to February, then remained constant to April, then increased to the end of the experiment (May) (Figure 4.13).

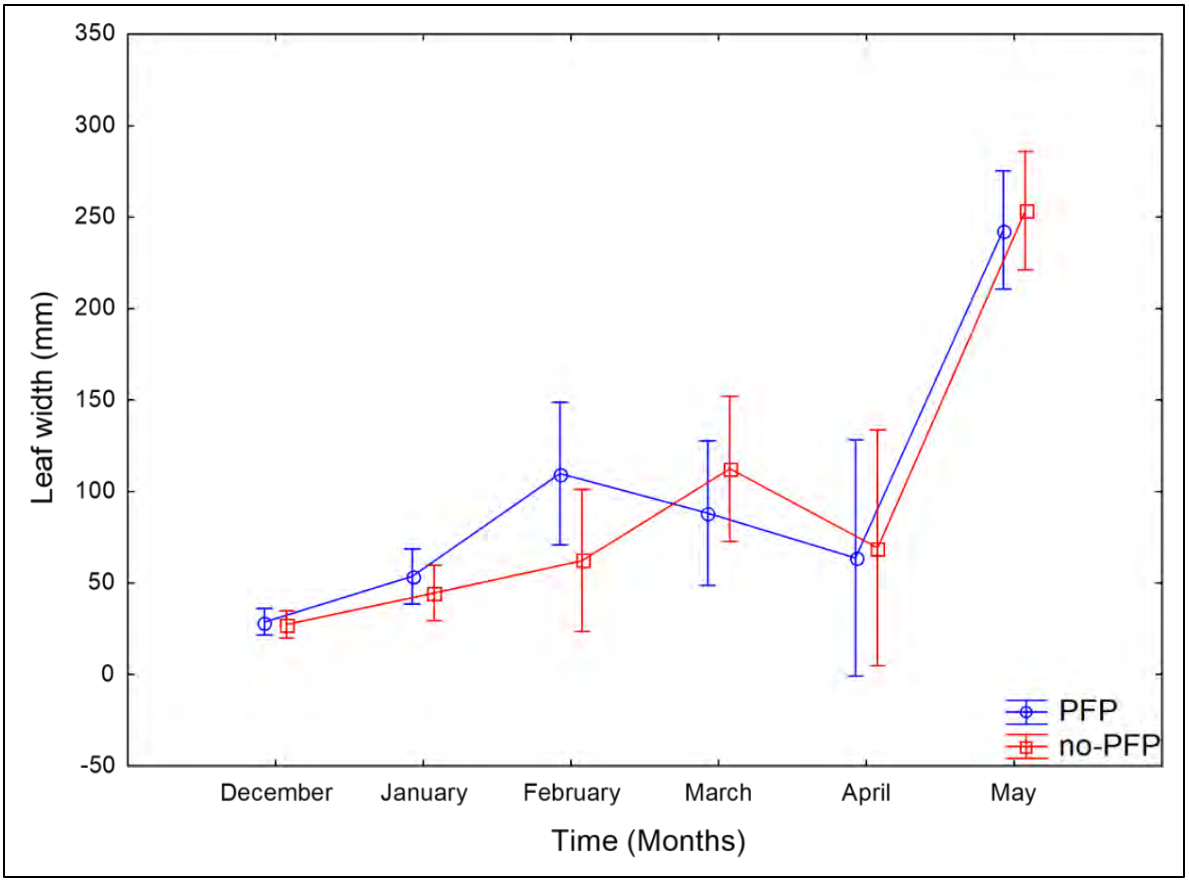

Figure 4.13: Comparison of mean (± standard error) leaf's width of plant by month in the no primary facultative ponds vs primary facultative ponds (PFP) treatments (RM-ANOVA $F_{(5,20)}$=2.49, p=0.06**).**

Swiss chard stem length was not affected by an interaction between time and treatments (RM-ANOVA $F_{(5,20)}$=2.07, p=0.11, Figure 4.14). However, stem length of Swiss chard was significantly influenced by the interaction with time (RM-ANOVA $F_{(5,20)}$=104.57, p<0.0001). Stem length over time was similar between these two treatments, and both increased from the beginning (December) until the end of the experiment (May) (Figure 4.14).

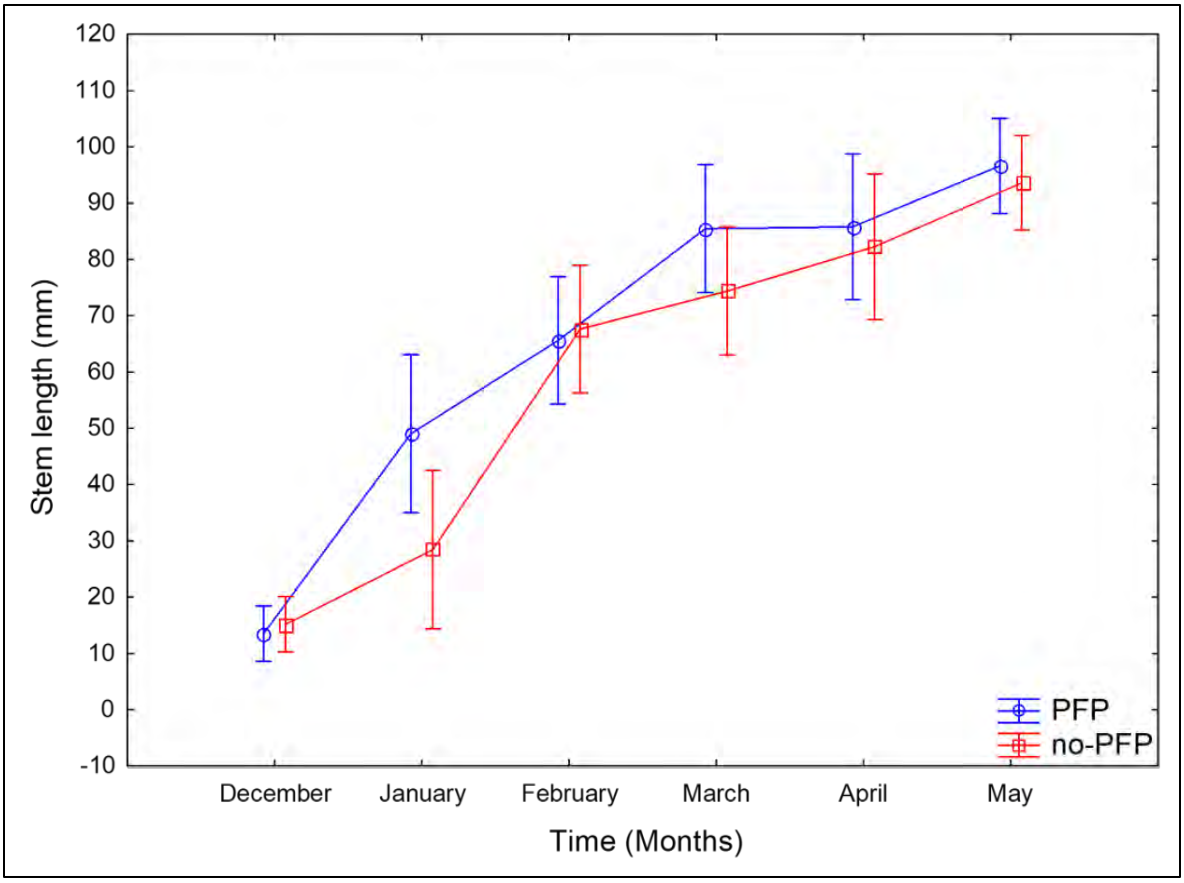

Figure 4.14: Comparison of mean (± standard error) stem length of the plants by month in the no primary facultative ponds vs primary facultative ponds (PFP) (RM-ANOVA $F_{(5,20)}$=2.0758, p=0.11).

Plant biomass

Wet biomass of Swiss chard was not significantly different between the no-PFP and the PFP treatment (Student's t-test=-0.32, n=4, p=0.76, Table 4.5). The dry biomass also did not have a significant difference between the no-PFP and PFP (Student's t-test=0.01, n=4, p=0.99, Table 4.5). Similarly, the stem biomass in no-PFP and no-PFP treatments was similar (Student's t-test=0.54, n=4, p=0.61, Table 4.5). Root biomass was not significantly different between in the no-PFP and the PFP treatments (Student's t-test=-0.32 n=4, p=0.75, Table 4.5). The biomasses were similar in both PFP and no-PFP treatments, (Student's t-test, p>0.05, Table 4.5).

Table 4.5: Mean (± standard error) Swiss chard biomass (grams) in no primary facultative ponds (no-PFP) and primary facultative pond (PFP) treatments and the dry and wet leaves, stem and root biomass, and the total biomass over the five-month trial (Total wet biomass was sum of wet leaf, wet stem and wet root biomass).

	no-PFP	PFP	t value	*df	p value
Dry leaf biomass (g)	1801±40	1798±22	0.01	4	0.99
Wet leaf biomass(g)	18191±4765	21619±7806	-0.32	4	0.76
Wet stem biomass (g)	713±157	603±76	0.54	4	0.61
Wet root biomass (g)	635±49	707±186	-0.32	4	0.75
Total wet biomass (g)	**19538±4971**	**22929±8068**	**-0.08**	**4**	**0.93**

*Degrees of freedom (df).

Chapter 5. General discussion

After the introduction of PFPs in aquaponics, the next issue was to ensure the efficiency of fish solid waste treatment. Statistical comparison between PFPs and no-PFPs showed no significant difference regarding fish and vegetable production. This chapter addresses several points to demonstrate the importance of using drain-fill vs flooded models both carrying PFPs, for the successful production of fish and plants.

5.1. The use of drain-fill versus flooding and dealing with solids using primary facultative ponds

In addition to comparing these two experiments for an aquaponic system, the next step was to examine their effects on water quality, and fish production, as well as plant production.

5.1.1. Water quality

Temperature is a key element in fish production and plant cultivation (Xu *et al*., 2014). In this study, the water temperature was similar in the flooded and drained environments, except at the very end of the test. The total system water volume in the flooded treatments was higher and therefore with better heat retention than the smaller volume in drain-fill systems. In that system, the plant roots were also cooled by the atmosphere before refilling occurred.

The temperature variations between 15 and 30°C allowed for good growth of some fish species (Rakocy *et la*., 2006 and Bernstein, 2013). Catfish is not one of them because they do not grow well in cooler water (Hochmuth and Hanlon, 2010; Genello *et al*., 2015). The temperature was significantly higher in January and February but cooler thereafter. The fish growth trends in the current study levelled off towards May in all the treatments. As such, the temperature was probably too cold towards the end of the experiment in the current trail for optimal catfish growth. Overall, metabolic rates and fish/waste production was influenced by temperature changes towards winter. Future work that includes catfish in aquaponics should be limited to warmer seasons that are more suited to catfish growth. Catfish optimum temperature ranges from 20-26°C (Cowey, 1992) and 23.9-29°C (Hanssens, 2009); meaning that the bioassays in the three experimental aquaponics which varied between 19.2-28 °C were quite close to the optimum range, most of the time. It will be interesting to repeat the same bioassays in aquaponics when temperature will be in an increasing pattern, though still keeping the optimal range.

Plant growth was inflected by temperature. Since Swiss chards are terrestrial plants and their roots must be exposed air and to a temperature range of 10-30°C in the soil for growth (Bosire and Sikora, 2017); but even the highest temperature in this study was under 26 °C, which is within the acceptable range for Swiss chard (Sarker and Oba, 2019). Temperature was similar and followed a steeply decreasing trend in all the aquaponics systems in this work.

There were also other variables like a lack of root aeration in the flooded systems. Terrestrial plants require aerated roots (Rakocy, 1999 and Love *et al.*, 2014), which was the case with drain-fill treatments in the current study. The air that was sucked into the grow bed in the drain-fill system was beneficial to terrestrial plants like Swiss chard. Dissolved oxygen levels were significantly higher in the drain-fill as compared to the flooded design. The DO contributed to improved vegetable production in the drain-fill treatment. The DO was significantly higher in the drain-fill system compared to the flooded system at all sampling points, including the vegetable grow bed.

Similarly, plants can take up nitrate more efficiently when the plant roots are exposed to oxygen (Vymazal, 2007). The larger and dark greenery of the leaves of Swiss chard in the drain-fill, compared with those in the flooded system, could be due to more oxygen and the high concentration of nitrates coming from the denitrification of ammonia in the culture beds (Gopalakrishnan, 2007). This is why Swiss chard in drain-fill culture beds had a high concentration of chlorophyll-a compared to flooded ones by the end of the trial, and why the drain-fill system produced larger plants and more biomass.

The DO also accelerated the nitrification of ammonia (DeLong, 2009). Dissolved oxygen was 116% higher in the drain-fill water compared to that in the flooded. The higher DO in the drain-fill treatment was likely due to the tide and ebbing effect, where oxygen rich air was pulled into the grow-bed when the water drained, thus aerating the water when the bed filled up again. These results were similar to the 6-8 $mg \cdot L^{-1}$ found by Yildiz *et al.*, (2019) and Goddek (2017) when they studied the application of aquaponic system as a circular model. Higher Swiss chard and African catfish production were consistent with higher DO. Organisms grown in aquaponics prefer a high concentration of oxygen (Akan *et al.*, 2010), which helps to explain increased production in the drain-fill systems in this experiment.

The pH level was higher in the drain-fill during the three first month, before they became lower than those in the flooded system. The drain-fill treatment resulted in improved vegetable

production, because the pH was within a range similar to that reported by other authors who demonstrated good growth (between pH 6.5 to 7.8 and 14 to 35 °C) (Hochmuth and Hanlon, 2010; Genello *et al.*, 2015; Sarker and Oba, 2019).

Salinity has played a vital role in understanding the aquatic environment and determining EC. The results of this research showed that in flooded treatments, salinity was lower than in drain-filled treatments. This somewhat higher salinity (1.07-1.4‰) content in the drain-fill, may have contributed greatly to the growth of fish and plants (Rakocy *et al.*, 2006). In flooded growing beds, many predatory microorganisms in the hydroponics bed consume these salts. Nelson and Siegel (2014) discovered that there were living organisms that consumed salts in water but did not reduce salinity to its aquaponic threshold (1.4‰). The presence of these organisms was not monitored in the current study, but differences in their abundance in the different systems might account for differences in salinity. This should be investigated in future research.

The water top-up volumes were not recorded in the current study, and differences in the volume of water added to the different systems might account for the differences in salinity in the current study. These differences, could be due to differences in evapotranspiration and evaporation rates. The source of the salts and differences in evaporation and top-up rates need to be scrutinize in future work. Note should be taken to also record water top-up volumes in future research.

Increased salt concentration should not have had negative effects on fish production in this aquaponic system in the range observed. A smaller water system volume subject to greater water loss would have more concentrated salts. Added to this, since the fish were bigger in the drain fill system, they were also fed more feed which contributed to the dissolved salt contents of the system. Catfish prefer low salinity environments; also, their respiratory organ has been developed for atmospheric oxygen uptake and not osmoregulation (Palme *et al.*, 2018).

Nonetheless, water from drain-fill and flooded hydroponic beds treated with Swiss chard can be used in aquaponics in the grow out of African catfish as a water source despite differences in salinity level. The EC is a measurement of the salt ions dissolved in a solution, allowing them to conduct electrical current (Mongirdas and Kusta, 2006). As with salinity, the EC was higher in the drain-fill than in the flooded systems of drain-fill water was influenced by the salinity within these hydroponic beds because it is mainly an indicator of salinity and salt in water (Hargreaves *et al.*, 2005). For the case of this research, salinity and EC were within acceptable ranges for fish and plant production (Kim *et al.*, 2014).

The EC, salt and TDS values were consistently higher in the PFP system as opposed to the no PFP system during the experiment. The solids were removed from the no PFP systems, but were maintained in the PFP systems. Solids, such as uneaten feed and fish faeces, are a source of dissolved salts (Nicolas, 2014). These wastes are mineralised in the PFP (Li and Li, 2009). Every time post-PFP water was flushed back into the system, salts and dissolved solids would have been returned to the system. This would account for the higher salt, TDS and EC values in the aquaponics systems that included a PFP.

Nitrogen occurred as ammonia, nitrite and nitrate. They are always present in an aquaponic system since they come from fish food. The drain-fill treatment was able to reduce the ammonia nitrogen content more effectively in the waters of these hydroponic beds compared to flooded bed treatments. The drain-fill lowered the ammonia concentration to an average of 3.23 $mg \cdot L^{-1}$, close to the national discharge standard into municipal sewers of 2.89 $mg \cdot L^{-1}$ (Li *et al.*, 2014). The drain-fill treatment had an ammonia removal rate of 76.11% compared with the flooded which was 24.88%. The value for drain-fill is somewhat close to those of 99.7% reported in aerated wetland waters (Li *et al.*, 2014). This demonstrates that in the drain-fill, there was better nitrification than in the flooded, which is the main process of ammonia conversion by nitrifying bacteria (Palm *et al.*, 2018; Zwane, 2019).

Good nitrification of ammonia in aquaponics would be the basis for the effective conversion of ammonia into nitrites and then into nitrates, and the removal of nitrates by plants (Schneider *et al.*, 2005). The nitrate was very well sequestered by the Swiss chard in the culture beds of the drain-fill treatment compared to those of flooded. Thus, the Swiss chard treated the water and sequestered more nitrates as they grew.

Nitrification was enhanced in the drain-fill treatment trial. The bed media also contain bacterial films, as well as the root system. Drain-fill promotes aeration, hence the amplification of nitrification processes. Nitrite can be also toxic to fish if found at concentration levels over 5 ppm (Love *et al.*, 2014). The improved conversion of ammonia to nitrate in the drain-fill treatment can be attributed to the higher DO due to water movement in the system (Vymazal, 2007). It can be a factor which positively affected the growth and as well as the fish and plant production in the drain-fill than in the flooded system.

Chemical oxygen demand (COD) is the consumption of dioxygen by organic materials (chemical oxidants) which degrades the oxygen dissolved in the water (Moore *et al.*, 1951; Li and Li, 2009; Antonio *et al.*, 2013). In aquaponics, which is an aerobic environment, COD can

be reduced by bacteria, which use organic materials as nutritional substrate. The COD was similar in both the flooded and the drain-fill; algae were oxygen generators during daylight hours (Wang *et al.*, 2009). In aquaponics, nutrient levels during the system water cycle increased with culture time (Gopalakrishnan, 2007; Li and Li, 2009).

In the case of this research, this high rate of COD reduction may have played an important role for the improved production of fish and plants observed in the drain-fill compared to the flooded one. Algae and bacteria have coexisted since the earliest stages of evolution and are still involved in reducing COD in each aquatic ecosystem (Ramanan *et al.*, 2016). This co-evolution has revolutionized life in aquaponics, thus promoting high production (Ramanan *et al.*, 2016). In conclusion, micro-organisms (bacteria) make nutrients more bioavailable to plants.
The COD strongly fluctuated in both aquaponic designs; but it remained consistently higher in the no-PFP system. When the overall water quality results of the five experimental months were compiled for the comparison of the drain-fill and the flooded systems, on the one hand, the DO, EC, salinity, NH_3^+, NO_3^-, NO_2^- were significantly higher in the drain-fill system (Table 5.1). On the other hand, the temperature, the TDS and the COD were at similar levels. But PO_4 and pH had higher values in the flooded design. Phosphate concentrations were similar both in PFP and the no-PFP treatments.

Table 5.1: Monthly dynamics tables of water quality parameter between three treatments in aquaponic system with primary facultative ponds (PFP) and no primary facultative ponds /Drain or Flooded. (TDS = total dissolved solids).

Flooded / PFP	Drain-fill / PFP	Drain-fill / no-PFP
Temperature average from December to February, steadily increased to high levels from March to May.	Temperature started at high levels in December then decreased to unstable medium-high levels in January to February, then increased in March back to high, then decreased to low in April and May.	Temperature remained low since December to April, then increased steadily to high levels in May.
Salinity was very high from December to February then decreased steadily from March to May.	Salinity remained low from December to March, then increased to high from April to May.	Salinity remained high from December until April then decreased to low in May
TDS was very high from December to February, then decreased to average from March to May. NH_3 remained average from December to May all over the experimental time.	TDS was high in December, then steadily decreased to low levels in January and February, then gradually increased to average in March and reached high levels in April and in May. NH_3 was high in December, then decreased to low in January and in February, then increased to high in March-April and May.	
NO_2 was low from December to February, then steadily increased to high levels in March-April-May.	The NO_2 levels were high in December, then decreased to average levels in January-February, then increased to high levels in March then decreased to low levels in April-May.	NO_2 levels remained high from December to April, then steadily decreased to low levels in May.
PO_4 remained low from December to May all over experimental time.	PO_4 remained low all over time the experiment from December to May.	PO_4 remained high from December to April, then steadily decreased to low in May.

5.1.2. Fish production

The fish harvest weight from the drain-fill was greater than that in the flooded treatment, so the drain-fill performed significantly better than flooded. Catfish final and total biomass and increment in biomass were significantly higher in the drain-fill system as opposed to the flooded design. Fish final weight and length were significantly higher in the drain-fill treatment, with respective increments of 13.9% and 16.2%. The drain-fill trial had a final fish survival rate of 88.33%, while the African catfish reared in the flooded trial had a survival rate of 80.83%. So, 23 fish died in the flooded systems and 14 in the drain-fill systems. The initial and the final

environmental conditions were similar both in the drain-fill and the flooded aquaponics design. During this research, the progressive growth of fish over the course of five months was illustrated by the increase in weight on specimens above 300 g. The drain-fill did not subject the fish to environmental stress such as temperature variations and water quality conditions, and these were in acceptable ranges for African catfish (Timmons and Ebeling, 2010). These results corroborate those of Nawwar (2016) for aquaponics in Perlis (Malaysia). Their research focused on the production of catfish and vegetables (three types of plants: red and green-red amaranth *Amaranthus* spp. and water spinach *Ipomoea Aquatica*) in an aquaponic system. Similar results were also found in commercial aquaponics studies in which African catfish were produced by Rakocy (1989). Their research outcomes were consistent with those found in the drain-fill treatment of the aquaponic system in the current study. The drain-fill treatment was better compared to the flood system, demonstrated by the better production of plants and fish in the drain-fill treatment (Table 5.2).

African catfish were better able to tolerate stresses related to water quality, when reared in the drain-fill system, and were more resistant than those reared in the flooded design. In the drain-fill, they could withstand adverse water conditions such as NH_3 which was as high as 29 mg·L^{-1} at the outlet of the vegetable grow bed (Vymazal, 2007 and Bosire and Sikora, 2017). The NH_3 levels at the fish tank inlet were generally lower than this. The NH_3 levels include the ammonium ion N; however, it was not adjusted for pH and temperature so this was not determined in the current study (Li and Li, 2009, Nawwar, 2016).

Furthermore, catfish do not have good appetite at high ammonia levels, which could also cause broken head disease (Vymazal, 2007). The ammonia in the systems might have been responsible for poor growth, because the fish did not always feed well in the current study.
Fish still grew better in the drain-fill and this is perhaps linked to improved oxygenation (due to water movement) and associated accelerated nitrification (Shava and Gunhidzirai, 2017). There were higher oxygen concentrations in drain-fill water compared to flooded. However, in flooded beds, the elevated NH_3 levels can cause reduced growth and mortality. A lack of autotrophic nitrification and inadequate microbial activity, which is integral to water treatment, could be responsible for the poor fish growth (Rakocy, 1989; Rakocy *et al.*, 2006; Hargreaves *et al.*, 2005; Maucieri *et al.*, 2018). Hydroponic vegetable beds in the drain-fill treatment were better at reducing the ammonia concentration than those in the hydroponic vegetable beds that were constantly flooded, which is indicative of the importance of proper aeration of the grow bed for improved nitrification.

The SGR and FCR for African catfish obtained in the drain-fill and in the flooded for this research were very close to those obtained in a similar study (Maucieri *et al.*, 2018). Values of 1.19% body weight gain day^{-1} for SGR and 2.58 for FCR were measured for African catfish reared in water containing 5.50 to 7.50 mg L^{-1} DO and pH from 5.8 to 8.5 (Maucieri *et al.*, 2018). For African catfish, Xu *et al.* (2014) reported a SGR of up to 1.96% day^{-1} and an FCR of 1.07. These SGR and FCR were better than those obtained in the drain-fill and the flooded treatments of this study; the poorer growth and FCR in the current study were probably due to lower water temperatures.

Table 5.2: Fish growth monthly dynamics tables in the three treatments in aquaponic system with primary facultative ponds (PFP) and no primary facultative ponds in drain-fill or flooded grow-beds.

Flooded / PFP	Drain-fill / PFP	Drain-fill / no-PFP
Fish number weight remained low from December to February, then steadily increased to high levels from March to May.	Fish number weight was low in December to February. It reached average levels in March and then increased to high levels in April-May.	Fish number weight was low in December, January; it kept average levels in February, March, and April and increased to high levels in May.
Fish weight remained low from December until January, then it increased to average in February and became high from March to May.	The fish weight was low in December, average January, and February, then high in March-April-May.	Fish weight was low in December it became average in January then high in February, March, April, and May.
Fish length was low in December-January, then average in February. It reached high levels in March, April, and May.	Fish length was low in December, then average in January and February. It reached high levels in March, April, and May.	Fish length was low in December reached average in January and increased to high levels in February, March, April, and May.

African catfish grow best in warm water (25-33°C) and can exhibit a high growth rate in this temperature range (Britz and Hecht, 1987; Somerville, 2014). The temperature variation in the aquaponic systems reported by Somerville *et al.*, (2014) was optimal, varying between 26 to 28°C. Catfish growth in this experiment was not as good as it could have been because the temperature was not always optimal during the trial. It was often too cold, even below 18°C. These results were similar to those found in catfish farming with the cultivation of vegetables (*Lettuce* and *Amaranth*) (Love *et al.*, 2014). They were also the same as those found in aquaponic systems of *Tilapia niloticus* and those of catfish at very high temperatures (38°C) and in extreme conditions at certain times (Britz and Hecht, 1987). According to Love *et al.*, (2015), catfish and *Oreochromis* tilapia had similar results and it was concluded that they are

thermophilic fish and known to tolerate a wide range of water temperatures (14-31°C even 7-41°C). However, the treatments in the current experiment resulted in adequate conditions for production in an aquaponic system, producing acceptable levels of African catfish. Nevertheless, fish growth could probably have been improved further if the temperatures in the current study were kept warmer in the cooler periods of the trail.

Specific growth rate (SGR) was reduced with increasing stocking density whereas food conversion ratio (FCR) increased with stocking density (Ricker, 1979; Cowey, 1992). Aquaponic systems with the lowest stocking densities (150/m^3) performed better than 300 and 450 fish/m^3 respectively. The FCR is more often applied to fish than to other animals, primarily due to their energy requirements; maintenance is lower compared to other warm-blooded animals. In fish, the decrease in energy requirements can be explained by four reasons, including (Ricker, 1979):

- Fish do not have the ability to maintain constant body temperature;
- Fish exert less energy in maintaining their position (balance);
- Energy is lost in small quantities during urinary and gill excretions;
- There is a lower energy cost associated with the assimilation of the food that is ingested.

The growth of African fish as well as their production were represented in Table 5.2, to evaluate the performance between these two experiments and their treatments. However, in this research, the progressive fish growth over the five months was illustrated by the incremental increase in weight, and a number of fish grew beyond 300 g in both experiments. When data from May were compared between the two experiments, in Experiment 2 (no-PFP and PFP), the average fish weight (119% increment) and length were not significantly higher.

The levelling off and drop in mean individual fish length and weight in the final months of the experiment were possible. This was not due to the fish getting smaller. Fish were harvested when they reached 300 g. As such, the larger fish were removed (i.e. harvested) after the preceding month's weigh and measure, which accounts for the levelling off and in some instances the drop in individual fish size towards the end of the experiment.

5.1.3. Plant production

The Swiss chard leaves were consistently larger in the drain-fill bed culture as opposed to the flooded system where the leaves remained almost constant in size during the six months of the

experiment. In the drain-fill bed culture, the chard growth kept increasing from month to month except for a trough in April. The Swiss chard total and wet weights were much higher in the drain-fill as opposed to the flooded aquaponics design. The drain-fill system produced more leaves, longer leaves and wider leaves. Vymazal (2007), the drain-fill system produced bigger Swiss chard platns, as compared to the flooded system. This gap in terms of leaf size in the flooded system is most likely due to constant water movement in the flooded bed leaving no room for roots fixation and plant stability (Somerville *et al.*, 2014).

The improved growth and health of Swiss chard in the drain-fill compared to the flooded has a possible explanation; that is, well oxygenated roots and improved nutrient availability. Schneller (2015) mentioned that difference in growth may be due to good nutrient cycling linked to the system, which was a result of more optimal water quality conditions. In terms of Swiss chard parameters and water quality, the two farming methods were clearly different during the five months of the current investigation. The drain-fill treatment produced more Swiss chard compared with the lower production in the flooded system. Inside the culture beds of the drain-fill system, dissolved and solid waste from fish, containing a considerable contribution of ammonia-rich nutrients, were more rapidly sequestered by accelerated nitrification processes in the presence of increased aeration. The leaves of Swiss chard in the drain-fill were dark green in colour with a higher chlorophyll-a concentration index and were larger than those in the flooded system (Table 5.3). They also showed no trace of necrotic spots which are often found in plants that are deprived of specific nutrients (Wang *et al.*, 2009). Improved growth was probably due to the link between DO and availability of nitrogen. The conversion of ammonia to nitrate increases at high DO concentrations (Kadlec and Wallace, 2008; Vymazal, 2014), which in turn promoted plant growth. The number and the length (148%) and the width (157%) and the stem and the roots weight of spinach leaves are also significantly higher in Experiment 2. Thus, the production and yield of fish and vegetables is higher in Experiment 2.

The PFP/drain-fill design encompasses chard individuals with higher leaf numbers, stem length, leaf length, leaf width, chlorophyll values, temperature and COD. The no-PFP aquaponic design includes samples with less chlorophyll-a, shorter stems, smaller number of leaves, less PO_4 and alkalinity, more NO_2 and NO_3, lower temperatures, and less DO, as compared to the no-PFP/drain-fill; thus, the no-PFP presents an intermediate performance. At the other extreme, the flooded design is characterized by lower temperature, lower COD, shorter stem as opposed to the drain-fill.

Table 5.3: Plant growth monthly dynamics tables by treatments for experiment in aquaponic system with primary facultative ponds (PFP) and no primary facultative ponds with drain-fill or flooded grow-beds.

Flooded / PFP	Drain-fill / PFP	Drain-fill / no-PFP
Numbers of leaves per plant was low from December to February and steadily increased to high levels from March to May	Numbers of leaves per plant were low in December, they steadily increased to high January and February, then decreased steadily to low levels in March and again increased to high levels in April and May.	Numbers of leaves per plant remained low from December until April then increased steadily to maximum in May.
Leaves length remained low from December to May.	Leave length increased steadily from low levels in December to high levels January and February, then decreased steadily to low levels in March, then increased steadily to high levels in April and May.	
Stem length remained at low levels from December to February and then increased steadily to high levels from March to May.	Stem length increased steadily from low levels in December to high levels in January and February, then decreased steadily to low levels in March then increased steadily to high levels from April to May	Stem length remained at low levels all over the experiment from December to May.
Chlorophyll-a remained at low levels from December to February, then increased to high levels and remained high from March to May.	Chlorophyll-a was at low levels in December, then increased steadily to high levels from January to February, then decreased steadily to low levels in March, then increased to high levels in April-May.	Chlorophyll-a levels remained low all over the experiment from December to May.

The benefit to plants, of maintaining nutrients in the systems using a PFP, might have been masked by too much nutrient produced by the fish. If nutrient balance is not maintained, then there is either too much nutrient for the plants or not enough nutrients for the plants (Schneider *et al*., 2005; Bernstein, 2013; Nicolae, 2014). In the current experiment, the size of the plant grow-bed and subsequent plant biomass, relative to the fish biomass in the system of the current study, might not have been adequately balanced. It is possible that due to there being too much fish biomass in the system, relative to the plant biomass, the benefit of the PFP (and the additional nutrients in the system) might not have been seen in the plant production results of the current study. This needs to be addressed in future work by re-doing the PFP vs no-PFP experiment after addressing this possible nutrient balance concern.

Overall system metabolic rates and fish/waste product yield will be variably influenced by temperature changes towards winter. The fish growth trends were declined towards May in all

the trials. Plant growth was less affected since leaf growth was supported by the widening thereof and increased chlorophyll production as a result of the shortened photoperiod cycle towards the winter.

5.2. Comparisons of the efficiency of the three experimental aquaponics: drain-fill, flooded and no-PFP

The drain-fill treatment produced better results in many regards: temperature, NO_2, NH_3, salinity, leaf width, chlorophyll-a, leaf length, leaf numbers, fish weight, fish length, K-condition factor, PO_4. Thus, the drain-fill resulted in more fish and plant production, with more fertility (PO_4, NO_3) and less toxicity (NH_3 to NO_2 conversion). This drain-fill aquaponics design was moderate in TDS, pH, DO and NO_3 and dry plant matters.

As discussed for fish production, the production of Swiss chard was best in the drain-fill aquaponics system (Hanssens, 2009). The drain-fill mechanism exposed air to the roots of terrestrial plants, like chard, whilst nitrification was enhanced by the mixing of air with the rising water in the media bed, which also served as a substrate for nitrifying bacteria (El Bouhy *et al.*, 2006; Mongirdas and Kusta, 2006). The nitrification end product (nitrate) was then rapidly taken up by the roots of the plants, hence the fastest growth of the plants for the applied trial (drain-fill).

A further notable feature was the ability of the plants to adapt to the gradual change in photoperiod and lowered ambient/water temperature towards winter, and without losing any biomass. Widening of the leaves increases surface area for photosynthesis, and the proliferative production of chloroplasts (Chlorophyll-a) compensated for the shortened photoperiod and apparent light intensity (Genello *et al.*, 2015; Palm *et al.*, 2018). This phenomenon makes chard a possible selection of choice for winter production in aquaponics.

The intervention of ammonifying microorganisms and plants is then necessary in a PFP treatment (Mancuso, 2013; Akan *et al.*, 2010). The results obtained in this work were comparable to those obtained in a free-flow system, in which ammonia does not pose a problem due to mixing and turbulent water flow is optimal (Yang and Kim, 2019). The high nutrient concentration levels, as well as similar oxygenation found in the PFP and no-PFP treatments were equal to those found in aquaponics in which the microorganisms and plants act as filters favouring improved function (Akan *et al.*, 2010). These nutrient levels are sufficient to support good production of hydroponic plants and were close to those found in this study (Resh, 2013). The hydraulic retention time in the PFP might not have been long enough, and this might be a

possible reason for the similarity between PFP and no-PFP in this experiment. The time that effluent spends in bioreactors dictates if the bioreactor will convert protein into ammonia (Aguilar *et al.*, 2012). In an aquaponic system, the hydraulic retention time depends on the water flow in the cultivation and breeding tanks (Yang and Kim, 2019). This is how we can have two types of flow or hydraulic retention (high and low flow). The high flow rate, which is recommended in aquaponics and is equal to a low hydraulic retention time. It is mainly used to prevent the accumulation of solid fish waste which can thus produce ammonia, which is toxic to aquatic life, as well as plants (Danaher *et al.*, 2013; Yang and Kim, 2019). For the high flow case (i.e. a low hydraulic retention time) it is suggested to use 38.40 $m^3 \cdot day^{-1}$ compared with 13.3 $L \cdot h^{-1}$ in this research, which is 5.91 times lower than that normally suggested in hydroponic production. In addition to this, in this test the PFP tank of the system was not continually connected to the loop which formed it. Based on this, in an aquaponic system the optimization of water flow needs more in-depth examinations, to optimally convert nitrogen in these three dimensions (ammonia, nitrites and nitrates) and for growth and agricultural production and fish farming (Li *et al.*, 2005; Danaher *et al.*, 2013). To this extent, aquaponics can be seen in the form of a wastewater treatment system, because it involves both nitrification and the elimination of solid wastes in these waters (Gräber and Junge, 2014). Thus, in order to optimise the performance of the aquaponic system operating conditions, PFPs were introduced for the treatment of solid waste from fish. Future research aimed at possibly optimizing how a PFP should be loop-connected in an aquaponic system and determining optimal hydraulic retention time should be considered so as to optimise the mineralisation of nutrients in the PFP.

The drain-fill was high in leaf width, chlorophyll-a, fish weight, fish length, PO_4, NO_3 and DO. There was no apparent benefit, based on the data presented in this work, for including a PFP. The differences with the drain-fill design were clearcut and it outperformed the flooded system. Thus, the best choice, regarding fish, vegetable and water quality was the drain-fill. The PFP had minimal influence on the performance of the system. The last choice was the flooded design.

5.3. Effects of water quality on fish and vegetable production

There are factors which directly correlate with the fish and vegetable production and indirect factors which influence the direct ones. Figure 5.1 presents Spearman (rs) correlations for the direct factors.

Of course, the vegetable production indicators were correlated between themselves: leaf length, leaf width, stem length, and chlorophyll production ($p<0.001$). Interestingly, these vegetable production factors were also correlated with catfish production, i.e. fish length and width. So, while the chemistry effects the plant, the fish were influenced by the toxicity levels in their environment as well as temperature and the dissolved oxygen levels for gill respiration. The pH negatively impacted vegetable production. When the pH diminished, the Swiss chard production increased.

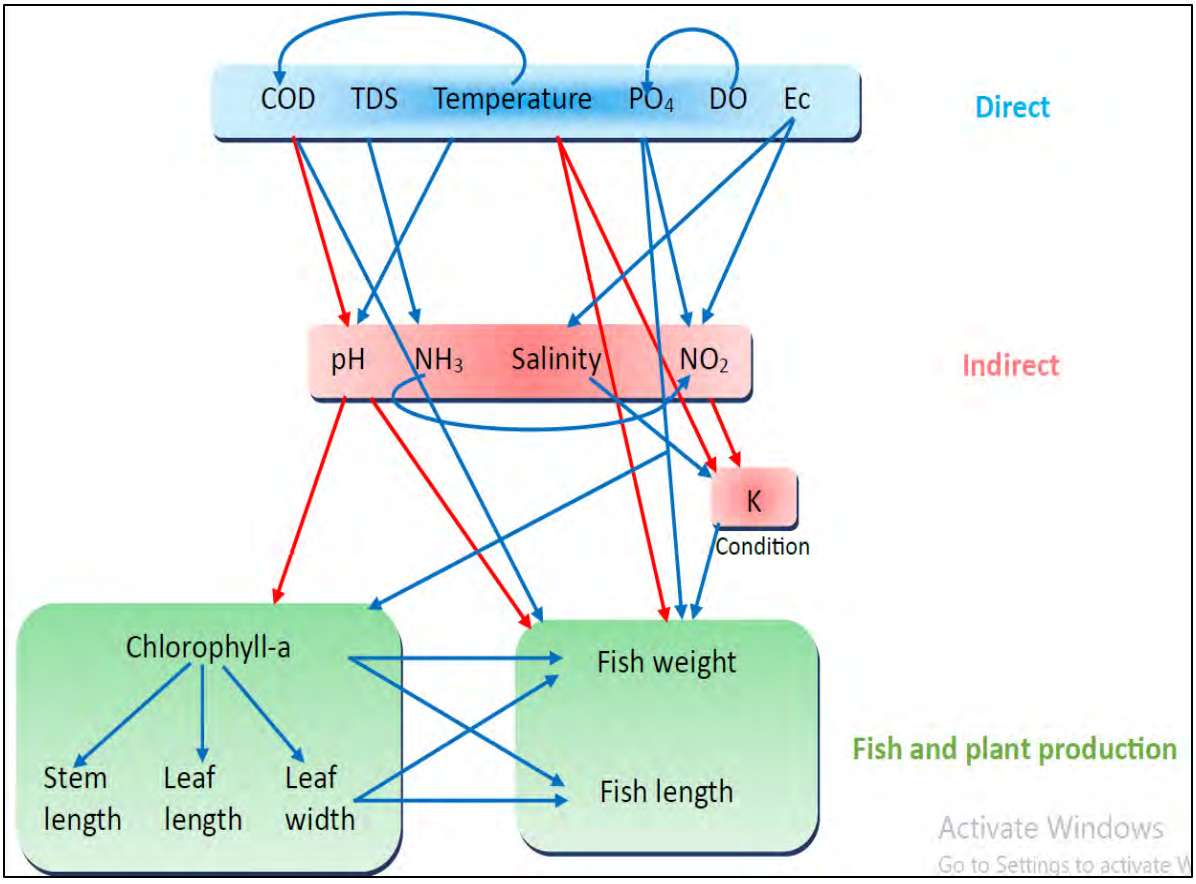

Figure 5.1: Direct and indirect effects of water quality parameters (Chemical oxygen demand, Total dissolved solids, Phosphates, Dissolved oxygen, Electrical conductivity, Hydrogen potential, Ammonia, Nitrite) on vegetable and fish production (K is the condition factor) (red=inverse effect blue=positive)

When these relationships are checked in a multivariate approach, the principal components analysis using also the path diagram, it is remarkable that leaf length, leaf width, stem length, chlorophyll-a, fish weight and fish length are strongly correlated between each other and with the production component (RC1=principal component 1), where leaf number is not associated here. Further, fish weight and fish length are also correlated to a moderate extent with some chemical and physical parameters: i.e TDS, NH_3, PO_4 and negatively with pH and temperature (Figure 5.2).

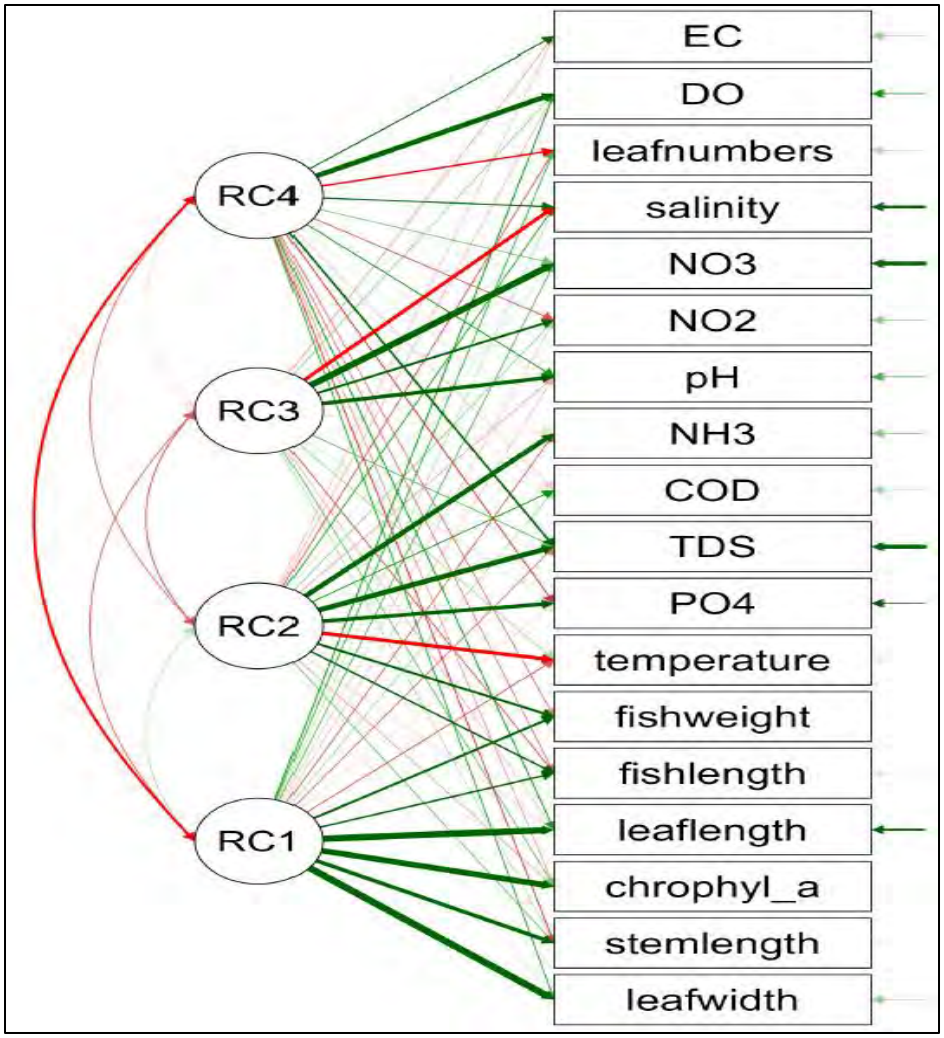

Figure 5.2: Path diagram showing the exploratory principal component analysis (PCA) components between the study variables of water quality parameters (including chemical oxygen demand (COD), total dissolved solids (TDS), phosphates, dissolved oxygen (DO), electrical conductivity (EC), hydrogen potential (pH), ammonia and nitrite and nitrate) on vegetable and fish production.

These water quality parameters are strongly correlated between themselves and also with the pH-nitrogen-salinity component (RC 2=PC 2); where pH and NO_2 play a positive role, while salinity plays a negative role therein. The nitrates (NO_3) are strongly correlated with the pH-nitrogen-salinity component (RC 3=PC 3), where pH and NO_2 play a positive role, while salinity plays a negative role (Figure 5.2). The fourth component moderately encompasses TDS, salinity and EC, and mainly DO. The latter component negatively correlates with leaf numbers per plant. This 4^{th} component is mostly, though negatively correlated with the production component (RC 1= PC 1) for Swiss chard and catfish (Figure 5.2). Fatima *et al.*, (2022), were doing the principal components analysis (PCA) to compare the sampling sites based on physico-chemical, metals, and biological parameters; the higher concentration of metals exceeding the biological parameters.

The chemical oxygen demand was significantly higher in the no-PFP aquaponics at certain times in the experiment. But simultaneously, higher COD levels stimulated the reduction of pH towards neutrality, thus aquaponic (El Bouhy *et al.*, 2006; Mongirdas and Kusta, 2006). However, pH was similar in all of the three aquaponic systems.

Whenever alkalinity increased, vegetable and fish production decreased (Hargreaves *et al.*, 2005; Maucieri *et al.*, 2018). There is a doubt on the way NH_3 data were collected without deducing the effects of acidity. When (NH_3-NO_2) toxicity increased, it negatively impacted on the fish K-condition factor, and subsequently reduced fish production (Schneider *et al.*, 2005; Bernstein, 2013). Thus NH_3-NO_2 toxicity is the complex which mostly affected fish production in the drain-fill aquaponic, given the fact that NO_2 levels were moderate in the flooded and the low in the no-PFP aquaponics.

Fish and plant growth were similar in PFP and non-PFP because water quality parameters were similar in both treatments. MacIntrye *et al.* (2008) and Hickey *et al.* (2018) focused on fish welfare in aquaponic systems: its relationship with water quality with a focus on feed and faeces, found that the parameters of water quality are the primary environmental consideration for optimizing aquaponics production and have a direct impact on fish welfare/health issues and plant needs. Their results were similar to those of this research (MacIntrye *et al.*, 2008; Hickey *et al.*, 2018). This similarity may be due to two conditions: (1) in no-PFP, the solid wastes present in these waters from fish tanks was removed from the system. (2) In the PFP, the solid waste of this water coming from the fish tanks was subjected to biodegradation or biotransformation of organic and/or inorganic compounds by an aerobic biomass, consisting mainly of microorganisms (bacteria and algae) (Delaide *et al.*, 2017). The PFP played the role of a "mechanical" solid settling filter. This treatment also contributed to nitrogen treatment in the water, whereafter this was removed in the hydroponic vegetable grow-beds (Somerville *et al.*, 2014; Delaide *et al.*, 2017). These two treatments were suitable for removing ammonia from an aquaponics system. They had similar plant and catfish growth, due to the similar water quality between the two.

Biological treatment systems that convert solid nitrogenous waste (i.e., protein) into dissolved ammonia, take long periods to mature, and this can be many months (Somerville *et al*, 2014). If the PFPs in this experiment were fully functional and mature, the solid waste would be converted into dissolved ammonia, and thus the dissolved ammonia coming out of the PFP should then be higher than the dissolved ammonia going into the PFP. In this trial, although it

appeared that post-PFP ammonia levels coming out of the PFP were starting to increase after about four months, by month five the ammonia levels coming out of the PFP were still not higher than the ammonia levels going into the PFP. This suggests that the PFP might not have been mature. Future research should look at running this trial for a longer period and optimising the hydraulic retention time in the PFP, and these might see a difference in PFP and no-PFP. On the other hand, there were always a surplus of nutrients available, which implied that more Swiss chard could have been maintained by the system and that the grow bed size was limited in the experimental design. Again, future work should also optimise the nutrient balance between vegetable grow-bed size and fish biomass in future work.

Chapter 6. General conclusions

In conclusion, this study, which focused on drain-fill technology and solids retained or removed from an aquaponic system, shows that aquaponics is a prospective solution to support food security. Aquaponics has the potential to solve both dietary and nutritional issues for good food security. This technology, once developed and implemented in a country, can contribute significantly to meeting its food needs, because it can produce plants and fish simultaneously. This was clearly demonstrated by the results provided in the studies of drain-fill technology and the removal of solids in aquaponics. The aim of this study was to evaluate the performance of primary facultative pond (PFP) and drain-fill technology in aquaponics. This was done by comparing water quality parameters, growth and health of African catfish (*Clarias gariepinus*) and Swiss chard (as a crop) between conventional systems, where solids are removed, and those with PFPs that maintain and treat solid waste as a loop in the system. In addition, flooded grow beds were compared to drain-fill grow beds using drain-fill technology. This will shed light on the nutrient balance in aquaponic systems.

The comparisons of the efficiency of the three experimental aquaponics: drain-fill, flooded and no-PFP became imperative. This design was medium in the TDS, pH, temperature, NO_2, NH_3, and salinity contents. The flooded design was clearly low both in fish and vegetable production. The drain-fill performed very well in many regards: temperature, NO_2, NH_3, salinity, leaf width, chlorophyll-a, leaf length, leaf numbers, fish weight, fish length, K-condition factor, PO_4. Thus, the drain-fill was dominant in fish and plant production, with more fertility (PO_4, NO_3) and less toxicity (NH_3 to NO_2 conversion). The addition of a PFP was mostly inconsequential, with no significant effect on fish and plant production.

Thus, the best choices, regarding fish, vegetable and water quality was the drain-fill. The last choice would be the flooded design.

The maintenance effort was similar between treatments, and the cost of an additional tank to form a primary facultative to retain solid waste, was low. In a commercial plant, the no-PFP will require much more time and cash investment for solid retaining as compared to the PFP. The chemical oxygen demand was significantly higher in the no-PFP aquaponics at certain times, implying negative impacts on water quality, which will mostly likely positively correlate with the biological oxygen demand, both being indicators of water pollution levels. In fact, there are biodegradable wastes from fish excreta and unused feed. But simultaneously, higher COD levels stimulated the reduction of pH towards neutrality, thus boosting vegetable

production and fish production within the same no-PFP aquaponic. However, pH was similar in all of the three aquaponic systems. The K-condition factor was significantly higher in the drain-fill, especially in April, when it went beyond 1.0. Salinity was higher in the drain-fill, boosting the K-condition vs the no-PFP. The drain-fill aquaponics yielded the biggest fish in length and weight, and the highest K-condition factor value, as well as the biggest Swiss chard and the highest number of leaves on a plant.

The specific growth rate (SGR) was 1.65 times higher in the drain-fill as opposed to the flooded. Notwithstanding, there were always a surplus of nutrients available, which implied that more Swiss chard could have been grown in the system and that the grow bed size was probably limited in the experimental design. The surplus of nutrients therefore might have masked the possible effect of the PFP nutrients, which could have made an impact on the gross production of Swiss chard should the grow bed have been bigger and the nutrient budget was more depleted. Added to this the increased footprint growth of chard and filter bed could also have minimized the recirculation content of toxic ammonia and nitrite that will impact the welfare and growth of fish otherwise. This adjustment is called nutrient balancing an important concept in aquaponics (Delaide *et al.*, 2017).

The benefit of adding a PFP, and the additional nutrients that are maintained in the system, might have been masked by the biomass of fish in the system relative to the biomass of the plants. In aquaponics, it is important to balance the size of the grow-bed (i.e. potential plant biomass) with the biomass of the fish (Li *et al.*, 2005; Danaher *et al.*, 2013). If there is too much fish biomass, there will be excess nutrients for the plants (Hanssens, 2009). In this experiment, plants grew equally well in the PFP and no-PFP treatment. There might have been too much fish biomass in both systems, so the plants in the no-PFP had sufficient nutrient to grow as well as those in the PFP treatment, and the additional nutrients available due to the PFP would therefore have had no influence on plant growth because the plants might already have had access to sufficient nutrients. Future work should look at first optimising the fish biomass with vegetable grow-bed size, before introducing the addition of a PFP. Furthermore, the PFP was functional for five months, i.e., the entire duration of the experiment. Since nutrient input rates influence the functioning of a PFP and since it takes time for a PFP to mature before it starts to operate optimally, further research should investigate inoculation frequency and the time it takes for a PFP to mature.

Future research

The following should be considered in future research:

- To increase the sampling size of the tanks, adjust flow rates to optimize the retention time of solid waste effluent in the PFP, and have the PFP included in the loop system with an optimized hydraulic retention time.
- To carry out some microbiological analyzes such as molecular identity of some isolated microorganisms (sanger sequencing) which colonize these wastewaters samples before and after treatment to investigate whether they may have plant growth promoting-like activities. The metagenomic analysis should also be performed to have an idea of all possible microbial contents in water sample.
- Nutrient balancing based on the findings of the current project in terms of reducing the surplus of nutrients by increasing the size of the plant grow bed. This will enhance plant biomass harvest, as well as improving water quality and concomitant fish growth. Fish stock densities must be optimized on trial-and-error basis, to achieve a nutrient balance, before other factors are investigated.
- Testing the real value of PFP when nutrient loads are depleted in the nutrient balanced system, showing the unmasked nutrient providing capacity of PFP's as a source of alternative mineralized waste product.
- Testing the drain-fill aquaponics model in winter, using value-added cold water fish species or carrying out the work in summer only using warm water species. Temperature should be given greater consideration in future work. A fish species such as value-added koi carp *(Cyprinus rubrofuscus*) or ornamental goldfish (*Carassius auratus*) can be used for all season aquaponics if unheated tunnels are used. African catfish should only be used form mid-spring to mid-autumn in tunnels in temperature regions such as South Africa. Thus, it will be interesting to repeat the same bioassays in October-April, when temperature will be in an increasing pattern, though still keeping the optimal range. Phosphate levels were high within all of the three aquaponics.

References

Akan, J.C., Abdulrahman, F.I., Yusuf, E. 2010. Physical and chemical parameters in abattoir wastewater sample, Maiduguri Metropolis, Nigeria. *The Pacific Journal of Science and Technology* 11(1), 640-648.

Al Bloushi, A. I. H. 2018. Effect of stocking density using tilapia *Oreochromis niloticuson* aquaponic system and cultivation of cherry tomato *Solanum lycopersicum* under UAE condition. MSc thesis, United Arab Emirates University.

Antonio, P., Chris, S., Christophe, S. 2013. Aquaponics- a fish-based intelligence solution to produce food using limited resources, and little water/*Smart Fish Program1*.

Aguilar, C., Zinnert, J. C., Polo, M. J., Young, D. R. 2012. NDVI as an indicator for changes in water availability to woody vegetation. *Ecological Indicators*, *23*, 290-300.

APHA. 2005. Standard methods for the examination of water and wastewater. 21st Edition, *American Public Health Association/American Water Works Association/Water Environment* Federation. Washington DC.

Artur, J.P. 1983. Notes on the design and operation of waste stabilization ponds in warm climates of developing countries. *The word bank + UNDP*, Washington.

Atwater, W.O., Bryant, A.P. 1900. The availability and fuel value of food materials. 12th Annual report (1899) of the Storrs, CT *Agricultural Experiment Station*, 73-110.

Barua, S.P., Khan, M.M.H., Ali, R. A.H.M. 2000. The Status of Alien Invasive Species in Bangladesh and their Impact on the Ecosystems. IUCN – *The World Conservation Union, Bangladesh*.

Bernstein, S. 2013. Aquaponic gardening, New Society Publishers. *Gabriola Island*, Canada.

Bolger, T., Connolly, P.L. 1989. the selection of suitable indices for the measurement

and analysis of fish condition. *Journal of Fish Biology* 34, 171-182.

Bosire, C.M., Sikora, T.A. 2017. Blue-green farms of the future: using aquaponics at primary school to foster sustainable development. Challenges of sustainable and commercial aquaponics. *Journal of Sustainability* 7, 4199-4224.

Bouma, T. J., De Vries, M.B., Low, E., Kusters, L. 2005. Flow hydrodynamics on a mudflat and in salt marsh vegetation: identifying general relationships for habitat characterization. *Hydrobiologia* 540, 259–274.

Bowers, D.G., Brett, H.L. 2008. The relationship between CDOM and salinity in estuaries: An analytical and graphical solution. A study published in the *Journal of Marine Systems* 73, 0924-7963.

Brikké, F., Bredero, M. 2003. Linking technology choice with operation and maintenance in the context of community water supply and sanitation: A reference document for planners and project staff. Geneva: *WHO and IRC Water and Sanitation Centre*, 27 p.

Britz, P.J., Hecht, T. 1987. Temperature preferences and optimum temperature for growth of African sharptooth catfish (*Clarias gariepinus*) larvae and post larvae. *Aquaculture* 63(1–4), 205–214.

Christensen, M.H., Harremoes, P. 1978. Nitrification and denitrification in wastewater treatment. The chemistry and microbiology of pollution, Edition Willey, N.Y., 391-364. De Cuyper K. et Loutz S. (1992).

Clunie, P., John, K. 2001. Freshwater catfish: A resource document. Freshwater ecology institute for the environmental research department of natural resources and environment, Final Report. *Heidelberg* VIC 3084.

Coche, A.G. 2014. Fish culture in rice fields is a worldwide synthesis. *Hydrobiological* 30 (1), 1-44.

Cowey, C.B. 1992. Nutrition: estimating requirements of rainbow trout. *Aquaculture* 100, 177- 189.

Cox, N. J. 2002. Speaking Stata: How to move step by: step. *Stata Journal* 2, 86–102.

Danaher, J.J., Shultz, R.C., Rakocy, J.E., Bailey, D.S. 2013. Alternative Solids

Removal for Warm Water Recirculating Raft Aquaponic Systems. *Journal of the World Aquaculture Society* 44, 374-383.

Delaide, B., Delhaye, G., Dermience, M., Gott, J., Soyeurt, H., Jijakli, M.H. 2017. Plant and fish production performance, nutrient mass balances, energy, and water use of the PAFF Box, a small-scale aquaponic system. *Aquacultural Engineering*, 78, 130-139.

DeLong, E.F. 2009. The microbial ocean from genomes to biomes. *Nature*, *459*, 7244, 200-206.

De Moor, I.J., Bruton, M.N. 1988. Atlas of alien and translocated indigenous aquatic animals in southern Africa. *South African National Scientific Programmes Report* 114, South Africa.

Diver, S. Rinehart, L. 2010. Aquaponics-integration of hydroponics with aquaculture; ATTRA NCAT: Butte, MT, USA, 28.

El-Bouhy, Z.M., Saleh, G.A., El-Nobi, G., Ahmed, R.R.M. 2006. Study on The Effect of Chlorine on Health and Growth of Some Ornamental Fishes. *Zagazig Veterinary Journal* 34(2), 164-172.

FAO (Food and Agriculture Organization of the United Nations). 2012. World agriculture towards 2030/2050. *Agricultural Development Economics Division*: Rome, Italy.

FAO (Food and Agriculture Organization of the United Nations). 2014. Waste management of fish and fish products. Goddek, S. 2017. Opportunities and challenges of multi-loop aquaponic systems. *Wageningen University*, Wageningen.

FAO (Food and Agriculture Organization of the United Nations). 2015. Land and Property Rights: Junior Farmer Field and Life School, facilitator's guide. Rome, Italy: *Aquaculture*, 1-64.

Fatima, A., Shafi, I., Afzal, H., Díez, I. D. L. T., Lourdes, D. R. S. M., Breñosa, J., Ashraf, I. 2022. Advancements in dentistry with artificial intelligence: current clinical applications and future perspectives. In *Healthcare* 10, 11, 2188. MDPI.

Fernandes, IM., Bastos, Y.F., Barreto, D.S., Lourenço, L.S., Penha, J.M. 2017. The efficacy of clove oil as an anesthetic and in euthanasia procedures for small-sized tropical fishes. *Brazilian Journal of Biology* 77 (3), 444-450.

Fowler, D., Coyle, M., Skiba, U., Sutton, M.A., Cape, J. N., Reis, S., Sheppard, L.J., Jenkins, A., Grizzetti, B., Galloway, J.N, Vitousek, P.L.A., Bouwman, A.F, Butterbach-Bahl, K., Dentener, F., Stevenson, D., Amann, M., Voss, M. 2013. The global nitrogen cycle in the twenty-first century. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 368 (1621), 20130164.

Fox, B.K., Tamaru, CS., Radovich, T.J.K., Klinger-Bowen, R., Mcgovern-Hopkins, K., Bright, L., Pant, A., Gurr, I., Sugano, J., Sipes, B., Lee, C. N. 2012. Beneficial use of vermicompost in aquaponic vegetable production. *The Food Provider*, 1-5.

Francis-Floyd, R., Watson, C., Petty, D., Pouder, D.B. 2009. Ammonia in aquatic systems. Gainesville: The University of Florida, *Institute of Food and Agricultural Sciences* (IFAS) Extension, 16.

Friedman, O., Dubinsky, Z., Arad, S. 1991. Effect of light intensity on growth and polysaccharide production in red and blue-green Rhodophyta unicells. *Bioresource Technology* 38, 105-110.

Genello, L., Fry, J.P., Frederick, J.A., Li, X., Love, D.C. 2015. Fish in the classroom: a survey of the use of Aquaponics in education. *European Journal of Health and Biology Education* 4 (2), 9-20.

Ghaly, A.E., Kamal, M., Mahmoud, N.S. 2005. Phytoremediation of aquaculture wastewater for water recycling and production of fish feed. *Environ. Int* 31, 1-13.

Goddek, S. 2017. Opportunities and challenges of multi-loop aquaponic systems. *ProQuest Dissertations & Theses Global.*

Goddek, S., Schmautz, Z., Scott, B., Delaide, B., Keesman, K.J., Wuertz, S., Junge, R. 2016. The effect of anaerobic and aerobic fish sludge supernatant on hydroponic lettuce. *Agronomy* 6 (2), 37.

Gopalakrishnan, T.R. 2007. Vegetable crops. New India Publishing.

Graber, A., Antenen, N., Junge, R. 2014. The multifunctional aquaponic system at ZHAW used as a research and training lab. *Third conference with international participation*. Conference Paper 245-255.

Greenfield, H., Southgate, D.A.T. 2003. Food Composition Data: Production, Management, and Use. *Rome*: FAO.

Hanssens, M. 2009. A review of the Clarias species (Pisces; Siluriformes) from the Lower Congo and the Pool Malebo. *Journal of Afrotropical Zoology* 5, 27-40.

Hargreaves, J.A., Tucker, C.S., Thornton, E.R, Kingsbury, S.K. 2005. Characteristics and sedimentation of initial effluent discharged from excavated levee ponds for channel catfish. *Agris* 33 (2), 96-109.

Hickey, G. L., Mokhles, M. M., Chambers, D. J., Kolamunnage-Dona, R. 2018. Statistical primer: performing repeated-measures analysis. *Interactive cardiovascular and thoracic surgery*, 26(4), 539-544.

Hochmuth, G.J., Hanlon, E.A. 2010. Commercial vegetable fertilization principles 17.

Hughey, T. 2005. Aquaponics for Developing Countries, 16–18. Innovative Aquaponics for Professional Applications (INAPRO).

Hussain, I., Barzilai, O., Reiner, A. S., DiStefano, N., McLaughlin, L., Ogilvie, S., Laufer, I. 2018. Patient-reported outcomes after surgical stabilization of spinal tumors: symptom-based validation of the Spinal Instability Neoplastic Score (SINS) and surgery. *The Spine Journal*, 18(2), 261-267.

Jensen, F.B. 2003. Nitrite disrupts multiple physiological functions in aquatic animals. *Comparative Biochemistry & Physiology*. A, 135, 9-24.

John, A.H., Craig, S.T., Ellen, R.T. 2005. Characteristics and sedimentation of initial effluent discharged from excavated levee ponds for channel catfish [J]. *Aquaculture Engineering* 33, 96-109.

Jones, S. 2002. Evolution of Aquaponics. *Aquaponics Journal* 6 (4), 23-54.

Joyce, A., Goddek, S., Kotzen, B., Wuertz, S. 2019. Aquaponics: Closing the Cycle on Limited Water, Land and Nutrient Resources, Aquaponics: Food Production Systems- Combined Aquaculture and Hydroponic Production Technologies for the Future, 19- 34.

Junge, R., König, B., Villarroel, M., Komives, T., Jijakli, M.H. 2017 Strategic points in aquaponics. *Water* 9, 182.

Junge, R., Wilhelm, S., Hofstetter, U. 2014. Aquaponic in classrooms as a tool to promote system thinking. In: Transmission of innovations, knowledge, and practical experience into everyday practice. Agriculture, environmentalism, horticulture and floristics, food production, and processing and nutrition. Conference Paper 1-11.

Kadlec, R.H., Wallace, S. 2008. Treatment Wetlands. CRC press, New York, United States of America.

Khandaker, M., Kotzen, B. 2018. The potential for combining living walls and vertical farming systems with aquaponics with special emphasis on substrates. *Aquaculture Research* 49 (4), 1454-1468.

Khokhar, T. 2017. Chart: Globally, 70% of fresh water is used for agriculture. *Data Blog*.

Kim, B., Kang, Z., Ramanan, R., Choi, J., Cho, D., Oh, M., Kim, H. 2014. Nutrient removal and biofuel production in high-rate algal pond using real municipal wastewater. *Journal of Microbiology and Biotechnology* 24 (8), 1123-1132.

Kiple, K.F. 2002. Cambridge World History of Food. Cambridge. The United Kingdom.

Koch, E.M. 2001. Beyond light: physical, geological, and geochemical parameters as possible submersed aquatic vegetation habitat requirements. *Estuaries* (24), 1-17.

Laidlaw, J., Magee, L. 2016. Towards urban food sovereignty: the trials and tribulations of community-based aquaponics enterprises in Milwaukee and Melbourne. *Local Environment* 21 (5), 573-590.

Lanl. 2016. The periodic table of Elements, Nitrogen. Chemistry Division U.S.

Department ofEnergy.

Lennard, W.A., Leonard, B.V. 2004. Comparison of Reciprocating Flow Versus Constant Flow in An Integrated, Gravel Bed, Aquaponic Test System, Aquaculture Int. *Kluwer Academic Publishers*, 12, 539-553.

Lennard, W.A. 2012. Aquaponic System Design Parameters: *Aquaponics* 3, 10.

Lennard, W.A., Leonard, B.V. 2004. Comparison of Reciprocating Flow Versus Constant Flow in An Integrated, Gravel Bed, Aquaponic Test System, *Aquaculture International. Kluwer Academic Publishers* 12, 539-553.

Li, W., Li, Z. 2009. In situ nutrient removal from aquaculture wastewater by aquatic vegetable Ipomoea aquatica on floating beds. *Water Science Technology* 59, 1937-1943.

Li, W., Liu, X., Khan, M.A., Yamaguchi, S. 2005. The effect of plant growth regulators, nitric oxide, nitrate, nitrite and light on the germination of dimorphic seeds of Suaeda salsa under saline conditions. *Journal of Plant Research* 118, 207–214.

Li, C., Zhang, B., Luo, P., Shi, H., Li, L., Gao, Y., Wu, W. 2019. Performance of a pilotscale aquaponics system using hydroponics and immobilized bio film treatment for water quality control. *Journal of Cleaner Production* 208, 274-284.

Li, F., Lu, L., Zheng, X., Zhang, X. 2014. Three-stage horizontal subsurface flow constructed wetlands for organics and nitrogen removal: effect of aeration. *Ecological Engineering* 68, 90-96.

Liddell, H., Scott, R. 1980. A Greek-English Lexicon (Abridged ed.). United Kingdom: *Oxford University Press*. ISBN 978-0-19-910207-5.

Lockhart, E.E., Tucker, C.L., Merritt, M.C. 1955. The effect of water impurities on the Flavors of brewed coffee. *Food Research* 20, 598.

Love, D.C., Fry, J.P., Genello, L., Hill, E.S., Frederick, J.A., Li, X., Semmens, K. 2014. An international survey of aquaponics practitioners. *PLoS One* 9 (7), e102662.

Love, D.C., Uhl, M. S., Genello, L. 2015. Energy and water use of a small-scale raft aquaponics system in Baltimore, Maryland, United States. *Aquacultural Engineering* 68, 19-27. aquaeng.

MacIntrye, M.C., Ellis, T., North, B.P., Turnbull, J.F. 2008 the Influences of water quality on the welfare of farmed rainbow trout: A Review. In Fish Welfare; Branson, E.J., Ed.; Blackwell Publishing Ltd.: Oxford, UK, pp. 150-178.

Mancuso, M. 2013. Fish welfare in aquaculture. *Aquaculture Research Development* 4, 107.

Mara, D.D. 1997. Design manual for waste stabilization ponds in India. Lagoon technology international Ltd., Leeds England.

Mateo-Sagasta, J., Zadeh, S., Turral, H., Burke, J. 2017. Water pollution from agriculture: a global review-Executive summary, Food, and Agriculture Organization of the United Nations Rome, Italy, *International Water Management Institute*, Colombo, Sri Lanka, 29.

Maucieri, C., Forchino, A.A., Nicoletto, C., Junge, R., Pastres, R., Sambo, P., Borin, M. 2018. Life cycle assessment of a micro aquaponics system for educational purposes built using recovered material. *Journal of Cleaner Production* 172, 3119-3127.

Mchunu, N., Lagerwall, G., Senzanje, A. 2019. Aquaponics model specific to South African conditions. *South African Journal of Agricultural Extension*. 47(1), 73-91.

Mchunu, N., Odindo, A., Muchaonyerwa, P. 2018. The effects of urine and urine-separated plant nutrient sources on growth and dry matter production of perennial ryegrass (Loliu perenne. L), *Agricultural Water Management*, 207, 37-43.

Miller, D.G., Brayton, S.V., Byles, W.T. 2001. Chemical oxygen demand analysis of wastewater using trivalent manganese oxidant with chloride removal by sodium bismuthate pretreatment. *Water environment research* (73), 1, 63-71.

Mitchell, M. N. 2010. Data Management Using Stata: A Practical Handbook. College Station, TX: Stata Press.

Mongirdas, V., Kusta, A. 2006. Oxygen mass balance in a recirculation aquaculture system for raising European Wels (Silurus glanis L.). *Ekologija* (4), 58-64.

Moore, W.A., Kroner, R.C., Ruchhoft, C.C. 1949. Dichromate Reflux Method for Determination of Oxygen Consumed. *Analytical Chemistry* 21, (8), 953-957.

Moore, W.A., Ludzack, F.J., Ruchhoft. C.C. 1951. Determination of Oxygen-Consumed Values of Organic Wastes Analytical Chemistry 23, (9), 1297-1300.

Naddafi, K., Hassanvand, M.S., Dehghanifard, E., Faezi, R.D., Mostofi, S., Kasaee, N.,Nabizadeh, R., Heidari, M. 2009. Performance evaluation of wastewater stabilization ponds in Arak-Iran. *Iranian Journal of Environmental Health Science and Engineering* 6 (1), 41-46.

Nawwar, Z.M. 2016. The Production of Catfish and Vegetables in an Aquaponic System, *Fisheries and Aquaculture Journal* 7 (4), 1-3.

Nelson, N., Siegel, D. 2014. CDOM in the Global Ocean. Presentation. Retrieved from SLS-2014/Siegel_CDOM_Nelson.pdf

Nicolae, C.G. 2014. Startup stages of a low-tech aquaponic system. *Scientific Papers*. Series D. Animal Science, LVII, 263-269.

Okeke, C.O., Igboanua, A.H. 2003. Characteristics and quality assessment of surface water and groundwater resources of Akwa Town, Southeast, Nigeria. *Journal of the Nigerian Association of Hydrology and Geology*. 14, 71-77.

Pagand, P., Plancheton, J.P., Casellas, C. 2000. A model for predicting the quantities of dissolved inorganic nitrogen released in effluents from a sea bass (Dicentrarchus Labrax) recalculating water system. *Aquaculture Engineering* (22), 137-153.

Palm, H., Knaus, U., Appelbaum, S., Goddek, S., Strauc, S., Vermeulen, T., Jijakli, M., Kotzen, B. 2018. Towards commercial aquaponics: a review of systems, designs, scales, and nomenclature, *Aquaculture International*, 26 (4), 813-842.

Papatryphon, E., Petit, J.S., Van Der W.H.M., Kaushik, J., Kanyarushoki, C. 2005. Nutrient balance modeling as a tool for environmental management in aquaculture: the case of trout farming in France. *Environmental Management* 35 (2), 16.

Peralta, G., Perez-Lloréns, J.L., Hernandez, I., Brun, F., Vergara, J.J, Bartual, A., Galvez, J.A., Garcia, C.M. 2000. Morphological and physiological differences between two morphotypes of Zostera noltii Hornem. from the south-western Iberian Peninsula. *Helgoland Marine Research* 54, 80-86.

Peralta, G., Perez-Lloréns, J.L., Hernandez, I., Vergara, J.J. 2002. Effects of light availability on growth, architecture, and nutrient content of the seagrass *Zostera noltii* Hornem. *Journal of Experimental Marine Biology and Ecology* 269 , 9-26.

Person-Le Ruyet, J., Labbé, L., Le Bayon, N., Sévère, A., Le Roux, A., Le Delliou, H., Quéméner, L. 2008. Combined effects of water quality and stocking density on welfare and growth of rainbow trout (*Oncorhynchus mykiss*). *Aquatic Living Resources* (21), 185-195

Post note 2006. Food security in developing countries. Parliamentary Office of Sciences and Technology 1-4.

Rakocy, J.E. 1989. Island perspectives; Virgin Islands Agricultural Experiment Station: Saint Croix, VI, USA, 5-10.

Rakocy, J.E. 1999. The status of aquaponics. Part 2. *Aquaculture Magazine* 25, 64-70.

Rakocy, J.E. 2012. Aquaponics-Integrating fish and plant culture; Wiley-Blackwell: Hoboken, NJ, USA, 344-386.

Rakocy, J.E., Bailey, D.S., Shultz, C., Thoman, E. 2004. Update on tilapia and vegetable production in the UVI aquaponic system. 676-690. In Bolivar R., Mair, G., Fitzsimmons, K., (Ed.). New dimensions in farmed tilapia: Proceedings from the 6th International Symposium on Tilapia in Aquaculture. Bureau of Fisheries and Aquatic Resources, Manila, Philippines.

Rakocy, J.E., Hargreaves, J.A. 1993. Integration of vegetable hydroponics with fish culture: A Review. In: Wang JK, Techniques for Modern Aquaculture.

American Society of Agricultural Engineers, 112-136.

Rakocy, J.E., Masser, M.P., Losordo, T.M. 2006. Recirculating aquaculture tank production systems: Aquaponics-Integrating fish and plant culture. Southern Regional Aquaculture Center, 454.

Ram, S. A., Irfan, Z.B. 2021. Application of system thinking causal loop modeling in the understanding water crisis in India: A case for sustainable integrated water resources management across sectors. *HydroResearch* 4, 1-10.

Ramanan, R., Kim, B.H., Cho, D.H., Oh, H. M., Kim, H. S. 2016. Algae-bacteria interactions: Evolution, ecology, and emerging applications. Review. *Biotechnology Advances* 34 (1), 14-29.

Resh, H.M. 2013. Hydroponic food production: A Definitive Guidebook for the Advanced Home Gardener.

Ricker, W.E. 1979. Growth rates and models. In: Hoar DJ, Randall DJ, Brett, JR (eds). Fish Physiology, Volume VIII. Academic Press, New York, United States of America.

Rodier, J. 2009. Water analysis. 9th edition, Dunod, Paris, 1365.

Rupasinghe, J.W., Kennedy, J.O.S. 2010. Economic Benefits of Integrating a Hydroponic- Lettuce System into a Barramundi Fish Production System. *Aquaculture Economics and Management*, 14, (2), 81-96.

Sabwa, A.J. 2021. Effects of stocking density on growth, water quality, and economic performance of monosex Nile Tilapia (Oreochromis niloticus) reared in an aquaponics system. PhD Thesis, University of Eldoret, Kenya.

Sánchez, H.J.A. 2014. Aquaponics and its potential aquaculture wastewater treatment and human urine treatment. (Master's thesis, Universidade NOVA de Lisboa (Portugal))

Sarker, U., Oba, S. 2019. Antioxidant constituents of three selected red and green color Amaranthus leafy vegetable. *Scientific reports* 9 (1), 18233.

Saxton, K.E., Rawls, W.J. 2006. Soil water characteristics by texture and organic

matter for hydrologic solutions. *Soil Science Society of America Journal*, 70, 1569-1578.

Scheidegger, B., Wilhelm, S. 2006. Bildungsarbeit mit Aquaponic-Systemen. Unpublished.

Schneider, O., Sereti, V., Eding, E.H, Verreth, J.A.J. 2005. Analysis of nutrient flows in integrated intensive aquaculture systems. *Aquacultural Engineering* 32, 379-40.

Schneller, A.J. 2015. A case study of indoor garden-based learning with hydroponics and aquaponics: evaluating pro-environmental knowledge, perception, and behavior change. *Applied Environmental Education and Communication* 14 (4), 256-265.

Schober, P., Vetter, T.R. 2018. Repeated measures designs and analysis of longitudinal data: if at first you do not succeed-try, try again. *Anesth Analg* 127(2), 569–575.

Shava, E., Gunhidzirai, C. 2017. Fish farming as an innovative strategy for promoting food security in drought risk regions of Zimbabwe. Jàmbá: *Journal of Disaster Risk Studies* 9 (1), 491.

Shilton, A.N., Mara, D.D. 2005. Computational Fluid Dynamics (CFD) modeling of baffles for optimizing tropical waste stabilization pond systems. *Water Science and Technology* 51 (12), 103-106.

Shi-Weng, L., Lingui, X., Shijian, X., Huyuan, F., Lizhe, A. 2009. Hydrogen peroxide acts asa signal molecule in the adventitious root formation of mung bean seedlings. *Environmental and Experimental Botany* 65, 63-71.

Simon, G., Boris, D., Utra, M., Kristin, V.R., Haissam, J., Ragnheidur, T. 2015. Challenges of sustainable and commercial aquaponics. *Sustainability* 7 (4), 4199-4224.

Somerville, C., Cohen, M., Pantanella, E., Stankus, A., Lovatelli, A. 2014. Small-Scale aquaponic food production. Integrated fish and plant farming. Food and Agriculture Organization of the United Nations (FAO) Rome. *Fisheries and Aquaculture Technical paper* 589, 262.

Sunarma, A. 2004. Peningkatan produktivitas usaha lele Sangkuriang (Clarias sp.). Temu Unit Pelaksana Teknis (UPT) dan Temu Usaha Direktorat Jenderal Perikanan Budidaya, Departemen Kelautan dan Perikanan. Bandung, 4-7 Oktober 2004. 13 pp.

Syeda U. F., Moazzam A.K., Farhan S., Nadeem M., Nasir S., Aamir A., Syed S.S. 2022. Geospatial assessment of water quality using principal components analysis (PCA) and water quality index (WQI) in Basho Valley, Gilgit Baltistan (Northern Areas of Pakistan).

Thorarinsdottir, R.I., Kledal, P.R., Skar, S.L.G., Sustaeta, F., Ragnarsdottir, K.V., Mankasingh, U., Pantanella, E., Ven, R.V.D., Shultz, C. 2015. Aquaponics Guidelines, University of Iceland, aquaponics 1-63.

Timmons, M.B., Ebeling, J.M. 2010. Recirculating Aquaculture. *Nrac*, 205-258.

Tura, N., Hanski, J., Ahola, T., Ståhle, M., Piiparinen, S., Valkokari, P. 2019. Unlocking circular business: a framework of barriers and drivers, *Cleaner Production* 212, 90- 98.

Turcios, A.E., Papenbrock, J. 2014. Sustainable treatment of aquaculture effluents-what can we learn from the past for the future? *Sustainability* 6 (2), 836-856.

Van Den, D.R. 1974. Prediction of mineral nutrient status of trees by foliar analysis. *The Botanical Review* 40, 347-394.

Viladomat, L., Jones, P. 2011. Development of aquaponic systems for space and water- efficient food production in the occupied Palestinian territories -Pilot project final report. Aquaponics and social enterprise: INMED Partnerships for Children (South Africa and Jamaica).

Von Sperlin, M., de Lemos C.C.A. 2005. Biological wastewater treatment in warm climate regions, IWA, London (1), 495-656.

Vymazal J. 2014. Constructed wetlands for treatment of industrial wastewaters: a review. *Ecological Engineering* 73, 724-751.

Vymazal, J. 2007. Removal of nutrients in various types of constructed wetlands. *Science of the Total Environment* 380, 48-65.

Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., Liu, Y., Wang, Y., Ruan, R. 2009. Cultivation of green algae Chlorella sp. in different wastewaters from municipal wastewater treatment plant. *Applied Biochemistry Biotechnology* 162 (4), 1174-1186.

Williams, S.R., Long, S. 1997. Nutrition and diet therapy, center 8th Edition MPH RD MREd, Sue Rodwell Hardcover.

Xu, J., Deng, Y., Yao, L. 2014. Sustainable development-oriented industrial restructuring modeling and analysis: a case study in Leshan. *Clean Technologies and Environmental Policy* 16 (2), 267-279.

Yang, T., Kim, H.J. 2019. Nutrient management regime affects water quality, crop growth, and nitrogen use efficiency of aquaponic systems. *Scientia Horticulturae 256*, 108619.

Yavuzcan, Y.H., Robaina, L., Pirhonen, J., Mente, E., Domínguez, D., Parisi, G. 2017. Fish Welfare in Aquaponic Systems: Its Relation to Water Quality with an Emphasis on Feed and Faeces-A Review. *Water* 9, 13.

Yildiz, Y.H., Radosavljevic, V., Parisi, G., Cvetkovikj, A. 2019. Insight into Risks in Aquatic Animal Health in Aquaponics, Aquaponics Food Production Systems-Combined Aquaculture and Hydroponic Production Technologies for the Future, Editors: Goddek, S., Joyce, A., Kotzen, B., Burnell, G.M., SNS., A.G. 435-452, 978-3-030- 15942-9.

Zwane, E.M. 2019. Impact of climate change on primary agriculture, water sources, and foodsecurity in Western Cape, South Africa. *Jàmbá: Journal of Disaster Risk Studies* 11(1), 562.