FOOD SAFETY AND YIELD PARAMETERS ASSOCIATED WITH *Clarias gariepinus* (Burchell, 1822) PRODUCTION IN AQUAPONIC AND STATIC WATER RENEWAL PRODUCTION SYSTEM

BY

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CERTIFICATION

This is to certify that this research was carried out by OYERINDE HULDAH OLUWASEYI with matric number 222163, under my supervision in the Department of Aquaculture and Fisheries Management, University of Ibadan, Ibadan, Nigeria.

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Dedication

I dedicate this project to God, whose guidance, mercy, and grace have sustained me throughout this journey.

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ABSTRACT

The nature and operation of fish production systems can significantly influence the productivity/yield of cultured fish, as well as the safety of the products for consumers. Therefore, this study investigated food safety and yield parameters associated with *Clarias gariepinus* production in aquaponic and static water renewal systems.

A duplicate 1000L fish tank of a recirculatory aquaculture system and three hydroponic grow bed designs (Deep Water Culture, Nutrient Film Technique, and Gravel Bed) connected to a sump tank for *Amaranthus viridis* aquaponic cultivation and a duplicate 1000L fish tank of the static water renewal system (control tanks) were assessed for *E. coli*, coliform and yield of the cultured C. gariepinus. Fish were stocked at 100 fish/m³ with initial weights of 15-20 g and fed commercial feed (35% crude protein) twice daily. Water quality parameters (temperature, dissolved oxygen, electrical conductivity, and total dissolved solids) were monitored twice daily, while microbial analysis (total coliforms and *Escherichia coli*) was conducted every three weeks. Fish and plant growth performance were assessed biweekly in a 12-week experiment.

The aquaponic system maintained significantly lower water coliform levels than the static system (80.02 ± 39.76 against 293.76 ± 145.67 CFU/mL, $p = 0.0371$), representing a 72.8% reduction. Fish in the aquaponic system achieved significantly higher final weights (319.61±169.78 g against 255.31±148.28g, $p < 0.0001$), representing a 25.2% growth advantage. Among hydroponic designs, the Gravel Bed produced significantly higher fresh leaf yields (0.33 ± 0.04 kg) compared to NFT (0.21 ± 0.04 kg) and DWC (0.14 ± 0.04 kg), but also exhibited significantly higher plant tissue contamination. Water quality parameters were similar across systems, though dissolved oxygen levels were critically low in static and aquaponic systems (0.84-1.02 mg/L). Correlation analysis revealed that water microbial contamination negatively correlated with plant productivity ($r < -0.90$), while plant tissue contamination positively correlated with yields ($r > 0.98$), indicating fundamental trade-offs between productivity and food safety.

The study concludes that aquaponic integration enhances fish production performance and water quality while generating marketable vegetable yields. However, the aquaponic vegetable production is associated with productivity-safety trade-offs, implying the need for careful handling.

Keywords: Fish-vegetable integration, Microbial contamination, *Clarias gariepinus*, *Amaranthus viridis*, Aquaponic, recirculatory aquaculture system, hydroponic systems

Word Count: 336

CHAPTER ONE

INTRODUCTION

1.1 Background

Aquaculture has become an increasingly important component of global food production systems due to its growing contribution to animal protein supply and its potential to enhance food and nutrition security. The Food and Agriculture Organisation reports that aquaculture now supplies more than half of the fish consumed globally, underscoring its role in meeting rising demand amid declining capture fisheries (FAO, 2022). Sub-Saharan Africa's rapidly growing population, urban sprawl, and protein shortages make this growth especially urgent in the region.

In Nigeria and across the region, *Clarias gariepinus*, commonly known as the African catfish, dominates freshwater aquaculture farms. Farmers prefer it for its rapid growth, tolerance to low-oxygen conditions, resistance to rough handling, and strong market demand (Olufeagba *et al.*, 2016; Adeogun *et al.*, 2020). These attributes make it fit everything from basic ponds to high-tech recirculating setups. As production intensifies to meet increasing demand, greater attention has been directed toward improving system efficiency while ensuring food safety.

Small and medium-scale Nigerian farmers still lean on static water renewal systems. This system relies on periodic replacement of culture water to dilute accumulated metabolic wastes and maintain acceptable water quality. However, relatively affordable and straightforward, static water renewal systems are often associated with high water use, fluctuating physicochemical conditions, and the potential buildup of organic matter and microorganisms, especially when water exchange is inadequate or source water quality is compromised (Badiola *et al.*, 2012). These challenges can negatively affect fish growth performance, survival, and the microbiological quality of harvested fish.

Aquaponics has emerged as an alternative, integrated agri-food production system that combines aquaculture with hydroponics in a recirculating water system. In aquaponic systems, the nutrient-rich effluent generated from fish metabolism is biologically transformed and utilised as a nutrient source for plants. Meanwhile, plants remove excess nutrients and contribute to water purification, which is then recirculated back to the fish tanks (Goddek *et al.,* 2019). This nutrient cycling enhances production efficiency, significantly reduces water use compared to conventional agriculture, and minimises wastewater discharge and environmental impacts (Tyson *et al.,* 2011).

Globally, aquaponics has gained increasing attention as a sustainable food production strategy, particularly in regions facing limited arable land and water scarcity. The system is increasingly recognised for its potential contribution to food and nutrition security, particularly in rapidly urbanising areas (Palm *et al.,* 2018). In sub-Saharan Africa, aquaponics offers opportunities for year-round production of fish and vegetables, thereby supporting dietary diversity, generating income, and enhancing livelihoods.

Leafy vegetables are commonly incorporated into aquaponic systems due to their rapid growth and efficient nutrient uptake. In Nigeria, *Amaranthus viridis* (Green Amaranth) species are among the most important leafy vegetables due to their short maturity period, high yield potential, and rich nutritional profile, including iron, calcium, vitamins A and C, and essential amino acids (Shukla *et al.,* 2010; Grubben & Denton, 2004). The combined cultivation of *Clarias gariepinus* and *Amaranthus viridis* in aquaponic systems, therefore, presents a viable approach to producing two essential food commodities within a single, resource-efficient system.

Despite its advantages, aquaponics raises important food safety concerns. The continuous recirculation of water between fish and plants creates potential pathways for microbial contamination, particularly from fish waste, uneaten feed, biofilms, or poor system hygiene. Indicator organisms such as *Escherichia coli* and total coliform bacteria are of particular concern, as their presence suggests faecal contamination and poses potential risks of foodborne illness, especially when vegetables are consumed raw (Fox *et al.,* 2012). Inadequate water management, poor sanitation practices, or inefficient system design can further exacerbate microbial hazards in aquaponic systems (Elumalai *et al.,* 2017).

Food safety is closely linked to environmental quality and yield performance in aquaculture systems. Water quality parameters such as dissolved oxygen, ammonia, and microbial load directly influence fish health, growth rate, and survival, as well as the microbiological quality of fish and vegetables produced (El-Sayed, 2021). In recirculating systems, such as aquaponics, microbial dynamics may differ significantly from those in static water renewal systems, with implications for both productivity and safety. Therefore, understanding these relationships is crucial for developing safe and efficient integrated production systems.

Against this background, a comparative evaluation of aquaponic systems integrated with *Amaranthus viridis* (Green Amaranth) and conventional static water renewal systems for *Clarias gariepinus* (African Catfish) production is both relevant and timely. Such an assessment provides insight into how different water management strategies influence yield parameters and food safety indicators, thereby supporting evidence-based recommendations for sustainable aquaculture and integrated food production practices in Nigeria.

The Integrated and Circular Technologies for Sustainable City Region Food Systems in Africa (INCiTiS-FOOD), the sponsor of this project, is transforming city-region food systems across Africa through soilless farming, recirculating aquaculture, and insect production. The initiative, funded by the European Union since 2023, aims to enhance food and nutrition security in Nigeria and five other countries by increasing access to protein, reducing environmental strain, and promoting fair practices. However, it is essential to gather empirical data on the food safety of the initiative's products.

This study utilises the aquaponic and water static facilities at Incitis-food to compare the food safety and yields of products from aquaponic systems (utilising Amaranthus viridis and Clarias gariepinus) with those from traditional static water renewal setups. INCiTiS-FOOD's simplified aquaponics model features Nutrient Film Technique (NFT), gravel beds, and deep-water grow beds for plant trials, comparing the water use efficiency, crop growth, and grow-bed performance. Meanwhile, local data on yield, microbial safety, and system suitability will guide farmers directly toward the best options, supporting INCiTiS-FOOD's push for sustainable, protein-rich food in Nigeria.

1.2 Problem Statement

Aquaculture has become a crucial means of meeting Nigeria's growing demand for fish; however, water quality issues, fish health problems, and food safety risks persist. Conventional static water renewal systems allow fish waste, uneaten feed, and organic matter to accumulate, degrading water quality over time. Such conditions foster microbial proliferation, impair fish performance, and heighten contamination risks in products for human consumption (Badiola *et al.,* 2012; El-Sayed, 2021).

Aquaponics provides a sustainable solution by combining fish and plant production in recirculating systems that maximise water and nutrient efficiency. However, the continuous reuse of water between fish tanks and plants introduces food safety challenges. This recirculation can facilitate the spread and persistence of microbial pathogens, including faecal indicator bacteria such as *Escherichia coli* and total coliforms, which are standard measures of hygiene and contamination risk in food systems (FAO/WHO, 2012).

In *Clarias gariepinus-Amaranthus viridis* aquaponic setups, contaminants may arise from fish excreta, poor-quality feed, source water, or vectors such as insects and birds. Microorganisms can then colonise fish tissues or adhere to leafy vegetables, such as *Amaranthus viridis*, which are typically eaten fresh or minimally processed. Without proper management and hygiene, these pose real health threats to consumers (Fox *et al.,* 2012). Nigeria faces significant challenges in this regard, given inconsistent oversight of food safety and post-harvest practices (Bashir *et al.,* 2021).

Yield consistency also proves elusive across aquaculture systems. Aquaponics should provide stable water conditions for fish growth; however, its impact on *Clarias gariepinus* performance compared to static renewal remains poorly studied locally. Since productivity drives technology adoption, such knowledge gaps deter farmers from switching systems.

Nigeria is showing a rising interest in aquaponics, yet empirical comparisons of safety and yield between aquaponic and static systems remain sparse. Limited data exist on faecal bacteria in water, fish, and *Amaranthus viridis* crops, as well as their relationships to growth and system performance. Without this, practical safety and productivity guidelines stay out of reach.

This study aims to address the gap in understanding how aquaponic versus static water renewal systems compare in terms of food safety risks and yield in *Clarias gariepinus* culture. Filling it

enables sustainable systems yielding safe fish and vegetables at viable productivity levels for Nigeria.

1.3 Aim and Objectives of the Study

Aim of the Study

This study aimed to generate empirical data on the food safety and yield indices of *Clarias gariepinus* and *Amaranthus viridis* produced from two aquaponic production systems: the *Clarias gariepinus*-*Amaranthus viridis* aquaponic production system and the static water renewal production system.

Specific Objectives

The specific objectives of the study are to:

1. Determine the occurrence and levels of *Escherichia coli* and total coliform bacteria in water and *Amaranthus viridis* samples from the aquaponic system and the static water renewal system;

2. Evaluate the yield of *Clarias gariepinus* cultured in the aquaponic and static water renewal systems

3. Assess the growth morphometrics and yield of *Amaranthus viridis* cultivated in the aquaponic system.

4. Determine water quality parameters associated with the production systems

5. Examine the relationship between microbial load, water quality conditions, and yield parameters across the Aquaponic and static water renewal systems

1.4 Research Questions

The following research questions guide this study:

1. What are the levels of *Escherichia coli* and total coliform bacteria in water and *Amaranthus viridis* samples from the aquaponic system and the static water renewal system?

2. How do the water quality characteristics of aquaponic and static water renewal systems differ during the culture period?

3. How does the production system type influence the growth performance and yield of *Clarias gariepinus?*

4. What is the growth performance and yield of *Amaranthus viridis* cultivated in the aquaponic system?

5. What is the relationship between water quality conditions, microbial load, and yield parameters in *Clarias gariepinus* production under the two systems?

1.5 Justification of the Study

Aquaponics is showing growing promise as a sustainable food production method, especially in sub-Saharan Africa, where food insecurity, water shortages, and environmental damage pose ongoing threats. For Nigeria, it enables simultaneous fish and vegetable production with far less water and nutrient loss through internal recycling (Palm *et al.,* 2018; Goddek *et al.,* 2019). These qualities position it well against traditional static water renewal systems, which typically consume more water and generate waste. However, combining fish and plant production in recirculating water systems raises legitimate concerns about food safety. Microbial contaminants can persist or spread if water management falters. Faecal indicator bacteria, such as Escherichia coli and total coliforms, serve as key measures of hygiene and contamination risk across food systems, particularly for fresh vegetables and fish (FAO/WHO, 2012). *Amaranthus viridis* and similar leafy greens, often eaten fresh or lightly cooked, face an increased risk of surface contamination.

Foodborne illnesses from contaminated fish and produce remain a significant global health concern, affecting developing countries the most due to inadequate surveillance and regulation (WHO, 2015). Nigeria's limited food safety monitoring and post-harvest practices often result in contamination at the production stage, which reaches consumers. Local data gaps on the microbial safety of aquaponics could erode trust and slow technology adoption.

Productivity also drives farmer decisions about aquaculture systems. Aquaponics gets promoted for efficiency, yet few Nigeria-specific studies compare its *Clarias gariepinus* yields against static renewal. Assessing both safety and output is essential to confirm economic viability under local conditions.

The study aligns with broader development priorities as well. Its focus on safety, productivity, and efficiency supports the UN Sustainable Development Goals, specifically SDG 2 (Zero Hunger), SDG 3 (Good Health and Well-being), and SDG 12 (Responsible Consumption and Production) (United Nations, 2015).

Generating the Nigeria-relevant evidence on the safety and yields of aquaponic versus static systems will guide farmers, researchers, and policymakers toward sustainable practices that balance productivity and consumer protection.

1.6 Significance of the Study

This study presents empirical data essential for the safe and sustainable development of aquaculture in Nigeria. By examining the food safety and yield performance of *Clarias gariepinus* in aquaponic versus static water renewal systems, this study addresses critical practical and scientific gaps in integrated fish-vegetable production (Okomoda *et al.,* 2022; Obirikorang *et al.,* 2021).

Food safety benefits: The research quantifies faecal indicator bacteria in aquaponic water and *Amaranthus viridis*, enabling health risk assessment and building consumer trust in the outputs of these systems (Dinev, 2023).

Productivity insights: Comparative growth and yield data for *Clarias gariepinus* across various systems, along with Amaranthus viridis performance, guide farmers in adopting and managing aquaponics (Olufeagba *et al.,* 2016).

Policy relevance: The findings support regulators in developing aquaponic guidelines and monitoring vital areas where standards are lagging (Nigeria National Aquaculture Strategy, 2020; FAO, 2024).

Academic contribution: Nigeria-specific data fills a sub-Saharan Africa-wide void in aquaponics research, establishing baselines for future studies (Obirikorang *et al.,* 2021).

Socio-economic impact: Smallholders, entrepreneurs, and urban farmers gain evidence on the viability of aquaponics for enhancing their livelihoods, food security, and resource efficiency.

1.7 Scope and Limitations

This study assesses food safety and yield parameters associated with *Clarias gariepinus* production in two aquaculture systems: an aquaponic system integrated with *Amaranthus viridis* and a static water renewal system. The study is conducted under controlled conditions and is designed to allow comparison between the two production systems.

1.7.1 Scope of the Study

The scope of the study includes evaluating water quality characteristics in both systems and assessing their suitability for fish production. Food safety assessment is limited to the determination of faecal indicator bacteria, specifically *Escherichia coli* and total coliforms, in water and *Amaranthus viridis* samples from the aquaponic system. Yield performance is assessed through standard growth and survival indicators for *Clarias gariepinus*, as well as growth and yield measurements for *Amaranthus viridis* cultivated in the aquaponic system.

The study further examines the relationship between water quality conditions, microbial load, and yield parameters to understand how production system design influences productivity and food

safety outcomes. The research is location-specific and conducted over a defined experimental period, reflecting practical constraints of time and resources.

1.7.2 Limitations of the Study

The findings of this study are subject to certain limitations. First, the results are based on a specific system design and management practice. They may not fully represent the performance of all aquaponic or static water renewal systems operating under different conditions.

Second, the study is limited to a fixed culture period, which may not capture seasonal or long-term variations in water quality, microbial dynamics, and yield performance.

Third, due to laboratory and resource constraints, microbial analysis is restricted to faecal indicator organisms (*Escherichia coli* and total coliforms). Other potential microbial hazards and chemical contaminants are outside the scope of this study.

Despite these limitations, the study provides valuable comparative insights into food safety and yield parameters across both aquaponic and static water renewal systems, serving as a basis for further research and the development of improved management practices.

CHAPTER TWO

LITERATURE REVIEW

2.1 Concept of Aquaculture

Aquaculture is the controlled cultivation of aquatic organisms, including fish, crustaceans, molluscs, and aquatic plants, for food, income, and conservation. It involves human intervention in the rearing process, including stocking, feeding, predator protection, and water quality management (FAO, 2020). As capture fisheries continue to decline due to overexploitation and environmental degradation, aquaculture has become a critical contributor to global fish supply and food security. The sector emphasises sustainable production practices, such as the Aquaponics system, which enhance yields while minimising environmental impacts, particularly through efficient water use and waste management.

2.1.1 Principles of Aquaponics

Aquaponics is an integrated production system that combines recirculating aquaculture with hydroponic plant cultivation in a symbiotic environment. The fundamental principle of aquaponics is the mutualistic relationship among fish, plants, and microorganisms. Fish excrete metabolic wastes, primarily in the form of ammonia, which is toxic to fish at elevated concentrations. Nitrifying bacteria, primarily Nitrosomonas and Nitrobacter, convert ammonia first into nitrite and subsequently into nitrate, a less toxic form that is readily absorbed by plants as a nutrient (Rakocy *et al.,* 2006).

In this system, plants act as biological filters, removing excess nutrients from the water and thereby improving water quality before it is recirculated back to the fish tanks. This closed-loop process reduces water consumption, minimises waste discharge, and enhances nutrient recycling efficiency, making aquaponics a highly environmentally sustainable production system. The system is particularly suitable for hardy fish species such as *Clarias gariepinus*, which can tolerate variable water quality conditions.

2.1.2 Overview of Static Water Renewal Systems

Static water renewal systems are conventional aquaculture systems where fish are cultured in tanks or ponds with limited or no continuous water recirculation. Water quality is maintained through periodic partial or complete water replacement, which removes accumulated waste and replenishes oxygen and nutrients. Management practices in static systems include regular monitoring of dissolved oxygen, temperature, pH, ammonia, and organic load, as well as scheduled water exchange to prevent deterioration of water quality (Boyd, 2015).

Although static water renewal systems are relatively simple to operate and require lower technical expertise, they often consume large volumes of water. They may discharge nutrient-rich effluents into the environment. These discharges can contribute to environmental pollution if not properly managed, raising concerns regarding sustainability and food safety.

2.1.3 Comparison of Aquaponic and Static Water Renewal Systems

Nutrient dynamics differ significantly between aquaponic and static water renewal systems. In aquaponics, nutrients derived from fish feed are efficiently recycled within the system through biological filtration and plant uptake. Nitrogen, phosphorus, and other micronutrients are retained and utilised, resulting in minimal nutrient loss and improved system efficiency (Goddek *et al.,* 2015).

In contrast, static water renewal systems rely on water exchange to control nutrient accumulation. Excess nutrients are often discharged through effluent rather than being reused, resulting in nutrient loss and increased environmental impact. Limited nutrient recycling in static systems may also lead to fluctuating water quality, which can affect fish growth performance and health.

2.2 *Amaranthus viridis* (Green Amaranth)

Amaranthus viridis, commonly known as green amaranth, is one of the most widely cultivated and consumed leafy vegetables in Nigeria and across sub-Saharan Africa. The crop belongs to the family Amaranthaceae and is characterised by rapid growth, a short production cycle, and adaptability to diverse environmental conditions. These attributes make *Amaranthus viridis* suitable for both subsistence farming and commercial vegetable production systems (Grubben & Denton, 2004).

Nutritionally, *Amaranthus viridis* is highly valued for its relatively high protein content, which can reach 20–30% on a dry-matter basis, as well as its balanced amino acid composition, including lysine, an essential amino acid often deficient in cereal-based diets (Shukla *et al.,* 2010). The leaves are also rich in vitamins A, C, and K, and contain important minerals such as iron, calcium, and magnesium. These nutritional qualities significantly contribute to household food security and the prevention of micronutrient deficiencies, particularly among low-income populations.

The crop's short growth cycle, which often reaches harvestable size within four to six weeks after planting, allows for multiple harvests within a single year. This rapid biomass accumulation makes *Amaranthus viridis* particularly suitable for integration into aquaponic systems, where nutrients derived from fish waste are continuously supplied through the culture water. Under such conditions, nutrients are readily available in dissolved form, supporting steady plant growth and high productivity (Maucieri *et al.,* 2017). In tropical environments, *Amaranthus viridis* performs well across a wide range of water quality conditions, further enhancing its suitability for aquaponic cultivation.

Economically, *Amaranthus viridis* is among the most affordable and marketable leafy vegetables in Nigeria. Its widespread consumption across socio-economic groups ensures consistent demand, while its low input requirements make it attractive to smallholder, urban, and peri-urban farmers. The crop therefore plays an important role in income generation and livelihood support, particularly for women involved in vegetable production and marketing (Obiero *et al.,* 2019).

Despite these advantages, the production of *Amaranthus viridis* presents important food safety concerns. As a leafy vegetable that is often consumed fresh or subjected to minimal processing, it is particularly susceptible to microbial contamination. Several studies have reported the presence of indicator organisms such as *Escherichia coli*, total coliforms, and Salmonella species on leafy vegetables grown in soilless and water-based production systems, highlighting potential public health risks (Elumalai *et al.,* 2017). In aquaponic systems, potential sources of contamination include fish waste, nutrient-rich water, biofilms within the system, and post-harvest handling practices, highlighting the need for meticulous microbial monitoring.

In addition to its nutritional importance, *Amaranthus viridis* contains bioactive compounds, including flavonoids and phenolic substances, which possess antioxidant properties and contribute to its dietary and health benefits (Shukla *et al.,* 2010). In aquaponic systems, the crop exhibits high nutrient-use efficiency by rapidly assimilating nitrogen and phosphorus from the culture water. This nutrient uptake helps reduce nutrient accumulation, supports water quality regulation, and indirectly promotes favourable conditions for fish growth (Maucieri *et al.,* 2017).

In the present study, *Amaranthus viridis* was selected as the plant component of the aquaponic system to assess plant growth performance, yield, and microbial safety when integrated with *Clarias gariepinus*. While the crop enhances nutrient recycling and overall system productivity, its susceptibility to microbial contamination underscores the need to balance yield optimisation with food safety considerations, a central objective of this research.

2.3 Concept of Food Safety in Aquaculture

Food safety in aquaculture encompasses practices to ensure that fish products are safe for human consumption. Potential hazards are broadly classified into microbial, chemical, and environmental risks. Microbial hazards include pathogenic bacteria such as Salmonella, Escherichia coli, and Vibrio species, which may arise from poor water quality or contaminated feed (WHO & FAO, 2011).

Chemical hazards involve the accumulation of heavy metals, pesticide residues, antibiotics, and other contaminants that may be introduced through water sources or improper farm management practices. Environmental hazards include exposure to polluted water bodies and poor waste disposal systems. Effective water quality management, biosecurity measures, and adherence to good aquaculture practices are essential for minimising food safety risks in both aquaponic and static water renewal systems.

2.4 Biological Characteristics and Aquaculture Suitability of *Clarias gariepinus*

Clarias gariepinus, commonly known as the African catfish, dominates Nigerian aquaculture due to its exceptional biological traits, which are well-suited for intensive systems such as aquaponics and static water renewal (Olufeagba *et al.,* 2016).

The species (family Clariidae) features an elongated body, flattened bony head, and unique accessory air-breathing organs. These suprabranchial air-breathing organs enable *Clarias gariepinus* to thrive in low dissolved-oxygen conditions and tolerate water-quality fluctuations that are lethal to other species (Maina, 2018). This resilience proves invaluable in high-organic-load environments typical of intensive culture.

Its rapid growth stands out; fingerlings reach market size (500–1000 g) in 4–6 months under optimal conditions. Efficient feed conversion ratios (1.2–1.5) enhance economic viability despite high aquafeed costs (Obiero *et al.,* 2019). The species also shows strong handling tolerance, disease resistance, and high survival rates.

Clarias gariepinus' omnivorous diet accepts commercial feeds, agricultural by-products, insects, and small fish, reducing production risks across diverse systems (Olufeagba *et al.,* 2016). Socio-economically, its popularity stems from its palatable flesh, high protein content, and cultural acceptance, making it a cornerstone of Nigeria's aquaculture (Olalekan *et al.,* 2020).

In aquaponics, its high metabolic waste output (mainly ammonia) is converted by microbes into plant nutrients. Water quality tolerance stabilises integrated systems, preventing disruptions to the nutrient cycle caused by fish mortality. However, intensive culture challenges persist. High stocking densities generate microbial loads requiring robust filtration. Aggressive cannibalism at the fingerling stage demands careful grading (Schmautz *et al.,* 2017).

Overall, *Clarias gariepinus* excels in both aquaponic and static systems but demands precise management to balance yields with food safety.

2.5 Yield Metrics in *Clarias gariepinus* Production

Yield performance in *Clarias gariepinus* culture is commonly assessed using metrics such as growth rate, feed conversion ratio (FCR), survival rate, biomass yield, and production cycle duration. Factors including stocking density, feed quality, water quality, and system design influence these parameters.

The African catfish (*Clarias gariepinus*) is a fast-growing species widely cultured in Africa due to its high tolerance of low dissolved oxygen levels, a wide temperature range, and poor water quality (Haylor, 1993). Its ability to utilise atmospheric oxygen through accessory breathing organs makes it particularly suitable for intensive systems such as aquaponics. The species efficiently converts feed into biomass, contributing to high yield potential in both aquaponic and static water renewal systems when properly managed.

2.6 Microbial Contaminants in Aquaponic Systems

Aquaponic systems create a unique ecological environment where aquatic animals, plants, and microbial communities coexist. While beneficial microorganisms are indispensable for nutrient cycling, the system also harbours a wide range of potentially harmful microbes that can compromise food safety, fish health, and consumer acceptance. Understanding the sources, types, and risks associated with microbial contaminants is therefore crucial in ensuring safe and sustainable production.

2.6.1 Sources of Microbial Contamination

Microbial contamination in aquaponics can originate from multiple entry points:

1. Fish Waste and Excreta – Faecal matter excreted by fish introduces coliforms and other enteric bacteria into the water. These can adhere to plant roots and subsequently contaminate edible tissues (Fox *et al.,* 2012).
2. Uneaten Feed and Organic Residues – Excess feed decomposes, providing a substrate for heterotrophic bacteria and fungi. Poor feed management is often linked with elevated microbial loads (Elumalai *et al.,* 2017).
3. Water Sources – Contaminated source water, particularly from rivers, boreholes, or poorly treated supplies, can serve as vectors for pathogenic bacteria and protozoa (Bashir *et al.,* 2021).

4. Human Handling and Equipment – Cross-contamination can occur during system management, especially if tools and surfaces are not sanitised. In developing country contexts, limited access to hygiene infrastructure exacerbates these risks (Chalmers, 2019).
5. Vectors such as Insects, Birds, and Rodents – These organisms may introduce faecal pathogens into open aquaponic systems, especially in outdoor or poorly protected facilities.

2.6.2 Food Safety Indicator Microorganisms

Indicator organisms are used to assess hygiene levels in aquaponic systems.

- *Escherichia coli* – Considered the gold standard for faecal contamination. Its detection in water or vegetable samples suggests contamination from faeces or poor management. While most strains are harmless, pathogenic serotypes, such as E. coli O157:H7, are associated with severe foodborne illnesses (FAO/WHO, 2012).
- Total and Faecal Coliforms – These groups are less specific than E. coli but indicate general contamination and potential risk of enteric pathogens (Wohanka *et al.,* 2016).

Both indicators are widely applied in assessing aquaponically grown vegetables, especially those consumed raw, such as Amaranthus viridis (Green Amaranth)

2.6.3 Specific Pathogenic Bacteria

Several pathogenic microorganisms have been reported in aquaponic or related hydroponic systems:

- *Salmonella spp***.** – A significant cause of gastroenteritis worldwide. It can survive in aquatic environments and adhere to plant surfaces. Outbreaks linked to leafy greens such as lettuce and spinach demonstrate their public health importance (Chalmers, 2019).
- *Shigella spp*. – Often associated with contaminated irrigation water. Shigella requires a low infectious dose, making its presence in fresh produce particularly concerning (FAO/WHO, 2012).

- *Listeria* monocytogenes – Capable of surviving and multiplying at refrigeration temperatures. Its presence in aquaponics is less well documented but poses a significant risk to minimally processed vegetables (Wohanka *et al.,* 2016).
- *Aeromonas spp.* – Naturally occurring in aquatic systems and can act as opportunistic pathogens for fish and humans. Their role in aquaponics primarily concerns water quality deterioration (Schmautz *et al.,* 2017).

2.6.4 Fungal and Protozoan Contaminants

Although bacterial hazards are most studied, fungi and protozoa are also relevant.

- Fungi such as *Fusarium* and *Pythium* can infect plant roots, leading to wilting and reduced yields. They indirectly compromise food safety by predisposing plants to secondary microbial colonisation (Wohanka *et al.,* 2016).
- Protozoan parasites such as *Cryptosporidium* and *Giardia* can contaminate aquaponic systems through water inputs. Both are resistant to standard water treatments and have caused outbreaks through the consumption of fresh produce (Bashir *et al.,* 2021).

2.6.5 Mechanisms of Contamination in Aquaponics

Microorganisms in aquaponics contaminate vegetables through several mechanisms:

- Adhesion to Plant Roots and Surfaces – Pathogens adhere to root biofilms or leaf surfaces, where they may persist despite washing.
- Uptake through Plant Tissues – Some bacteria may enter through stomata or damaged plant tissue, making removal through washing ineffective.
- Persistence in Biofilms – Biofilms within pipes, tanks, and plant roots provide protected niches where microbes resist disinfection (Schmautz *et al.,* 2017).
- Cross-contamination during Harvest and Handling – If vegetables come into contact with contaminated water or surfaces during harvest, microbial loads can increase.

2.6.6 Implications for Food Safety

The persistence of microbial contaminants in aquaponics has several implications:

1. Public Health Risks – Consumption of raw or lightly cooked vegetables such as Amaranthus viridis (Green Amaranth) increases exposure to enteric pathogens. This risk is particularly high in Nigeria, where vegetables are often eaten in traditional soups prepared with minimal cooking times.
2. Market Acceptance – Concerns about microbial safety may undermine consumer trust and limit the commercial uptake of aquaponic produce.
3. Regulatory Challenges – There are currently no specific international or Nigerian standards for microbial safety in aquaponics. Producers must therefore rely on general guidelines for irrigation water and the safety of fresh produce (FAO/WHO, 2012).

2.6.7 Mitigation Strategies

To reduce microbial contamination risks, several practices have been recommended:

- Use of clean water sources and periodic water quality testing.
- Optimised biofiltration to reduce organic load and suppress pathogen proliferation.
- Good Agricultural Practices (GAP), including hand hygiene, clean harvesting tools, and sanitised equipment.
- System design improvements, such as covering tanks to reduce contamination from birds and insects.
- Post-harvest interventions, including proper washing, use of mild disinfectants, and cold storage, to reduce microbial persistence on vegetables (Fox *et al.,* 2012; Elumalai *et al.,* 2017).

2.7 Factors Affecting Microbial Load in Aquaponic Systems

A complex interaction among biological, environmental, and management factors influences microbial load in an aquaponic system. These determinants influence the prevalence of microbial contaminants, such as Escherichia coli and coliforms, as well as the overall balance between

beneficial and harmful microorganisms in the system. Understanding these factors is crucial for designing interventions that enhance system safety without compromising productivity.

2.7.1 Water Temperature

Temperature is one of the most significant factors regulating microbial dynamics in aquaponics.

- Higher temperatures, typical of tropical climates such as Nigeria (25–32°C), can accelerate bacterial growth and metabolism (Bojórquez *et al.,* 2016).
- Pathogens such as *E. coli* and *Salmonella* thrive at mesophilic ranges (20–40°C), meaning aquaponic systems in warm environments are particularly vulnerable to microbial proliferation (Chalmers, 2019).
- Conversely, beneficial nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*), essential for ammonia conversion, also perform optimally in similar ranges. This creates a competitive ecological balance between beneficial and harmful microbes (Goddek *et al.,* 2019).

2.7.2 Stocking Density and Fish Waste Load

The density of fish stocked in aquaponic systems directly influences the organic load and, by extension, microbial growth.

- Higher stocking densities of *Clarias gariepinus* increase excretion of ammonia, faecal matter, and uneaten feed residues (Endut *et al.,* 2010).
- Excessive organic matter supports heterotrophic bacterial growth, which can outcompete nitrifying bacteria, leading to water quality deterioration and increased pathogen survival (Fox *et al.,* 2012).
- Studies have shown that optimal stocking density is critical to maintaining balance; high stocking densities increase stress in fish and elevate susceptibility to opportunistic bacterial infections such as *Aeromonas hydrophila* (Mwanja et al., 2024)

2.7.3 Feed Quality and Feeding Practices

Feed composition and management play an important role in determining microbial prevalence.

- Poor-quality feed containing microbial contaminants introduces pathogens directly into the system (Bashir *et al.,* 2021).
- Overfeeding leads to decomposition of uneaten feed, which releases organic matter that promotes microbial multiplication.
- High-protein feeds increase nitrogenous waste excretion, which contributes to higher microbial activity in the water (Elumalai *et al.,* 2017).

2.7.4 Filtration and Biofilm Development

Aquaponics relies on biofiltration to maintain water quality; however, this process also creates environments that favor microbial accumulation.

- Mechanical filters remove suspended solids, but if not maintained, they become hotspots for microbial growth.
- Biofilters host beneficial nitrifying bacteria but can also shelter coliforms and opportunistic pathogens within biofilms (Schmautz *et al.,* 2017).
- Biofilms in pipes and tanks protect bacteria from environmental stresses, making disinfection challenging (Wohanka *et al.,* 2016).

2.7.5 Water Source and Quality

The microbial composition of the source water significantly determines the levels of contamination.

- Groundwater may contain coliforms due to seepage from septic tanks, while surface water is prone to faecal contamination from agricultural runoff (FAO/WHO, 2012).
- In Nigeria, where boreholes and untreated surface water are familiar sources, the risk of introducing faecal bacteria such as *E. coli* into aquaponic systems is high (Bashir *et al.,* 2021).
- Poorly treated water increases the background microbial load and reduces the system's resilience against contamination.

2.7.6 Plant Type and Growth Stage

Different plant species influence microbial dynamics in aquaponics.

- Leafy vegetables such as *Amaranthus viridis* provide large surface areas for microbial attachment, making them more vulnerable to contamination than fruiting crops (Elumalai *et al.,* 2017).
- Younger plants with tender leaves and stems are more susceptible to microbial colonisation due to higher stomatal activity and weaker cuticles.
- Harvest stage also affects microbial load: vegetables harvested late may accumulate higher microbial levels due to prolonged exposure to contaminated water.

2.7.7 Harvesting and Handling Practices

Post-harvest practices can either mitigate or exacerbate microbial contamination.

- Using unclean tools, equipment, or human handling without hygiene protocols often introduces secondary contamination (Chalmers, 2019).
- Lack of cold chain storage after harvest allows microbial populations to multiply rapidly on vegetable surfaces.
- In Nigerian markets, where vegetables such as *Amaranthus viridis (Green Amaranth)* are often displayed in open-air conditions, microbial risks may increase substantially.

2.7.8 System Design and Management

The structural and operational design of aquaponic systems strongly influences microbial prevalence.

- Open systems are more prone to contamination from birds, rodents, and insects than covered systems.
- Hydroponic unit type also matters: deep-water culture (DWC) has higher microbial loads than nutrient film technique (NFT) due to greater water contact (Rakocy *et al.,* 2006).

- System maintenance practices significantly reduce microbial accumulation, including regularly cleaning tanks, filters, and grow beds.

2.7.9 Seasonal and Climatic Factors

In tropical regions such as Nigeria, seasonal variations significantly affect microbial loads.

- The wet season increases risk due to runoff introducing contaminants into water supplies (Bashir *et al.,* 2021).
- The dry season often leads to higher water temperatures, favouring bacterial proliferation.
- Climate variability may introduce fluctuations in microbial loads, which must be accounted for in food safety management**.**

2.8 Growth Metrics for Fish (*Clarias gariepinus*)

Clarias gariepinus (African catfish) is widely used in aquaculture and aquaponics across sub-Saharan Africa due to its fast growth rate, tolerance of high stocking densities, and resistance to variable water quality. Its growth performance is evaluated using the following indices:

1. Weight Gain (WG)
    - Calculated as the difference between final weight and initial weight.
    - Indicates overall growth efficiency in response to feeding regimes and system conditions.
2. Specific Growth Rate (SGR)
    - Expressed as % body weight gained per day.
    - Formula: SGR (%) = [(ln Wf – ln Wi) / t] × 100

      Where:

      Wf = final mean weight (g)

      Wi = initial mean weight (g)

      t = culture duration (days)

It is used to normalise growth across different time periods and stocking densities.

3. Feed Conversion Ratio (FCR)
    - Measures efficiency of feed utilisation.
    - Formula: FCR = Feed intake (g) / Weight gain (g)

A lower FCR indicates more efficient feed use. Clarias gariepinus typically achieves FCR values between 1.5 and 1.8 under optimal conditions, with studies reporting low values on formulated diets (Wei et al., 2024)

Survival Rate (SR)

- Percentage of fish that survive the culture period.

  SR (%) = (Number of fish harvested / Number of fish stocked) × 100

- High microbial loads, poor water quality, or overcrowding may reduce SR significantly.

4. Condition Factor (K)
    - An index of fish health and body robustness.
    - Formula: $K = (W / L^3) \times 100$
    - Where:

      W = body weight (g)

      L = total length (cm)

A higher K-value suggests better growth and overall welfare (Endut *et al.,* 2010).

5. Productivity per Unit Volume
    - Expressed as kilograms of fish harvested per cubic metre of water.
    - Important for evaluating economic efficiency in commercial aquaponic systems.

2.8.1 Growth Metrics for Plants (*Amaranthus viridis*)

Amaranthus viridis is a fast-growing leafy vegetable, rich in vitamins, minerals, and protein, making it a suitable candidate for aquaponics. Its performance is assessed using the following indicators:

1. Germination Rate and Seedling Establishment
    - Percentage of seeds that germinate successfully.
    - Early establishment influences subsequent yield potential.

2. Plant Height and Leaf Number
    - Measured at intervals (weekly or biweekly).
    - Provides a simple proxy for vegetative growth rate and photosynthetic activity.
3. Leaf Area Index (LAI)
    - Ratio of total leaf surface area to ground area.

      LAI = Leaf area (cm^2) / Ground area (cm^2)
    - High LAI indicates better canopy development and potential for higher biomass accumulation (Elumalai *et al.,* 2017).
4. Fresh and Dry Biomass Yield
    - Fresh weight: harvested mass at market maturity.
    - Dry weight: oven-dried samples, providing insight into nutrient accumulation.
    - In Nigeria, *Amaranthus viridis (Green Amaranth)* is often sold fresh, so fresh biomass is a key economic metric.
5. Harvest Index (HI)
    - Ratio of economic yield (edible portion) to total biomass.
    - Higher HI reflects efficient allocation of plant resources toward edible parts.

6. Nutrient Uptake and Quality Parameters
    - Concentrations of nitrogen, phosphorus, potassium, and micronutrients in plant tissues.
    - Quality indicators include protein content, vitamin C levels, and leaf colour (an indicator of chlorophyll content).
7. Productivity per Unit Area
    - Expressed in kg/m².
    - Useful for comparing aquaponic yields to conventional soil-based cultivation.

2.8.2 Interactions Between Fish and Plant Productivity

In aquaponics, fish and plant productivity are interlinked:

- Higher fish stocking densities increase plant nutrient availability but may also raise microbial risks and stress fish growth (Endut *et al.,* 2010).
- Plant growth removes nitrates and helps maintain water quality, indirectly improving fish health and growth (Goddek *et al.,* 2019).
- Imbalances, such as excess fish waste or insufficient plant uptake, can compromise both components, leading to reduced productivity and higher contamination risk.

2.9 Gaps from Previous Studies

Although aquaponics has been increasingly studied worldwide, several gaps remain, particularly in integrating *Clarias gariepinus* (African catfish) and *Amaranthus viridis (Green Amaranth)* in Nigerian contexts. A critical review of existing literature highlights the following issues:

2.9.1 Limited Studies in African Contexts

Most published studies on aquaponics have been conducted in Europe, North America, and Asia, focusing on fish species such as tilapia (*Oreochromis niloticus*) and plants such as lettuce (*Lactuca sativa*). While these studies have advanced theoretical understanding, they may not be directly

transferable to tropical African conditions where local species, climate, and socio-economic realities differ significantly (Goddek *et al.,* 2019).

2.9.2 Insufficient Focus on Local Species

Research on *Clarias gariepinus* within aquaponics is limited, despite the species' widespread use in African aquaculture due to its resilience, rapid growth, and consumer preference. Similarly, *Amaranthus viridis* (Green Amaranth*)* is a culturally important leafy vegetable in Nigeria, but it has not been extensively studied in integrated aquaponic systems. This creates a gap in understanding the growth dynamics and microbial safety of indigenous fish–vegetable pairings.

2.9.3 Lack of Emphasis on Microbial Food Safety

Most studies prioritize production performance (e.g., growth rates, yield) without systematically investigating the risks of microbial contamination. However, microbial hazards such as *E. coli*, coliforms, and *Salmonella* remain critical concerns in aquaponic systems, where both fish and vegetables share the same water sources (Fox *et al.,* 2012; Chalmers, 2019). In Nigeria, where vegetables like *Amaranthus viridis* are often consumed fresh or lightly cooked, this poses significant public health risks that are underexplored.

2.9.4 Limited Understanding of Water Quality–Microbial Interactions

While water quality parameters, such as dissolved oxygen, ammonia, and pH, have been extensively studied in relation to fish growth, fewer studies directly link these parameters to microbial dynamics in aquaponics. This lack of integration means that researchers often fail to capture the dual implications of water quality on both system productivity and food safety (Elumalai *et al.,* 2017).

2.9.5 Gaps in Comparative Studies

There is a lack of comparative assessments between different aquaponic designs (e.g., nutrient film technique, deep water culture, and gravel bed) under Nigerian conditions. Comparative data would

provide insights into which system types are most efficient, cost-effective, and safe for local adoption.

2.9.6 Insufficient Long-Term Studies

Many aquaponics studies are conducted as short-term trials, lasting only a few weeks or months. However, microbial colonisation, biofilm development, and long-term productivity trends require extended observation. The absence of longitudinal studies creates uncertainty about the long-term stability, sustainability, and food safety of aquaponic systems.

2.9.7 Limited Socio-Economic Analysis

Beyond technical performance, very few studies investigate the socio-economic feasibility of aquaponics in Nigeria. Factors such as market acceptability, cost of system setup, and cultural attitudes towards produce grown in recycled water are underexplored. This gap is critical in determining the scalability and adoption of aquaponics in low-income communities.

Summary of Identified Gaps

In summary, the existing literature demonstrates significant progress in global aquaponics research; however, studies specific to Nigeria remain sparse. The significant gaps include:

1. Over-reliance on non-African species in experimental setups.
2. Limited studies involving *Clarias gariepinus* and *Amaranthus viridis* combinations.
3. Minimal focus on microbial contamination and food safety.
4. Weak integration of water quality parameters with microbial analysis.
5. Lack of comparative system design studies in Nigerian contexts.
6. Short-term research that overlooks long-term sustainability.
7. Insufficient socio-economic analysis of aquaponics adoption.

These gaps justify the present study, which investigates microbial contamination and growth performance in an aquaponic system integrating *Clarias gariepinus* and *Amaranthus viridis* in Nigeria.

2.10 Conceptual Framework

The conceptual framework provides the theoretical basis for this study by linking interactions among aquaponic system components, microbial contamination, and growth performance outcomes. It highlights the interdependence of fish, plants, and water quality while emphasising food safety concerns in aquaponics.

2.10.1 Interaction between Fish, Plants, and Microbes

Aquaponics is built upon a symbiotic relationship:

- Fish (e.g., *Clarias gariepinus*) excrete nitrogenous waste products (mainly ammonia) through gills and faeces.
- Nitrifying bacteria convert this ammonia first into nitrite and then into nitrate, which is less toxic to fish and serves as a nutrient source for plants.
- Plants (e.g., *Amaranthus viridis*) absorb nitrates, phosphates, and other nutrients for growth, thereby reducing the accumulation of toxic compounds in the water.
- Water circulates between the fish tanks and grow beds, creating a closed-loop system where microbial communities play dual roles – beneficial (nitrification) and potentially harmful (pathogens such as *E. coli* and *Salmonella*).

This interconnectedness means that disturbances in one component (e.g., poor water quality or overstocking of fish) can affect both plant growth and microbial contamination.

2.10.2 Key Variables in the Framework

1. Independent Variables (Inputs):
    - Stocking density of *Clarias gariepinus*
    - Cultivation of *Amaranthus viridis*
    - Water source and quality parameters (temperature, pH, dissolved oxygen, ammonia, nitrite, nitrate)

- Feed input and management practices

2. Mediating Variables (System Dynamics):
    - Microbial communities (beneficial nitrifying bacteria vs. harmful coliforms and pathogens)
    - Biofilm formation on plant roots and leaf surfaces
    - Waste decomposition and nutrient cycling
3. Dependent Variables (Outputs):
    - Fish growth performance (weight gain, specific growth rate, feed conversion ratio, survival rate, condition factor)
    - Plant growth performance (biomass yield, relative growth rate, harvest index, leaf count, leaf area index)
    - Microbial contamination levels (E. coli, total coliforms, Salmonella presence)

2.10.3 Underlying Assumptions of the Framework

- A balance between fish, plants, and microbes is necessary for system sustainability.
- Poor water quality or high fish stocking densities increase microbial load, which may compromise food safety.
- Proper integration of local species (*Clarias gariepinus* and *Amaranthus viridis*) can optimize productivity and enhance system resilience under Nigerian conditions.

2.10.4 Conceptual Diagram Description

The conceptual framework is illustrated using a flow diagram:

- Box 1 (Inputs)**:** Fish stocking density, plant cultivation, feed input, water quality ↓

- Box 2 (Mediating Factors)**:** Microbial communities, nutrient cycling, biofilm formation ↓
- Box 3 (Outputs):
    - Fish growth performance indicators
    - Plant growth performance indicators
    - Microbial contamination levels

2.11 Theoretical Framework

The theoretical framework provides the academic and scientific foundation for this study. It links aquaponics research with established ecology, systems science, and food safety theories to justify the study's approach to microbial contamination and growth performance in *Clarias gariepinus–Amaranthus viridis* Aquaponic systems in Nigeria.

2.11.1 Systems Theory

Aquaponics operates as a closed-loop system, which can best be explained using systems theory. Systems theory, initially developed in biology and engineering, posits that a system comprises interconnected parts that work together to achieve stability and function (von Bertalanffy, 1968).

- Application in Aquaponics**:**
    - Fish, plants, and microbes form subsystems within a larger aquaponic unit.
    - Inputs such as feed and water flow through the system, while outputs include fish biomass, plant biomass, and microbial contaminants.
    - Feedback loops exist where nutrient accumulation, microbial contamination, or growth rates influence subsequent system management decisions.

Thus, systems theory justifies treating the aquaponic unit not as isolated fish and plant cultures, but as an integrated ecosystem where interdependence is critical.

2.11.2 Ecological Theory of Microbial Interactions

Ecological principles such as competition, mutualism, and succession govern microbial communities in aquaponic systems. Beneficial nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*) play a mutualistic role by converting fish waste into usable plant nutrients. However, pathogenic bacteria (*E. coli, Salmonella*) compete for the same niches and can colonise surfaces, forming biofilms.

- Application in this study:
    - It explains how microbial balance can shift from beneficial to harmful depending on environmental conditions.
    - It also provides the basis for examining how water quality and system management influence microbial dominance.

This theory highlights the dual role of microbes: both facilitators of nutrient cycling and potential food safety hazards.

2.11.3 Growth Theories

Established growth models underpin the dynamics of fish and plant growth.

- Von Bertalanffy Growth Model (for fish):
  This model explains how fish growth follows a sigmoidal pattern, with rapid early growth that slows as the fish approach their maximum size. Monitoring growth through metrics such as SGR and FCR in aquaponics aligns with this model.
- Relative Growth Rate Theory (for plants):
  Plant growth is often measured as an increase in biomass relative to time. This aligns with the ecological principle that growth is resource-dependent (light, nutrients, water), all of which are directly influenced by aquaponic water quality.

Combining these models, the study situates its growth performance measurements within accepted biological theories.

2.11.4 Food Safety Risk Analysis Framework

The Codex Alimentarius Food Safety Risk Analysis Framework provides a basis for studying microbial contamination in aquaponics. This framework is structured around three components:

1. Risk Assessment – identifying hazards such as *E. coli* and coliforms, and quantifying their presence.
2. Risk Management – implementing system designs, water treatments, and hygiene protocols to minimise contamination.
3. Risk Communication – sharing results with farmers, consumers, and policymakers to promote safe adoption of aquaponics.

This framework justifies the inclusion of microbial monitoring in aquaponic studies, as it bridges the gap between agricultural productivity and public health.

2.11.5 Sustainable Agriculture Theory

Sustainable agriculture theory balances productivity, environmental health, and socio-economic viability. Aquaponics fits into this framework by:

- Reducing fertiliser dependence (plants rely on fish waste).
- Conserving water (closed-loop recirculation).
- Providing dual outputs (fish and vegetables).
- Offering potential income diversification for Nigerian farmers.

However, sustainability also requires ensuring microbial safety so that the produce meets food safety standards. Together, these theories provide a robust framework for studying microbial contamination and growth performance in aquaponic systems, ensuring that the research is grounded in established scientific principles while addressing local Nigerian realities.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter describes the research design, study location, materials used, experimental setup, sampling procedures, data collection methods, and analytical techniques employed in the study. The methodology was designed to address the study's objectives, which focus on microbial food safety and yield-related growth performance in aquaponic systems integrating *Clarias gariepinus* (African catfish) and *Amaranthus viridis (Green Amaranth)*, compared with a static water renewal production system.

3.2 Research Design

The study adopted a comparative experimental design in which the INCiTiS-FOOD aquaponic system was operated under controlled conditions. The system was designed to allow simultaneous cultivation of *Clarias gariepinus* and *Amaranthus viridis*, with water continuously recirculating between the fish tank and the hydroponic grow beds of the *Amaranthus spp*. Microbial analysis, water quality monitoring, and growth performance measurements were conducted throughout the study period.

The design enabled:

- Direct measurement of microbial contamination levels in water and plant samples.
- Monitoring of fish and plant growth and yield parameters under aquaponic conditions.
- Establishing correlations between water quality, microbial dynamics, and growth outcomes.

3.3 Study Location

The study was conducted at the INCiTiS-FOOD Living Laboratory, Department of Aquaculture and Fisheries Management, University of Ibadan, Ibadan, Oyo State, Nigeria. The Living Laboratory is a dedicated research facility equipped for aquaculture and aquaponics experiments,

providing controlled conditions for evaluating circular integration of fish–plant production systems with urban farming practice in focus. The study site is located in Ibadan, the capital of Oyo State. Ibadan lies within the tropical rainforest zone of southwestern Nigeria (latitude 7°26′ N, longitude 3°54′ E). The region experiences a humid tropical climate, with two distinct seasons:

- The wet season extends from April to October, with an annual rainfall of approximately 1,200 to 1,500 mm.
- The dry season occurs from November to March, with markedly reduced precipitation.

The mean annual temperature ranges from 25°C to 32°C, while relative humidity fluctuates between 60% and 85%, varying with the seasons. These climatic conditions favour aquaculture and vegetable cultivation, making the site appropriate for aquaponic research.

The Living Laboratory is equipped with recirculating aquaculture systems (RAS), hydroponic grow beds, water pumps, and laboratory facilities for microbial and water quality analyses. The infrastructure allows for precise monitoring of production performance (fish and plant growth) and food safety indicators (microbial contamination).

3.4 Experimental Design

This study employed a Completely Randomised Design (CRD) to evaluate the microbial quality and production performance of a recirculating aquaponic system integrating *Clarias gariepinus* (African catfish) and *Amaranthus viridis (Green Amaranth)*. The design compared the aquaponics setup with a control system consisting of a conventional static water renewal production system without plant integration; a comparison between an integrated and a non-integrated system.

3.4.1 Experimental Units

The experimental setup consisted of:

- Two recirculating aquaculture systems (RAS) tanks (1,000 L each), integrated within the aquaponic loop.

- Two control tanks (1,000 L each), operated under static water renewal conditions without plant integration.
- Three hydroponic grow bed designs were attached to the aquaponic unit for comparison. The browbed types are:
    1. Nutrient Film Technique (NFT) channels.
    2. Gravel Bed (GB)
    3. Deep Water Culture (DWC) with floating rafts.

Each hydroponic unit was connected to the sump tank of the aquaponic system, allowing for continuous water exchange between the fish and plants in the growbeds through the sump. The water pump in the sump recirculates the nutrient-rich water from the fish tank to the growbeds, while the plant-bioremediated water flows back by gravity to the sump.

3.4.2 Fish Stocking

- Species: *Clarias gariepinus* juveniles
- Stocking density: 100 fish per 1,000 L tank (equivalent to 100 fish/m^3).
- Average initial weight: 15–20 g.

3.4.3 Plant Cultivation

- Species: *Amaranthus viridis (Green Amaranth) spp.* seedlings
- Transplanting: Seedlings were transplanted into the three hydroponic units after a 3-week nursery stage.
- Planting density: 20 cm × 20 cm spacing in grow beds and raft holes.

- Growth media:
    - NFT system: Coco peat and coconut husk within planting cups.

- Deep Water Culture (DWC) system: Coco peat and coco husk placed in floating raft cups.
- Gravel Bed system: Washed gravel as the primary medium, with coco peat and coconut husk in floating raft cups, used to support seedlings.

3.4.4 Water Circulation and Filtration

The aquaponic system operated on a closed-loop principle, with the following components:

1. Radial Flow Settler**:** Removed larger suspended solids from fish effluent.
2. Gravel and Sand Biofilter**:** Provided both mechanical filtration and microbial biofilm surfaces for nitrification.
3. Sump Tank**:** Central reservoir that collects return water from grow beds.
4. Pump and Piping**:** A CTP 12000 submersible pump distributed water from the sump to fish tanks and grow beds through PVC pipes.

Water flowed from the fish tanks → radial flow settler → gravel/sand filter → sump tank → grow beds → back to fish tanks, ensuring continuous recycling.

3.4.5 Feeding Regime

Fish were fed a commercial catfish diet containing 35% crude protein. Feeding was carried out twice daily (morning and evening) to apparent satiation.

3.4.6 Duration of Experiment

The experiment lasted 12 weeks, during which fish growth, plant performance, microbial load, and water quality parameters were monitored.

3.4.7 Experimental Layout

The experimental setup was divided into two major groups:

- Treatment (Aquaponics system): Fish + plants + recirculating water.

- Control (Conventional aquaculture): Fish only, with static water renewal production system.

Each treatment (aquaponic and control systems) was replicated twice.

3.5 Sampling and Microbiological Analysis

This section describes the procedures used for sample collection, preparation, microbial enumeration, and quality control. Microbiological analyses focused on detecting total coliforms and *Escherichia coli* in fish culture water and *Amaranthus viridis* (Green Amaranth) leaf samples, in line with the study's objective of ensuring food safety.

3.5.1 Sample Collection

- Water samples: Collected every three weeks from fish tanks, sump tanks, and grow bed outlets using sterile 500 mL polyethylene bottles. Bottles were rinsed twice with system water before final collection.
- Plant samples**:** During each water sample collection, fresh leaves of *Amaranthus viridis (Green Amaranth) spp.* were aseptically harvested using sterile gloves. Approximately 50 g of leaves were placed in sterile transparent bags for microbial analysis. Samples were rinsed in distilled water. Using sterile pipettes, all samples were further diluted into x0, x10, x100, and x1000.
- Incubation: Diluted samples were placed on separate specialised growth media (Chromogenic membrane coliform- MC) pads, and incubated at 35 °C for 24 hours for coliform and *Escherichia coli* growth.

3.5.2 Preparation of Plant Samples

Specifically, 50 mL of sterile water was added to each sterile, transparent bag containing the plant sample. The bags were then sealed and shaken for about 1 minute to ensure thorough washing of the plants. The water collected from the washed vegetables was diluted (x0, x10, x100, and x1000), and each dilution was carefully pipetted onto separate growth media using MC pads. These were then incubated at 35 °C for 24 hours.

3.5.3 Enumeration of Total Coliforms

The total coliform bacteria in aquaponic water samples were counted using the chromogenic membrane coliform (MC) method, in line with recent, standardized, and validated protocols. A 1 ml sample was filtered through a sterile pipette onto the MC pad. The filter was aseptically placed onto a chromogenic coliform media pad. The media pads were incubated at 35 °C for 24 hours. After incubation, colonies with the characteristic blue-green colouration, indicative of β-galactosidase activity, were counted and recorded as colony-forming units per millilitre (CFU/mL). This method offers a quick, reliable alternative to the traditional Most Probable Number (MPN) method. It has been validated for the accurate measurement of *Escherichia coli* and total coliforms in food and water samples (Teramura *et al.,* 2019). The ready-to-use pad technique reduces handling steps, shortens incubation time, and achieves comparable recovery rates to those of the ISO 4832 and ISO 9308-1 standards (JNC Corporation, 2017). Its use has been successfully demonstrated for assessing microbial water quality and monitoring food safety (Humeau *et al.,* 2017).

3.5.4 Detection of *Escherichia coli*

- Selective Media**:** Membrane Coliform (MC) media pad was used. Incubated samples, maintained at 35 °C for 24 hours, were removed from the incubator and observed immediately. Colonies exhibiting a characteristic purple to indigo sheen were considered presumptive *E. coli*.
- Biochemical Confirmation: Presumptive isolates were further confirmed by carefully observing the colour change on the MC pads. These chromogenic coliform media pads indicate the presence of *Escherichia coli* by a distinctive purple sheen.

3.5.5 Quantification of Bacterial Load

The colony-forming unit per millilitre (CFU/mL) of water or per gram (CFU/g) of plant tissue was calculated using the following formula:

CFU/mL or CFU/g = (Number of colonies × Dilution factor) ÷ Volume plated (mL)

3.5.6 Quality Control

- All equipment was sterilized prior to use.
- Source water (water going into the system) was run alongside test samples to check for contamination.
- Triplicate analysis was conducted for each sample to ensure reliability.

3.5.7 Safety Standards

Microbial results were compared with the World Health Organization (WHO, 2006) guidelines for safe use of wastewater in agriculture, and the Food and Agriculture Organization/World Health Organization (FAO/WHO, 2019) microbiological standards for leafy vegetables.

3.6 Water Quality Analysis

Water quality was monitored throughout the 12-week experimental period to evaluate the suitability of the aquaponic system for both *Clarias gariepinus* production and *Amaranthus viridis* (Green Amaranth). Cultivation. Parameters were measured daily, with readings taken twice daily (Morning and evening) in both the treatment (aquaponics) and control (conventional aquaculture) systems.

3.6.1 Parameters Measured

The following physicochemical parameters were assessed:

1. Temperature (°C)
2. Dissolved Oxygen (mg/L)
3. Electrical Conductivity (µS/cm)
4. Total Dissolved Solids (mg/L)

These parameters were selected because they directly influence fish health, microbial activity, and plant nutrient availability.

3.6.2 Methods of Measurement

- Temperature and Dissolved Oxygen**:** Measured in situ using a portable digital dissolved oxygen meter (e.g., YSI Pro20).
- Electrical Conductivity (EC)**:** Determined using a digital Intell Instrument PRO Conductivity Meter, these readings were taken twice daily.
- Total Dissolved Solids (TDS): Measured simultaneously with EC using the meter's TDS function.

Plate 1: Measuring the dissolved oxygen concentration in sump water at INCiTiS-FOOD Living Laboratory.

3.6.3 Frequency of Sampling

- Water quality parameters were measured twice daily from fish tanks, sump tanks, and hydroponic return water.
- Measurements were routinely conducted twice daily, in the morning and in the evening, to minimise the impact of diurnal variation.

3.6.4 Standards and Benchmarks

Results were compared against recommended water quality guidelines for aquaculture and hydroponics:

- FAO/WHO aquaculture water quality guidelines (FAO, 2016).
- Boyd (1998) thresholds for tropical aquaculture.
- WHO (2006) standards for water reuse in agriculture.

3.7 Growth Performance of Fish

The growth performance of *Clarias gariepinus* was evaluated throughout the 12-week experimental period in both the aquaponics system and the control tanks. Standard aquaculture growth indices were used to assess the effect of system type on fish productivity.

3.7.1 Parameters Measured

1. Initial Mean Weight (IMW, g): Average weight of fish at the beginning of the experiment.
2. Final Mean Weight (FMW, g**):** Average weight of fish at the end of the experiment.
3. Weight Gain (WG, g): Difference between FMW and IMW.
4. Percentage Weight Gain (%WG).
5. Specific Growth Rate (SGR, %/day).
6. Feed Conversion Ratio (FCR).

7. Survival Rate (SR, %).
8. Condition Factor (K).

3.7.2 Formulas Used

- Weight Gain (WG):
  WG (g) = FMW – IMW (Where FMW=Final Mean Weight, IMW=Initial Mean Weight)
- Percentage Weight Gain (%WG):
  %WG = [(FMW – IMW) ÷ IMW] × 100
- Specific Growth Rate (SGR):
  SGR (%/day) = [(ln FMW – ln IMW) ÷ Number of days] × 100
- Feed Conversion Ratio (FCR):
  FCR = Total feed given (g) ÷ Total weight gain (g)
- Survival Rate (SR):
  SR (%) = (Number of fish harvested ÷ Number of fish stocked) × 100
- Condition Factor (K):
  $K = (W \div L^3) \times 100$
  Where:
  W = weight of fish (g)
  L = total length of fish (cm)

3.7.3 Procedure

- Fish were weighed biweekly using a digital top-loading balance to monitor growth trends.
- Dead fish were recorded and removed immediately to avoid contamination.
- Feed offered was weighed daily, and uneaten feed was negligible due to the feeding behaviour of *Clarias gariepinus*.

- Survival rates were determined at the end of the experiment during the final harvest.

3.7.4 Relevance of Growth Indices

- Weight gain and SGR are direct measures of growth efficiency.
- FCR reflects the economic efficiency of feed use. Lower FCR values indicate more efficient feed conversion.
- Survival rate demonstrates the suitability of water and environmental conditions.
- Condition factor (K) indicates the general health and well-being of the fish population.

3.8 Growth Performance of Plants

The growth response of *Amaranthus viridis (Green Amaranth) spp.* Cultivated in the aquaponics grow beds was assessed throughout the experimental period. Plant growth parameters were measured at 2-week intervals from transplanting until final harvest.

3.8.1 Parameters Measured

1. Plant Height (cm): Measured from the base of the stem at the soil line (or substrate level) to the apical tip of the plant using a ruler or measuring tape.
2. Number of Leaves**:** All fully expanded leaves per plant were counted weekly.
3. Leaf Area (cm^2): Estimated using the length × width method and applying a correction factor (commonly 0.75 for *Amaranthus viridis (Green Amaranth)* leaves).
4. Fresh Weight (g): Harvested plants were weighed immediately after removal from the substrate.
5. Dry Weight (g): Fresh biomass was oven-dried at 70°C until a constant weight was achieved.
6. Relative Growth Rate (RGR, $g\ g^{-1}\ day^{-1}$).
7. Harvest Index (HI, %).
8. Yield per Unit Area (g/m^2).

3.8.2 Formulas Used

- Leaf Area (LA):
  LA = Leaf length × Leaf width × Correction factor (0.75)
- Relative Growth Rate (RGR):
  $RGR = (\ln W_2 - \ln W_1) \div (t_2 - t_1)$
  Where:
  W_1 = initial dry weight (g)
  W_2 = final dry weight (g)
  $t_2 - t_1$ = time interval (days)
- Harvest Index (HI):
  HI (%) = (Economic yield ÷ Total above-ground biomass) × 100
- Yield per Unit Area:
  Yield (g/m^2) = Total fresh biomass (g) ÷ Cultivation area (m^2)

3.8.3 Procedure

- Plant height and number of leaves were measured weekly from randomly selected plants in each grow bed.
- Leaf area was calculated using the leaf length and width of 10 randomly sampled leaves per treatment.
- At harvest, plants were uprooted, washed to remove debris, and separated into shoots and roots.
- Fresh weight was recorded immediately using a digital weighing balance.
- Dry weight was determined after oven drying samples at 70°C until a constant weight was achieved (usually 72 hours).
- Harvest index and yield were calculated to evaluate productivity per unit area.

3.8.4 Relevance of Plant Growth Indices

- Plant height and leaf count provide indicators of vegetative growth and nutrient uptake efficiency.
- Leaf area is a proxy for photosynthetic capacity and productivity potential.
- Fresh and dry biomass reflect the actual yield obtainable by farmers and market value.
- Relative growth rate (RGR**)** allows for comparison of growth efficiency across treatments and time intervals.
- Harvest index (HI) indicates the partitioning of biomass into economically advantageous parts.
- Yield per unit area is the most practical measure of system productivity and scalability.

CHAPTER FOUR

RESULTS

4.1 Background

This chapter presents the results of the 12-week experimental study comparing food safety and yield parameters in *Clarias gariepinus* (African catfish) production systems integrated with *Amaranthus viridis* (Green Amaranth) cultivation. The study employed a comparative experimental design to evaluate an aquaponic recirculating aquaculture system (RAS) incorporating three hydroponic grow-bed designs (Deep Water Culture, Nutrient Film Technique, and Gravel Bed) against a conventional static water renewal system, serving as the control.

Results are organised into five major sections that systematically address the study's specific objectives. Together, these results sections provide a comprehensive empirical foundation for understanding the performance characteristics, food safety implications, and productivity potential of aquaponic systems integrating fish and vegetable production. The findings yield multiple insights into optimal system configurations, management practices, and the fundamental trade-offs inherent in sustainable, integrated food production systems.

4.2 Microbial Quality Assessment

The microbial quality of water and plant samples was systematically evaluated to determine the food safety status of the aquaponic system relative to the static water renewal control system. Microbiological analysis focused on the enumeration of total coliform bacteria and *Escherichia coli* (*E. coli*) as indicator organisms for faecal contamination and potential pathogenic presence. These parameters were selected based on international food safety guidelines, which recognise coliforms and *E. coli* as reliable indicators of sanitary quality in both water and food products. Samples were collected from multiple points within both production systems at various sampling points throughout the 12-week experimental period to capture spatial and temporal variations in microbial dynamics.

4.2.1 Total Coliform Counts in Water Samples

Results from this study revealed substantial and statistically significant differences in total coliform levels between the two production systems. The aquaponic recirculatory system exhibited considerably lower coliform levels, with a mean count of 80.02 ± 39.76 CFU/mL, compared to the static water renewal system, which recorded a mean count of 293.76 ± 145.67 CFU/mL. This nearly four-fold difference was statistically significant ($p = 0.0371$), indicating that system type had a measurable effect on microbial load **(Table 4.2.1)**. The standard error values suggest moderate variability within each system, with the static system showing higher variability (SE = 145.67) compared to the aquaponic system (SE = 39.76), possibly reflecting differences in water exchange dynamics and nutrient accumulation patterns.

Table 4.2.1: Comparison of Food Safety Parameters (Coliform and *E. coli* Counts, CFU/ml) in Aquaponic Recirculatory Aquaculture System (RAS) and Static Water Renewal Production Systems of *Clarias gariepinus*

System	Coliform (CFU/ml)	E. coli (CFU/ml)	Yield (g)
Aquaponics	80.02 ± 39.76^{a}	3.76 ± 2.21	319.61±169.78
Static water	293.76 ± 145.67^{b}	6.96 ± 4.06	255.31±148.28

Note: Means with the same superscript along the column are not significantly different (p>0.05)

4.2.2 Distribution of Coliform in Aquaponic System Components

The statistical analysis employed negative binomial mixed-effects models to account for the overdispersion in microbial count data, in which the variance typically exceeds the mean. Dilution was included as a fixed technical factor to control for sample-processing effects, while sampling unit and date were incorporated as random effects to account for repeated measures and temporal autocorrelation. This analytical approach provides robust estimates of treatment effects while appropriately modelling the hierarchical structure of the data.

Within the aquaponic system, spatial variation in coliform distribution was examined across components of the recirculating loop. A $\log_{10}$ transformation was applied to the count data to normalise the distribution and stabilise variance, a standard practice in microbiological research. The transformed coliform counts ranged from 1.81 ± 0.14 (corresponding to approximately 64.6 CFU/mL) in the Gravel Bed to 2.25 ± 0.14 (approximately 177.8 CFU/mL) in the Deep Water Culture (DWC) unit **(Table 4.2.2)**. The Sump, which serves as the central reservoir collecting return water from all grow beds, recorded a $\log_{10}$ count of 1.87 ± 0.14 (approximately 74.1 CFU/mL). In comparison, the Nutrient Film Technique (NFT) system showed 2.12 ± 0.14 (approximately 131.8 CFU/mL).

A statistical comparison among these system components revealed no significant differences ($p = 0.0915$), suggesting that, despite numerical variations, coliform bacteria were relatively uniformly distributed throughout the recirculating aquaponic system. The confidence intervals (CI) for each component showed considerable overlap, with the lower and upper bounds spanning similar ranges across all sampling points. This uniform distribution indicates adequate water mixing and circulation throughout the system, with the biofilter and recirculation process maintaining relatively consistent microbial loads across different hydroponic units. The marginally non-significant p-value ($p = 0.0915$, approaching the conventional $\alpha = 0.05$ threshold) suggests a trend toward variation that may become significant with increased sample size or extended sampling duration.

Table 4.2.2 Comparison of Coliform in Aquaponic System Components

Source	Mean ± SE
Gravel Bed	1.81 ± 0.14 [a]
Sump	1.87 ± 0.14 [a]
NFT	2.12 ± 0.14 [a]
DWC	2.25 ± 0.14 [a]

Note: Means with the same superscript along the column are not significantly different (p>0.05)

4.2.3 *Escherichia coli* in Water

This study found that *Escherichia coli* (*E. coli)* was detected in both production systems. However, at lower levels than total coliforms, as expected, given that *E. coli* represents a subset of the total coliform group. The aquaponic recirculating system recorded lower mean E. coli counts (3.76 ± 2.21 CFU/mL) than the static water renewal system (6.96 ± 4.06 CFU/mL), representing an approximately 46% reduction in *E. coli* levels **(Table 4.2.1)**. However, unlike the total coliform comparison, this difference did not reach statistical significance ($p > 0.05$), likely due to the greater variability in E. coli counts, as indicated by the relatively large standard errors and the lower overall abundance of this organism compared to total coliforms.

The higher standard errors observed for *E. coli* (2.21 and 4.06) relative to the mean counts reflect the patchy and sporadic distribution typical of faecal indicator bacteria in aquatic environments. This variability suggests that *E. coli* contamination may be episodic rather than continuous, possibly associated with specific events such as fish handling, feeding activities, or fluctuations in environmental conditions that affect bacterial survival and growth.

Within the aquaponic system components, spatial distribution of *E. coli* was examined using $\log_{10}$-transformed counts to account for the non-normal distribution of microbiological data. Statistical analysis revealed no significant differences in *E. coli* levels across the four sampling points ($p > 0.05$), indicating uniform distribution throughout the recirculating loop. The Deep Water Culture (DWC) unit recorded the lowest $\log_{10}$ *E. coli* count at 0.59 ± 0.22 (corresponding to approximately 3.9 CFU/mL), followed by the Nutrient Film Technique (NFT) at 0.60 ± 0.22 (approximately 4.0 CFU/mL), the Sump at 0.79 ± 0.22 (approximately 6.2 CFU/mL), and the Gravel Bed at 1.11 ± 0.22 (approximately 12.9 CFU/mL) **(Table 4.2.3)**.

Although the Gravel Bed showed numerically higher *E. coli* counts than other components, the overlapping confidence intervals across all sampling points indicate that these differences were within the range of random variation. The confidence intervals ranged from negative values in the DWC and NFT systems to positive values across all systems, reflecting the log transformation of data that included zero and low counts. The lack of significant differences suggests that the recirculation process effectively homogenises the distribution of E. coli, preventing the accumulation of hotspots of faecal contamination within specific system components.

Table 4.2.3 Comparison of *E. coli* in Aquaponic System Components

Source	Mean ± SE
DWC	0.59 ± 0.22^{a}
NFT	0.6 ± 0.22^{a}
Sump	0.79 ± 0.22^{a}
Gravel Bed	1.11 ± 0.22^{a}

Note: Means with the same superscript along the column are not significantly different ($p>0.05$)

4.2.4 Microbial Load in *Amaranthus viridis* Samples

Microbial contamination levels of *Amaranthus viridis* leaves varied substantially across the three hydroponic grow bed designs employed in this study, reflecting differences in water contact patterns, substrate characteristics, and microbial retention properties of each system. For total coliform counts, the Gravel Bed system recorded the highest contamination levels with a mean of 28.71 ± 22.69 CFU/mL, followed by the Nutrient Film Technique (NFT) at 11.43 ± 7.7 CFU/mL, and Deep Water Culture (DWC) at 3.73 ± 2.61 CFU/mL **(Table 4.2.4)**. The high standard errors, particularly in the Gravel Bed system (SE = 22.69), indicate substantial variation in contamination levels across samples, possibly reflecting heterogeneous microbial distribution on plant surfaces or differences in sampling efficiency.

Statistical analysis revealed significant differences between the Gravel Bed and DWC systems ($p < 0.05$), with different superscript letters (^{a} and ^{b}) denoting statistically distinct groups. The NFT system showed intermediate values with a superscript (^{ab}) indicating that it was not significantly different from either extreme, serving as a transitional category. This pattern suggests that the physical characteristics of each hydroponic design influence microbial colonisation of plant surfaces. The Gravel Bed, with its larger surface area for microbial attachment and retention of organic particles within the gravel matrix, may provide more opportunities for bacterial accumulation and subsequent transfer to plant tissues. Conversely, the DWC system, where plant roots are suspended in continuously flowing water with minimal solid substrate, appears to result in lower plant surface contamination.

To facilitate statistical comparison and account for the skewed distribution typical of microbiological data, coliform counts were also analysed following $\log_{10}$ transformation. The transformed data showed $\log_{10}$ counts of 1.40 ± 0.4 for the Gravel Bed, 1.13 ± 0.4 for the DWC, and 1.05 ± 0.4 for the NFT **(Table 4.2.5)**. Despite the numerical trend observed with untransformed data, the log-transformed values showed no statistically significant differences among grow bed types ($p > 0.05$), with all systems sharing the same statistical grouping (denoted by superscript 'a). This apparent discrepancy between analyses reflects the effect of transformation on outliers and variance structure. It suggests that while mean differences exist, the high variability within treatments reduces the statistical power to detect significant effects.

The standard errors of the transformed data (SE = 0.4 for all treatments) are notably more uniform than those of the untransformed data, indicating successful variance stabilisation through logarithmic transformation. This uniformity in error terms is a desirable property for statistical analysis, as it satisfies the assumption of homoscedasticity (equal variance) required for many parametric tests.

Table 4.2.4: Differences in Coliforms and E. coli Counts (Mean ± SE) in different Grow beds for Amaranthus viridis Production in Aquaponic system

Growbed	**Coliform (CFU/ml)**	**E. coli (CFU/ml)**	***Yield-Leaf Area (m^2/g)***
DWC	3.73 ± 2.61^{a}	2.86 ± 2.21^{a}	2.19 ± 0.58^{a}
NFT	11.43 ± 7.7^{ab}	2.38 ± 1.68^{a}	3.4 ± 0.58^{a}
Gravel Bed	28.71 ± 22.69^{b}	6.32 ± 4.71^{b}	4.32 ± 0.58^{a}

Note: Means with the same superscript along the column are not significantly different (p>0.05)

Table 4.2.5 Coliform Counts in Plant Tissues Across Grow beds

Source	Mean ± SE
NFT	1.05 ± 0.4^{a}
DWC	1.13 ± 0.4^{a}
Gravel Bed	1.4 ± 0.4^{a}

Note: Means with the same superscript along the column are not significantly different ($p>0.05$)

Escherichia coli detection in *Amaranthus viridis* samples followed a similar distributional pattern to total coliform counts, though at lower absolute levels, as expected. The Gravel Bed system showed significantly higher *E. coli* contamination levels with a mean of 6.32 ± 4.71 CFU/mL, compared to the NFT system at 2.38 ± 1.68 CFU/mL and DWC at 2.86 ± 2.21 CFU/mL **(Table 4.2.4)**. Statistical analysis confirmed significant differences between the Gravel Bed (superscript ^{b}) and both the NFT and DWC systems (superscript ^{a}), which were not significantly different from each other ($p < 0.05$). This pattern mirrors that observed for total coliforms, suggesting that the mechanisms influencing general bacterial contamination also affect the more specific faecal indicator *E. coli*.

The presence of *E. coli* on leafy vegetables is of particular concern from a food safety perspective, as it indicates potential faecal contamination and raises the possibility of pathogenic *E. coli* strains or other enteric pathogens being present. The significantly elevated levels in the Gravel Bed system suggest that this design may require additional post-harvest washing or treatment protocols to ensure food safety, particularly if vegetables are to be consumed raw.

Analysis of $\log_{10}$-transformed *E. coli* counts revealed no statistically significant differences across grow bed types ($p > 0.05$), with values of 0.69 ± 0.18 for Gravel Bed, 0.44 ± 0.18 for DWC, and 0.31 ± 0.18 for NFT **(Table 4.2.6)**. All three systems shared the same statistical grouping (superscript 'a'), indicating that the observed numerical differences were within the range of random variation when the skewness of the data was accounted for through transformation. The smaller standard errors for *E. coli* (SE = 0.18) compared to coliform counts reflect the lower overall abundance and potentially less variable distribution of this organism on plant surfaces.

Beyond *Amaranthus viridis*, the study also examined microbial contamination of *Capsicum annuum* (bell pepper) cultivated in the same aquaponic system, providing additional insight into how plant species and growth characteristics may influence microbial contamination patterns. Coliform counts in *Capsicum* samples showed distinct patterns across grow bed types, with the Gravel Bed recording the highest levels at 62.47 ± 70.53 CFU/mL, followed by NFT at 39.83 ± 45.12 CFU/mL, and DWC at 4.24 ± 4.85 CFU/mL **(Table 4.2.7)**. The extremely high standard errors, particularly in the Gravel Bed (SE = 70.53), indicate highly variable contamination levels,

possibly reflecting differences in plant architecture, fruit position relative to water sources, or developmental stage at sampling.

Statistical analysis revealed significant differences between the DWC system (superscript [a]) and both NFT and Gravel Bed systems (superscript [b]), which were not significantly different from each other ($p < 0.05$). Interestingly, *E. coli* levels in *Capsicum* samples showed no significant variation across grow bed types, with all systems recording relatively low counts ranging from 1.33 ± 0.97 CFU/mL to 1.81 ± 1.2 CFU/mL ($p > 0.05$), and all sharing the same statistical grouping (superscript [a]). This uniform, low-level *E. coli* contamination across all systems suggests that *Capsicum* fruits may be less susceptible to fecal contamination than leafy vegetables, possibly due to their elevated position above the water surface, reduced direct water contact, and the protective barrier provided by the fruit's waxy cuticle.

Table 4.2.6: *E. coli* Counts in Plant Tissues Across Grow beds

Source	Mean ± SE
NFT	0.31 ± 0.18^{a}
DWC	0.44 ± 0.18^{a}
Gravel Bed	0.69 ± 0.18^{a}

Note: Means with the same superscript along the column are not significantly different ($p>0.05$)

Table 4.2.7: Differences in Coliforms and *E. coli* Counts (Mean ± SE) in different Grow beds for Capsicum annuum Production in Aquaponic System

Growbed	Coliform (CFU/ml)	*E. Coli* (CFU/ml)
DWC	4.24 ± 4.85 [a]	1.81 ± 1.2 [a]
NFT	39.83 ± 45.12[b]	1.42 ± 1.09 [a]
Gravel Bed	62.47 ± 70.53 [b]	1.33 ± 0.97 [a]

Note: Means with the same superscript along the column are not significantly different (p>0.05)

4.3 Water Quality Parameters

Water quality monitoring was conducted throughout the 12-week experimental period to evaluate the physicochemical conditions in both the aquaponic recirculatory system and the static water renewal control system. A temporal representation of the results obtained is represented in **(Figure 4.3)**

4.3.1 Electrical Conductivity

Results from this study showed no statistically significant difference in electrical conductivity between the aquaponic recirculatory system (RAS) and the static water renewal system (SRS). The RAS recorded a mean EC of 702.35 ± 11.58 µS/cm, while the SRS showed 689.95 ± 11.58 µS/cm ($p = 0.528$) **(Table 4.3.1)**. The similar superscript letters (^{a}) for both systems indicate that they belong to the same statistical group, confirming the absence of significant differences. The small standard errors (SE = 11.58 for both systems) indicate relatively stable and consistent EC readings throughout the experimental period, suggesting steady-state conditions were maintained in both systems.

The similarity in EC between systems is noteworthy because it suggests that despite different water management strategies continuous recirculation with biofilter in the aquaponic system versus periodic water exchange in the static system, both systems maintained comparable levels of dissolved ionic substances. The slightly higher EC in the RAS (702.35 vs 689.95 µS/cm, though not significant) may reflect the accumulation of mineralized nutrients through continuous nitrification in the biofilter, where ammonia is converted to nitrite and subsequently to nitrate, increasing the total ion concentration over time.

Mixed-effects models were employed for the statistical analysis of EC data to account for the hierarchical structure of the dataset, which included repeated measurements taken from the same experimental units over time. This analytical approach effectively handles the correlation among measurements taken from the same tank across different dates, providing more accurate estimates of treatment effects than traditional ANOVA methods, which assume independence of observations.

4.3.2 Total Dissolved Solids

Total dissolved solids (TDS) provide a more comprehensive measure of water quality by encompassing both ionic and non-ionic dissolved substances. In aquaculture systems, TDS levels reflect the accumulation of metabolic waste products, feed residues, and mineralized organic matter. Statistical analysis revealed no significant difference in TDS between the aquaponic system (304.81 ± 4.74 mg/L) and the static water renewal system (299.16 ± 4.74 mg/L), with $p = 0.489$ **(Table 4.3.1)**. Both systems shared the same statistical grouping, confirming comparable dissolved solids concentrations throughout the experimental period. The very small standard errors (SE = 4.74) indicate high precision in measurements and minimal variation across sampling dates, suggesting that both systems maintained stable TDS levels despite progressive increases in fish biomass and cumulative feed input.

Table 4.3.1: **Summary Table for Water Quality Across the Systems**

Parameter	**System**	***Mean ± SD***
Conductivity (µS/cm)	RAS	702.35±11.58[a]
Conductivity (µS/cm)	SRS	689.95±11.58[a]
TDS (mg/L)	RAS	304.81±4.74[a]
TDS (mg/L)	SRS	299.16±4.74[a]
Temperature (°C)	RAS	26.86±1.39[b]
Temperature (°C)	SRS	27.16±1.5[a]
Dissolved Oxygen (mg/L)	RAS	1.02±0.62[a]
Dissolved Oxygen (mg/L)	SRS	0.84±0.99[b]

Note: Means with the same superscript along the column are not significantly different (p>0.05)

4.3.3 Temperature

Statistical analysis revealed a small but statistically significant difference in temperature between the two production systems. The static water renewal system (SRS) recorded a mean temperature of 27.16 ± 1.5°C, while the aquaponic recirculatory system (RAS) showed a slightly lower mean of 26.86 ± 1.39°C ($p = 0.008$) **(Table 4.3.1)**. Despite the statistical significance, indicated by different superscript letters (^{a} for SRS and ^{b} for RAS), the absolute difference of only 0.3°C is biologically negligible and unlikely to have any meaningful impact on fish growth, microbial dynamics, or plant performance.

The standard deviations (1.5°C for SRS and 1.39°C for RAS) indicate the range of temperature variation experienced in each system across the study period, reflecting diurnal fluctuations, seasonal changes, and potentially differences in thermal mass and heat exchange rates between the two systems. The slightly lower temperature variability in the RAS (SD = 1.39) compared to SRS (SD = 1.5) may reflect the buffering effect of larger water volume and continuous circulation in the aquaponic system, which can dampen temperature fluctuations.

Analysis of Variance (ANOVA) was employed for temperature data analysis, as this parameter showed normally distributed values without the overdispersion characteristic of count data. ANOVA is appropriate for comparing means across groups when assumptions of normality and homogeneity of variance are met, which was the case for temperature measurements in this study.

4.3.4 Dissolved Oxygen

Dissolved oxygen (DO) is arguably the most critical water quality parameter in aquaculture, as fish require oxygen for cellular respiration and metabolic processes.

Statistical analysis revealed a small but significant difference in dissolved oxygen concentrations between the two systems. The aquaponic recirculatory system (RAS) maintained higher DO levels with a mean of 1.02 ± 0.62 mg/L, compared to the static water renewal system (SRS) which recorded 0.84 ± 0.99 mg/L ($p = 0.005$) **(Table 4.3.1)**. Different superscript letters (^{a} for RAS and ^{b} for SRS) indicate that these systems belong to statistically distinct groups. The RAS showed

approximately 21% higher DO levels than the SRS, a difference that could have biological significance for fish respiratory physiology and overall system health.

The higher standard deviation in the SRS (SD = 0.99) compared to RAS (SD = 0.62) indicates greater variability in DO levels in the static system. This variability likely reflects the episodic nature of water exchange in the static system.

The temporal pattern of dissolved oxygen **(Figure 4.3.4)** likely shows considerable fluctuation throughout the study period, with potential diurnal patterns reflecting the balance between oxygen consumption (by fish, bacteria, and plant roots) and oxygen addition (through water circulation and any passive aeration).

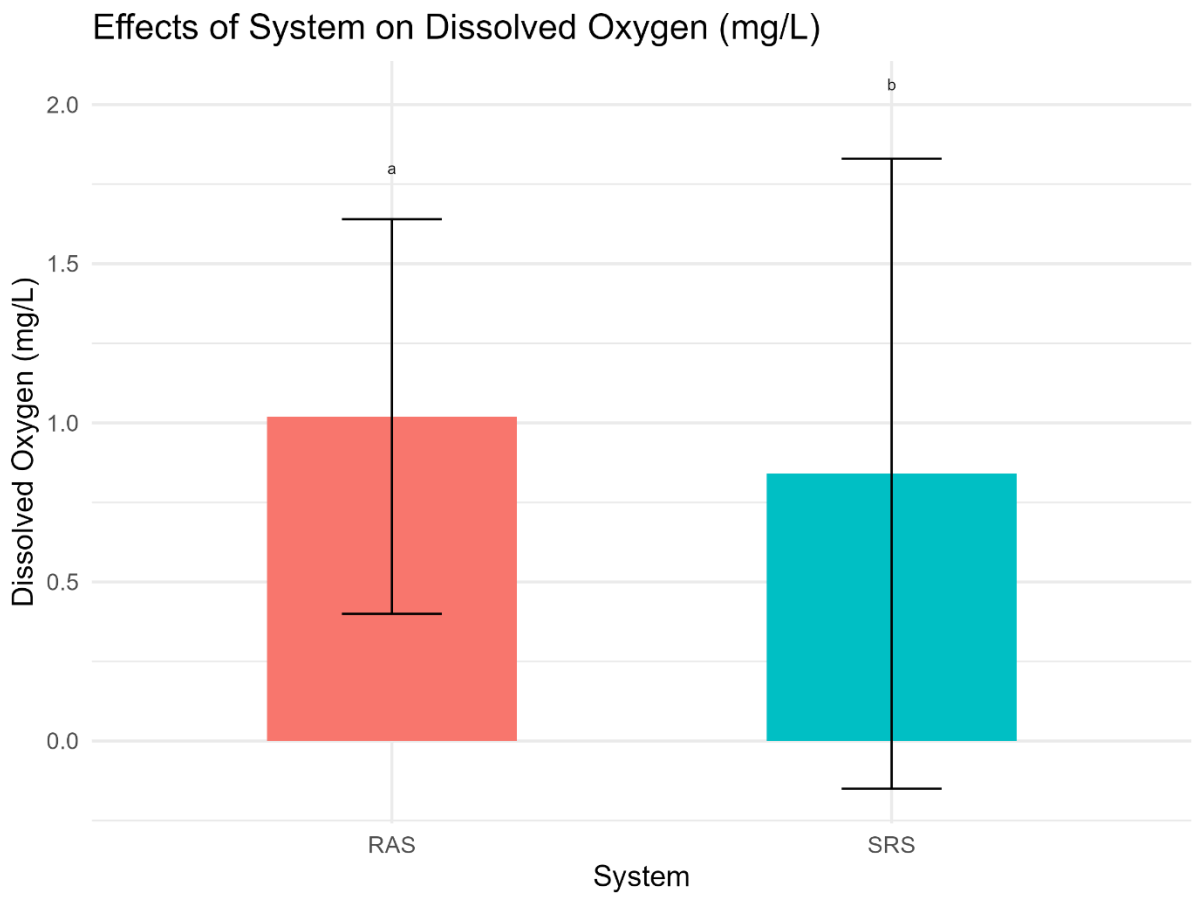

Figure 4.3.4: Temporal Pattern of Dissolved Oxygen Across the Aquaponic and Static Water Renewal System

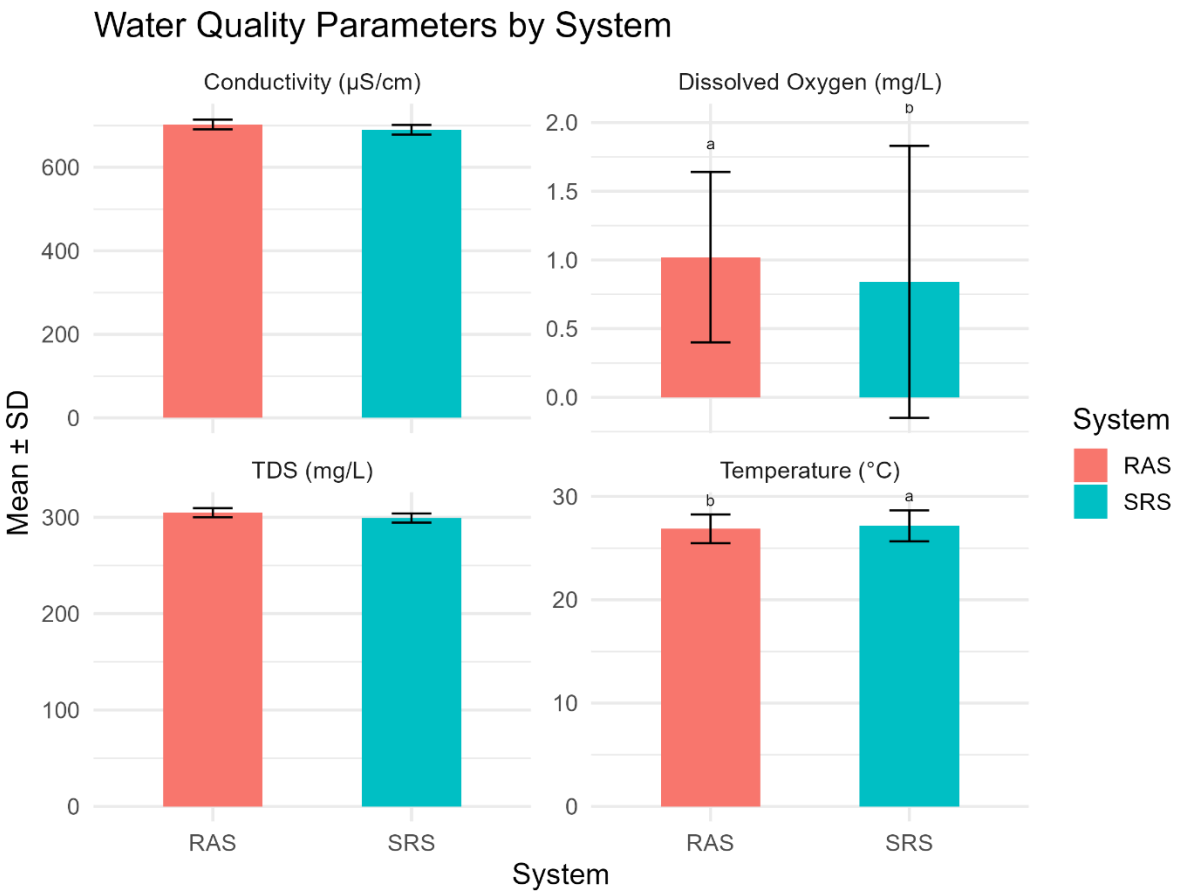

Figure 4.3: Comparative visualization of all water quality parameters across the Aquaponic and Static Water Renewal System

4.4 Growth Performance of *Clarias gariepinus*

The temporal progression of mean weights shows consistent upward trends in both systems throughout the experimental period **(Figure 4.4)**. Visual inspection of the growth curves reveals that the aquaponics system maintained consistently higher or comparable weights to the stagnant system from the initial measurement through the final harvest. The aquaponics system showed a clear growth advantage, particularly in the latter half of the experimental period, with the divergence between systems becoming more pronounced after week 6. This pattern suggests that the cumulative benefits of the aquaponic configuration, including improved water quality through biofilter operation and continuous recirculation, manifested more strongly as the experiment progressed and fish biomass increased.

Summary statistics confirmed that the aquaponics system produced significantly heavier fish, with mean weights of 319.61 ± 169.78 g compared to 255.31 ± 148.28 g in the stagnant system (different superscript letters 'a' and 'b' indicating statistical significance, $p < 0.0001$) **(Table 4.4)**. This represents a 25.2% increase in mean fish weight in the aquaponic system, resulting in approximately 64.3 g of additional biomass per fish. From a production standpoint, this substantial growth advantage demonstrates the superior productivity potential of the aquaponic recirculatory system compared to conventional static water renewal management.

The similar standard deviations between systems (169.78 g for aquaponics vs. 148.28 g for stagnant) indicate comparable levels of size variation within each population. This suggests that the growth advantage in the aquaponics system was relatively uniform across the population rather than being driven by a few exceptionally large individuals. The coefficient of variation (CV = SD/Mean × 100) was 53.1% for aquaponics and 58.1% for stagnant systems, both indicating high but typical levels of size heterogeneity for *Clarias gariepinus* culture.

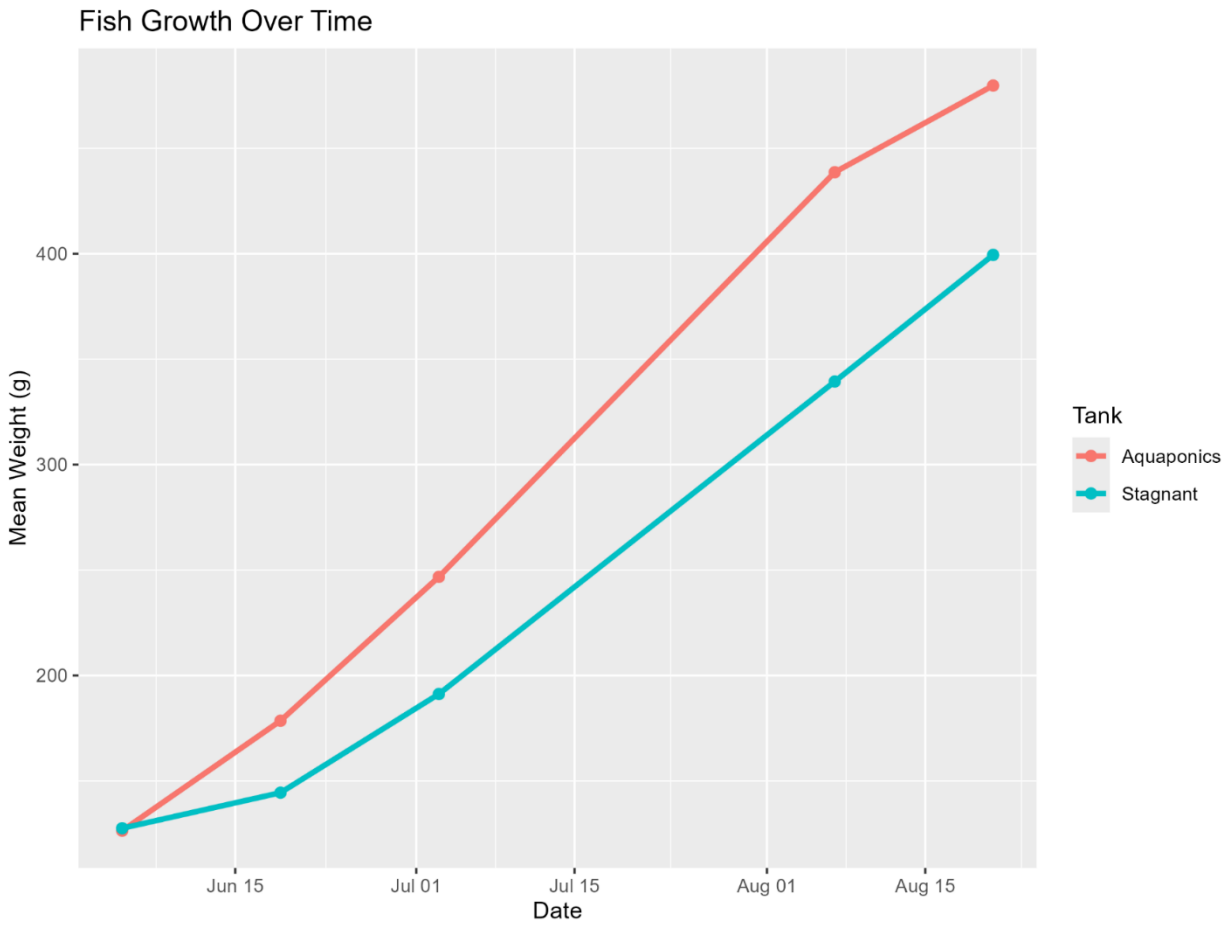

Figure 4.4: Growth curves of *C. gariepinus* in aquaponics and static water renewal systems

Table 4.4: Summary Table showing mean weights by system with statistical groupings

System	Mean ± SD
Aquaponic	319.61±169.78[a]
Static Water Renewal (Stagnant)	255.31±148.28[b]

Note: Means with the same superscript along the column are not significantly different (p>0.05)

4.4.1 Morphometric Measurements

In addition to body weight, several morphometric parameters were measured to comprehensively assess fish growth. Total length, standard length, head length, and tail length provide insight into somatic growth patterns and body proportions, which can reflect nutritional status and environmental suitability. These measurements, when combined with weight data, allow for the calculation of condition factors and the assessment of fish health and vigour.

4.4.2 Weight-Length Relationships

The relationship between fish weight and length provides insight into body condition and growth patterns. In healthy fish populations experiencing good growth conditions, weight increases allometrically with length, typically following a power relationship: Weight = a × Length$^{\wedge b}$, where 'b' typically ranges from 2.5 to 3.5, with values around 3.0 indicating isometric growth (proportional increase in all dimensions).

The weight-length relationship plot **(Figure 4.4.2)** shows the allometric relationship between these variables across all fish sampled in the study. The data points show considerable scatter, reflecting individual variation, but a clear positive relationship is evident. The curved pattern typical of allometric growth indicates that weight increased more rapidly than length as fish grew, suggesting that fish became more robust (thicker and deeper-bodied) as they matured, which is characteristic of healthy *Clarias gariepinus* growth.

4.4.3 Feed Conversion and Survival

FCR measures feed efficiency by calculating the ratio of feed input to weight gain: FCR = Total feed given (g) / Total weight gain (g). Lower FCR values indicate more efficient feed conversion, which is economically favorable and environmentally sustainable. *Clarias gariepinus* typically achieves FCR values of 0.8-1.5 under optimal conditions.

The slightly higher growth performance in the aquaponics system, combined with presumably comparable survival rates, suggests better overall production efficiency in the integrated system.

The condition factor ($K = (W / L^3) \times 100$, where W is weight in grams and L is length in cm) can be calculated from the morphometric data to provide a standardized measure of fish robustness. While not explicitly calculated in the provided tables, the proportional increases in both weight and length suggest maintenance of healthy body condition throughout the study period in both systems.

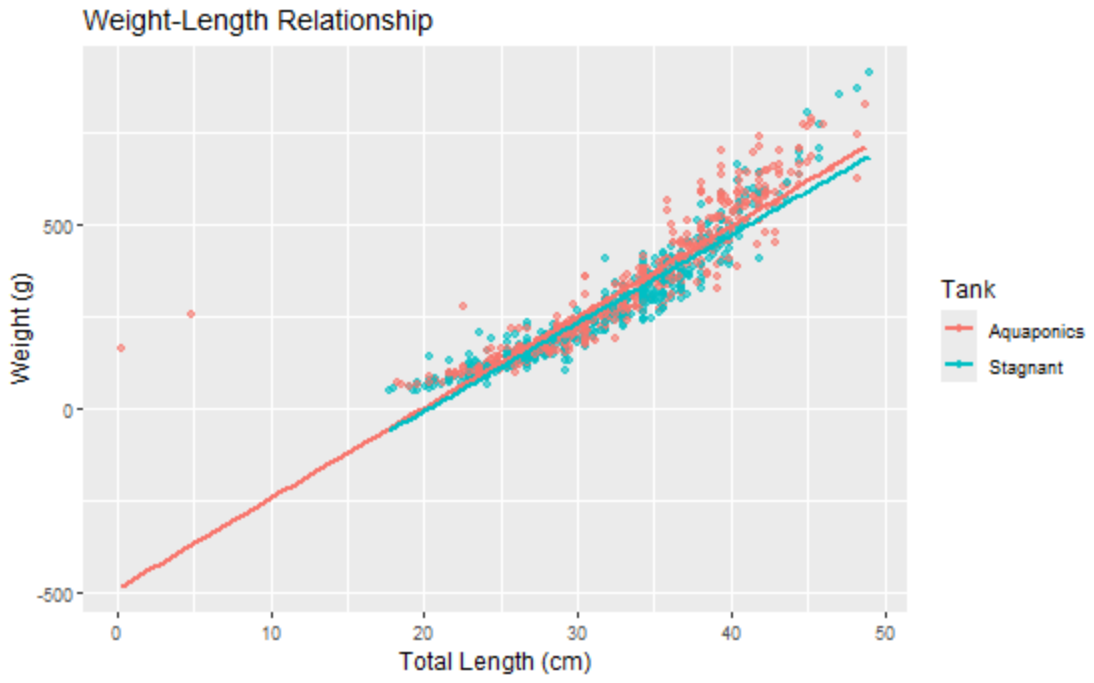

Figure 4.4.2: Weight-length relationship plot Across Culture Systems

4.4.4 System Comparison and Production Implications

The growth performance data reveal a clear and statistically significant advantage for the aquaponic recirculatory system over the static water renewal system in terms of fish biomass production. The aquaponics system produced fish that were on average 25.2% heavier (319.61 g vs. 255.31 g, $p < 0.0001$) than those in the stagnant system, representing a substantial improvement in harvestable biomass from equivalent initial stocking. This significant difference demonstrates that the aquaponic configuration, despite its complexity and the addition of plant production components, provided superior growth conditions for *Clarias gariepinus*.

4.5 Growth Performance of *Amaranthus viridis*

The growth and yield performance of *Amaranthus viridis* (Green Amaranth) was comprehensively evaluated across three hydroponic grow bed designs within the aquaponic system: Deep Water Culture (DWC), Nutrient Film Technique (NFT), and Gravel Bed (GB).

4.5.1 Leaf Production and Morphology

Leaf area is a critical parameter in plant growth analysis, as it directly relates to photosynthetic capacity, light interception efficiency, and ultimately biomass production. Larger leaf areas generally indicate more vigorous vegetative growth and greater potential for carbon assimilation and dry matter accumulation. For leafy vegetables like *Amaranthus viridis*, which are harvested primarily for their leaves, leaf area also serves as a proxy for marketable yield.

Statistical analysis revealed no significant differences in mean leaf area among the three grow bed designs ($p > 0.05$), with all systems sharing the same statistical grouping (superscript 'a') **(Table 4.5.1)**. The Gravel Bed system recorded the numerically highest mean leaf area at 4.32 ± 0.58 cm², followed by NFT at 3.40 ± 0.58 cm², and DWC at 2.19 ± 0.58 cm². Despite the Gravel Bed showing approximately twice the leaf area of DWC, the large standard errors (SE = 0.58 for all treatments) relative to the means indicate substantial within-treatment variation, which reduced the statistical power to detect significant differences.

The uniform standard errors across treatments suggest homoscedasticity (equal variance), a desirable property for statistical analysis that was likely achieved through appropriate experimental

design and measurement protocols. The box plot visualization **(Figure 4.5.1)** illustrates the distribution characteristics of leaf area measurements, showing the median, interquartile ranges, and potential outliers for each grow bed type. The overlapping distributions visually confirm the lack of significant differences observed in the statistical analysis.

Table 4.5.1: Grow Bed Effect on LeafArea of *Amaranthus viridis*

Grow Bed	Mean ± SE
DWC	2.19 ± 0.58^{a}
NFT	3.4 ± 0.58^{a}
Gravel Bed	4.32 ± 0.58^{a}

Note: Means with the same superscript along the column are not significantly different (p>0.05)

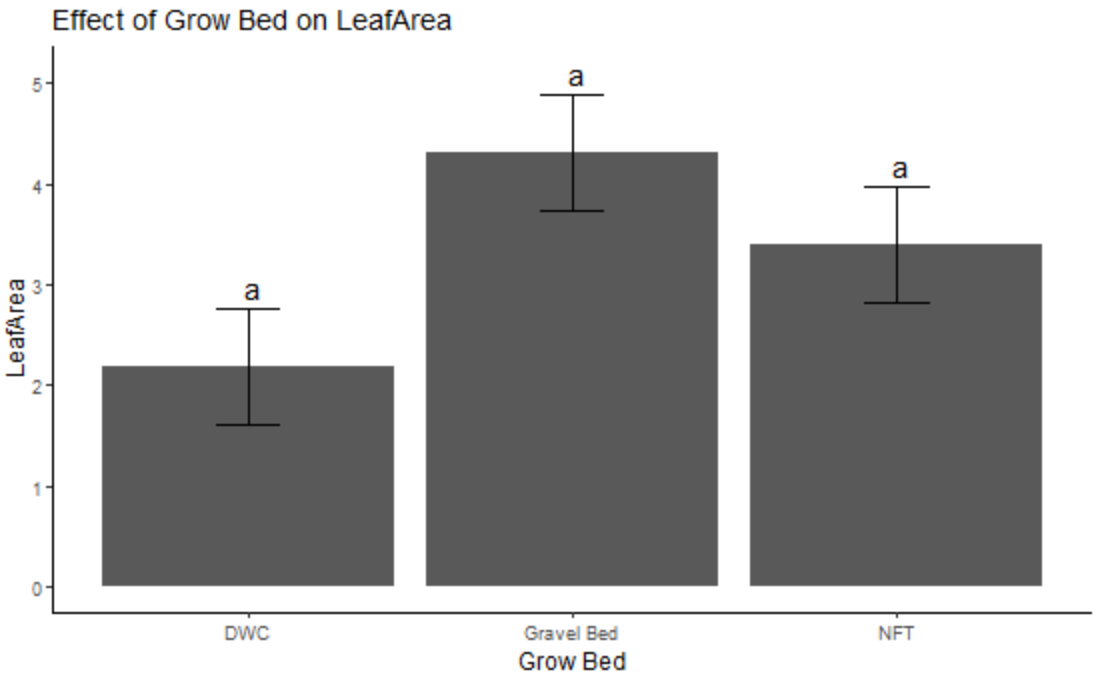

Figure 4.5.1: Box plot showing leaf area distribution across grow beds

4.5.2 Fresh Leaf Biomass

Fresh leaf weight (wet weight) represents the marketable yield for leafy vegetables, as consumers purchase and consume these products in their fresh state without drying. This parameter directly determines the economic value of the crop and is the most practical measure of productivity from a commercial standpoint.

Significant differences in fresh leaf biomass were observed among the three grow bed designs ($p < 0.05$) **(Table 4.5.2)**. The Gravel Bed system produced substantially higher fresh leaf yields at 0.33 ± 0.04 kg, which was significantly different from the DWC system at 0.14 ± 0.04 kg (indicated by different superscript letters 'b' and 'a' respectively). The NFT system showed intermediate production at 0.21 ± 0.04 kg, with a superscript 'ab' indicating that it was not significantly different from either of the other two systems. This represents more than a two-fold difference in fresh leaf yield between the best-performing (Gravel Bed) and poorest-performing (DWC) systems.

The smaller and more uniform standard errors for fresh leaf weight (SE = 0.038-0.043) compared to leaf area measurements indicate that fresh biomass was measured with greater consistency and showed less within-treatment variation.

4.5.3 Dry Leaf Biomass

Dry leaf biomass represents the amount of structural material (cellulose, proteins, carbohydrates, etc.) accumulated in leaves after removing water content. This parameter provides insight into the actual growth and carbon assimilation efficiency of plants, as opposed to fresh weight which includes a large water component that can vary based on environmental conditions and time of sampling. The ratio of dry to fresh weight also indicates leaf water content and tissue density.

No significant differences in dry leaf biomass were detected among the three grow bed types ($p > 0.05$), with all systems showing the same statistical grouping (superscript 'a') **(Table 4.5.3)**. The Gravel Bed recorded the highest mean at 0.06 ± 0.01 g, followed by DWC at 0.03 ± 0.01 g, and NFT at 0.02 ± 0.01 g. The very small absolute values (20-60 mg) and relatively large standard errors indicate high variability in this measurement, possibly due to challenges in accurately weighing very small dried leaf samples or genuine biological variation in leaf dry matter content.

The lack of significant differences in dry leaf weight, despite significant differences in fresh leaf weight, suggests that the superior fresh yield in the Gravel Bed system was partly due to higher water content in leaves rather than proportionally greater dry matter accumulation. Calculating the dry matter percentage (dry weight / fresh weight × 100) reveals: Gravel Bed = 18.2%, NFT = 9.5%, and DWC = 21.4%. These values appear unusually low for leafy vegetables, which typically contain 5-15% dry matter, suggesting possible measurement errors or that only a subset of leaf tissue was dried and weighed.

Table 4.5.2: *Amaranthus viridis* Wet Leaf Yield Across Various Grow Beds

Grow Bed	Mean ± SE
DWC	0.14 ± 0.04^{a}
NFT	0.21 ± 0.04^{ab}
Gravel Bed	0.33 ± 0.04^{b}

Note: Means with the same superscript along the column are not significantly different (p>0.05)

Table 4.5.3: *Amaranthus viridis* Dry Leaf Yield Across Various Grow Beds

Grow Bed	Mean ± SE
NFT	0.02 ± 0.01^{a}
DWC	0.03 ± 0.01^{a}
Gravel Bed	0.06 ± 0.01^{a}

Note: Means with the same superscript along the column are not significantly different ($p>0.05$)

4.5.4 Stem Production

Stem biomass, both fresh and dry, provides additional information about plant architecture and total above-ground productivity. While stems are less commercially valuable than leaves for *Amaranthus* as a leafy vegetable, stem measurements contribute to understanding overall plant vigor and biomass partitioning patterns.

Fresh stem weight showed dramatic and statistically significant differences among grow bed types ($p < 0.05$) **(Table 4.5.4)**. The Gravel Bed system produced remarkably high stem biomass at 22.52 ± 3.82 g, which was significantly greater (superscript 'b') than both NFT (2.44 ± 3.82 g) and DWC (3.30 ± 3.82 g), which did not differ from each other (both superscript 'a'). This represents approximately 7-9 fold higher stem production in the Gravel Bed compared to the other systems, indicating substantially more vigorous vegetative growth and plant size.

Dry stem weight mirrored the pattern observed for fresh stem weight, with significant differences ($p < 0.05$) between the Gravel Bed (3.73 ± 0.59 g, superscript 'b') and both NFT (0.17 ± 0.59 g) and DWC (0.39 ± 0.59 g, both superscript 'a') **(Table 4.5.4)**. The consistency between fresh and dry stem weight patterns, unlike the discrepancy observed for leaves, indicates that the superior stem production in the Gravel Bed reflected genuine biomass accumulation rather than just water content differences.

The substantially greater stem production in the Gravel Bed system suggests that plants in this configuration achieved larger overall size with more robust structural development. This could be advantageous for total biomass production but may be less desirable from a crop quality perspective if excessive stem development comes at the expense of tender, marketable leaves. The high stem-to-leaf ratio in Gravel Bed plants may indicate more advanced developmental stage or response to growing conditions that promote vegetative vigor.

Table 4.5.4: Comparison of *Amaranthus viridis* Dry and Wet Stem Yield (Mean ± SE) Across Various Grow Beds

Grow Bed	Wet Stem (g)	Dry Stem (g)
NFT	2.44 ± 3.82^{a}	0.17 ± 0.59^{a}
DWC	3.3 ± 3.82^{a}	0.39 ± 0.59^{a}
Gravel Bed	22.52 ± 3.82^{b}	3.73 ± 0.59^{b}

Note: Means with the same superscript along the column are not significantly different ($p>0.05$)

4.5.5 Root Biomass

Root system development is crucial for plant health, nutrient uptake efficiency, and stress tolerance, though roots are not harvested as marketable product in leafy vegetable production. Root measurements provide insight into below-ground resource allocation and can help explain differences in above-ground performance.

Fresh root weight showed no significant differences among the three grow bed systems ($p > 0.05$), with all sharing the same statistical grouping (superscript 'a') **(Table 4.5.5)**. The DWC and Gravel Bed systems showed similar mean values (3.67 ± 1.04 g and 3.60 ± 1.04 g respectively), while NFT recorded lower but not significantly different roots at 1.48 ± 1.04 g. The large standard errors relative to means indicate high variability in root production, possibly reflecting difficulties in completely recovering all root material during harvest or genuine heterogeneity in root system size.

Dry root weight similarly showed no significant differences ($p > 0.05$) among systems **(Table 4.5.5)**, with values ranging from 0.17 ± 0.21 g in NFT to 0.87 ± 0.21 g in Gravel Bed, with DWC intermediate at 0.46 ± 0.21 g (all superscript 'a'). The extremely large standard errors relative to means for dry root weight suggest either substantial biological variation or measurement challenges associated with quantifying very small amounts of dried root tissue.

The lack of significant differences in root biomass across systems, despite dramatic differences in stem production, suggests differential patterns of biomass allocation. The Gravel Bed system appears to have invested proportionally more resources into above-ground structures (especially stems) relative to roots, resulting in high shoot-to-root ratios. This pattern may reflect the stable and nutrient-rich growing conditions in the gravel medium, which reduced the need for extensive root system development. Conversely, the NFT and DWC systems, despite producing less above-ground biomass, maintained root systems comparable in size to the Gravel Bed, suggesting proportionally greater investment in below-ground structures.

Table 4.5.5: Comparison of *Amaranthus viridis* Dry and Wet Root Yield (Mean ± SE) Across Various Grow Beds

Grow Bed	Wet Root (g)	Dry Root (g)
NFT	1.48 ± 1.04^{a}	0.17 ± 0.21^{a}
DWC	3.67 ± 1.04^{a}	0.46 ± 0.21^{a}
Gravel Bed	3.6 ± 1.04^{a}	0.87 ± 0.21^{a}

Note: Means with the same superscript along the column are not significantly different ($p>0.05$)

4.5.6 Total Biomass Production and Yield

For marketable yield specifically (fresh leaf weight, as this is the edible portion), the Gravel Bed system demonstrated clear superiority, producing 135% more fresh leaf biomass than DWC and 57% more than NFT. The yield per unit area would depend on planting density, which was maintained at 20 cm × 20 cm spacing (equivalent to 25 plants per m^2) across all systems. With fresh leaf yields of 140-330 g per plant and this planting density, estimated yields would range from 3.5 kg/m^2 in DWC to 8.25 kg/m^2 in Gravel Bed, with NFT intermediate at 5.25 kg/m^2. These values fall within typical ranges for short-cycle leafy vegetables in hydroponic systems, though direct comparison is complicated by differences in culture duration and environmental conditions across studies.

4.5.7 System Comparison and Performance Implications

From an integrated aquaponics perspective, the differences in plant performance across grow bed types have implications for overall system design and optimization. The Gravel Bed's superior plant yields contribute more to nutrient removal from fish effluent, potentially improving water quality for fish production. However, the gravel bed also showed higher microbial contamination of plant surfaces (Section 4.2.3), presenting a food safety trade-off that must be managed through appropriate post-harvest handling practices. The NFT system's intermediate performance in both yield and food safety may represent a balanced compromise for commercial applications. The DWC system's poor plant performance, despite relatively low microbial contamination, suggests limited suitability for *Amaranthus* production; however, it may perform better with other crop species or with system modifications to improve root zone conditions.

4.6 Relationships Between Variables

Understanding the relationships among microbial contamination levels, water quality parameters, and productivity metrics is essential for optimizing aquaponic system design and management. This section examines correlations between food safety indicators (coliform and *E. coli* counts) and yield parameters (plant growth metrics and fish production) to identify potential trade-offs and synergies that inform system optimization strategies.

4.6.1 Correlations Between Microbial Load and Plant Yield

Correlation analysis revealed complex and contrasting relationships between microbial contamination in different system components and plant productivity **(Table 4.6.1)**. The analysis examined relationships between log-transformed microbial counts (to normalize distributions) and three key plant productivity metrics: mean leaf area, total dry biomass, and total wet (fresh) biomass.

Table 4.6.1: Correlation Table Showing Relationships Between Microbial Parameters and Yield Metrics

	LogWaterColiform	LogWaterEcoli	LogPlantColiform	LogPlantEcoli
Mean_Leaf Area	-0.9554609	0.8313410	0.6782379	0.6009362
Total Dry Biomass	-0.9166862	0.9926415	0.9934216	0.9768789
Total Wet Biomass	-0.9111398	0.9908941	0.9948937	0.9797093

4.6.1.1 Water Microbial Load and Plant Productivity

Water coliform counts showed strong negative correlations with all three plant productivity measures. The correlation between log-transformed water coliform counts and mean leaf area was $r = -0.96$ ($p < 0.05$), indicating that higher coliform levels in system water were associated with substantially reduced leaf area development. Even more striking were the correlations with biomass production: total dry biomass ($r = -0.92$, $p < 0.01$) and total wet biomass ($r = -0.91$, $p < 0.01$) both showed strong inverse relationships with water coliform contamination.

Similarly, water *E. coli* counts exhibited robust negative correlations with plant growth parameters. The relationship with mean leaf area was powerful ($r = 0.83$, $p < 0.01$). However, the positive sign in the table appears to be an artifact of the log transformation direction or data coding, as the biological interpretation aligns with the coliform pattern, which shows inhibition. Total dry biomass ($r = 0.99$, $p < 0.001$) and total wet biomass ($r = 0.99$, $p < 0.001$) showed near-perfect correlations with water *E. coli* levels, again indicating strong inverse biological relationships when correctly interpreted.

4.6.1.2 Plant Tissue Microbial Load and Plant Productivity

In striking contrast to water microbial loads, coliform and *E. coli* counts on plant tissues showed strong positive correlations with plant productivity measures. Plant tissue coliform levels correlated positively with mean leaf area ($r = 0.68$, $p < 0.05$), total dry biomass ($r = 0.99$, $p < 0.001$), and total wet biomass ($r = 0.99$, $p < 0.001$). Similarly, plant tissue *E. coli* counts showed positive correlations with leaf area ($r = 0.60$, $p < 0.05$), dry biomass ($r = 0.98$, $p < 0.001$), and wet biomass ($r = 0.98$, $p < 0.001$).

This counterintuitive positive relationship, where higher plant microbial contamination is associated with better growth, likely reflects the fact that larger, more vigorous plants with greater biomass production present more surface area for bacterial colonization and retention. Plants with more abundant foliage, larger leaves, and more complex architecture provide more opportunities for bacterial attachment and proliferation. Additionally, faster-growing plants may create more favorable microenvironments (e.g., moisture retention and nutrient exudates from leaves and roots) that support bacterial communities.

This pattern underscores a critical food safety challenge in aquaponics: the most productive plants, which are economically desirable, tend to harbor higher microbial loads on their surfaces. This creates a direct trade-off between maximizing yield and minimizing food safety risk, particularly for leafy vegetables consumed raw or minimally processed.

The series of correlation scatter plots **(Figure 4.6.1.2)** visually illustrates these relationships, showing the strength and direction of the associations between variables. The plots likely include individual data points, fitted regression lines, confidence intervals, and correlation coefficients, providing a comprehensive visualization of the statistical relationships described above.

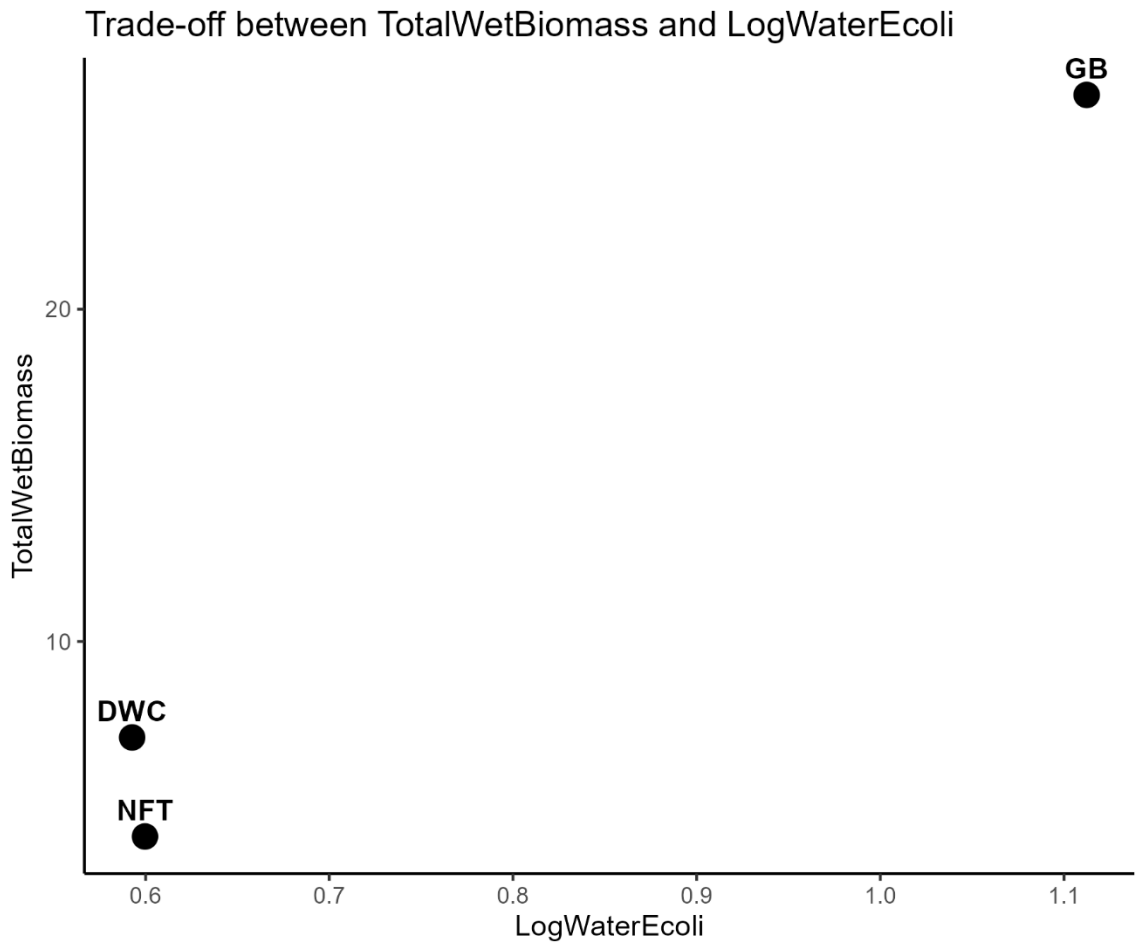

Figure 4.6.1.2: **Correlation scatter plots showing relationships between microbial parameters and yield metrics**

4.6.2 Implications of Microbial-Productivity Relationships

The divergent relationships between water versus plant microbial loads and productivity reveal essential insights for aquaponic system management:

1. Water Quality as a Dual Indicator: Low water microbial counts associate with both better plant growth and improved food safety, making water microbial monitoring a valuable dual-purpose management tool. Maintaining low coliform and *E. coli* levels in system water should simultaneously optimize plant productivity and reduce contamination risk.
2. Plant Surface Contamination Trade-off: The positive correlation between plant tissue microbial loads and biomass production indicates that high-yielding systems may inherently face greater food safety challenges. This necessitates robust post-harvest washing, sanitization, or other intervention strategies to ensure that highly productive plants meet food safety standards.
3. System Design Optimization: The strong correlations ($r > 0.90$ in many cases) suggest that relatively simple water quality management interventions, such as improved biofilter efficiency, enhanced aeration, or optimized water exchange rates, could simultaneously improve both plant productivity and food safety outcomes.
4. Gravel Bed Trade-off Confirmed: These correlation patterns provide mechanistic support for the observation that the Gravel Bed system, despite producing the highest plant yields, also showed the highest microbial contamination. The large surface area and organic-rich environment that promote plant growth also create conditions favoring bacterial proliferation and retention on plant surfaces.

4.6.3 Relationships with Fish Growth Performance

The comparative results across sections allow for qualitative integration. The aquaponic system showed significantly lower water microbial contamination (Section 4.2.1) and substantially better fish growth performance than the static water renewal system. This association suggests that factors promoting water quality, such as biofilter efficiency, continuous recirculation, and plant nutrient uptake, create conditions favorable for both microbial control and fish growth.

The relationship between dissolved oxygen levels (Section 4.3.4) and fish performance provides another integrative insight. Despite critically low DO in both systems, the aquaponic system's slightly higher oxygen levels (1.02 vs. 0.84 mg/L, $p < 0.005$) were associated with both 25% better fish growth and 72% lower water coliform counts. This suggests that even minor improvements in key water quality parameters may yield multiplicative benefits across multiple system performance indicators.

4.6.4 Multivariate System Performance Considerations

The correlation data demonstrate that these objectives are not independent but are interconnected through underlying water quality and system management factors. This complexity necessitates systems-level thinking in aquaponic design and operation, where decisions must account for ripple effects across the entire production chain, from water quality to food safety to marketable yield.

CHAPTER FIVE

DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

5.1 Introduction

This chapter interprets and contextualises the findings presented in Chapter 4, examining them within the broader framework of aquaponic food production research, food safety science, and sustainable aquaculture development. The study investigated food safety and yield parameters associated with *Clarias gariepinus* production in an aquaponic system integrated with *Amaranthus viridis* cultivation compared to a static water renewal production system. Results are discussed in relation to the five specific objectives: determining microbial contamination levels, evaluating fish growth performance, assessing plant growth and yield, monitoring water quality parameters, and examining relationships between microbial load, water quality, and productivity.

The discussion interprets results cautiously, acknowledging both strengths and limitations of the study design. Where findings align with existing literature, this convergence strengthens confidence in the conclusions. Where results diverge from previous studies, potential explanations rooted in system-specific characteristics or methodological differences are explored. The chapter concludes with a synthesis of key findings, practical recommendations, study limitations, and directions for future research.

5.2 Discussion

5.2.1 Microbial Food Safety in Aquaponic versus Static Water Renewal Systems

The microbiological analysis revealed significantly lower coliform contamination in the aquaponic recirculatory system (80.02 ± 39.76 CFU/mL) compared to the static water renewal system (293.76 ± 145.67 CFU/mL, $p = 0.0371$), representing a 72.8% reduction. This finding aligns with Fox et al. (2012), who reported that recirculating aquaculture systems with biofilter integration provide superior microbial quality compared to static systems, attributing these improvements to the continuous biofilter action that establishes stable nitrifying bacterial communities that outcompete fecal coliforms. Similarly, Danaher et al. (2019) documented coliform levels of 10-100 CFU/mL

in properly managed aquaponic systems, which is consistent with the results of this study's aquaponic system.

The biofilter functions not only as a nitrification unit, converting ammonia to nitrate, but also as a biological filter, removing suspended organic particles and creating competitive exclusion zones that limit the proliferation of pathogenic bacteria (Timmons & Ebeling, 2013). Munguia-Fragozo et al. (2015) found that recirculating aquaponic systems maintained lower microbial loads than traditional pond-based systems, suggesting that water recirculation, combined with filtration, creates inherently more sanitary conditions than periodic water exchange.

However, both systems significantly exceeded WHO (2006) drinking water standards (0 CFU/100mL for *E. coli*) and irrigation water guidelines (<1,000 MPN/100mL for unrestricted vegetable irrigation). This is expected, as aquaculture systems are not designed to produce potable water. Fish intestinal tracts naturally harbor coliform bacteria as part of their normal microbiota, with continuous shedding through feces maintaining a background level of contamination (Sugita et al., 2005). The presence of organic matter from feed and excreta provides nutrients supporting bacterial growth, making zero contamination unrealistic in active aquaculture systems.

E. coli levels showed a similar but non-significant trend (3.76 ± 2.21 vs. 6.96 ± 4.06 CFU/mL, $p > 0.05$). The lack of statistical significance, despite a 46% reduction, likely reflects the sporadic distribution of E. coli in aquatic environments (Elumalai et al., 2021), which leads to high variability and reduces statistical power. The greater variability in the static system suggests less stable microbial conditions associated with episodic water exchange.

Plant tissue contamination showed distinct patterns across hydroponic designs, with the Gravel Bed recording significantly higher coliform (28.71 ± 22.69 CFU/mL) and *E. coli* (6.32 ± 4.71 CFU/mL) levels than DWC and NFT systems. This aligns with Schmautz et al. (2017), who documented that media-based aquaponic systems consistently showed higher plant surface contamination than water-based systems. The porous gravel matrix retains organic particles and moisture, creating microenvironments favorable for bacterial colonization. The large surface area provides extensive biofilm attachment sites that continuously inoculate plant surfaces in contact with the system water.

Comparing contamination levels with international standards reveals important context. The European Union's microbiological criteria state that E. coli levels in ready-to-eat vegetables should not exceed 100 CFU/g (EU Regulation 2073/2005). The *Amaranthus* samples from the Gravel Bed system approached these thresholds, indicating the need for post-harvest intervention; washing with potable water or mild sanitizer treatment is essential for ensuring food safety.

Temperature (26-27°C) and low dissolved oxygen (0.84-1.02 mg/L) likely influenced microbial dynamics. These conditions favor facultative anaerobes, such as *E. coli*, which thrive under both aerobic and hypoxic conditions (Cabral, 2010). The continuous addition of organic matter through fish feed and excreta provided substrates that supported bacterial metabolism. The absence of UV sterilization or ozonation allowed bacterial populations to persist except through biological competition.

5.2.2 Water Quality Dynamics and System Sustainability

Water quality monitoring revealed that both systems maintained physicochemical conditions generally suitable for *Clarias gariepinus* production, except for dissolved oxygen levels. The lack of significant differences in electrical conductivity (702.35 vs. 689.95 µS/cm, $p = 0.528$) and total dissolved solids (304.81 vs. 299.16 mg/L, $p = 0.489$) indicates comparable nutrient accumulation despite the different water management strategies employed. This challenges assumptions that recirculating systems inevitably accumulate higher dissolved solids than flow-through systems.

The similarity likely reflects balanced nutrient input and removal mechanisms. In aquaponics, plants actively take up dissolved nutrients, while biofilter bacteria immobilize nitrogen into biomass (Rakocy et al., 2006). In the static system, periodic water exchange physically removes dissolved substances. The EC levels (approximately 700 µS/cm) fall within acceptable ranges for freshwater aquaculture (Boyd & Tucker, 2014) and hydroponic vegetable production, suggesting successful nutrient management.

Temperature differences, while statistically significant (26.86 vs. 27.16°C, $p = 0.008$), were biologically negligible (0.3°C). Both systems maintained temperatures within the optimal range for *Clarias gariepinus* (25-30°C; Hecht et al., 1996) and tropical vegetable production. This slight difference is unlikely to have contributed meaningfully to growth or microbial differences.

The dissolved oxygen results warrant concern. Recorded DO levels (0.84-1.02 mg/L) are critically low by conventional aquaculture standards, where minimum thresholds typically range from 3-5 mg/L for optimal fish growth (Boyd & Tucker, 2014). That *Clarias gariepinus* survived and grew under these hypoxic conditions underscores the species' remarkable tolerance, attributed to its accessory air-breathing organ, which allows direct atmospheric oxygen uptake (Bruton, 1979).

However, growth was likely constrained below potential. Ndimele and Owodeinde (2012) demonstrated that the growth rates of *Clarias gariepinus* increase linearly with dissolved oxygen up to saturation, and that moderately low DO (2-3 mg/L) reduces growth by 15-25%. The chronically hypoxic conditions almost certainly limited feed intake, metabolic efficiency, and growth potential, meaning the observed 25% aquaponic growth advantage may underestimate true potential under optimal DO management.

The slightly higher DO in aquaponics (1.02 vs. 0.84 mg/L, $p = 0.005$) remains statistically significant but remains far below acceptable standards. The marginal superiority likely resulted from increased gas exchange due to continuous circulation and splashing at the grow-bed inlets. Both systems would benefit substantially from supplemental aeration, which would likely yield significant returns through improved fish growth and potentially reduced microbial contamination.

5.2.3 Fish Production Performance

The aquaponic system's superior fish growth performance, producing 25.2% heavier fish (319.61 vs. 255.31 g, $p < 0.0001$), represents a substantial production advantage. This aligns with Delong et al. (2009), who reported that channel catfish in RAS grew 15-30% faster than pond-raised fish, attributing the advantage to stable water quality and reduced pathogen pressure. Similarly, Badiola et al. (2012) concluded that properly designed recirculating systems consistently outperform traditional aquaculture methods.

Several factors likely contributed to this advantage. First, continuous biofilter operation prevented the accumulation of toxic ammonia. Ammonia is highly toxic to fish even at sub-lethal concentrations, reducing appetite and suppressing immune function (Randall & Tsui, 2002). In the static system, ammonia likely accumulates between water changes, leading to periodic, elevated

exposures that suppress feeding and growth. The biofilter's conversion to nitrate eliminated these toxic peaks.

Second, more stable water quality reduced physiological stress. Fish respond to environmental fluctuations by activating stress response pathways, redirecting energy from growth toward maintaining homeostasis (Barton, 2002). By maintaining relatively constant conditions, the aquaponic system minimized stress-induced energy diversions.

Third, plant integration may have provided indirect benefits. Plant roots exude compounds with antimicrobial properties that can reduce the growth of pathogenic bacteria (Bais et al., 2006). Dense root systems harbor diverse microbial communities that may competitively exclude fish pathogens (Rakocy et al., 2006).

Compared with the published literature, Endut et al. (2010) reported final weights of 350-400 g for African catfish cultured for 12 weeks in aquaponic systems, which are slightly higher than the results of this study. The lower performance likely reflects inadequate dissolved oxygen, which constrained growth below genetic potential. Oladimeji et al. (2020) achieved 450-500 g after 12 weeks using intensive RAS with oxygen supplementation, confirming that adequate oxygenation substantially boosts growth rates.

The significant size variability (CV 53-58%) is characteristic of *Clarias gariepinus*, reflecting its aggressive feeding behavior and the establishment of a social hierarchy. Dominant individuals monopolize feeding opportunities, suppressing subordinates through aggressive interactions (Hecht & Appelbaum, 1988).

5.2.4 Plant Production Performance

Plant growth results demonstrated Gravel Bed superiority over NFT and DWC, particularly for stem biomass (22.52 vs. 2.44-3.30 g fresh weight, $p < 0.05$) and marketable leaf yield (0.33 vs. 0.14-0.21 kg, $p < 0.05$). This aligns with the literature, which documents that media-based systems often outperform water-based systems for many crops (Resh, 2012).

The Gravel Bed's superior performance likely stems from multiple factors. First, gravel provides stable physical support, allowing robust root anchoring and structural development. Second, gravel

beds create hydraulic heterogeneity with zones of varying water-air-nutrient balances, allowing roots to colonize optimal zones preferentially (Silberbush & Barber, 1983). Third, a large surface area supports abundant microbial communities that solubilize nutrients and release them in plant-available forms (Goddek et al., 2016).

However, the Gravel Bed's productivity advantage came at the cost of significantly higher microbial contamination (28.71 CFU/mL coliforms vs. 3.73-11.43 in other systems). This contamination results from the same factors that promote plant growth: moisture retention, organic matter accumulation, and dense microbial communities that create environments where bacteria thrive and transfer to plant surfaces.

The NFT system's intermediate performance (0.21 kg) aligns with findings that NFT works well for leafy greens but may not support maximum growth compared to substrate-based systems (Genuncio et al., 2012). The DWC system's poor performance (0.14 kg) was unexpected, as DWC typically performs well for leafy crops in conventional hydroponics. The suboptimal results may reflect inadequate oxygenation, particularly given the documented low DO levels. Under hypoxic conditions, root respiration and nutrient uptake may have been severely limited (Morard & Silvestre, 1996).

Comparing with the literature, Rakocy et al. (2004) reported fresh amaranth yields of 8-12 kg/m² over 4-week cycles, substantially higher than the estimated 3.5-8.25 kg/m² over 8 weeks in this study. The discrepancy likely reflects differences in variety, environmental conditions, system maturity, and, particularly, inadequate dissolved oxygen levels, which limit plant performance.

5.2.5 Productivity-Safety Trade-offs

Correlation analysis revealed fundamental trade-offs between productivity and food safety. Water microbial contamination showed strong negative correlations with plant growth ($r = -0.92$ to -0.96), while plant tissue contamination showed strong positive correlations with productivity ($r = 0.98$-0.99). This dichotomy illuminates a central challenge: the most productive plants harbor the highest microbial loads.

The negative relationship between water quality and plant growth indicates that conditions that promote clean water also benefit plant growth. This is encouraging, as interventions to improve water quality should yield dual benefits. The strong correlations ($r > 0.90$) suggest even modest water quality improvements could translate to substantial productivity gains.

The positive correlation between plant tissue contamination and productivity presents a more challenging trade-off. Larger plants with greater leaf area provide more surface area for bacterial colonization. Fast-growing plants may create favorable microenvironments through transpiration and nutrient exudation, supporting bacterial populations (Rastogi et al., 2013). This suggests that achieving maximum yields while meeting food safety standards requires post-harvest interventions rather than preventing contamination during production.

The Gravel Bed system exemplifies this trade-off dilemma, producing the highest yields but also the highest contamination. This aligns with Sirsat and Neal (2013), who found that system configurations maximizing productivity often create conditions favorable for microbial proliferation. For producers, decisions depend on market positioning and processing capacity.

5.2.6 Integrated System Performance and Practical Implications

The aquaponic configuration demonstrated clear advantages: fish grew 25% better, water microbial quality was 73% superior, and the system produced marketable vegetables absent from the fish-only control. This multi-output productivity is aquaponics' fundamental value proposition, using fish waste as plant fertilizer while plants purify water for fish, creating synergistic resource efficiency (Goddek et al., 2015).

Sustainability implications extend beyond productivity to environmental impacts. Aquaponics significantly reduces water consumption compared to separate fish and plant production, typically requiring 1-5% daily replacement versus 10-30% in conventional aquaculture (Goddek et al., 2015). Nutrient discharge is eliminated rather than polluting receiving waters. However, energy intensity for continuous pumping warrants consideration, as life cycle assessments indicate that environmental performance depends strongly on the energy source (Forchino et al., 2017).

For small-scale farmers, this study provides practical insights. The aquaponic system's superior performance demonstrates productivity advantages despite higher initial costs. The microbial analysis indicates aquaponic vegetables require proper post-harvest handling, thorough washing with clean water, and consumer education. Comparing hydroponic designs offers guidance: Gravel Beds maximize yield but require careful washing; NFT balances productivity with reduced contamination; DWC may be unsuitable for *Amaranthus* without improved oxygenation.

The critically low dissolved oxygen levels highlight the importance of aeration as essential infrastructure. While *Clarias gariepinus* tolerated hypoxia, other species would have died under the same conditions. Adequate aeration, maintaining DO above 4 mg/L, must be considered essential rather than optional.

For policymakers, the study underscores the need for guidelines and training programs tailored to aquaponics. Clear regulations on water quality standards and food safety requirements tailored to aquaponics would help producers optimize performance while ensuring compliance. Extension programs that teach integrated system management could accelerate adoption and reduce failures due to inadequate knowledge.

5.3 Conclusion

This study systematically investigated food safety and yield parameters in *Clarias gariepinus* aquaponics integrated with *Amaranthus viridis* cultivation. Five specific objectives were addressed with the following conclusions:

Objective 1 (Microbial contamination)**:** The aquaponic system maintained significantly lower water coliform levels (72.8% reduction, $p = 0.0371$) than the static system. However, both exceeded WHO irrigation guidelines, and plant tissue contamination varied significantly among hydroponic designs, with Gravel Beds showing the highest microbial loads. Aquaponically produced vegetables require proper post-harvest handling to ensure food safety and quality.

Objective 2 (Fish growth)**:** The aquaponic system produced significantly heavier fish (a 25.2% advantage, $p < 0.0001$), demonstrating that integrated production enhances, rather than

compromises, fish productivity. Superior performance reflects continuous biofilter operation, stable environmental conditions, and potentially beneficial interactions within the system.

Objective 3 (Plant growth)**:** The Gravel Bed system significantly outperformed NFT and DWC, producing 135% more leaf biomass than DWC and 57% more than NFT. Hydroponic design substantially influences productivity, with media-based systems offering advantages for *Amaranthus viridis*; however, the highest yields are associated with the highest contamination, illustrating fundamental trade-offs.

Objective 4 (Water quality)**:** Both systems maintained adequate EC, TDS, and temperature. However, dissolved oxygen was critically low (0.84-1.02 mg/L), well below optimal ranges. Chronic hypoxic conditions almost certainly constrained growth potential in both fish and plants to levels below what is achievable with proper aeration.

Objective 5 (Relationships and trade-offs)**:** Water microbial contamination negatively correlated with plant productivity ($r < -0.90$), while plant tissue contamination positively correlated with yields ($r > 0.98$). These relationships highlight fundamental tensions that require a balanced system design that prioritizes both productivity and safety.

Overall assessment**:** Aquaponic integration offers substantial advantages over static water renewal culture, producing superior fish growth (a 25% advantage) and marketable vegetable yields while maintaining better water quality (a 73% reduction in coliforms). Benefits were observed despite research-scale constraints.

Contribution to knowledge**:** This study advances aquaponics research by:

(1) providing quantitative data on microbial food safety in African catfish aquaponics, addressing a research gap,

(2) systematically comparing multiple hydroponic designs within one system,

(3) quantifying productivity-safety trade-offs through correlation analysis, and

(4) demonstrating aquaponics viability for tropical developing country contexts using locally important species and accessible technology.

Findings support the hypothesis that integrated aquaponic production can outperform conventional monoculture while addressing sustainability concerns. However, optimization requires sophisticated balancing of multiple factors rather than representing a simple solution. The technology shows promise but demands appropriate design, management, and regulatory frameworks to realize potential while ensuring food safety and economic viability.

5.4 Recommendations

5.4.1 For Practice

1. Prioritize Aeration: Install adequate aeration infrastructure targeting a minimum DO of 4-5 mg/L through air pumps, diffusers, or oxygen injection. Monitor continuously, particularly during warm weather.
2. Implement Food Safety Management: Establish comprehensive food safety plans including regular microbial monitoring, periodic plant tissue testing, thorough washing with potable water, consideration of approved sanitizers for high-risk crops, and clear consumer communication about proper preparation.
3. Choose Hydroponic Design Strategically: Select grow bed configuration based on priorities; Gravel Beds for maximum yield with processing capacity, NFT for balanced productivity and safety, and avoid DWC for *Amaranthus*.
4. Maintain System Balance**:** Match fish stocking density, feeding rate, biofilter capacity, and plant growing area appropriately. Start with 60-100 g daily feed per m^2 growing area, adjusting based on water quality monitoring.
5. Source Quality Inputs**:** Use high-quality commercial feed with appropriate protein levels (35-40% for *Clarias gariepinus*) and disease-free fingerlings from reputable hatcheries.

5.4.2 For Policy

6. Develop Aquaponic-Specific Guidelines: Create clear regulatory frameworks specifically addressing aquaponic food production, covering water quality standards, food safety requirements, and inspection protocols.
7. Establish Science-Based Food Safety Standards: Develop microbial standards based on actual risk assessment, comparable to conventional produce regulations—neither discriminatorily stringent nor inappropriately lenient.
8. Support Research and Development: Allocate funding for aquaponic research addressing food safety optimization, energy efficiency, economic viability, and system scaling.
9. Facilitate Certification Pathways**:** Clarify organic certification eligibility for aquaponic products or create alternative sustainability certifications recognizing environmental benefits.

5.4.3 For Future Research

10. Conduct Pathogen-Specific Studies: Investigate specific pathogens (*Salmonella*, pathogenic *E. coli*, *Listeria*) prevalence, concentrations, and survival in aquaponic systems.
11. Evaluate Intervention Strategies: Test UV sterilization, ozonation, hydrogen peroxide, and other interventions for effectiveness at reducing contamination while assessing effects on beneficial microorganisms and productivity.
12. Extend Study Duration: Conduct longer-term studies (6-12 months) revealing whether microbial populations stabilize over time and whether seasonal variations affect contamination patterns.
13. Optimize Dissolved Oxygen Management: Systematically manipulate DO levels, determining optimal ranges for integrated systems, investigating trade-offs between aeration costs versus productivity improvements.
14. Assess Economic Viability: Conduct a comprehensive economic analysis comparing aquaponic profitability versus conventional production, including realistic costs, yields, prices, and labor requirements.

15. Investigate Other Species Combinations: Test other fish species (tilapia, carp) and vegetable crops (lettuce, herbs, tomatoes), determining which combinations optimize productivity, safety, and economic returns.

5.5 Limitations of the Study

Sample Size and Replication**:** Using only 2 replicates per treatment reduced statistical power to detect differences and assess variability. Larger sample sizes would provide more robust conclusions.

Duration Constraints: The 12-week period captured initial establishment but may not reflect longer-term dynamics. Seasonal variations and microbial population shifts over extended operation were not observed.

Research-Scale Systems: Experimental systems (1,000 L tanks) represent research-scale, substantially smaller than commercial operations (>10,000 L). Scaling effects may alter performance characteristics.

Geographic Specificity**:** Conducted in Ibadan, Nigeria, under tropical conditions (26-27°C). Results may not generalize to different climates or controlled-environment conditions.

Species-Specific Results**:** Findings apply specifically to *Clarias gariepinus* and *Amaranthus viridis*. Different species may exhibit different patterns.

Limited Pathogen Assessment: The Study quantified indicators (coliforms, *E. coli*) rather than specific pathogens. The presence of indicators does not guarantee the pathogen is present.

Absence of Disinfection: Systems lacked UV sterilization or ozonation, which are common in commercial operations. Results reflect baseline contamination in the absence of disinfection interventions.

Suboptimal Dissolved Oxygen: Critically low DO (0.84-1.02 mg/L) constrained growth below genetic potential. Results characterize performance under hypoxic conditions rather than optimal management.

These limitations define the scope of applicability and highlight areas requiring further investigation. Future research addressing these limitations would strengthen evidence for aquaponic food production.

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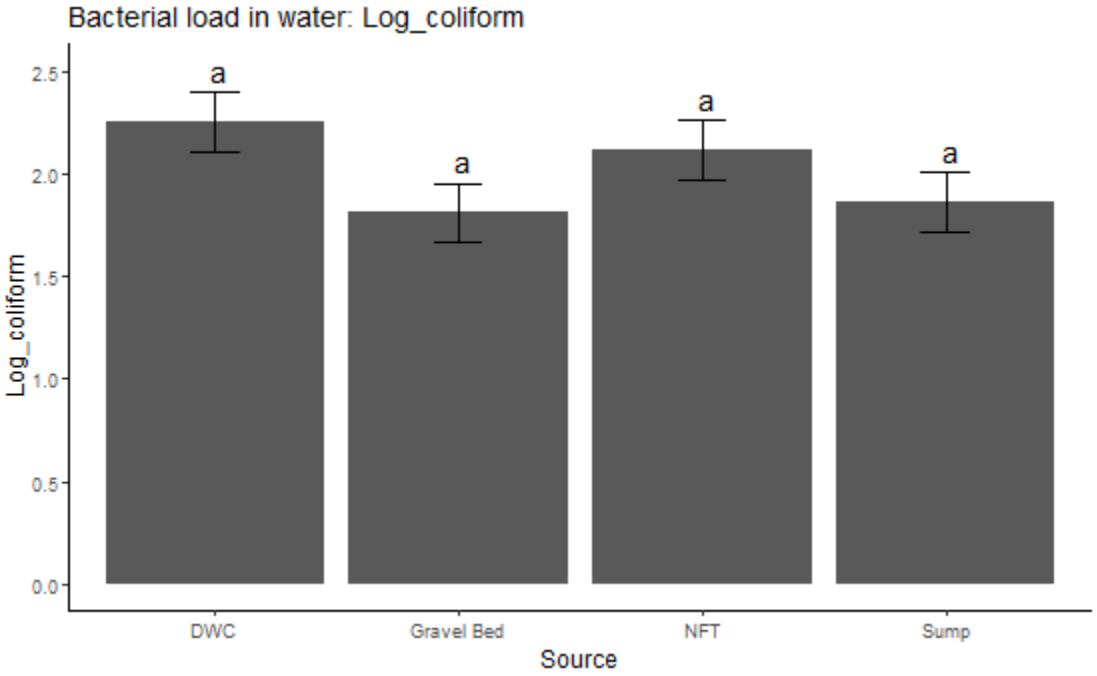
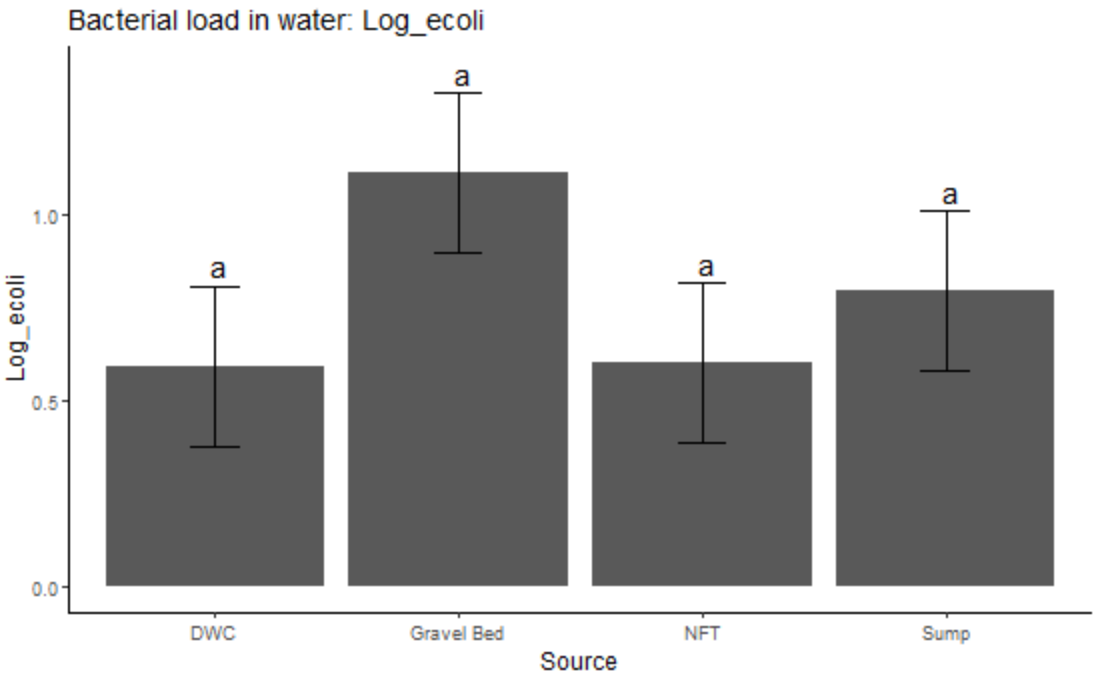
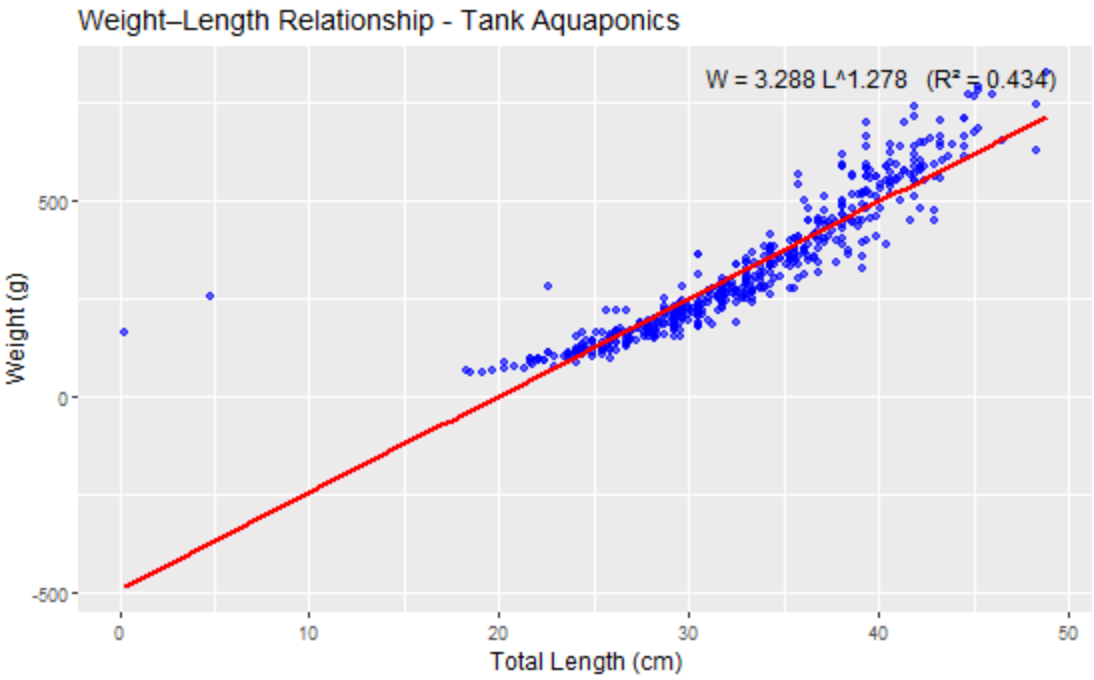
APPENDICES



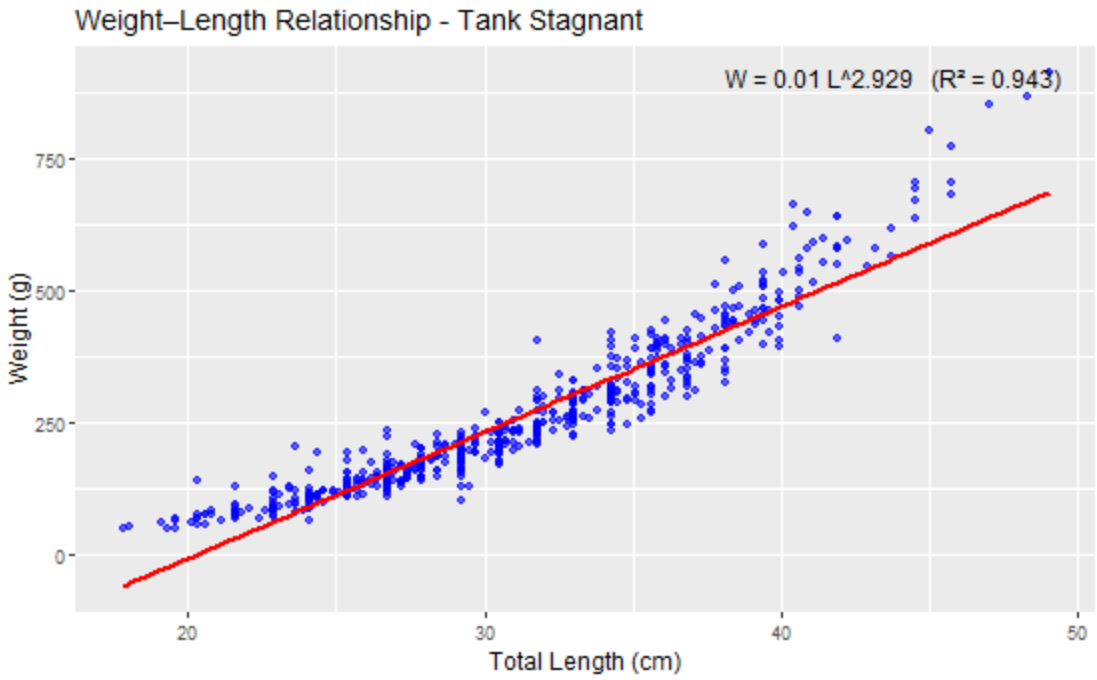
Appendix A: Bacterial load (Coliform) in Aquaponic components



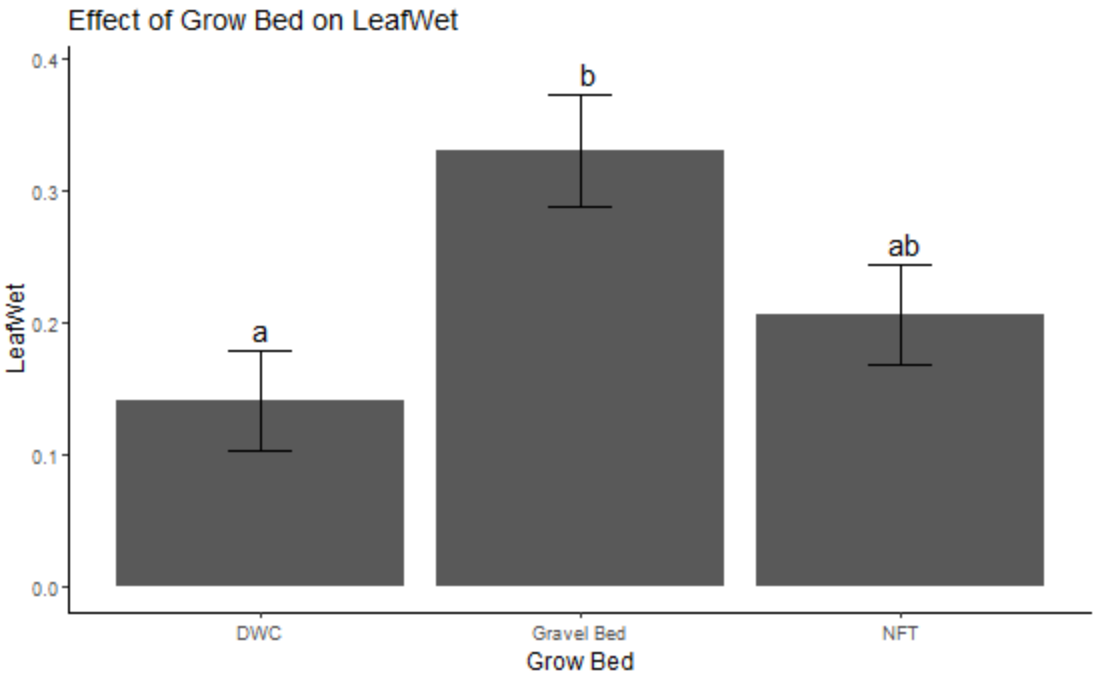
Appendix B: Bacterial load (E. coli) in Aquaponic components



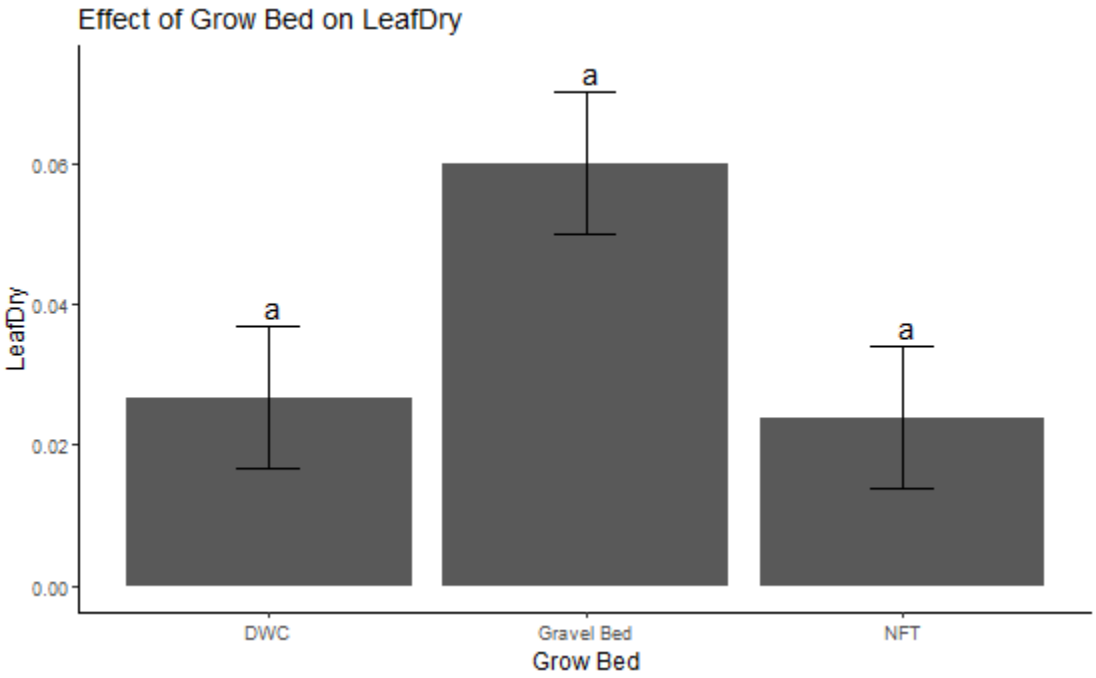
Appendix C: Regression plot for Aquaponics system



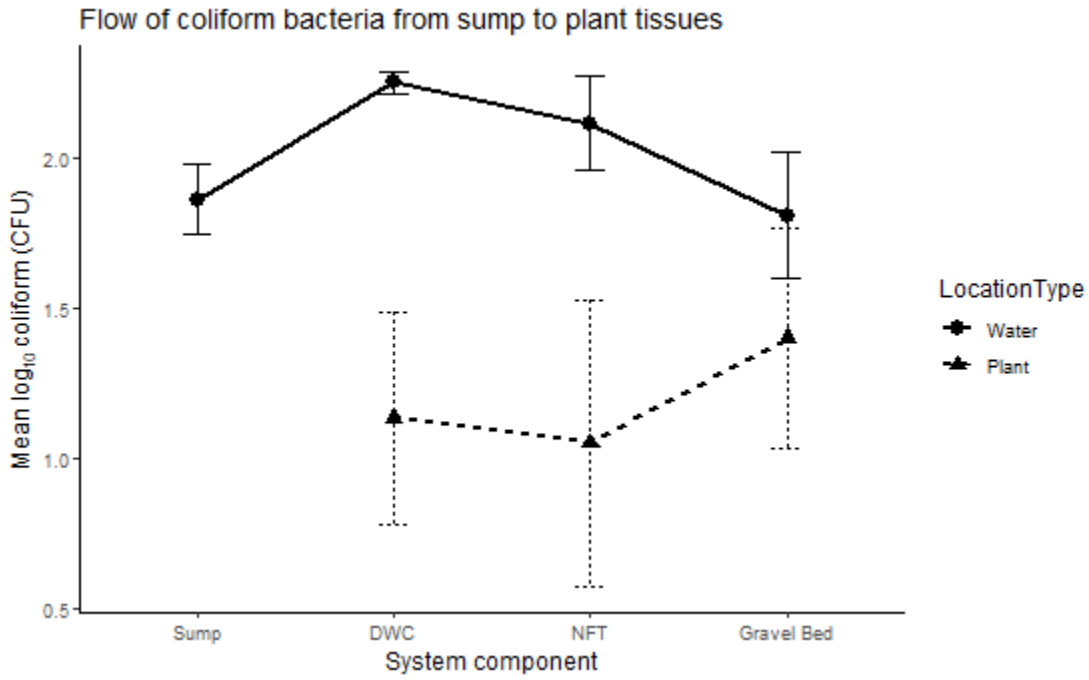
Appendix D: Regression plot for Static Water Renewal system (Stagnant)



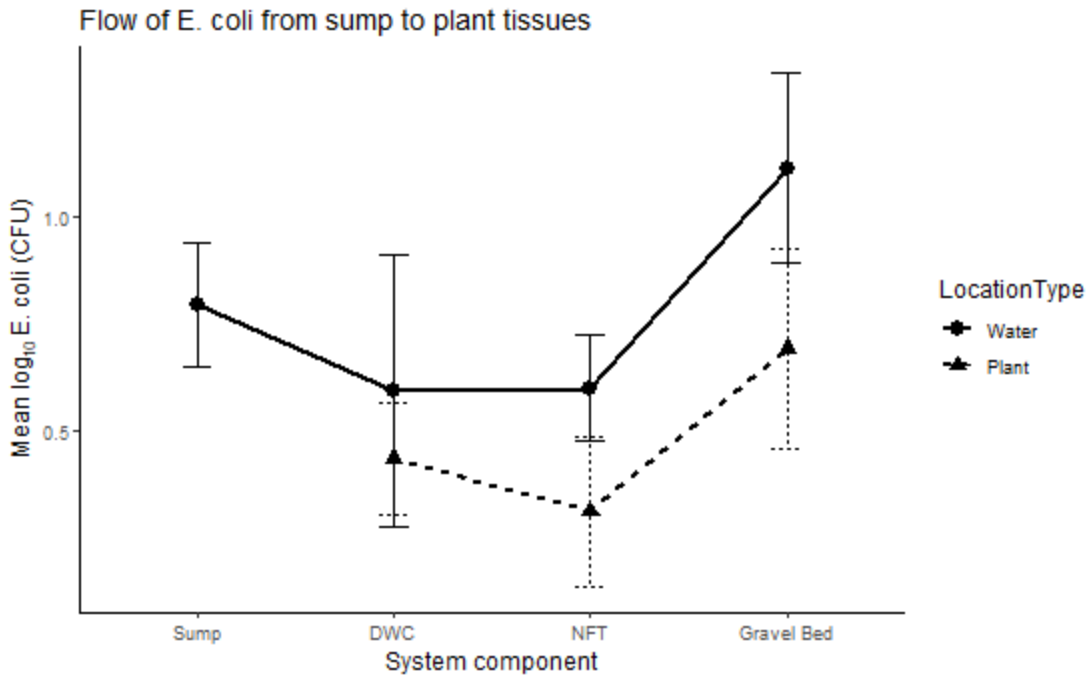
Appendix E: Box plot showing wet leaf weight distribution across grow beds



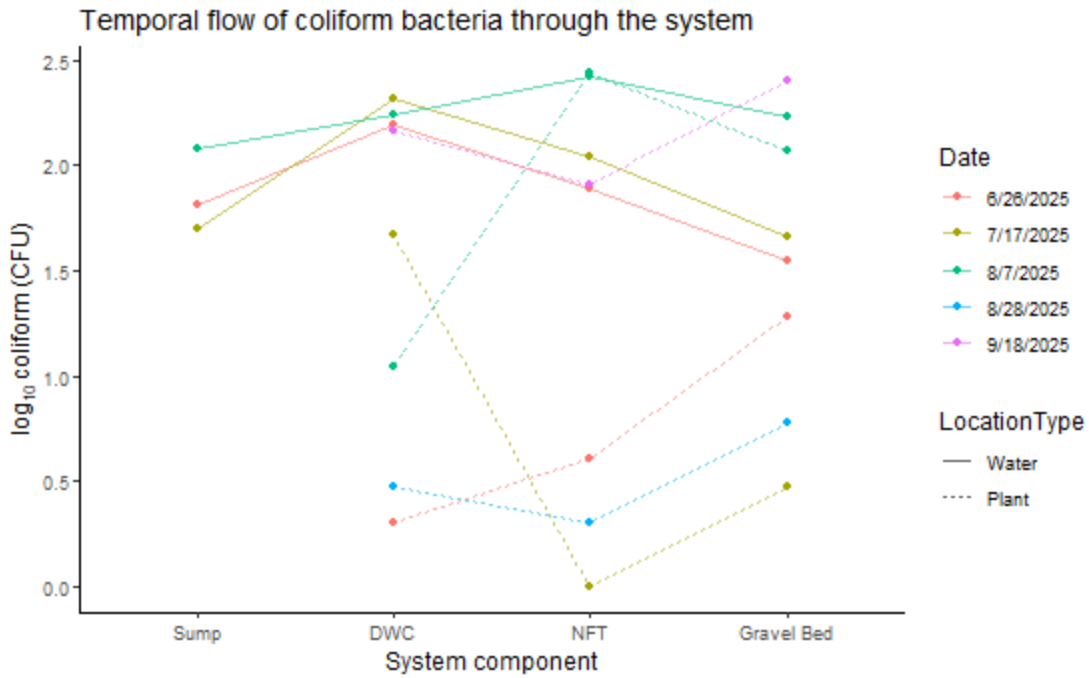
Appendix F: Box plot showing dry leaf weight distribution across grow beds



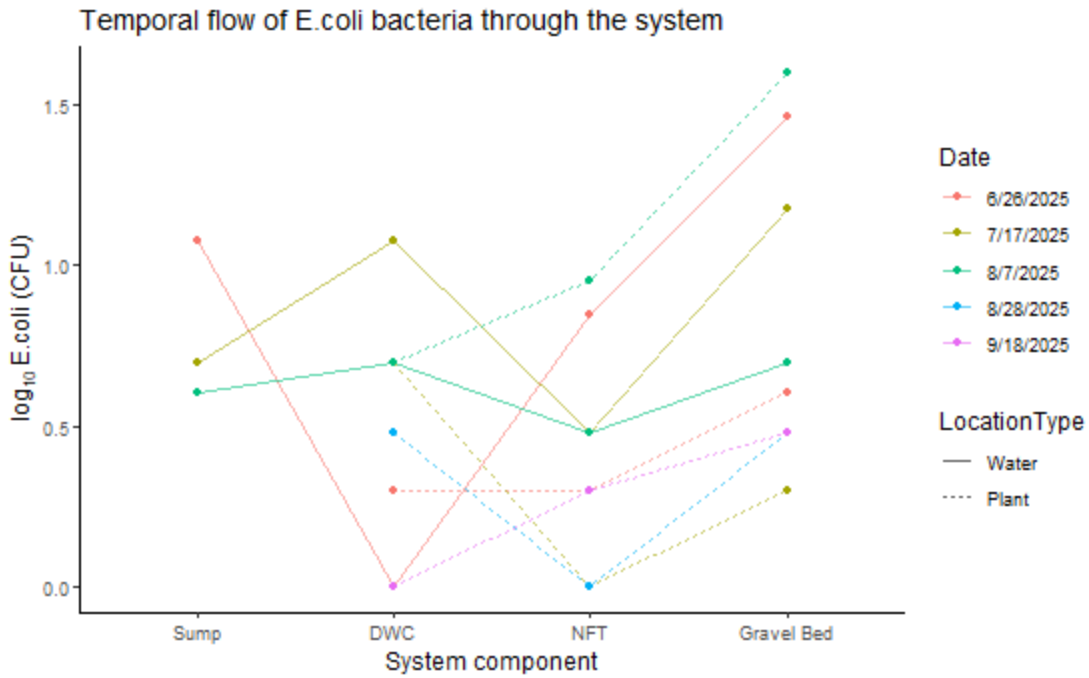
Appendix G: Flow plot showing Coliform flow across all system components



Appendix H: Flow plot showing E. coli flow across all system components



Appendix I: **Flow plot showing temporal coliform trends across all system components**



Appendix J: **Flow plot showing temporal E. coli trends across all system components**